COPPER AND ZINC IN WATER, SEDIMENT AND GASTROPODS IN THE HARBOURS OF THE CAPE TOWN METROPOLE, SOUTH AFRICA

WINSTON FRU

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SUPERVISOR: PROF J.P. ODENDAAL

CO-SUPERVISOR: PROF R.G. SNYMAN

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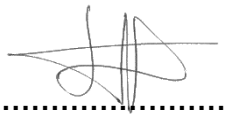
DECLARATION

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

Signed  **…….** **Date**……**10/03/2020**……...

ABSTRACT

The harbours in Cape Town are an important hub for development and socio-economic activities, some of which include shipping traffic, ship repair and maintenance, commercial and artisan fishing, construction (dredging and reclamation) and recreational vessel activities. These harbour related activities have contributed enormously to the influx of contaminants such as metals into the coastal environment. The semi-enclosed nature of the harbours associated with limited water exchange is conducive for water pollution. Thus, harbours are increasingly becoming a hotspot for metal loading into coastal ecosystems.

Copper and zinc are metal-based biocides in present-day antifouling (AF) paints. Concerns have been raised over the effect of these metal ions in the marine environment with high levels being detected in areas of intense vessel activities such as harbour. The aim of the study was to determine concentration levels of copper and zinc in seawater, sediment and gastropods (*Burnupena* spp. *and Nucella* spp.) from selected harbours and reference sites in the Cape Town metropole. Also, to determine the suitability of the two gastropods for use as biomonitors of metal contamination as well as whether there was a causal relationship between copper and zinc content in the gastropods and the concentrations in water and sediment from the harbours.

Samples were collected once-off seasonally in March (dry season) and September (wet season) 2016 from sampling point(s) in the harbours and reference sites at spring low tides. Samples of seawater, sediment and gastropods (soft tissue and shell) were acid digested and metal concentrations analysed in quintuplicate using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Statistical analyses were conducted using the SigmaPlot 13 software. Statistically significant differences in copper and zinc concentrations between sampling points in harbours and the reference sites were evaluated using a Kruskal–Wallis One-Way ANOVA on Ranks and Student Newman Kuels Method for post hoc tests as datasets were non-parametric. Dunn's Method was used for Post hoc test after the ranked based ANOVA to evaluate significant differences in copper and zinc concentrations between harbours (unequal datasets). The Mann Whitney Rank Sum Test was used for comparisons in copper and zinc concentrations between the two seasons per sampling point, per sampling sites and between soft tissue and shell per sampling point. The Spearman's Rank Order Correlation was used to determine if there was a relationship between the metal concentrations in the ambient samples (seawater and sediment) and soft tissue and shells of the gastropods.

The results showed that the mean copper and zinc concentrations (mg/L) in seawater ranged between not detected (ND) to 0.0818±0.0494 and ND to1.7679±0.639, respectively. The corresponding mean concentrations (mg/kg dry weight) of copper and zinc in sediment were

ND to 3432.16±2306.68 and 1.20±1.53 to 2380.43±1456.79, respectively. The highest mean copper and zinc concentrations (mg/kg dry weight) were found in *Nucella* soft tissue with a range of 19.84±6.43 to 2211.61±3168.07 and 77.20±15.14 to 5045.44±2447.15, respectively. The mean copper and zinc concentrations in seawater, sediment and gastropods (soft tissue and shell) were found to be generally higher in the dry season than the wet season. Generally, the mean copper and zinc concentrations in the soft tissue of the gastropods were higher than in the shells. The findings indicated that variations in copper and zinc concentrations in seawater, sediment and gastropods (soft tissue and shells) at sampling points in the harbours could be attributed to proximity to contamination sources, the rate of water exchange, metal handling strategies of gastropods as well as changes in environmental factors. The highest mean copper and zinc concentrations were found at sampling points close to areas of intense vessel-related activities in the harbours. Therefore, it could be suggested that AF paints are a predominant source of copper and zinc in seawater, sediment and the gastropods in the harbours. The correlation analyses revealed that there were generally no significant correlations between copper and zinc contents in the soft tissue or shell of the gastropods (*Nucella* spp.) and the ambient environmental concentrations in the harbours and reference sites although some distinct trends were observed. It was shown that copper and zinc concentrations in the soft tissue or shell of *Nucella* spp. may not be directly affected by those of the ambient seawater and sediment. It may, therefore, be presumed that the changes in copper and zinc loading in seawater and sediment were not the only factors that influenced the level of bioavailability of these metals to the *Nucella* spp. It is possible that the bioaccumulation of copper and zinc in the soft tissue or shell of *Nucella* spp. may have been influenced by many physicochemical and biological parameters. By comparing the data with water and sediment quality guidelines, it was observed that mean copper and zinc concentrations in seawater from some of the sampling points in the harbours exceeded the South African Water Quality Guidelines (SAWQGs). Likewise, the mean copper and zinc concentrations in sediment from some sampling points in the harbours were moderately or seriously polluted based on the Benguela Current Large Marine Ecosystem Sediment Quality Guidelines for southern Africa (BCLME-SQGs). It is, therefore, strongly suggested that source identification and continuous monitoring of copper and zinc in water, sediment and biota in the harbours is imperative.

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"It takes a whole village to raise a child."

(African proverb)

DEDICATION

This thesis is dedicated to the memory of my beloved mother

Mangie Bih Rose (1957-2008)

Her omnipresence continues to regulate my life.

"While we do our good works let us not forget that the real solution lies in a world in which charity will have become unnecessary."

(*Chinua Achebe, Anthills of the Savannah*)

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GLOSSARY

Terms/Acronyms/Abbreviations	Definition/Explanation
AF	Antifouling
BCLME	Benguela Current Large Marine Ecosystem
CBD	Central Business District
DEAT	Department of Environmental Affairs and Tourism
DEA	Department of Environmental Affairs
HELCOM	Baltic Marine Environment Protection Commission - Helsinki Commission
Mg/kg DW	Microgram per kilogram dry weight
Mg/L	Milligram per Litre
PEL	Probable Effect Level
SAWQGs	South African Water Quality Guidelines
SQGs	Sediment Quality Guidelines
TBT	Tributyltin
TEL	Threshold Effect Level
UNEP	United Nations Environmental Programme
US EPA	United States Environmental Protection Agency
IUCN	International Union for Conservation of Nature
Bioaccumulation	It is defined as the process by which organisms accumulate chemicals both directly from the abiotic environment and from dietary sources (Leblanc, 2004).
Biocides	Biocides are active substances that can deter or kill the microganisms responsible for biofouling (EC, 1998)
Biomonitor	A biomonitor is an organism (or a part of an organism or community of organisms) that contains information on the quantitative aspects of the quality of the environment (Markert et al., 2003).
Gastropod	A mollusc of the large class Gastropoda, which includes snails, slugs and whelks (Soanes & Stevenson, 2004).
Harbour	A place on the coast where ships may moor in shelter, either naturally formed or artificially created (Soanes & Stevenson, 2004).

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE PROBLEM

Marine pollution resulting from anthropogenic activities has now become a global environmental concern. According to Pielke (2005), coastal environments have become hubs of anthropogenic activities due to their significant natural resources and favourable geographical locations. Increasing urbanization, industrial development and tourism, coupled with coastal population growth, have resulted in the degradation of coastal ecosystems. The coastal and marine environments are exposed to an array of anthropogenic pollutants including metals. Metals occur naturally in the marine environment, however anthropogenic activities may increase metal influx into the coastal environments through a variety of point and non-point sources, including industries, wastewater and domestic effluents, agricultural runoffs, atmospheric deposition, boating and recreational uses, oil and chemical spills as well as anti-fouling paints on vessel hulls (Birch et al., 1998; Fu & Wang, 2011; Berto et al., 2012). Metals are considered as one of the major anthropogenic pollutants in coastal areas worldwide (Ruilian et al., 2008). They pose a serious threat to human health, living organisms, the intrinsic structure and functioning of ecosystems and the goods and services these ecosystems provide, due to their toxicity, persistence and bioaccumulation characteristic (DeForest et al., 2007; Roose et al., 2011). The current concerns on metal pollution in the marine environment was brought to the limelight after the Minamata incident in Japan, where humans were directly exposed to the deleterious effects of mercury after consuming contaminated fish, and the 'Itai Itai' disease in Niigata caused by the consumption of cadmium contaminated food (Nammalwar, 1983). Over the past few decades, the state of the marine environment with respect to metal pollution has been a subject of growing international concern. The Mussel Watch Programme (MWP), initiated in the United States of America in 1976, was one of the first environmental monitoring programmes, which made use of living organisms in an extended geographical area (Goldberg, 1975; Goldberg et al., 1978). The MWP has been the basis of coastal environmental pollution monitoring worldwide (Cantillo, 1998).

In South Africa, the MWP was initiated towards the end of 1985 by the South African National Committee for Oceanographic Research (SANCOR) through which the Marine Pollution Research Programme (MPRP) was developed from 1985 to 1990 (SANCOR, 1985). The MPRP acted as a framework with the objectives to provide relevant and sound scientific advice to authorities on management and effects of pollutants such as metals in the marine

environment (SANCOR, 1985; Wepener & Degger, 2012). Within this framework, a data set was gathered on water, sediment and fauna accumulation to be used for comparative purposes both regionally and internationally (Hennig, 1985). There was no pollution monitoring programs after 1990 and research initiatives which focused on ecotoxicology and biomonitoring were for the most part done by universities and private consultancies (Donoghue & Marshall, 2003). From 1960-1990, the most commonly measured contaminants in South African marine pollution studies (i.e. 44% of studies) had been on metals. In the present-day, marine pollution monitoring in South Africa is conducted by Department of Environmental Affairs, Branch Oceans and Coasts (DEA: O&C). The scientific data gathered by this program is not made public; however, only limited results have been published in selected State of the Environment Reports. The South African marine environment is a rich and diverse national asset which provides important socio-economic opportunities for an ever-increasing population (Attwood et al., 2002). An estimated 30% of South Africa's population live along the coastline, which has led to rapid urbanization and industrialization of coastal areas (Taljaard et al., 2006). This rapid transformation has resulted to increase anthropogenic-derived pollutants such as metals which enter the coastal and marine environment. Coastal areas function as a sink for contaminants such as metals incoming from adjacent catchments, up-watershed and nearby land-based activities, but then again they are also a source for these contaminants to the adjoining coastal marine environments (Kennish & Fertig, 2012).

The Cape Town metropole (thereafter Cape Town) is situated along the coastline of South Africa and shares the same burdens as other coastal areas worldwide. It is estimated that one-fifth of South Africa's coastline has some form of development within 100m of the shoreline (SANBI, 2013). The propensity for urban and industrial development in South Africa, especially around major coastal cities such as Cape Town, raises concerns about the potential deterioration of the coastal marine environment from anthropogenic derived chemical pollutants such as metals. As a result, there has been an ever-increasing need to assess, monitor and predict the impacts that these pollutants have had and may have in future on the South African coastal and marine environment.

There is a wide range of monitoring methods being used to evaluate metal pollution status of the coastal and marine ecosystem of South Africa. Amongst these monitoring methods is the conventional chemical analysis of the abiotic matrices (water and sediment) as well as the use of living organisms (biological monitoring).

1.2 STATEMENT OF THE RESEARCH PROBLEM

The coastal and marine environment of Cape Town provides a wide range of ecological and socio-economic functions. Increasing urbanization and industrialization coupled with increasing population density along the coastline of Cape Town is threatening its coastal ecosystems. Cape Town's harbours are an important hub for development and socio-economic activities, some of which include shipping traffic, ship repair and maintenance, commercial and artisan fishing, construction (dredging and reclamation) and recreational vessel activities. These harbour activities have contributed enormously to the influx of contaminants such as metals into the coastal environment. The semi-enclosed natures of the harbours associated with limited water exchange are conducive for water pollution. Thus, harbours are increasingly becoming a hotspot for metal loading into coastal ecosystems (Bighiu, 2017). According to Long (2000), metals have been recognised as one of the most ubiquitous of contaminants in harbours worldwide. They may occur in water and sediments and bioaccumulate in the tissue of many organisms (Lahbib et al., 2013), and may pose a severe risk to the ecosystem and human health because of their toxicity, persistence and bioaccumulation characteristics (DeForest et al., 2007). Vessels have been found to be a potentially significant source of metal contaminants such as copper and zinc to coastal marine waters. The ban on tributyltin (TBT) antifouling (AF) paints in 2003, and its ratification in 2008 (IMO, 2002) resulted in the renewed use of metal-based AF paints such as copper-based AF paint (Schiff et al., 2004; Yebra et al., 2004; Paradas & Filho, 2007). For over a century copper has been the main biocide in antifouling marine paints and even with the introduction of TBT, copper was still used as a co-biocide (Blossom, 2015). Most present-day marine AF paints contain a core biocide in the form of copper oxide or, less commonly, copper thiocyanate (Turner, 2010) and a variety of co-biocides to enhance the overall toxicity or facilitate leaching. Zinc oxide is one of such co-biocides to copper and also by itself a core biocide in AF paints (Watermann et al., 2005).The gradual, controlled leaching of these biocides into the marine environment may exert unintended toxic effects on non-target organisms (Karlsson et al., 2010). Although copper and zinc are essential micronutrients for many organisms, used in enzymes involved in metabolic processes (White & Rainbow, 1985), they may become toxic depending on their concentration and speciation in an aquatic environment (Sunda, 1989). Concerns have been raised over the effect of these metal ions in the marine environment with high levels being detected in areas of intense boat activities (Srinivasan & Swain, 2007). Unfortunately, these levels are likely going to exacerbate with increasing harbour development and the increasing use of copper-based AF paints to replace the banned TBT paints. This may be coupled with other influxes such as industrial and wastewater discharge, surface run-off and atmospheric deposition. Studies in Europe (e.g., Hall & Anderson, 1999) and in the United

States (e.g., Flegal & Safiudo-Wilhelmyt, 1993; Schiff et al., 2007; Srinivasan & Swain, 2007), have documented copper contamination in marine environments from AF paints. Elevated concentrations of copper and zinc from AF in semi-enclosed areas such as harbours may pose a potential ecological risk to marine organisms. Other sources of metals (e.g., industrial and wastewater discharge) in the marine environment have been well documented (Alexander & Young, 1976; Al-Muzaini, 2013; Drira et al., 2017), but it is not generally recognized that vessel protective AF paints also constitute a potentially significant source of copper and zinc to coastal environments. High concentrations of copper and zinc in sediment from harbours in the west coast of Sweden, including natural harbours in pristine areas have been linked to the use of AF paints (Eklund et al., 2016). In the UK, Boxall et al. (2000) estimated that up to 2 kg copper per boat per year may leach from larger leisure vessel painted with copper-based antifouling products. Globally, it has been estimated that AF account for 1.5×10^6 Kg/year of copper input into seawater (Blossom, 2015), and according to Srinivasan & Swain (2007), most water quality assessments do not consider AF paints use as a potential source of copper contamination. According to the Biocide Directive (98/8/EC) (EC, 1998), zinc is not considered to be a biocide with respect to AF paints. To date, there is yet to be a suitably effective and environmentally safe antifouling biocide with no adverse environmental effects on non-target organisms (Lindgren et al., 2018). Considering the impact of metals on the marine environment and the lack of enough existing information on the impact of vessel activities in harbours of Cape Town, the determination of metal (copper and zinc) concentrations is imperative. This research, therefore, stems from the need for continuous and systematic monitoring of the health status of these fragile coastal marine ecosystems in relation to metal pollution to develop sound environmental management strategies and ensure responsible development while maintaining socio-economic benefits and ecological sustainability.

1.3 RESEARCH QUESTIONS

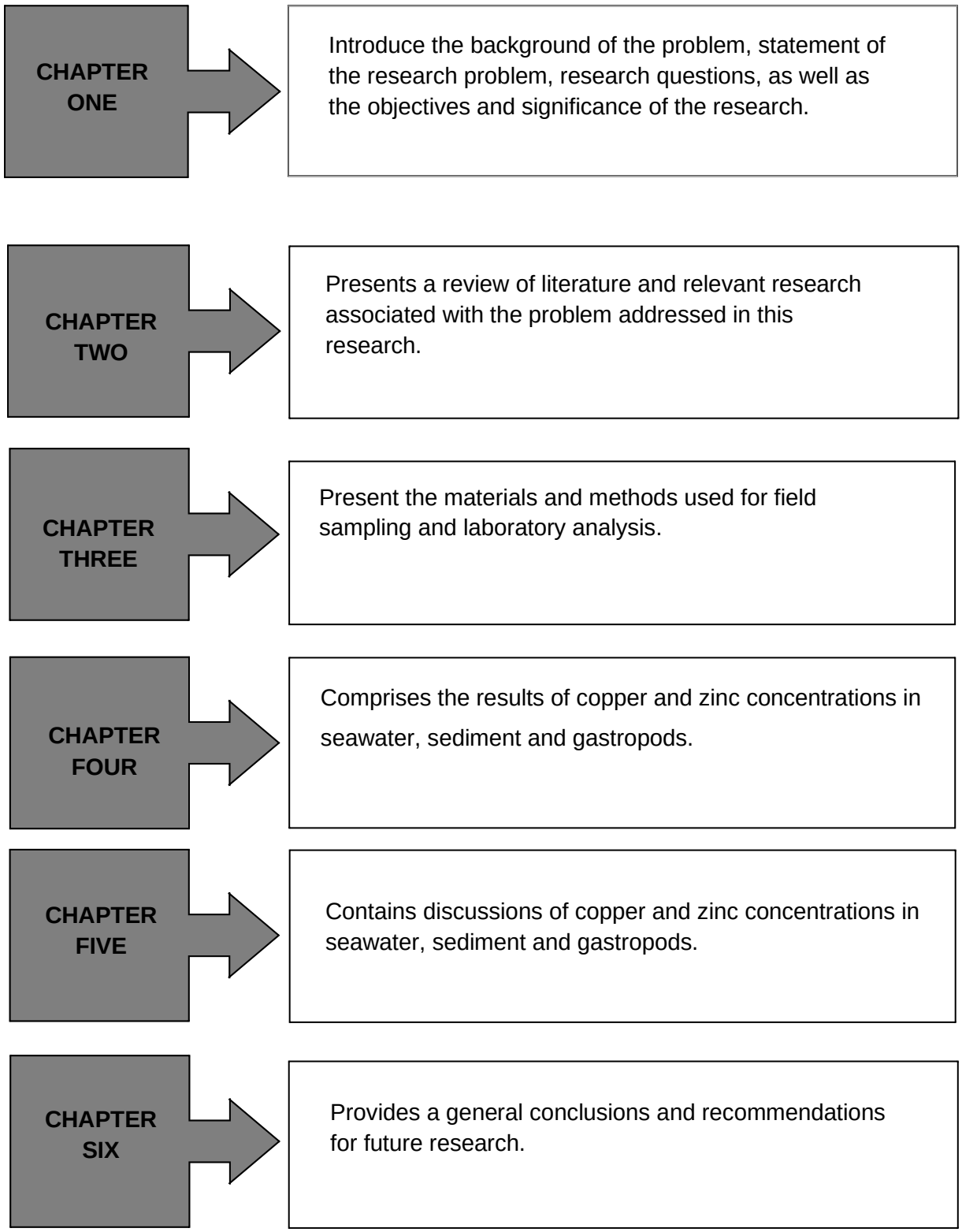
This study is aimed to answer the following research questions:

- What is the extent of copper and zinc contamination in water and sediment in selected harbours within the Cape Town Metropole?
- What are the levels of copper and zinc bioaccumulation in gastropods in selected harbours within the Cape Town Metropole?
- Are gastropod species suitable biomonitors?
- Is there a causal relationship between environmental copper and zinc concentrations and those found in gastropod species?

1.4 OBJECTIVES OF THE RESEARCH

1. To determine copper and zinc concentrations in surface water and sediment in selected harbours within the Cape Town Metropole.
2. To determine the levels of copper and zinc bioaccumulation in the two gastropods from the harbours under study.
3. To determine the suitability of the two gastropods for use as biomonitors of metal contamination.
4. To determine whether there is a causal relationship between copper and zinc content in the gastropods and the concentrations in water and sediment collected from the harbours.

1.5 ORGANISATION OF THE THESIS



CHAPTER TWO

LITERATURE REVIEW

2.1 SOUTH AFRICAN COASTAL AND MARINE ENVIRONMENT

The coastal and marine environment is an integral part of the global life support system and a positive asset that presents opportunities for sustainable development (UNCED, 1992). Although not always immediately apparent, our wellbeing as humans is influenced by the environmental state of our coastal and marine environment, as many aspects of our lives benefit from the goods and services provided by well-functioning coastal and marine ecosystems. In addition to the well-known economic value of fisheries, coastal and marine ecosystems support an array of related economic industries such as shipping, oil and gas industries, offshore wind energy and tourism. Also, the world's oceans and coasts provide for vital services in maintaining ecological diversity, climate regulation, nutrient cycling, carbon storage and more. It is estimated that two-thirds of the value of all the natural services provided by our natural environment is supplied by the seas and oceans (GESAMP, 2001). The South African coastal and marine environment spans two of the world's sixty-five Large Marine Ecosystems (LMEs); the Benguela Current LME and the Agulhas Current LME (NOAA, 2004). It is endowed with natural resources which are a rich and diverse national asset, providing momentous economic and social opportunities for an ever-growing population that has developed a strong dependence on these resources for their livelihood, economic gain, recreation and transport (Wynberg, 2000). The coastal and marine environment of South Africa is unique with two contrasting current systems (Figure 2.1); the warm Western Boundary Agulhas Current that flows southwards along the east coast from the Indian Ocean and the cold Eastern Boundary Benguela Current that flows northwards along the Atlantic coast to the west. The Agulhus current brings southward nutrient-poor tropical waters with very diverse biota from the rich Indo-Pacific region and coastal waters are characteristically blue and clear (Lombard et al., 2004). The average sea surface temperatures in the region show a decline of about 2^{o} C moving from north to south, with maximum average temperatures of about 28^{o} C in summer and 23^{o} C in winter in the north and 25^{o} C in summer and 21^{o} C in winter in the south (Lutjeharms, 2006). The productive Benguela current comprises a general equatorward flow of cool water in the South Atlantic Subtropical Gyre with an inshore dynamic wind-driven upwelling controlled by local weather systems, resulting in short-term upwelling cycles (Shannon, 1985). The mean monthly sea surface temperatures range from 15.4°C to 20.1°C

offshore (Taunton-Clark & Shannon, 1988), but in the upwelling region nearshore, variability is greater and temperatures range from 10°C to 18°C (Shannon et al., 1992). Upwelling is brought about by the predominant south-easterly winds that blow parallel to the west coast and pushes surface waters before it, and under the influence of Coriolis forces, the water is deflected offshore away from the coast resulting in deep cold waters rising to the surface (Branch & Branch, 1995) (Figure 2.2). These deep waters are nutrient-rich and are favourable for plant growth, having accumulated beneath where the absence of light has prevented plant life from utilizing the nutrients. Intense upwelling along the west coast may cause phytoplankton to flourish resulting in high biological productivity which in turn support an abundance of marine life thus underpinning large-scale fishing and mariculture industry along the coast (Branch & Branch, 1995).

Approximately 3650km in length (Lombard et al., 2004), the South African coastline is divided into three distinctly broad biogeographic regions: the cool temperate West Coast, the warm temperate South Coast and the subtropical East Coast (Stephenson & Stephenson, 1972; Brown & Jarman, 1978; Bustamante & Branch, 1996; Attwood et al., 1997; Lombard et al., 2004; Wepener & Degger, 2012) (Figure 2.1). The further classification has divided these biogeographic regions into six coastal and marine inshore and offshore ecoregions (Sink et al., 2012; SANBI, 2013). These six ecoregions which are bounded by the Exclusive Economic Zone (500m inland of the tide line to 200 nautical miles out to sea) are the Southern Benguela ecoregion; the southeast Atlantic ecoregion; the Agulhas ecoregion; the Natal ecoregion; the Delagoa ecoregion; and the Southwest Indian ecoregion. The distinct oceanographic variability, biological productivity, dissolved oxygen and the distinctiveness in temperatures is reflected in the division of the ecoregions. The coastline of South Africa consists of various types of benthic substrate including several sandy (42%), rocky (27%) and mixed substrata (31%) mostly characterized by sand on the upper shore, above a wave-cut rocky platform (Bally et al., 1984; Sink et al., 2012).

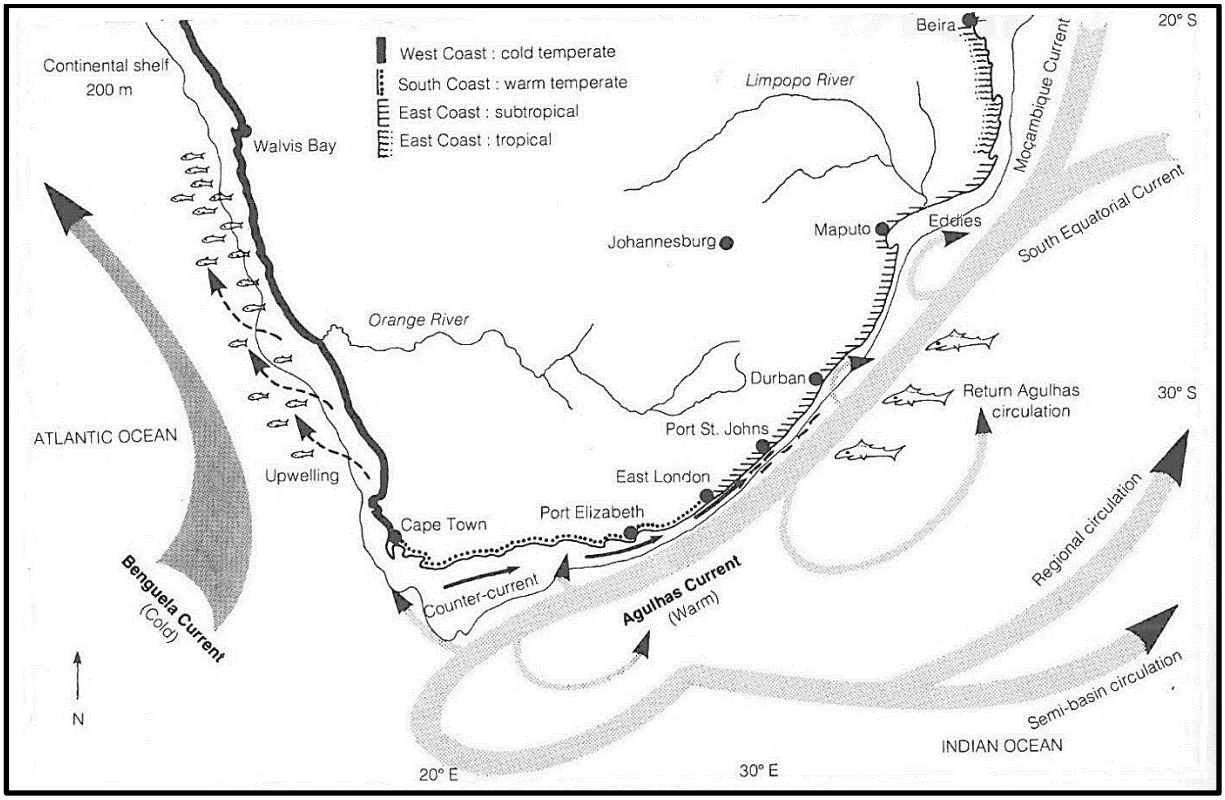


Figure 2. 1: Map of Southern Africa showing the two contrasting currents and the biogeographic regions. Source: (Branch & Branch, 1995)

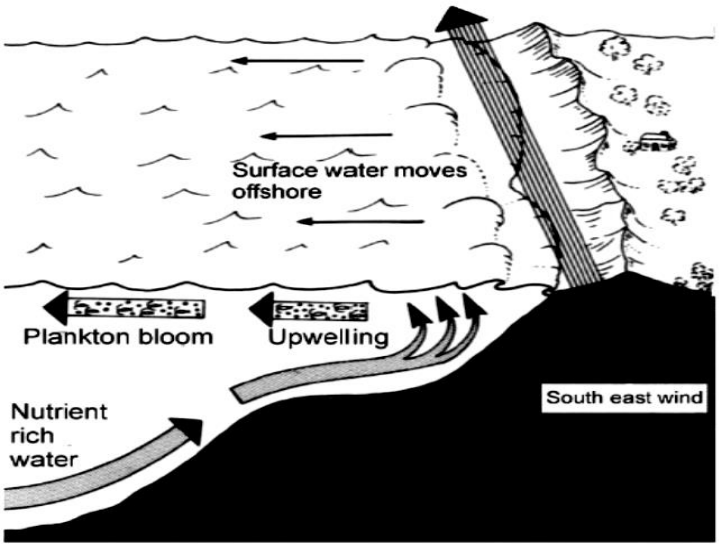


Figure 2. 2: An illustration of the upwelling process. Source: (Branch & Branch, 1995)

2.2 POLLUTION OF THE COASTAL AND MARINE ENVIRONMENT

2.2.1 Urbanization and industrialisation

Increased contamination of the marine environment, particularly in the coastal areas, has been associated with urbanisation and industrialisation along the shorelines. According to Forbes & Forbes (1994), approximately 70% of the human population resides within 60 km of the coastal area, and a significant proportion of the world's largest cities are linked either directly or indirectly, to the marine environment. South Africa has the largest and most industrialized economy in Africa, with a population of about 50 million and urbanization is estimated at 62% (Turok, 2012), of which half reside in the two major coastal cities of Cape Town and Durban (Prochazka et al., 2005). Over the past five decades, coastal cities around the world have grown dramatically and are predicted to likely continue to expand for the foreseeable future (Tibbetts, 2002). According to Tibbetts (2002), the main reasons for this expansion are the appeal of living in proximity to the coast, increase tourism, sufficient wealth for coastal retirement opportunities, an increase in coastal holiday-home purchases, and the quest for economic opportunities and basic livelihood. In coastal countries today, almost half of the total population live along the coastline and migration from often economically depressed rural inland areas to the coast is growing (DEA, 2012). As an interface between the land and the sea, the coastline has become a hotspot for urban concentration and intense anthropogenic activities. According to Costanza et al. (1997; 2014), coastal areas are among the most productive and valuable in the world, providing an array of essential goods and services to society, such as the provision of food, fuel, trade and recreational opportunities. The South African coastline with unique ocean current systems is highly productive and rich in biodiversity (DEA, 2012). According to Atkinson & Clark (2005), approximately 40% of South Africa's population resides within 100km of the coastline. In the Western Cape Province, the majority of people live within 25 km of the coast (DEADP, 2005), which has accelerated extensive urbanization and industrialization. Cape Town is one of the main coastal cities in South Africa with a population of over 3.5 million people (66% of the Western Cape population) (WESGRO, 2013). It has an extensive coastline of 307km (DEA, 2012), that stretches from Gordon's Bay to Atlantis, and characterized by a highly sensitive, complex and dynamic coastal environment. Cape Town's coastline is an important economic, social and environmental asset providing the communities and visitors with a multitude of social and economic benefits and opportunities as well as essential Ecosystem Goods and Service (CoCT, 2015). Cape Town is the economic and administrative hub of the Western Cape as well as the legislative capital of the Republic of South Africa. With such socio-economic and political status come urbanization, infrastructural development, land reclamation for port and industrial development, habitat

modification, tourism and recreational activities. These ongoing transformations have undoubtedly resulted in an increase in marine discharges from urban stormwater runoff, wastewater treatment plants, industries, power stations, agriculture, shipyard activities and recreational activities. Consequently, a total load of anthropogenic-derived pollutants (such as metals) being delivered into the coastal and marine environment has increased (Natesan & Seshan, 2010).

2.2.2 Marine pollution

Degradation of the coastal and marine environment has continued globally, and in many places even increased (UNEP, 2006a). The coastal and marine environments, which are among our most important food sources, are also an undisputable reservoir for pollutants. Pollution is one of the major stressors that influence the quality and health of the environment, posing potential threats to ecosystem services and living organisms (El-Shenawy et al., 2016). The major threats to the health, productivity, and biodiversity of the marine environment result from anthropogenic activities in both coastal and inland areas. The oceans are so vast and deep that it has been viewed for centuries to accommodate waste without significant changes and to have the ability to dilute toxic waste to innocuous levels or carry it away from the coastline with its currents (O'Neill, 1993). It has been the ultimate depository for humanity's wastes since before the dawn of civilisation. According to Brown (1978), it was believed that substances entering the sea simply become more and more diluted by this huge body of water until their concentrations are negligible, and so the sea was regarded as a vast sink into which anything could be dumped with impunity. Proponents of dumping in the oceans even had a catchphrase: "The solution to pollution is dilution." However, it is becoming increasingly certain that the increase in the rate of pollutant input is influencing coastal ecosystems. According to O'Neill, (1993), pollutants may be regarded as any introduced substance which may harm a resource and includes substances that are usually present in the environment but have exceeded natural levels due to anthropogenic input. The United Nations Convention on the Law of the Sea (UNCLOS) defines marine pollution as: "the introduction by man, directly or indirectly, of substances or energy into the marine environment, including estuaries, which results or is likely to result in such deleterious effects in harm to living resources and marine life, hazards to human health, hindrances to marine activities including fishing, impairment of the quality of use of sea water and reduction of amenities" (UNCLOS, 1982). William (1996) criticized the division of pollution into categories (e.g., air, water, land, etc.), and according to him pollution is only one, as every pollutant, whether it is in the air, or on land tends to find its way into the ocean (Shahidul Islam & Tanaka, 2004). Pollutants may enter the coastal and

marine environment through either point or non-point sources. Marine pollution from land-based sources poses one of the most serious threats to the quality and productivity of the coastal and marine environment (Williams, 1996). An estimated 80% of the pollution load in the coastal and marine environment originates from land-based sources, including municipal, industrial and agricultural run-off, as well as atmospheric deposition (UNEP, 2006b). Chemical such as oil-based products, pesticides, fertilizers, accidental oil spills, and antifouling paints are of major environmental concern as they may cause deleterious effects in the coastal and marine ecosystem.

The status of South Africa's coastal and marine environment was reported by Brown (1987), Griffiths et al. (2004) and DEAT (2006) to be in a moderately healthy state with respect to international trends. However, over the past decade, deterioration in the health of South Africa's coastal and marine environment is evident, suggesting that the status of this environment is now better classified as marginally healthy (DEA, 2012). Pollution of South Africa's coastal and marine environment stems mostly from land-based sources (e.g. industrial and municipal discharges, stormwater and agricultural runoff), atmospheric pollutants and maritime sources (e.g. accidental or deliberate discharges, dumping and antifouling coatings) (Wynberg, 2000), the traditional foci of attention regarding marine pollution. South Africa has a well-conserved coastline when compared with many other developing countries and marine pollution is limited predominantly to the densely populated KwaZulu Natal coast and the coastal urban cities of Port Elizabeth and Cape Town (Griffiths et al., 2010). There are approximately 75 outfalls within the coastal waters of South Africa of which 39 are in the Western Cape Province (DEA, 2012). These outfalls discharge wastewater either to the surf zone, inshore or directly into estuaries. According to DEA (2012), approximately 287 million cubic meters of wastewater per annum is discharged into the marine environment from land-based sources. These wastewater discharges comprise mainly municipal wastewater, effluent from fish processing operations, wastewater from chemical works, refineries and other industries, and cooling water (Sink et al, 2012). Many of South Africa's marine outfalls are monitored; though many surf zone and estuaries are not. However, urban stormwater runoff and untreated sewage from informal settlements are difficult to control or predict. Wastewater (sewage and industrial effluents) contains a diverse array of pollutants including metals, which may pose a direct or indirect effect to the coastal and marine ecosystem.

In recent years, maritime traffic on the world's oceans has increased dramatically thus increasing the risk of pollution caused by shipping (Tournadre, 2014). South Africa is a maritime nation positioned along one of the world's busiest shipping routes with several major ports including that of Cape Town. There is substantial shipping traffic in South Africa's coastal waters, with approximately 12,000 ships visiting its ports yearly (Rantsoabe, 2014). It is

estimated that about 120 million tons of oil and large volumes of bunker fuel sail through South African waters annually (IMO, 2005). This implies that South Africa has one of the highest concentrations of oil tankers and cargo ships in the world. This high shipping traffic coupled with the prevailing oceanographic conditions along the coast renders South Africa's waters vulnerable to pollution from operational and accidental discharges and ship groundings (IMO, 2005). The most recent major accidental discharges along the South African coast were the Treasure in 2000, the Apollo Sea in 1994, and the Katina-P in 1992, off the coast of Mozambique that travelled south with the Agulhus currents to the coastline of South Africa. Most of these accidents are from tankers transporting crude oil. The main sources of ocean-based pollution are from the shipping industry. These include accidental oil spills, deliberate discharge of ballast water, deliberate discharge of oily waste from vessels at seas and vessel maintenance activities. Also, the fact that South Africa is positioned along one of the world's shipping routes means a constant exposure to the leaching of biocides from antifouling paint used on vessel hulls into the ocean waters.

2.2.3 The legislative framework for marine pollution in South Africa

South Africa is committed to the protection of marine biodiversity, ecological integrity and the sustainable use of natural resources. These commitments have been sanctioned under several international conventions and agreements as well as embedded in national legislation and policies.

The ratification of the United Nations Convention on the Law of the Sea, in 1982 was the first momentous effort for a global response to the protection of the coastal and marine environment from pollution. This commitment was reinforced at the United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro, in 1992, with the adoption of Agenda 21 by participating countries including South Africa. Agenda 21 highlighted the need for a global response to environmental degradation and provided a blueprint for sustainable development. This is enshrined in the constitution of South Africa (108 of 1996, Section 24) which makes provision for the protection, conservation and sustainable use of the environment for present and future generations. The constitution also provides citizens with the right to an environment that is not harmful to their health or well-being and obliges the state to secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development.

Further commitment has been made by South Africa through the Global Plan of Action (GPA), an effort which was initiated by the United Nations Environment Programme (UNEP) to actively address the issue of land-based pollution sources in coastal areas globally. South Africa was

one of the 108 nations that adopted the GPA, of which its implementation was reinforced through the adoption of the Beijing Declaration in 2006. The GPA required governments and regional organizations to protect the coastal and marine environment from land-based pollution sources through the identification of the fates and impacts of the pollutants and the management and control thereof (Wepener & Degger, 2012).

South Africa is a signatory to several other international agreements and conventions on marine pollution some of which includes: the London convention for the prevention of marine pollution by Dumping of Waste and other Matter; the International Convention for the Prevention of Pollution from Ships (MARPOL); the International Convention on the Control of Harmful Anti-fouling Systems in Ships (AFS Convention); the Convention for the Conservation of Migratory Species; and United Nations Convention on Biological Diversity. International trends, public pressure and governments efforts to minimise or prevent the degradation of South Africa's coastal and marine environment have led to the promulgation of various legislative acts such as: the Marine Pollution Act (6 of 1981); the National Environmental Management Act (107 of 1998); the Environmental Conservation Act (73 of 1989); the Marine Living Resource Act (18 of 1998); the National Environmental Management: Biodiversity Act (10 of 2004) and the most recent, the Integrated Coastal Management Act (24 of 2008).

The Integrated Coastal Management Act is aimed at maximizing the benefits provided by coastal and marine environments and minimising the conflicts and deleterious effects of anthropogenic activities on human health, resources and on the environment. It promotes the conservation of the coastal environment, and the maintenance of the pristine characteristics of coastal landscapes and seascape while ensuring that the development and use of natural resources in the coastal zones are socially and economically justifiable, as well as being ecologically sustainable (Celliers et al., 2009). Although Glavovic, (2006) described the legislation that caters for the South African marine environment as extremely fragmented, significant progress has been made for an integrated approach through the Integrated Management Act. However, there is a need to establish a strong monitoring component within the marine legislation framework (Wepener & Degger, 2012).

2.3 METAL POLLUTION IN THE COASTAL MARINE ENVIRONMENT

Metals are found naturally in the coastal and marine environment at low concentrations (Ansari et al., 2004). However, increased anthropogenic activities have inevitably contributed to high levels of metal concentrations in the coastal and marine environment. Metals enter the coastal and marine environment through a variety of point and non-point sources. Anthropogenic

metals have been introduced into the coastal and marine environment directly by industrial activities, sewage treatments, urban discharges, atmospheric depositions and also by the use of biocides in anti-fouling paints on vessel hulls (Berto et al., 2012). As a result, the burden of metals has become a serious environmental concern to marine organisms and to human health.

Harbours are a repository of various contaminants including metals due to their proximity to land-based pollution sources and their associated vessel-related pollution sources. In coastal areas, harbours are often altered for commercial and recreational purposes (Johnston et al., 2011) and have become a hotspot for intense development associated with urbanization. This has resulted in an increase in anthropogenic activities such as commercial fishing, recreational yachting (marinas), vessel maintenance and repair, and constructions (dredging and reclamation). According to Denton et al. (2005), harbours are often areas of severe marine pollution due to their associated vessel activities. Their semi-enclosed nature may restrict water circulation which may contribute to high pollutant concentrations (Schiff et al., 2007). This is because pollutant inputs might not be sufficiently flushed into open oceans but rather accumulate to ecologically harmful levels (Owen & Sandhu, 2000; Schiff et al., 2007). Studies such as Matthiessen et al. (1999) and Hall & Anderson (1999) have found concentrations of metals in the water column from vessel harbours to be much higher than from sheltered estuarine or open coastal areas. The 307km stretched coastline of Cape Town is no exception to international trends. The coastline is endowed with natural harbours most of which have been transformed into calm and sheltered waters for navigation and mooring of commercial, recreational and naval vessels. The most noticeable of such transformation is the Port of Cape Town which is the second busiest container port in South Africa and several commercial and recreational fishing fleets, marinas and naval bases. These anthropogenic transformations may contribute enormously to the release of contaminants such as metals into the coastal and marine environment. Metals in water and sediment as well as through trophic transfer can have deleterious effects on marine organisms (Zyadah, 1995). Metals such as copper and zinc have been found to occur at high concentrations in areas of intense vessel traffic such as harbours worldwide (e.g. Young et al., 1979; Barber & Trefry, 1981; Claisse & Alzieu, 1993; Debourg et al., 1993; Madsen et al., 1998; Matthiessen et al., 1999; KEMI, 2006; Jones & Bolam, 2007; Paradas & Amado Filho, 2007; Karlsson et al., 2010; Gadd & Cameron, 2012; Berto et al., 2012). For example, a study by Schiff et al. (2007) in San Diego Bay vessel traffic areas, dissolved copper concentrations in surface waters ranged from 0.001-0.021mg/L with an average of 0.0085mg/L which was above the Environmental Quality Standard of 0.005mg/L copper in 86% of the sampled areas. Elevated zinc concentrations of 0.01-0.04mg/L have been observed in areas of intense vessel activities in some estuaries in the United Kingdom

(e.g. Matthiessen et al., 1999; Boxall et al., 2000). In South Africa, Okoro et al. (2014) reported mean copper and zinc concentrations in sediment from Cape Town Harbour in the range of 54.808±8.64 to 668.48±212.00mg/kg and 226.70±93.06 to 7429.64±3082.75mg/kg, respectively. Also, Fatoki & Mathabatha (2001) reported mean copper concentrations in seawater and sediment from East London Harbour in the range of 0.001 to 0.0204mg/L and 17.9 to 106mg/kg, respectively, whereas those in Port Elizabeth Harbour ranged between 0.0013 to 0.0064mg/L and 22.7 and 68.5mg/kg, respectively. Mean zinc concentrations in seawater and sediment from East London Harbour ranged between 0.0013 to 0.0133mg/L and 42.5 to 246mg/kg, respectively, whereas mean zinc concentrations in Port Elizabeth Harbour ranged between 0.0027 to 000.94mg/L and 41.7 to 132 mg/kg, respectively, during the same study.

2.3.1 Copper

Copper is a transition metal with atomic number and atomic weight of 29 and 63.54, respectively, and with two stable isotopes: Copper-63 and Copper-65 with relative abundances of 69.2% and 30.8%, respectively (Adriano, 1986a; Wright & Welbourn, 2002). It belongs to group IB of the periodic table (Adriano, 1986a). copper is a ubiquitous metallic element widely distributed especially in sulfide, arsenide, chloride and carbonate deposits (Sadiq, 1992), and is ranked 25th in abundance among the elements in the earth's crust (Taylor, 1964).

2.3.1.1 Sources of copper in the environment

The history of copper use is undoubtedly as old as human civilization and presently there are multifarious sources to the environment. In the marine environment, these sources include natural weathering of rocks and minerals containing copper, release from sediment back into the water column, release from organisms and release from anthropogenic inputs (Srinivasan & Swain, 2007). Studies have revealed that anthropogenic inputs are major sources of copper contamination (Nriagu, 1979 cited Sadiq, 1992). Copper for many years has been effectively employed in controlling algae growth and fish parasites in aquatic systems (Yanong, 2010). Major industrial inputs include mining, refining and smelting industries, copper wire mills, coal production and iron and steel production (CCME, 2008). Copper is also used in construction, in roofing materials and brass and copper plumbing. Its compounds are used in fertilizers and as biocides in antifouling paints for vessel hulls. Copper can enter the marine environment through groundwater and stormwater runoff, sewage effluents and by industrial atmospheric emissions. An estimated 100000 metric tons of copper is released into the atmosphere annually, of which 10000 metric tons is deposited into the oceans through both wet and dry

deposition (Sadiq, 1992). Other sources of copper input into the coastal and marine environment may include road surface and parking lots runoff (wear of tires, brake pad and exhaust), and domestic and industrial effluents (Nicolau et al., 2012; Khan et al., 2014). Approximately 0.34 billion metric tons of copper presently exists in the marine environment based on a concentration of 0.25µg/L and a volume of 1.338×10^9 km^3. Copper inputs into the ocean include atmospheric depositions which account for 22×10^6 kg/year, riverine influx (including dissolved copper) at 58×10^6 kg/year and particulate copper at 1500×10^6 kg/year and antifouling copper estimated at 15×10^6 kg/year (Blossom, 2015).

2.3.1.2 Ecotoxicity of copper

Copper is an essential micronutrient in all living organisms and plays a catalytic role in many biological enzymes systems, the most notable of which are cytochrome oxidase, and the electron carrier plastocyanin (Coale & Bruland, 1988; Flemming & Trevors, 1989; Sadiq, 1992; Khan et al., 2014). Copper is important to processes such as cellular respiration, free radical defence and cellular iron metabolism (Kwok et al., 2008). Most molluscs in the marine environment depend on the copper-based blood protein hemocyanin for oxygen transport rather than the iron-based haemoglobin. Although copper is an essential micronutrient used in enzymes involved in several metabolic processes (White & Rainbow, 1985), it may have deleterious effects on organisms at concentrations higher than physiologically necessary (Ytreberg et al., 2010). Therefore, copper concentrations in natural environments and its bioavailability are important. Copper is present in all compartments of the marine environment and may exist in various chemical forms such as cupric ions, or complexes with inorganic or organic ligands or as suspended particles (Mance et al., 1984; Jones & Bolam, 2007). The free cuprous (+1) and cupric (+2) ions are the most toxic forms in marine ecosystems, with toxicity increasing in the order, organic copper > inorganic copper >copper (I) and copper (II) (Jones & Bolam, 2007). The composition of the various copper forms depends on pH and on the presence of other inorganic and organic ligands in water. Copper is generally more soluble in acidic waters, and precipitates as copper(II) hydroxide ($Cu(OH)_2$) at pH values above 6.5 (CCME, 2008). The bioavailability and concentration of dissolved copper (such as the cupric ion concentration) is controlled by several factors which include salinity, the presence of organic ligands and pH (Bryan & Langston, 1992). Though the free cupric (+2) ion accounts for only a small proportion (less than 1%) of the total dissolved copper in seawater (Bryan & Langston, 1992), it is the most biotoxic form of copper as it readily migrates through cell membranes (Campbell, 1995; Srinivasan & Swain, 2006). A number of marine organisms have detoxification systems that are inducible (Bryan & Langston, 1992). These organisms have

adapted to survive different copper concentrations, even to the extent that similar species are able to adapt and tolerate different concentrations at different locations (Zhou et al., 2003). Despite the presence of such detoxifying systems for copper in marine organisms, it is the most toxic metal, after mercury and silver to a wide range of marine organisms (Ansari et al., 2004). According to Neff (2002), it is difficult to isolate adverse effects of excess copper in the marine environments, as most copper contaminated marine environments are also contaminated with other metals and organic contaminants.

The toxicity of copper is dependent upon its availability and the physicochemical characteristics of the specific environment that significantly influence metal speciation (Flemming & Trevors, 1989). Experimental studies, have shown that dissolved ionic copper concentrations of 0.001-0.02mg/L produce a variety of toxic effects in marine organism (Bryan & Langston, 1992; Ansari et al., 2004) For example, concentrations of 0.002mg/L were observed to have major effects on young bay scallops and surf clams (Nelson et al., 1988). High copper concentrations have been shown to reduce the filtration rate of marine bivalve (Hall et al., 1998), impair or inhibits the settlement of coral larvae (Reichelt-Brushett & Harrison, 2000) exhibit reduced growth in marine diatoms (Cid et al., 1995), and in macroalga, *Ceramium tenuicorne* (Karlsson et al., 2010; Ytreberg et al., 2010), cause cell abnormalities due to oxidative stress (Rijstenbil et al., 1994), hinder normal larvae development of mussels *Mytilus galloprovincialis* (Rivera-Duarte et al., 2005), and decrease population biomass in amphipods, *Allorchestes compressa* (Ahsanullah & Williams, 1991). Dissolved copper concentration of 0.005mg/L in a 48 hours exposure causes development abnormalities in embryos of mussel *Mytilus edulis* and oyster *Crassostrea gigas* (Martin et al., 1981). Copper with an LC_{50} of 0.006mg/L in a 48 hour exposure significantly reduced the survival of the abalone *Haliotis midae* (Stofberg et al., 2011). It has also been suggested that copper can induce imposex in gastropods (Nias et al., 1993). However, this effect has not been experimented in the laboratory for copper.

2.3.2 Zinc

Zinc is a bluish-white metal with an atomic number of 30, and an atomic weight of 65.37 with a melting point of 419.6°C, and a boiling point of 907°C (Adriano, 1986b). It is the first element of group IIB in the periodic table. Zinc is the 24th most abundant element found in the earth's crust. It is ubiquitously present in nature with concentrations in the earth's crust ranging between 10 and 300mg/kg with an average of 70mg/kg (Malle, 1992). Zinc is divalent in all its compounds (Vardatsikos et al., 2013) with a composite of five stable isotopes; Zinc-64, Zinc-66, Zinc-67, Zinc-68, and Zinc-70 and relative abundances of 48.89%, 27.81%, 4.11%,

18.56% and 0.62%, respectively (Adriano, 1986b). It is distributed in a variety of forms such as zinc carbonate, zinc chloride, zinc oxide, zinc acetate and zinc sulphide (Simon-Hettich et al., 2001).

2.3.2.1 Sources of zinc in the environment

Zinc ranks fourth after iron, aluminium and copper, in the most widely used metals globally. An estimated 13.2 million tons of zinc was produced in 2014 worldwide (Tolcin, 2014). Naturally, it is rare for zinc to occur in its metallic state, however, many minerals contain zinc as a major component from which it may be economically extracted (Simon-Hettich et al., 2001). Due to weathering processes, soluble compounds of zinc are formed and may be made available to the aquatic environment. Natural background total zinc concentration in seawater are between 2×10^{-6}mg/L and 1×10^{-4}mg/L (Simon-Hertich et al., 2001), although concentrations as high as 0.02mg/L have been measured in areas of vessel activities with poor water circulation (Bird et al., 1996). The largest natural release of zinc to the aquatic environment is from erosion and with natural inputs to the atmosphere mainly through volcanic activities and forest fire. Zinc inputs from anthropogenic sources far exceed that from natural sources (Fishbein, 1981; Adriano, 1986b; Callender, 2005). The main anthropogenic sources of zinc are mining and smelting, municipal sewage sludge, corrosion of galvanized structures, coal and fuel combustion, waste incineration, and the use of zinc-containing fertilizers and pesticides (Adriano, 1986b). A large proportion of zinc that enters the world's ocean is derived from atmospheric deposition (Neff, 2002), with an estimated 11,000 to 60,000 metric tons/year of dissolve and particulate zinc deposited from the atmosphere into the marine environment (Jickells, 1995). Zinc is extensively used as a protective coating of other metals such as iron and steel (35% of the global production of zinc), an alloy for die casting (25%) and the construction industry (CCME, 2008). The inorganic forms of zinc have numerous applications, such as for automobile equipment, storage and dry-cell batteries, and dental, medical and domestic uses (Simon-Hertich et al., 2001). They are also used in the manufacture of adhesives, as a flux in metallurgical processes, and as a wood preservative. Zinc compounds are used as anticorrosive pigments in vessels parts (e.g., sacrificial anodes) and like copper are also used as a biocide in antifouling paints for vessel hulls. All these uses may contribute to zinc input in the environment.

2.3.2.2 Ecotoxicity of zinc

Zinc is an essential element to all marine organisms, being an integral component of about 300 enzymes and nearly all enzymes involved in metabolism (Vallee & Auld, 1990; Simon-

Hertich et al., 2001). It can become toxic to many marine organisms at high concentrations and cause different permanent and severe damages. The zinc ion is naturally persistent, though it can be converted into other species and can form complexes with a range of organic and inorganic ligands such as zinc-chloro complexes or zinc hydroxide with chemicals present in the marine environment (Yung et al., 2014). The physical and chemical forms of zinc, the toxicity of each form and the degree of inter-conversion among the different forms are vital to the question of zinc toxicity. All forms of zinc that can be sorbed or bounded by biological tissue are potentially toxic and most often than not, zinc will not be sorbed or bound unless it is dissolved. The free zinc (II) ion is the most abundant of the dissolved forms of zinc and the most bioavailable (Pagenkopf, 1983; O'Brien et al., 1990; Rainbow et al., 1993), hence the source of toxicity. Like copper, elevated concentrations of zinc can have an adverse effect on marine organisms. For example, dissolve zinc ions can prevent growth and reduce photosynthesis ability in marine diatoms *Thalassiosira psedonnana* after 48 hours exposure (Wong et al., 2010) cause morphological abnormalities in embryos of the sea urchin *Lytechinus pictus* after 96 hours exposure at the low mg/L range (Fairbairn et al., 2011) and hinder growth rate of *Ceramium tenuicorne* by 50% at 0.025mg/L after 7 days exposure (Ytreberg et al., 2010). Also, zinc concentrations between 0.2 and 1mg/L range, in a chronic exposure for 100 days, retarded growth, delayed sexual maturity, and reduced reproduction in the amphipod *Corophium volutator* (Fabrega et al., 2012). Concentrations in the range of 0.005 to 0.02mg/L of dissolve zinc interfere with normal fertilization and early development of some molluscs, crustaceans and fish (Ojaveer et al., 1980; Verriopoulos & Hardouvelis, 1988; Hunt & Anderson, 1989). Zinc with an EC_{50} of 0.1023mg/L after 48 hours exposure significantly affected larval development in *Haliotis midae* (Stofberg et al., 2011).

2.3.3 Copper and zinc deficiency and surplus

Despite copper and zinc being essential micronutrients used in enzymes involved in several metabolic processes, they may become toxic to organisms at concentrations higher than physiologically needed (Sunda, 1989; Matthiessen et al., 1999; Ytreberg et al., 2010; Karlsson et al., 2010). According to Rainbow (1995), a metal becomes toxic to living organisms when, in its bioavailable form in the ambient environment, it exceeds the threshold concentration defined by the organism. The concept of toxicity of a substance being dependent on the magnitude of exposure to an organism is attributed to Paracelsus. He wrote: 'All substances are poison; there is none which is not a poison. The right dose differentiates a poison from a remedy' (Ansari et al., 2004). Each biologically essential metal in an organism has a specific optimal concentration range, which can be determined by the natural concentration range of the metal in the organism's natural environment and its homeostatic capacity (Muyssen &

Janssen, 2002). Studies in relation to the toxicant effect of essential metals follow the general trend that concentrations below or above the optimum range, lead to a deficiency or toxicity (Förstner & Wittmann, 1981; Fu et al., 2016). Figure 2.3 depicts a dose-response curve for essential metals such as copper and zinc showing the range of concentrations spanning deficiency, optimal and toxicity. The area between A and B represents concentration for optimal growth, health and reproduction known as the window of essentiality. Concentrations before point A indicate deficiency and that after point B have toxic to lethal effects.

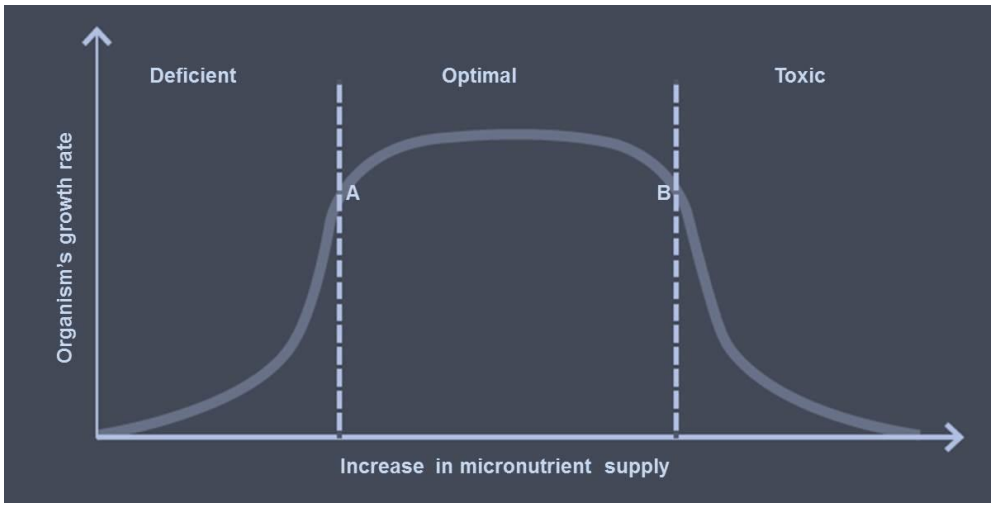


Figure 2. 3: A representation of a concentration-response for a micronutrient such as copper and zinc (Adapted from Alloway, 2013)

The ecotoxicant effect of metals in the marine environment is generally determined by whether it is in the form that an organism can directly absorb or ingest. The fraction of the concentration of a chemical in the environment that is potentially available for biological action such as uptake by an aquatic organism is said to be bioavailable (Ansari et al., 2004). This bioavailable fraction is the critical factor for toxicity (Fent, 2004). Bioavailability includes not only the characteristics of the chemical and environmental speciation but also the behavioural and physiological aspects of the organism (Rand et al., 1995). The ecotoxicant effects of metals such as copper and zinc can be influenced by several parameters such as its geochemical behaviour and the physiology of the target organism. Some of the most important of these parameters are:

i. Speciation of metal in the marine environment: This refers to the various physical and chemical forms in which a metal may exist in a system. It may be found in the form of free ions or organometallic molecules and be transported in the dissolved or particulate phase (Alzieu, 1988). In each phase, speciation occurs between specific ligands, defined by ligand concentrations and the strength of each metal-ligand association.

Hence, an organism is never exposed to metal as a single entity but rather to a variety of physicochemical forms, which may differ in its availability to the organism (Luoma, 1983).

ii. The presence of other metals or toxicants: This may antagonize (reduce) or synergize (increase) the additive toxicity of each metal (Ansari et al., 2004).

iii. Environmental parameters: Physicochemical factors such as temperature, pH, dissolved oxygen, light and salinity may influence the physiology and metabolism of the organism as well the possible form of metal in water. These may render the organism vulnerable to the effects of toxicants (Ansari et al., 2004).

iv. Condition of organisms: The sensitivity of the organisms differs giving to several factors such as stage in life cycle (eggs, larvae, juveniles, adults), changes in life cycle (e.g., moulting, reproduction), age and size, sex, food (starvation), additional protection (e.g., shell), etc (Ansari et al., 2004).

v. Adaptation of organisms to metal absorption in the marine environment: Metal bioaccumulation studies in marine organisms have revealed the existence of detoxification mechanisms. Hence, metals can be: stored in specialised cells (e.g., oyster amebocytes with copper and zinc); blocked by complexation with low molecular weight thio-proteins (metallothioneins); and immobilised by the formation of stable compounds based on antagonistic elements (Alzieu, 1988; Förstner & Wittmann, 1981). The presence of these mechanisms in organisms is to avoid the effect of chronic exposure (Alzieu, 1988).

2.3.4 Copper and zinc in antifouling paints

Metal contaminants in harbours can emanate from several sources as previously mention. However, several studies have reported the use of antifouling paints on vessels as a major source of metals in harbours (Schiff et al., 2007; Cassi et al., 2008; Dafforn et al., 2008; Daehne et al., 2017). The undesirable accumulation of living organisms on submerged artificial surfaces such as vessel hulls by adhesion, growth and reproduction is known as biofouling (WHOI, 1952; Cao et al., 2011). Biofouling is ubiquitous in the coastal and marine environment and is a huge problem in the maritime industry (Cao et al., 2011; Dafforn et al., 2011). The growth of organisms on vessel hulls causes increased frictional resistance and fuel consumption (Abbott et al., 2000). Additionally, hull maintenance is costlier and time-consuming, because dry-docking operations need to be more frequent and longer with biofouling. These cleaning processes generate paints particulates enriched with contaminants (such as copper and zinc) and through runoff and wash down or as airborne dust enter near-

shore waters (Turner et al., 2008) (Figure 2.4). The financial cost of vessel hull biofouling has led to the development of antifouling technologies such as the use of antifouling paints. Antifouling paints are applied to vessel hulls to prevent the growth of fouling organisms. These paints have both biocidal and solvent components (Okamura & Mieno, 2006), which leaches into the environment being toxic to fouling organisms (Boxall et al., 2000; Callow & Callow, 2002). However, the biocide release from a vessel's hull can be harmful to non-target organisms (Tornero & Hanke, 2016).

For many years, organotin tributyltin (TBT) has been the most widely used active biocide in antifouling paints. A global ban on the use of TBT-paints was sanctioned in 2008 (IMO, 2002), and copper became the principal biocide in antifouling paints (Warnken et al., 2004; Readman, 2006; Jones & Bolam, 2007; Srinivasan & Swain, 2007). According to Schiff et al. (2004), these paints may contain 20% to 76% copper in the form of copper oxide (Cu_2O). Zinc (as zinc oxide) is also a common component in antifouling paints as an anticorrosion additive (Lahbib et al., 2013), and by itself a core biocide in antifouling paints (e.g., zinc pyrithione, zineb, zinc acrylate copolymers, etc.). It has both physical and chemical mode of actions, functioning as a binder and as a pigment (Yebra et al., 2004; KEMI, 2006; Singh & Turner, 2009). Studies have documented the leaching of copper and zinc from vessel hulls into coastal and marine environments with areas of vessel traffic and maintenance as major sources of copper and zinc release (Matthiessen et al., 1999; Costa & Wallner-Kersanach, 2013; Lee et al., 2018) Copper and zinc are present in elevated concentrations in antifouling paints (Watermann et al., 2005). Most antifouling paints contain copper in the form of cuprous oxide with concentrations ranging from 10-25% to 40-50% by weight and zinc in the form of zinc oxide with concentrations ranging from 1-10% to 10-25% by weight (Readman, 2000; Penttila, 2017).

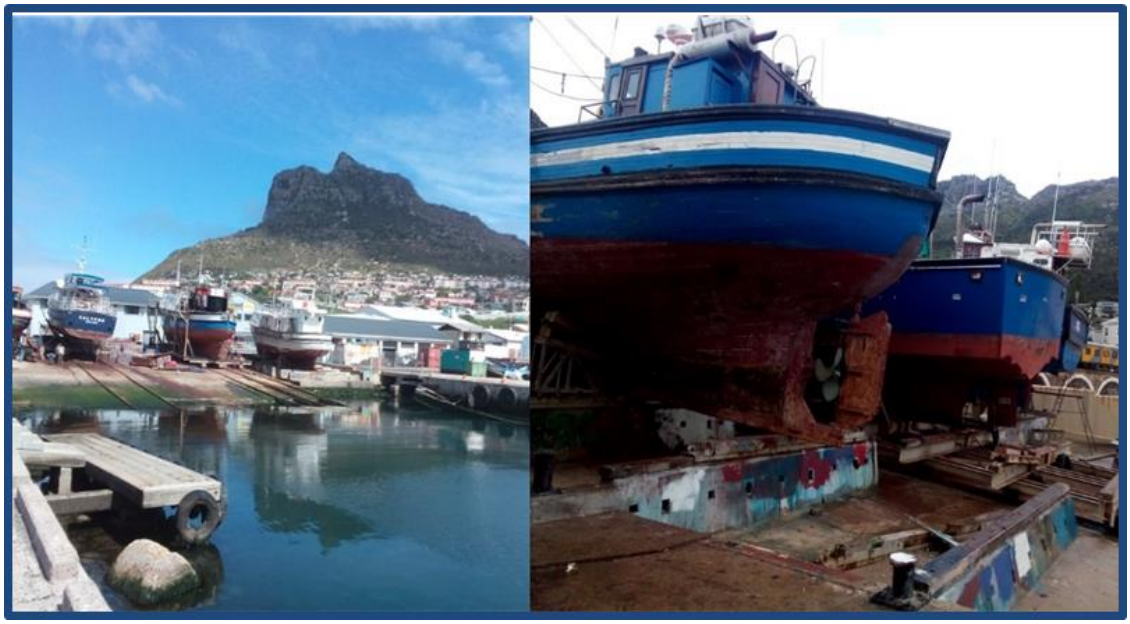


Figure 2. 4: Maintenance and repair of fishing vessels at a vessel repair facility adjacent to a slipway in Hout Bay Harbour (antifouling paint residue may enter near shore waters through runoff or wash down) (Source: Fru. W)

2.4 ENVIRONMENTAL MONITORING

In recent years, the fate of the environment has become a critical issue worldwide. Population growth and industrialization are creating a burden on society by requiring continued development and associated resource use. There is enough evidence to show that such development has led to deleterious impacts on the environment. It is without a doubt that increased anthropogenic activities and insatiable demands are changing the soil, water, air, climate, and resources in unforeseen ways. Hence, there is an increased need to protect the environment with focused attention on the concept of environmental monitoring (Artiola et al., 2004). Monitoring is the methodical measurement of variables and processes over time with respect to a specific problem (Spellerberg, 2005). Environmental monitoring, therefore, involves the systematic sampling of the abiotic (air, water, soil) and biotic (living organisms) components of a target environment in order to assess its status (Artiola et al., 2004). The metal pollution status of the coastal and marine environment can be assessed by the chemical analysis of water, sediment and/or indigenous biota (Phillips, 1977; Rainbow, 1995).

2.4.1 Water

Until in recent times, the chemical analysis of water has been used as the conventional method for assessing the metal pollution status in the environment (e.g., Goldberg, 1965; Brooks et al., 1967; Chester & Stoner, 1974; Fukai & Huynh-Ngoc, 1976). Metals in water can be divided into two main components: metals in dissolved form and in suspension bound to organic or inorganic particulate matter (e.g., phytoplankton, zooplankton, debris, clay, silts) (Phillips, 1977; Arfin et al., 2012). The chemical analysis of water for metals presents certain limitations. Dissolved metal concentrations are low, often near to analytical limit of detection and may need pre-concentration. This is costly, time-consuming and liable to contamination during or before analysis. Furthermore, the large variation of metal concentrations in water with seasonal differences, time of day, the magnitude of freshwater influx, depth of sampling, the regular flow of industrial effluent and hydrological factors such as tides and currents make it difficult comparing locations for their level of metal pollution (Phillips, 1977; Rainbow, 1995). This can be overcome by implementing extensive monitoring over time that can account for such variation. Such extensive monitoring programmes are feasible but expensive. Additionally, a strong disadvantage to the analysis of water for metals arises from the fact that metal concentrations provide an assessment of total metal present (solute and particulate form), not the bioavailable fraction. It is the bioavailable fraction that is potentially toxic and of ecotoxicological relevance (Rainbow, 2006).

2.4.2 Sediment

Several studies have reported the use of sediment to delineate areas of metal pollution (e.g. Chester & Stoner, 1975; Jaffé & Walters, 1977; Cosma et al., 1982; Zhuang & Gao, 2014 and Qian et al., 2015). The analysis of sediment overcomes some of the limitations encountered with water. Sediments are considered as a reservoir for various contaminants. Metals accumulate in sediment over time, and their concentrations are therefore high, easy to measure and less liable to contamination before and during analysis (Phillips, 1977; Rainbow, 1995; Rainbow, 2006). Moreover, metals integrate with sediment over time, thus the concentration of metal in the sediment reflects the metal that has accumulated over a period. This overcomes the effect of temporal variation of metal availability and minimises the need for extensive monitoring programmes. Again, there are still problems associated with the use of sediment metal concentration to assess the magnitude of metal pollution. The physicochemical characteristics of sediment that vary spatially affect metal accumulation (Luoma, 1989; Bryan & Langston, 1992). For example, metal accumulation in sediment is dependent on the organic content (measured as total carbon) and particle size of the sediment. Sediment rich in organic content will bind more metals than those with poor organic content,

and sediments with particles of large surface areas will absorb more metals than those of small surface area. Hence, mud which is characterized by small particle size and high organic carbon content binds more metals than sand of large particle size and low organic carbon content (Phillips, 1977; Rainbow, 2006).

Like with the chemical analysis of water, the metal concentrations analysed in sediments are that of total and not the bioavailable metal, except for sediment from deposit feeders (sediment-ingesting organisms). Sediment through trophic transfer can also be a source of metals to deposit feeders, and the trophic bioavailability of metals in ingested sediments will also vary with the physicochemical characteristics of the sediment (Rainbow, 2006). Therefore, once again, total metal concentrations in sediment may not be good measure of the relative bioavailability of metals in different sediment, in this instance as a source of trophic transfer available metal for biota. In circumstances where the metal concentrations in sediment are high, there is a possibility that a very small fraction is bioavailable in the overlying water column (Phillips, 1977). Thus, high metal concentrations in sediment may be indicative of a low rate of mobilization of metals from sediment, as well as the prevalence of the removal of metals from the water column by precipitation or sedimentation. Total metal concentrations in sediment are not accurate relative measurements of metals bioavailability in compared environments. It is worth noting that the bioavailability of metals in a target environment should be of utmost interest, to predict the ecotoxicant effects of metals.

2.4.3 Biological monitoring

Biological monitoring (biomonitoring) has become the basis of modern ecotoxicological assessment (Connell et al., 1999). The chemical analysis of environmental matrices such as water and sediment has been the conventional method used to measure metal pollution status in the environment, though it does not provide strong evidence on the combined influence and potential toxicant effect of such pollution on the organisms and ecosystem (Zhou et al., 2008). According to Zhou et al. (2008), biomonitoring is a scientific method for monitoring the environment which includes human exposures to natural and man-made chemicals, based on the sampling and analysis of an organism's tissue or fluid. It may take many forms based on different aims and demands. Biomonitoring takes advantage of the understanding that chemicals that have entered the organisms leave biomarkers reflecting this exposure. The chemical itself may be the biomarker. Biomonitoring can directly reveal information on the possible effects and actual combined toxicities of pollutants, owing to the homogeneity between the selected organisms and their habitats, thereby mirroring the resultant harmful impact in the environment (Zhou et al., 2008). It displays strong advantages when compared

with the conventional chemical analysis of abiotic matrices (water and sediment). Some of these advantages include the following: biomonitoring (i) reveals the most direct biological changes of organisms affected by exogenous chemicals which cannot be determined by the conventional chemical analysis; (ii) effectively shows the combined effects of the complex pollutants on the organisms in the environment; (iii) is more suitable when cumulative effect of environmental factors which extend over a long period of time has to be observed; (iv) has high sensitivity due to the rapid responses induced in the organisms exposed to pollutants, and this helps to necessitate prompt precautions; (v) overcomes the difficulties of monitoring of pollutants at low levels which were below detection limits of the instrumental analytical methods; (vi) is less expensive and less time consuming on like the conventional chemical analysis which requires expensive instruments and continuous sampling over a long period of time (Zhou et al., 2008).

Since it offers significant advantages in comparison to the conventional analysis of abiotic matrices, biomonitoring has established itself as an excellent tool for the evaluation of environmental pollution (Conti et al., 2004), especially for metal pollution status in the coastal and marine environment.

2.4.3.1 Biomonitors

Biomonitors are organisms (or parts of organisms or communities of organisms) that accumulate contaminants in their tissue which may, therefore, be used to provide a relative measure of the total amount of contaminants in the environment integrated over a period of time (Hatje, 2016). A biomonitor contains information on the quantitative aspects of the quality of the environment and the nature of environmental changes (Markert et al., 2003). This information can be the occurrence of certain elements or chemical compounds and/or changes in the morphological, histological or cellular structure, metabolic-biochemical processes, and behaviour or population structure, of the organism. When assessing the metal pollution status of an aquatic environment (e.g., marine environment), a biomonitor will denote, an organism that bioaccumulates metals in its tissue, which may be analysed to provide a measure of the bioavailable metals in the ambient environment (Rainbow, 1995). These bioaccumulated metal concentrations are measured easily, not susceptible to contamination and provide a time-integrated measure of the bioavailable metal over a long period (Phillips, 1997). Therefore, the fraction of metal that may have ecotoxicological effects is measured unambiguously. Suitable biomonitors usually provide great help to the monitoring of metal pollution in the coastal and marine environment and are required to possess the following attributes (Phillips, 1977; Rainbow, 1995; Connell et al., 1999; Zhou et al., 2008; Hamza-Chaffai, 2014):

- be sedentary, hence a representative of the local area and pollution;
- accumulate high levels of the pollutants without being killed;
- abundant and with wide distribution for repetitive sampling and assessment;
- relatively long-lived for the comparisons between various ages;
- sizable, providing adequate tissue for analysis;
- afford suitable target tissue or cell for further research at the microcosmic level;
- occupy an important position in the food chain
- a relationship should exist between pollutant concentrations in the surrounding environment, and the levels present in the tissue of the living organism.

Conventionally, the mention of biomonitors simply means the use of an organism's soft tissue. Nonetheless, metal concentrations in soft tissue are dependent on several physiological, environmental and temporal factors which may influence the total metal concentrations in the soft tissue. The use of shells of biomonitor organisms has received less attention than soft tissue. However, some studies have suggested its use as a biological condition independent factor of metal concentration which thus reflects a more transparent picture of the ambient metal concentration (e.g., Fischer, 1983, 1988; Broman et al., 1991; Badran, 1998; Yasoshima & Takano, 2001; Cravo et al., 2002, 2004; Palpandi et al., 2010; Kesavan et al., 2013; Yap, 2014; Piwoni-Piórewicz et al., 2017). Most studies have been directed either to the soft tissue or to the shells but very few have simultaneously examined metal concentrations in both soft tissue and shells. According to Langston et al. (1998), it is commonly accepted that soft tissue accumulates higher metal concentrations than the shells. However, few studies have shown molluscs shells to accumulate higher concentrations than the soft tissue (e.g. Szefer & Szefer, 1985,1990; Puente et al., 1996; Fishelson et al., 1999; Szefer et al., 2002). The analysis of metals in shells has several practical advantages as compared to soft tissue such as (i) shells can be preserved for longer periods before analyses; (ii) they are easy to detach neatly from the whole organism, both onsite and in the laboratory and the problem of depurating the organisms before analysis is avoided (Koide et al., 1982); (iii) they exhibit less variability unlike soft tissue (Badran 1982; Lingard et al., 1992); (iv) shells may provide a geologic record of anthropogenic changes in metal content in the environment with this record preserved even after death of the organism (Bertine & Goldberg, 1972; Carell et al., 1987). Consequently, shells may afford a more accurate indication of the magnitude of pollution and environmental change.

One of the most used approaches in biomonitoring is the determination of pollutant levels (i.e. residue analysis) in bioaccumulator organisms (Reguera et al., 2018). This study employs this approach, which can provide information on the accumulation, distribution and transfer properties of the pollutants in the selected organisms by the chemical analysis due to the occurrence of bioaccumulation for many chemicals such as metals in aquatic organisms.

2.4.3.2 Metal bioaccumulation

Among the different biomonitoring methods used for assessing an aquatic environment, the approach based on the bioaccumulation capacity of some chemical species such as metals is among the most important (Conti & Iacobucci, 2008). Bioaccumulation is an important process by which chemicals can affect living organisms. Metals can accumulate in the marine food chain up to concentrations that are harmful to marine organisms, particularly predators, and may pose a health risk to humans (HELCOM, 2007). Living organisms can bioaccumulate contaminants through two routes; bioconcentration, defined as the accumulation and sequestration of contaminant materials by organisms directly from the ambient environment; and biomagnification, the accumulation of contaminant materials by the twofold processes of bioconcentration and trophic transfer (Connell et al., 1999). The concentration of a chemical in an organism can increase over a time-period relative to that in the surrounding environment. According to Leblanc (2004), bioaccumulation is the process by which organisms accumulate chemicals both directly from the abiotic environment (i.e., water, air, and soil/sediment) and from dietary sources (trophic transfer). Several processes some of which include, uptake, storage, and elimination are involved during bioaccumulation. Dynamic equilibrium between exposure to a chemical from the surrounding environment and uptake, excretion, storage, and degradation within an organism leads to bioaccumulation (Zhou et al., 2008). The coastal and marine environments are exposed to a variety of contaminants, amongst them metals, which are persistent, non-biodegradable and can accumulate in organisms. In recent years, metal bioaccumulation by marine organisms has been a subject of considerable interest because of the serious concern that high levels of metals may have deleterious effects on these organisms, as well as pose a risk to human health. Marine organisms have the potential to bioaccumulate high levels of metals from their environment (Fowler, 1990; Rainbow & Phillips, 1993; Szefer et al., 1999). The magnitude of bioaccumulation of metals is dependent on the total concentration, the bioavailability of each metal in the environmental medium and the route of uptake, storage and excretion mechanisms (Valavanidis & Vlachogianni, 2010). Other aspects that may influence the bioaccumulation of metals by marine organisms include: abiotic factors such as water currents, water flow, metal speciation, temperature, pH, salinity, dissolve oxygen, the presence of other pollutants and seasons and biotic factors like age, body size,

nutritional, and reproductive status (Oehlmann & Schulte-Oehlmann, 2003; Ansari et al., 2004; Zhou et al., 2008; Gupta & Singh, 2011). Therefore, all these factors must be carefully considered before an organism can be used as a biomonitor for metal pollution. Bioaccumulation assessment is part of a global effort to identify and control chemicals (e.g., metals) of environmental concern. It is a general consensus that substances such as metals, which are persistent, bioaccumulative, and deleterious and subject to long-range transport are of particular concern (Gupta & Singh, 2011), and should be continuously monitored. The bioaccumulation of metals by living organisms is often a good integrative indicator of exposure and has been extensively used to assess contamination levels of metals in polluted ecosystems (Phillips & Rainbow, 1994).

2.4.3.3 Gastropods as biomonitors

Specific biomonitors will exhibit different responses to different sources of metal bioavailability such as in solution, in sediment or in the diet. Therefore, in order to have complete information of the total bioavailable metal in a marine environment, it is necessary to use different biomonitors, which reveals metal bioavailability in all existing sources (Rainbow, 1993; Rainbow & Phillips, 1993; Rainbow, 1995). According to Phillips & Rainbow (1988, 1993), such relative use of different biomonitors should help in revealing the precise source(s) of the contaminant metal to that particular biomonitor. Gastropods are the largest (with over 60,000 species) of the seven classes in the species-rich phylum Mollusca and make up more than 80% of the total species (Markert et al., 2003). Due to their widespread distribution and vast species number, gastropods play vital ecological roles in different ecosystems worldwide. They are key species for ecosystems functioning, such as litter decomposition and as well as their contribution to an enormous amount of the biomass on the different trophic levels in ecosystems (Oehlmann & Schute-Oehlmann, 2003). Gastropods are known to actively accumulate metals under natural environments through water or their diet and for that reason are commonly studied around the world from the ecotoxicological point of view (Elder & Collins, 1991). They have long been identified as natural accumulators of high level of metals (Zhou et al., 2008). Different gastropods species can reveal different accumulative abilities for various metals, thus may offer various likely biomonitors for the assessment of metal pollution in the coastal and marine environment (Liang et al., 2004). Even though gastropods have not been exhaustively used in biomonitoring as compared to other Mollusca species such as bivalves, they however, fulfil the requisite attributes to be good biomonitors (Ireland & Wootton, 1977; Phillips, 1977; Gay & Maher, 2003; Chelazzi et al., 2004; Taylor & Maher, 2006). When compared with other invertebrate groups (e.g., arthropods) and particularly vertebrates, gastropods exhibit low or under detectable enzyme activity which metabolizes pollutants and

has limited ability to physiologically inactivate metals (e.g., intracellular compartmentalization, or binding to metallothioneins) (Oehlmann & Schute-Oehlmann, 2002). Consequently, gastropods achieve high bioaccumulation for toxicants than other systematic groups. Therefore, toxicants might show adverse effects on gastropods at lower environmental concentrations as compared to other invertebrates or vertebrates, enabling their use as sentinel organisms for environmental monitoring (Oehlmann & Schute-Oehlmann, 2002).

Several studies have reported the use of gastropods as biomonitors for metal pollution in the coastal and marine environments (e.g. Ireland & Wootton, 1977; Bat et al., 1994; Kang et al., 1999; Leung et al., 2001; Campanella et al., 2001; Liang et al., 2004; Maher et al., 2016; Krupnova et al., 2017). Therefore, it is widely established that gastropods fulfil the standards for excellent biomonitors and can accumulate high concentrations of metals relative to concentrations gradient of these metals in the ambient environment.

CHAPTER THREE

MATERIALS AND METHODS

3.1 CHOICE OF GASTROPOD SPECIES

To assess the metal pollution status of an environment using biota, it is important that the selected organisms reflect the status quo of the environment from which they are collected. Several contaminants, including metals, may bioaccumulate in the tissue of organisms and the chemical analysis of this biological tissue can be used to show that such an organism has been exposed to contaminants and, in some cases, to monitor the bioavailability of that contaminant over space and time in the aquatic ecosystem. Therefore, such organisms provide integrated measures of the ecotoxicological significant portion of the metal in the ambient environment (Rainbow & Phillips, 1993).

Two gastropod molluscs, *Burnupena* spp. *and Nucella* spp. were used in this study. They were selected based on the following reasons: they exhibit a very limited mobility over a wide range and therefore represent the contamination of their habitat; they are robust and tolerant of high metal concentrations and large ranges in salinity; they are widely distributed, ranging from Namibia on the west coast to northern KwaZulu-Natal on the east coast of South Africa (Dempster & Branch, 1999); they are easy to identify and collect, and provides sufficient tissue for analysis of metal concentrations. Moreover, these two gastropods are ecologically relevant as they represent top predators in marine benthic food chains and also breakdown dead organisms (Wang, 2002; Wang & Tse, 2009). Gastropods may prey directly on bivalves or barnacles which are filter feeders or scavenge dead animal tissue. This attribute makes them easy targets for contamination of metals. It is worth noting that there are no published studies on metal bioaccumulation in these two gastropods (*Burnupena* spp. *and Nuccella* spp.).

3.1.1 *Burnupena* spp.

Burnupena is a genus of sea snails, marine gastropod molluscs in the family Buccinidae, the true whelks (Iredale, 1918) (Figure 3.1). They are 30 to 60mm in length and inhabit the mid-to high-intertidal zones to subtidal zones on rocky shorelines from the west coast to the east coast of South Africa (Dempster & Branch, 1999) (Figure 3.2). They are predators (prey on barnacles, mussels, and *Littorina* spp.) or scavengers of dead animals on the lower shore and in the subtidal zones (McQuaid, 1982; Barkai & McQuaid, 1988; Branch et al., 2010). Their taxonomic classification is given below (Gofas & Bouchet, 2014):

Phylum: *Mollusca*; **Class:** *Gastropoda*; **Subclass**: Prosobanchia; **Order:** *Neogastropoda*; **Family:** *Buccinidae;*

Genus: *Burnupena*

3.1.2 *Nucella* spp.

Nucella (common name dog whelk) is a widely distributed, predatory marine gastropod genus whose species are found along rocky shorelines of the South African coast (Figure 3.1). They are members of the family Muricidae with sizes ranging from 20 to 40mm. Both ends of *Nucella* shells are pointed and can be of different colours; black, grey, orange, purple with stripes, and grey with stripes (Abbott & Haderlie, 1980). Most species live in the intertidal or subtidal zone and feed on mussels, limpets, barnacles and *Littorina* (Branch et al., 2010). They drill neat cylindrical holes through mussel shells using enzymes to allow feeding (Rovero et al., 1999; Branch et al., 2010). Some species of *Nucella* have been used as biomonitors of metal pollution in coastal and marine ecosystems (e.g. Miller & Pondick, 1984; Leung et al., 2001; Leung et al., 2005). Their taxonomic classification is given below (Bouchet et al., 2017):

Phylum: *Mollusca*; **Class:** *Gastropoda;* **Subclass**: Prosobanchia; **Order:** *Neogastropoda*; **Family:** *Muricidae*;

Genus: *Nucella*

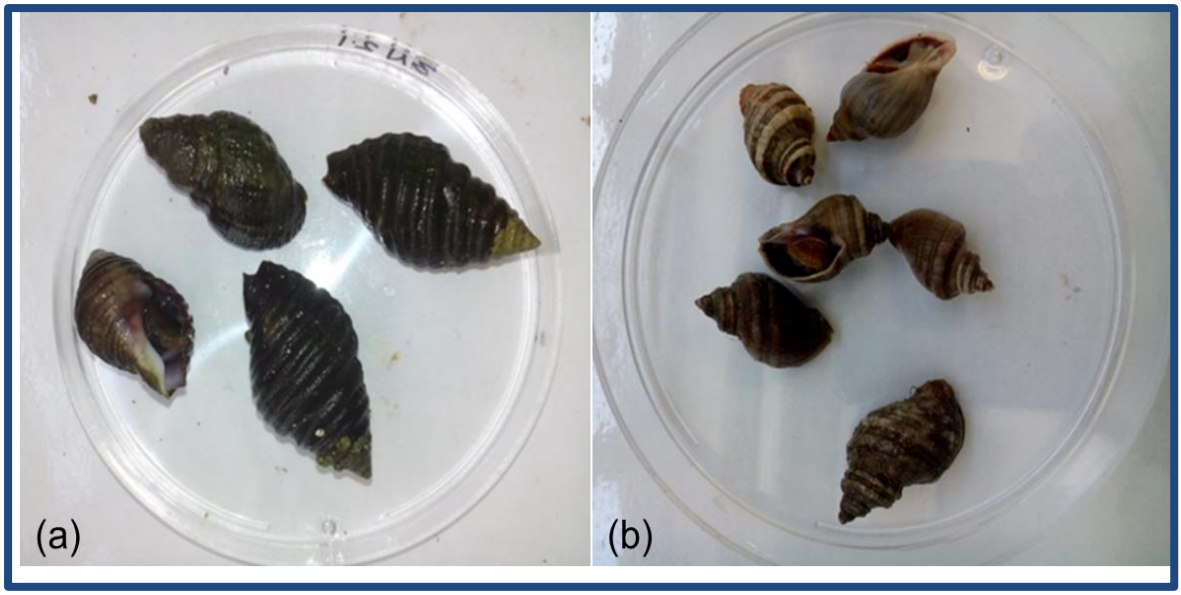


Figure 3. 1: (a) *Burnupena* spp. and (b) *Nucella* spp. samples (Source: Fru. W)

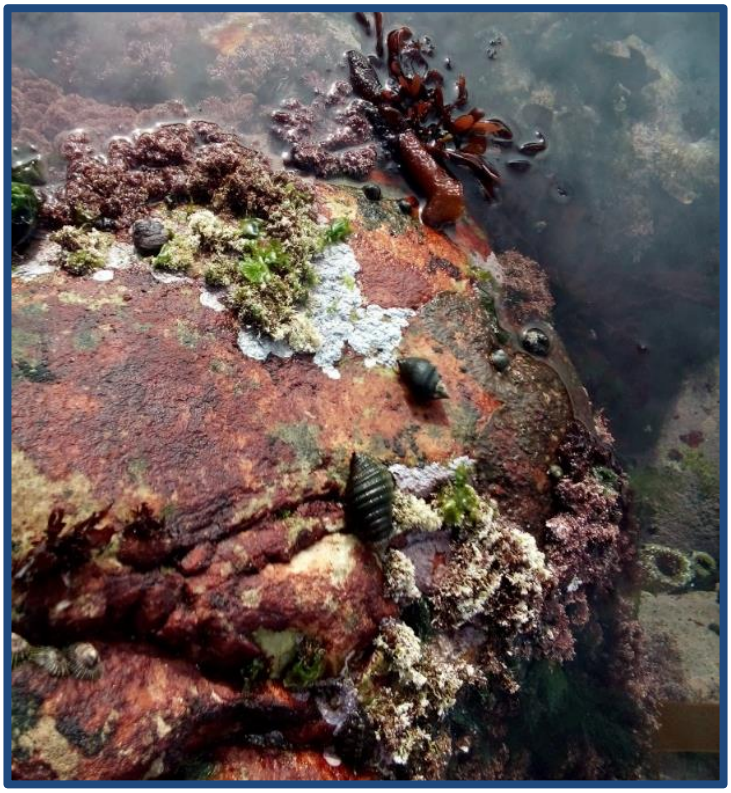


Figure 3. 2: *Burnupena* spp. found on rocks in the intertidal zone in Kalk Bay Harbour during sampling at spring low tide (Source: Fru. W)

3.2 DESCRIPTION OF STUDY AREA

The Cape Town metropole (hereafter Cape Town) is in the Western Cape province of South Africa and considered as one of the most beautiful cities in the world. It is the second most populated urban area in South Africa (STATS SA, 2011). Cape Town has a Mediterranean-type climate with mild, moderately wet winters and dry, warm summers. The annual average temperature in the region is 17°C (range ± 10°C) (Shannon, 1985). Cape Town has an extensive coastline, rocky mountain ranges which are dominated by the Table Mountain chain and connected to the mainland by a low-lying sandy plain known as the Cape Flats. The slopes of Table Mountain has historically been the centre of urban development, starting initially around Table Bay and then progressively expanding southwards, mainly along the eastern sides of the Table Mountain chain (Van Herwerden & Bally, 1989). Its coastline is dominated by rocky shores interspersed with pockets of sandy beaches or mixed sand and rock (Bally et al., 1984). The geology of the area comprises of three main rock formations which are of varying ages; the late-Precambrian Malmesbury Group, the Peninsula granite and the Table Mountain group (UCT, 2018).

3.2.1 Sampling sites

This study was carried out in six selected sites around Cape Town. The sites which included four harbours and two marine protected areas (MPA) were selected based on the hydrodynamics, the magnitude of anthropogenic activities as well as the presumed or confirmed presence of the two gastropods. The two marine protected areas were reference sites and metal inputs of anthropogenic origin were minimal given the absence of nearby impacting human activities. The four harbours are either fishing or recreational harbours (marinas) that may be affected by metal inputs associated with anthropogenic activities (e.g. discharge of untreated municipal wastewaters and polluting spills from marine traffic). It should be noted that no data exist on boat usage for the different harbours. Therefore, distinctions were based on observation during sampling periods. The six sampling sites are: Granger Bay Harbour (GRB); Hout Bay Harbour (HB); Kalk Bay Harbour (KB); Gordon's Bay Harbour (GB); Betty's Bay Marine Protected Area (BB: MPA), and Cape of Good Hope Marine Protected Area (CGH: MPA) (Figure 3.3).

Figure 3. 3: Map of study area showing the six sampling sites (four harbours and two reference sites) (Source: Google Earth, 2019; Fru. W)

3.2.1.1 Granger Bay Harbour (33°53’59.9” S 18°24’50.5” E)

The Granger Bay Harbour is situated on the Table Bay coastline next to the Victoria & Alfred Waterfront and is approximately 500m from the Port of Cape Town (Figure 3.4). Within the harbour precinct, is the Granger Bay Residential Area and the Granger Bay marina, which provides access to the Victoria & Alfred marina that caters for a wide range of vessels and yachts. Also present in the harbour are two slipways, the Granger Bay slipway used for boat surveys, repairs and maintenance and the Oceana Power Boat club slipway that caters for a wide variety of pleasure boaters, recreational fisherman and commercial fisherman. The harbour is potentially exposed to many pollutants from Table Bay and the two large rivers that open into the bay (the Diep River and the Salt River). Also, GRB is adjacent to Green Point which is highly urbanized with well-established commercial and residential development. Some of these developments include the Cape Town Stadium and the Metropolitan Golf Club.

Figure 3. 4: An aerial view of Granger Bay Harbour showing the sampling points (GRB1, GRB2 and GRB3). (Source: Bing Map, 2017)

3.2.1.2 Hout Bay Harbour (34°02’56.1” S 18°20’50.9” E)

Hout Bay Harbour is located 22 kilometres from the Cape Town Central Business District (CBD) (Figure 3.5). It is the closest proclaimed fishing harbour to Cape Town and lies between Chapman’s Peak and Mount Sentinel. It is a large harbour with industrial fishing and processing facilities, traditional small-scale fishing vessels, a yacht basin, recreational motor

fishing boats and ski boats slipways. Due to its proximity to Cape Town, it has become a tourist hotspot and recreational fishing charters are a popular activity. There are several commercial fishing companies and to the south of the harbour, there is a fish meal factory that occupies a large part of the harbour. Furthermore, there is a vessel repair facility that provides for vessel repairs and maintenance (Kaiser Associates, 2012).

Figure 3. 5: An aerial view of Hout Bay harbour showing the sampling points (HB1, HB2 and HB3). (Source: Bing Map, 2017)

3.2.1.3 Kalk Bay Harbour (34°07'45.3" S 18°26'57.3" E)

Kalk Bay is a small town wedged between the ocean and sharply rising mountainous heights close to Muizenberg and only 30 kilometres from the Cape Town CBD (Figure 3.6). It has a unique setting as the railway from Cape Town CBD to Simon's Town cuts through the town. At the southern part of Kalk Bay is a small harbour near the railway with only one jetty where several fishing boats are moored. The harbour is mainly used for fishing and small tourist boat activities. It serves as the home base of False Bay's commercial line fishing fleet. In recent times Kalk Bay has become a touristic hub as it can be easily reached either by road or rail from Cape Town. It also has a functional cradle and slip facility to provide vessel maintenance. Adjacent to the harbour, there is undeveloped land used as parking space for visitors coming to the harbour (Kaiser Associates, 2012).

Figure 3. 6: An aerial view of Kalk Bay Harbour showing the sampling points (KB1, KB2 and KB3). (Source: Bing Map, 2017)

3.2.1.4 Gordon's Bay Harbour (34°09'49.7" S 18°51'33.0" E)

Gordon's Bay is situated on the eastern edge of False Bay where part of the Hottentots Holland Mountains dips its toes in the ocean. It is approximately 50 kilometres from the Cape Town CBD and adjacent to Somerset West and Strand. It has two operational harbours, namely Harbour Island (a) and the Old Harbour (b) (Figure 3.7). The Harbour Island is a marina development for yacht moorings and has a slipway owned by the Gordon's Bay Boat Angling Club. The Old Harbour is a mixed-use harbour, with most of the water space being used for a yacht marina by the Gordon's Bay Yacht Club, which has a large foothold in the harbour. The South African Navy (Gordon's Bay Academy) also has a facility in the harbour. A small fishing boat quay with a slipway and cradle is also active with a ship repair haul located next to the harbour (Kaiser Associates, 2012).

Figure 3. 7: An aerial view of the two harbours in Gordon's Bay and their sampling points (GB1, GB2 and GB3); (a) Harbour Island, and (b) Old Harbour. (Source: Bing Map, 2017)

3.2.1.5 Betty's Bay Marine Protected Area (34°21'45.2" S 18°54'14.1" E)

The Betty's Bay Marine Protected Area (MPA) is situated in the Kogelberg Nature Reserve Complex and lies approximately 29 km south-east of the coastal town of Gordon's Bay (Figure 3.8). It is adjacent to the coastal town of Betty's Bay, which lies along the Atlantic Ocean on the Southern Cape coast of South Africa. This MPA covers 3 km of coastline and includes the inshore marine environment between two rocky promontories, one on the west at Stony Point and the other on the east of Jock's Bay (Figure 3.9). The habitats within the MPA are diverse

and comprise of rocky shores, exposed sandy beaches, estuaries, sub-tidal reefs and kelp forests. The area is productive and supports a rich diversity of fish, invertebrate and algal species as well as populations of the African penguin and bank cormorant both of which are IUCN Red Data species. There are a variety of touristic attractions in and associated with the MPA including recreational shore angling, surfing and kite surfing, visits to the penguin colony and the whaling station, swimming and bathing, hiking and boating. All marine organisms are protected with no fishing allowed off a vessel within the boundaries of the MPA, with the exception of shore angling subject to valid permits (Chadwick et al., 2014; Marine Conservation Institute, 2018a).

Figure 3. 8: An aerial view of Betty's Bay MPA showing the sampling point (BB: MPA). (Source: Bing Map, 2017)

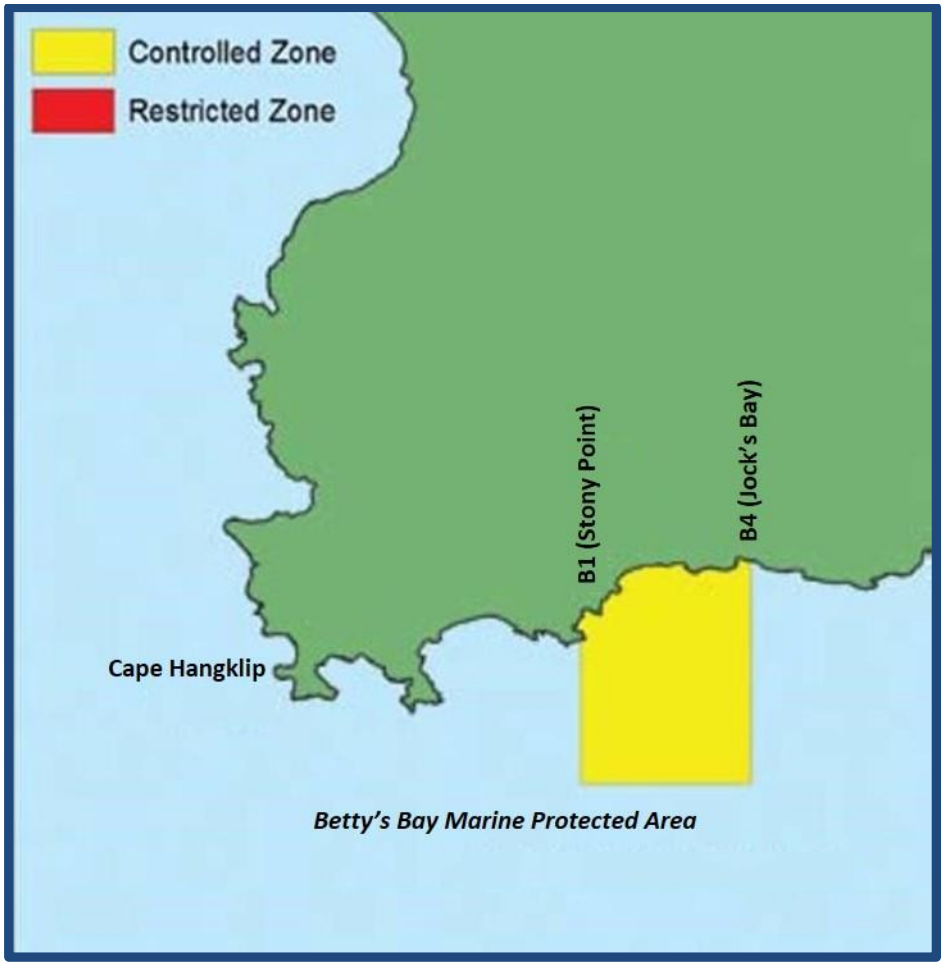


Figure 3. 9: The inshore boundaries of the Betty's Bay MPA (extend from the western boundary, B1 situated at Stony Point, to eastern boundary, B4 situated to the east of Jock's Bay, extending two nautical miles seawards from the high-water mark) (Source: Chadwick et al., 2014)

3.2.1.6 Cape of Good Hope Restricted Zone (34°21'25.5" S 18°28'24.9" E)

The Cape of Good Hope Restricted Zone is a 'no-take' zone situated within the Table Mountain National Park MPA, which curves around the long, thin Cape Peninsula from Mouille Point near Cape Town's centre in the west to Muizenberg in False Bay in the east (Figure 3.10). The Cape of Good Hope Restricted Zone stretches from between Hoek van die Bobbejaan and the fence at Scarborough and includes rocky cliffs and shores, sandy beaches, a safe haven for lobsters, and a number of 'no-take' zones that act as nurseries for the depleting fish species (Marine Conservation Institute, 2018b) (Figure 3.11). There is a high level of tourism, recreational activities as well as research and education with the area because of its proximity to Cape Town.

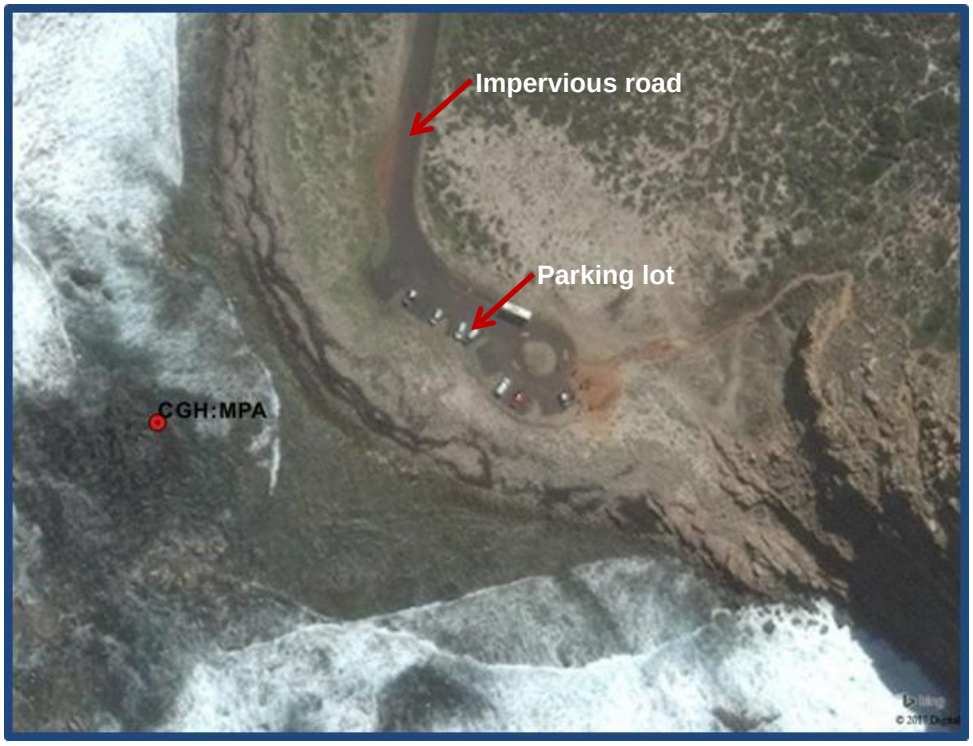


Figure 3. 10: An aerial view of the Cape of Good Hope Restricted Zone showing the sampling point (CGH: MPA). (Source: Bing Map, 2017)

Figure 3. 11: The inshore boundaries of the Cape of Good Hope Restricted Zone MPA (between Hoek van die Bobbejaan and the fence at Scarborough and extending approximately one nautical mile seawards). (Source: Chadwick et al., 2014)

3.3 COLLECTION OF SAMPLES

The sampling periods were divided into the dry season and wet season. All samples (seawater, sediment and gastropods) were collected once during spring low tides in the dry and wet season over a period of one year. The dry season and wet season sampling were carried out in a once-off trip per site in March and September 2016, respectively. The sampling events were scheduled to ensure that samples collected had fully experienced the respective seasons and therefore differing boat usage and weather conditions. In each of the six sampling sites (i.e., four harbours and two reference sites), three sampling points were chosen, except for the two reference sites with only one sampling point per site. The selections were based on water exchange and circulation (i.e. open and semi-enclosed waters) as well as vessel activities in the harbours and their potential as pollution point sources (e.g., vessel repair and maintenance areas and vessel anchorage areas). The distance between each sampling point ranged from 50-100m with all samples collected within a radius of 10m near to the shoreline. The temperature and pH of seawater at each sampling point were measured using a multi-parameter instrument (Hanna instruments: HI9811-5 Portable pH/EC/TDS/Temperature Meter). The equipment was checked and calibrated according to the manufacturers' specifications.

3.3.1 Gastropods

The two marine gastropods, *Burnupena* spp. and *Nucella* spp. (hereafter gastropods) were randomly picked by hand in the intertidal rocky shores. The gastropods were found at low tides attached to the rocks and in the crevices as well as attached to artificial structures (pier-pilings, pontoons, breakwaters, etc.). Five specimens each were collected at each sampling point for all sites. Every attempt was made to find both gastropods at all sampling points but at some sampling points only one gastropod could be found (Table 3.1). Age and size-related differences in metal bioaccumulation (Bourgoin, 1990) were circumvented by selecting individuals of similar shell length between 35-40mm and 20-30mm for *Burnupena* spp. and *Nucella* spp., respectively. Sex was not determined as the variability of copper and zinc concentrations due to sex difference was not part of the study. The gastropods were identified based on the description by Branch et al. (2010). The collected specimens were immediately rinsed with seawater and kept in plastic containers in an icebox. The gastropods were not subjected to depuration as it might lead to contamination (Phillips & Rainbow, 1988; Blackmore, 2000; Yap & Cheng, 2010). The specimens were then transported to the laboratory and frozen at -20°C until analysis.

Table 3. 1: Gastropods found at each sampling points in the harbours and reference sites

Harbours and Reference sites	Sampling points	Organisms found	
		Dry season	**Wet season**
BB: MPA	BB_{MPA}	*Burnupena* spp*., Nucella* spp.	*Burnupena* spp*, Nucella* spp.
CGH: MPA	CGH_{MPA}	*Burnupena* spp*., Nucella* spp.	*Burnupena* spp*., Nucella* spp.
GRB	GRB1	*Burnupena* spp*., Nucella* spp.	*Burnupena* spp.
	GRB2	*Burnupena* spp.	*Burnupena* spp.
	GRB3	*Nucella* spp.	*Nucella* spp.
HB	HB1	*Burnupena* spp*., Nucella* spp.	*Burnupena* spp*., Nucella* spp.
	HB2	*Nucella* spp.	*Nucella* spp.
	HB3	*Nucella* spp.	*Nucella* spp.
KB	KB1	*Nucella* spp.	*Nucella* spp.
	KB2	*Nucella* spp.	*Burnupena* spp*., Nucella* spp.
	KB3	*Nucella* spp.	*Nucella* spp.
GB	GB1	*Burnupena* spp.	*Burnupena* spp.
	GB2	*Burnupena* spp.	*Burnupena* spp.
	GB3	*Nucella* spp.	*Burnupena* spp*., Nucella* spp.

3.3.2 Sediment

Sediment samples were collected within a 10 x 10m area, using a small plastic scoop. Five replicates of surface sediment to a depth of 2cm were collected at each sampling point and placed into polyethylene ziplock bags. The samples were immediately kept in an icebox, transported to the laboratory and stored at -20°C until further analysis. All sampling equipment (e.g., scoops, bags) were made of non-contaminating material cleaned with distilled water before and after each sampling occasion.

The particle size distribution of the sediment samples collected from the harbours and reference sites were determined using the hydrometer method at Bemlab Testing Laboratory. Chemical dispersion was done using Sodium hexametaphosphate and three sand fractions (fine sand, coarse sand, and gravel) were determined through sieving. Silt and clay were then determined using sedimentation rates at 20°C, using an ASTM E100 (152H-TP) hydrometer. Sediment particle-size analysis is important as the surface areas of sediment depends on the grain size which influences the adsorption and desorption of metals in sediment and may

modify sediment metal chemistry and bioavailability in aquatic ecosystems (Simpson et al., 2005).

3.3.3 Seawater

Seawater samples from a depth of approximately 30cm were collected with a 200ml jug at the same area as the sediment samples. The 200mL jug was rinsed twice with seawater and used to collect five replicate samples of 100mLeach in plastic containers. The samples were kept in an icebox during transportation to the laboratory and stored at 4°C until further analysis. It should be noted that the sampling jug and plastic containers were first cleaned by soaking with 10% nitric acid for 2 days and rinsed with distilled water until neutral pH.

3.4 SAMPLE PREPARATION FOR METAL ANALYSIS

The detailed process for acid digestion was carried out by following the procedure outlined by Odendaal & Reinecke, (1999). Acid blanks were routinely digested and diluted in the same way as the samples and were analysed along with them. Glassware and equipment used in the metal analysis were acid-washed by soaking in 10% nitric acid for 24 hours and then rinsed several times with distilled water and dried to avoid possible contamination.

3.4.1 Gastropod soft tissue, shells and sediment

The gastropods were thawed, and their soft tissue were removed from the shells by using pre-cleaned stainless steel and plastic forceps, to avoid any contamination. Each sample (soft tissue and shell) was then rinsed with distilled water, to remove any contaminants. Frozen sediment samples were also allowed to thaw at room temperature. All samples (gastropods soft tissue/shells, and sediment) were dried in an oven (Memmert drying oven) for 48 hours at 60°C. Thereafter, whole soft tissue, shells and sediment were ground separately using a glass mortar to obtain a 0.1-0.3g subsample using a Precisa XB 220A balance. Samples were digested in 10ml 65% nitric acid at 40 °C in a Grant UBD heating block for one hour, thereafter to 120 °C for 3 hours. The digestates were allowed time to cool and then filtered through Whatman No.6 (90mm) filter paper into labelled volumetric flasks. Each digestates was diluted to 20ml with distilled water and then filtered through 0.45µm cellulose nitrate membrane micro-filter (Millipore) paper into pill vials using syringes. Thereafter, 1ml subsamples were placed in plastic centrifuge tubes and diluted to 10ml with distilled water. The samples were then stored in the refrigerator for analysis

3.4.1 Seawater

10ml aliquots of seawater were digested in 5ml of 65% nitric acid at 40 °C in a Grant UBD heating block for one hour, thereafter to 120 °C for 3 hours. The digestates were allowed time to cool and then filtered through Whatman No.6 (90mm) filter paper into labelled volumetric flasks. Each digestates was diluted to 20ml with distilled water and then filtered through 0.45µm cellulose nitrate membrane micro-filter (Millipore) paper into pill vials using syringes. Thereafter, 2ml subsamples were placed in plastic centrifuge tubes and diluted to 10ml with distilled water. The samples were then stored in the refrigerator for analysis.

3.5 RAINFALL DATA

The rainfall data at weather stations within the sampling sites from January to December 2016 was obtained from the South African Weather Service (SAWS). For sampling sites where no weather station was located, rainfall data from the closest weather station were used.

3.6 MARINE WATER AND SEDIMENT QUALITY EVALUATION

Water quality guidelines play an important role in protecting water uses as well as in evaluating the impact of environmental contaminants on the quality and uses of aquatic resources. The South African Water Quality Guidelines for Coastal Marine Waters (SAWQGs) was first published in 1995 by the Department of Water Affairs and Forestry (DWAF) and has currently been updated by the Department of Environmental Affairs (DEA). The SAWQGs consists of the guidelines for industrial uses, marine aquaculture, recreational and the protection of the natural environment. These guidelines are used in setting site-specific water quality objectives in the marine environment. For a water body, the water quality objectives are the target values of the different water quality constituents which have been set for the designated beneficial uses. The target values of copper and zinc in seawater recommended in the South African Water Quality Guidelines for Coastal Marine Waters (Volume 1: Natural Environment and Mariculture Use) have been used for the purpose of this study (DEA, 2018)(Table 3.2).

Sediment Quality Guidelines (SQGs) are also important due to the profound influence of sediment on the health of aquatic organisms, which may be exposed to chemicals through their immediate interactions with bottom sediments. In depositional areas, sediment tends to integrate chemical contaminant inputs which may associate with particulate matter and eventually incorporated into bottom sediment over time. As a result, sediment may become a long-term source of chemical contaminants to the aquatic environment, not only to benthic organisms but also to the overlying water column (BCLME, 2006). Therefore, the comparison of sediment concentrations with corresponding SQGs provides a very useful approach in

assessing sediment contamination in the aquatic environment (MacDonald et al., 2000). The use of SQGs for evaluating the toxicological significance of sediment-associated chemicals such as metals has become an integral component in the management and protection of aquatic ecosystems. There are currently no SQGs for the marine environment in South Africa. However, the sediment metal concentrations in this study were compared to the SQGs for the protection of marine aquatic ecosystems for the Benguela Current Large Marine Ecosystem (BCLME) in southern Africa (BCLME, 2006) (Table 3.2).

Table 3. 2: Water and sediment quality guideline values used in this study

Metals	Seawater(mg/L)[1]	Sediment (mg/kg DW)[2]	
		TEL	PEL
Copper	0.003	18.7	108
Zinc	0.02	124	271

TEL= the threshold effects level; PEL=Probable effects level; [1]DEA, 2018; [2]BCLME, 2016

3.7 METAL ANALYSIS

All analyses were carried out at the Central Analytical Facilities (CAF) at Stellenbosch University. The concentrations of copper and zinc were analysed in quintuplicate using an Agilent 8800 Triple Quadrupole Inductively Coupled Plasma – Mass Spectrometry (ICP- MS). Detection limits for all metals analysed were 0.1 ppb. The concentrations of copper and zinc were computed using the following two formulae:

I. Metal concentrations in soft tissue, shells and sediment

$$= \frac{[\mathbf{ICP\ Value - Blank}]\mathbf{X}\ [\mathbf{200}]}{\mathbf{Sample\ Mass}\ (\mathbf{g})}$$ Unit= mg/kg dry weight (DW)

II. Metal concentrations in seawater

$$= [\mathbf{ICP\ Value} - \mathbf{Blank}]\mathbf{x}\ [\mathbf{10}]$$ Unit=mg/L

3.8 STATISTICAL ANALYSIS

All statistical analyses were conducted using SimaPlot 13 software (SYSTAT Software Inc.) and data are presented as mean (±SD). Normality of data was tested using a Shapiro–Wilk test and median values were used for analyses as data sets were shown to be non-parametric. Statistically significant differences in copper and zinc concentrations between sampling points within each harbour and the two reference sites for the two seasons were evaluated using a Kruskal–Wallis One-Way ANOVA on Ranks and Student Newman Kuels Method for post hoc tests. The Dunn's Method was also used for Post hoc test after the ranked based ANOVA to evaluate significant differences in copper and zinc concentrations between sampling sites (pooled data sets) as their pooled data sets were unequal. The Mann Whitney Rank Sum Test was used for comparisons in copper and zinc concentrations between the dry and wet seasons per sampling point, the dry and wet seasons per sampling sites (pooled data sets) and between soft tissue and shell per sampling point for the two seasons. The Spearman's Rank Order Correlation was used to determine if there was a relationship between copper and zinc concentrations in ambient samples (seawater and sediment) and soft tissue of the gastropods as the data was non-parametric. For all statistical analyses, the condition for the significant difference was set at $p < 0.05$.

3.9 ETHICAL STATEMENT

This study was carried out after the issuance of an ethical clearance by the Animal Ethics Committee (AEC) and the Higher Degrees Committee (HDC) of the Cape Peninsula University of Technology. All samples (water, sediment and gastropods) were collected with permissions from the harbours (approval by Harbour Masters) and reference sites (Permit No. CRC/2015/025/--2015/V1 and Permit No. 0052-AAA008-00029) under an integrated research permit issued jointly by the Department of Environmental Affairs and Department of Agriculture, Forestry and Fisheries (Appendix C). The gastropods collected are not currently listed as endangered or protected.

CHAPTER FOUR

RESULTS: COPPER & ZINC

The results of this study are expressed in milligrams of metal (copper and zinc) per litre (mg/L) for seawater; milligrams of metal per kilogram of dry weight (mg/kg DW) for sediment and gastropods and are the mean value of five replicates. It should be highlighted that because the *Burnupena* spp. and *Nucella* spp. were not available at all sampling points in each harbour and at all sampling seasons (Table 3.1), comparisons of copper and zinc concentrations in the gastropods between the harbours (pooled metal concentrations of sampling points in each harbour) were not possible.

4.1 PHYSICO-CHEMICAL PARAMETERS

4.1.1 Temperature

In this study, the seawater surface temperatures of the sampling points were measured for each season (Table 4.1). In the West Coast area of South Africa, the seawater surface temperature ranges are usually between 11 and 18°C, depending on upwelling conditions (DEA, 2018). The seawater surface temperatures recorded at sampling points were in the range of 15.1 and 17.8°C, which was within the yearly mean seawater surface temperature ranges for the South African west coast area.

4.1.2 pH

A measure of the concentration of hydrogen ions in a solution is known as the pH. It is measured on a scale from 0.0 to 14.0. Many chemical and biological processes in water are affected by changes in pH. Water with a pH less than 7 is acidic, whereas alkaline water has a pH of greater than 7. The pH of seawater ranges between 7.9 and 8.5 (DEA, 2018). The pH values measured at the sampling points for each season (Table 4.1), ranged between 7.0 and 8.2 and indicate the moderately alkaline nature of the seawater.

Table 4. 1: Physico-chemical measurements (Temperature and pH) taken from the sampling points in each harbour and the two reference sites during the dry and wet season sampling occasions

Harbours and Reference sites	Sampling points	Dry season		Wet season	
		Temperature (°C)	pH	Temperature (°C)	pH
BB:MPA	BB_{MPA}	17.2	7.9	15.1	8.2
CGH:MPA	CGH_{MPA}	17.3	8.2	16.3	8.1
GRB	GRB1	16.0	7.9	16.4	8.1
	GRB2	15.5	7.8	16.0	8.0
	GRB3	15.8	7.8	15.9	7.9
	Mean	15.8	7.8	16.1	8.0
HB	HB1	16.8	7.2	14.6	7.9
	HB2	16.1	7.3	15.9	7.9
	HB3	15.9	7.0	14.3	7.6
	Mean	16.3	7.2	14.7	7.8
KB	KB1	18.0	8.0	17.0	8.0
	KB2	17.9	7.9	17.1	8.0
	KB3	17.6	8.0	17.3	8.0
	Mean	17.8	8.0	17.1	8.0
GB	GB1	16.0	7.9	16.0	7.6
	GB2	16.3	7.9	16.4	7.7
	GB3	16.6	7.7	15.9	7.7
	Mean	16.3	7.8	16.1	7.7

4.2 SEDIMENT CHARACTERISTICS

In this study, sand-sized particles were predominant in the harbours and the two reference sites and contain less than 8% clay-sized particles (Table 4.2). Sediment from GRB, KB, GB, and CGH: MPA had the highest proportion of sand-sized particles (93%). A silt component was present in small amounts (2%) in GRB, HB, and BB: MPA but not found in KB, GB, and CGH: MPA.

Table 4. 2: Sediment characteristics related to particle-size for the four harbours and the two reference sites based on Shepard's sediment classification

Harbours and Reference sites	Clay %	Silt %	Sand %
BB:MPA	7	2	91
CGH:MPA	7	0	93
GRB	5	2	93
HB	7	2	91
KB	7	0	93
GB	7	0	93

4.3 RAINFALL DATA

Table 4. 3: Monthly mean rainfall (mm) from weather stations located adjacent to the sampling sites during the study period (2016)

Months	Royal Yacht Club* (GRB)	Hout Bay (HB)	Fish Hoek (KB)	Strand* (GB)	Betty's Bay (BB: MPA)	Cape Point (CGH: MPA)
January	5.8	10.6	18.4	5.6	24.9	4.8
February	3.0	4.4	6.4	20.0	26.2	8.0
March	27.6	44.2	49.9	65.8	152.0	19.8
April	28.0	43.6	53.7	45.0	83.5	19.8
May	17.8	34.2	28.3	27.6	27.5	21.2
June	78.4	88.6	94.9	97.2	204.7	52.8
July	136.6	133.8	116.4	128.0	157.0	75.0
August	53.2	75.2	93.0	71.2	129.0	57.8
September	29.6	72.6	80.6	56.8	89.0	60.4
October	15.0	35.2	14.4	13.8	20.9	20.2
November	3.2	8.2	7.0	1.4	8.5	3.2
December	29.2	39.2	46.6	11.8	6.5	11.8

*Closest weather station to sampling site; Source: SAWS

As previously mentioned in section 3.5, the annual rainfall data recorded at weather stations in the sampling sites or close to the sampling sites during the sampling period was obtained from the SAWS (Table 4.3).

4.4 COPPER CONCENTRATIONS

4.4.1 Seawater

The mean copper concentrations (mg/L) measured in seawater samples from the four harbours (Granger Bay Harbour [GRB], Hout Bay Harbour [HB], Kalk Bay Harbour [KB] and Gordon's Bay Harbour [GB]) and the two reference sites (Betty's Bay Marine Protected Area [BB:MPA] and Cape of Good Hope Marine Protected Area [CGH:MPA]) for the study period are displayed in Table 4.4.

Table 4. 4: Mean copper concentrations (mg/L) (±SD) in seawater from sampling points in the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

		Seawater (mg/L)	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	0.0863 (±0.0735)^{D}	0.0105 (±0.0054)
CGH:MPA	**CGH_{MPA}**	0.0262 (±0.0306)	0.0100 (±0.0080)
GRB	**GRB1**	ND	0.0520 (±0.0934)
	GRB2	0.0027 (±0.0050)^{A}	0.0053 (±0.0079)
	GRB3	ND	0.0210 (±0.0241)
BB:MPA	**BB_{MPA}**	0.0863 (±0.0735)	0.0105 (±0.0054)^{FGH}
CGH:MPA	**CGH_{MPA}**	0.0262 (±0.0306)	0.0100 (±0.0080)
HB	**HB1**	0.0246 (±0.0194)	0.0020 (±0.0028)^{A}
	HB2	*0.0818 (±0.0494)	0.0011 (±0.0013)^{A}
	HB3	0.0377 (±0.0278)	0.0009 (±0.0016)^{A}
BB:MPA	**BB_{MPA}**	0.0863 (±0.0735)	0.0105 (±0.0054)
CGH:MPA	**CGH_{MPA}**	0.0262 (±0.0306)	0.0100 (±0.0080)
KB	**KB1**	ND	0.0393 (±0.0748)
	KB2	ND	0.0013 (±0.0028)
	KB3	ND	0.0086 (±0.0142)
BB:MPA	**BB_{MPA}**	0.0863 (±0.0735)^{LN}	0.0105 (±0.0054)
CGH:MPA	**CGH_{MPA}**	0.0262 (±0.0306)	0.0100 (±0.0080)
GB	**GB1**	0.0023 (±0.0051)^{A}	0.0182 (±0.0180)
	GB2	*0.0017 (±0.0039)	0.0134 (±0.0089)
	GB3	0.0123 (±0.0269)^{A}	0.0228 (±0.0114)

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference between seasons per sampling point is indicated by an asterisk (*). ND=Not Detected; Dotted underline numbers =Exceed SAWQGs (0.003mg/L); *n*= number of replicates.

4.4.1.1 Comparisons of copper concentrations in seawater from sampling points within each harbour and the two reference sites for the dry and wet seasons

4.4.1.1.1 ***Dry season***

4.4.1.1.1.1 Granger Bay Harbour

The pairwise multiple comparisons of copper concentrations in seawater revealed that GRB2 had a significantly lower ($p<0.05$) copper concentration than BB_{MPA}. There was no statistically significant difference ($p>0.05$) between GRB2 and CGH_{MPA}. Copper was not detected at GRB1 and GRB3 and therefore, no comparisons could be done.

4.4.1.1.1.2 Hout Bay Harbour

All pairwise multiple comparisons of copper concentrations in seawater at HB indicated that there were no statistically significant differences ($p>0.05$) between each of the three sampling points (HB1, HB2, and HB3) and the two reference sites.

4.4.1.1.1.3 Kalk Bay Harbour

No statistical analyses could be done at KB as copper was not detected in seawater collected from all three sampling points.

4.4.1.1.1.4 Gordon's Bay Harbour

Pairwise multiple comparisons of copper concentrations in seawater at GB revealed that GB1 and GB3 had significantly lower ($p<0.05$) copper concentrations than BB_{MPA}. There were no statistically significant differences ($p>0.05$) between each of the three sampling points and CGH_{MPA}.

4.4.1.1.2 ***Wet season***

4.4.1.1.2.1 Granger Bay Harbour

Copper concentrations for seawater at GRB, in pairwise multiple comparisons showed no statistically significant differences ($p>0.05$) between each of the three sampling points and the two reference sites.

4.4.1.1.2.2 Hout Bay Harbour

Pairwise multiple comparisons of copper concentrations in seawater at HB revealed that HB1, HB2, and HB3 had significantly lower ($p<0.05$) copper concentrations than BB_{MPA}. No statistically significant differences ($p>0.05$) were found between each of the three sampling points and CGH_{MPA}.

4.4.1.1.2.3 Kalk Bay Harbour

Multiple comparisons of copper concentrations in seawater at KB revealed no statistically significant differences ($p>0.05$) between each of the three sampling points and the two reference sites.

4.4.1.1.2.4 Gordon's Bay Harbour

All pairwise multiple comparisons of copper concentrations in seawater at GB showed no statistically significant differences ($p>0.05$) between each of the three sampling points (GB1, GB2, and GB3) and the two reference sites.

4.4.1.2 Comparisons of copper concentrations in seawater between sampling seasons per sampling point

4.4.1.2.1 Granger Bay Harbour

Comparisons of copper concentrations in seawater revealed no statistically significant seasonal differences ($p>0.05$) at GRB2. No comparisons were done for GRB1 and GRB2 as copper was not detected during dry season sampling (Figure 4.1a).

4.4.1.2.2 Hout Bay Harbour

Comparisons of copper concentrations in seawater showed that HB2 had a significantly higher ($p<0.05$) copper concentration in the dry season than in the wet season. There were no statistically significant seasonal differences ($p>0.05$) at HB1 and HB3 (Figure 4.1b).

4.4.1.2.3 Kalk Bay Harbour

No seasonal comparisons could be executed in KB as copper was not detected in all three sampling points during the dry season sampling (Figure 4.1c).

4.4.1.2.4 Gordon's Bay Harbour

Comparisons of copper concentrations in seawater indicated that GB2 had a significantly lower ($p<0.05$) copper concentration in the dry season than in the wet season. There were no statistically significant seasonal differences ($p>0.05$) at GB1 and GB3 (Figure 4.1d).

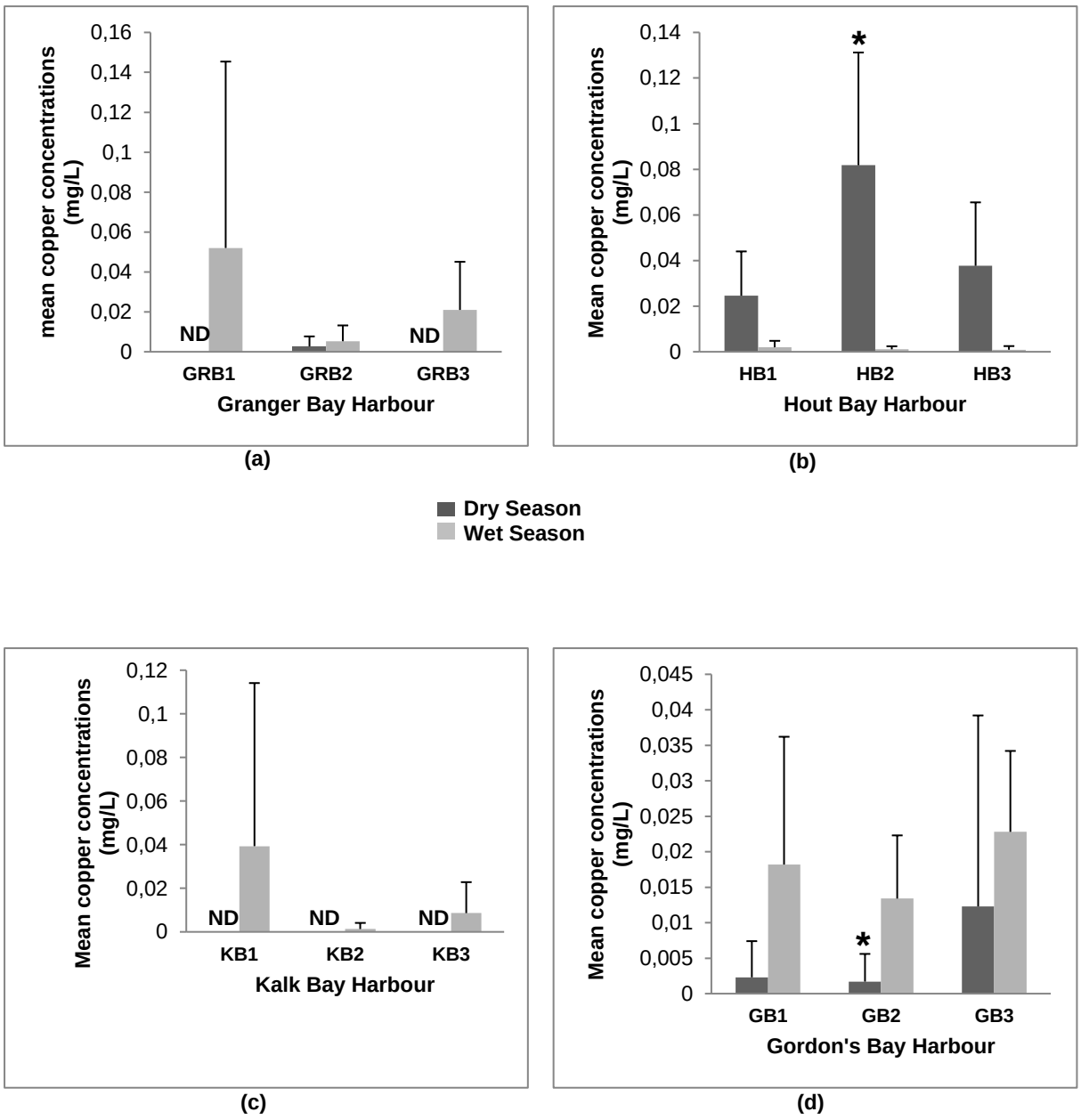


Figure 4. 1: Comparisons of mean copper concentration in seawater between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; ND=Not Detected; (a): mean copper concentrations in GRB; (b): mean copper concentrations in HB; (c): mean copper concentrations in KB; (d): mean copper concentrations in GB.

4.4.1.3 Comparison of the pooled copper concentrations in seawater between the harbours and the two reference sites for different sampling seasons

To compare the copper concentrations in seawater between the harbours and the two reference sites, datasets of the three sampling points in each harbour were pooled for the dry and wet season sampling occasions. The pooled datasets were used for statistical analysis (Table 4.5).

Table 4. 5: Pooled mean copper concentrations (mg/L) (±SD) in seawater from the four harbours and the two reference sites for dry and wet season sampling occasions

	Seawater (mg/L) (±SD)	
Harbours and reference sites	**Dry Season**	**Wet Season**
BB:MPA (*n*=5)	0.0863 (±0.0735)^{FC}	0.0105 (±0.0054)
CGH:MPA (*n*=5)	0.0262 (±0.0306)^{C}	0.0100 (±0.0080)^{D}
GRB (*n*=15)	*0.0009 (±0.0030)^{ABD}	0.0261 (±0.0555)^{D}
HB (*n*=15)	*0.0480 (±0.0408)^{CF}	0.0013 (±0.0019)^{BCF}
KB (*n*=15)	ND	0.0164 (±0.0442)
GB (*n*=15)	*0.0054 (±0.0156)^{AD}	0.0181 (±0.0129)^{D}

To compare copper concentrations in seawater between harbours, letters (A-F) were used to denote significant differences (significant difference from: BB: MPA=A; CGH: MPA=B; GRB=C; HB=D; KB=E and GB=F). A significant difference between seasons per harbour is indicated by an asterisk (*) on the left; ND=Not Detected; Dotted underline numbers =Exceed SAWQGs (0.003mg/L); *n*= number of replicates.

*4.4.1.3.1 **Dry season***

All pairwise multiple comparisons of copper concentrations in seawater revealed that GRB had a significantly lower ($p<0.05$) copper concentration than HB and the two reference sites. Likewise, multiple comparisons showed that GB had a significantly lower ($p<0.05$) copper concentration than HB and BB_{MPA}. No statistically significant differences ($p>0.05$) were found between HB and the two reference sites, and between GB and CGH_{MPA}. Copper was not detected in seawater from KB; therefore, comparisons could not be done.

*4.4.1.3.2 **Wet season***

All pairwise multiple comparisons of copper concentrations in seawater indicated that HB had a significantly lower ($p<0.05$) copper concentration than GRB, GB and reference site CGH_{MPA}. There were no significant differences ($p>0.05$) in copper concentrations between the four harbours and $BB_{MPA.}$ Furthermore, except for HB, the four harbours showed no significant difference ($p>0.05$) when compared to $CGH_{MPA.}$ Also, no statistically significant differences ($p>0.05$) were found between KB and GB, HB and KB, GRB and KB, and between GRB and GB.

4.4.1.4 Comparisons of the pooled copper concentrations in seawater between sampling seasons per harbour

Seasonal comparisons of pooled mean copper concentrations in seawater per harbour are displayed in Figure 4.2.

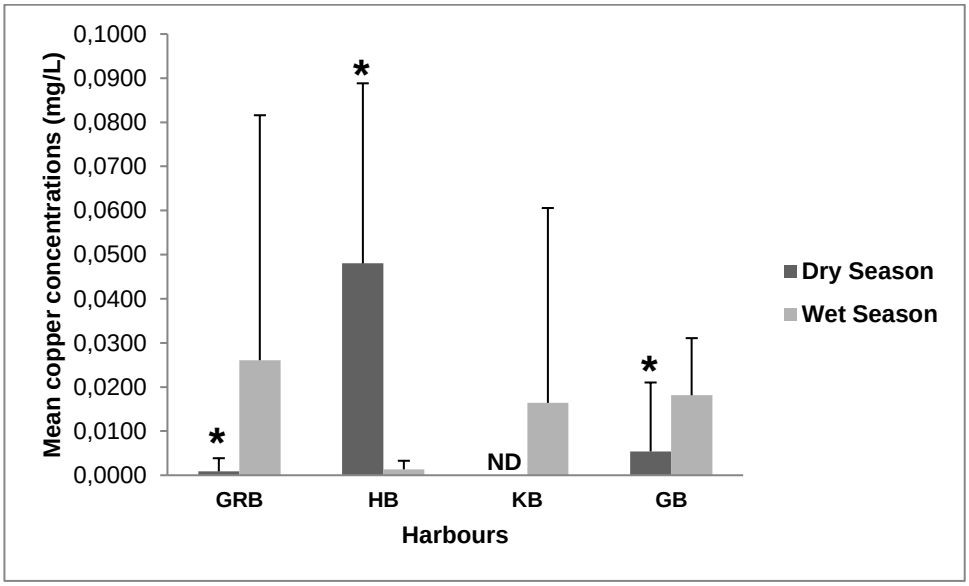


Figure 4. 2: Comparisons of pooled mean copper concentrations in seawater between seasons per harbour. An asterisk (*) above the bar showed a significant seasonal difference. Error bars = ±SD; ND=Not Detected

4.4.1.4.1 Granger Bay Harbour

Seasonal comparisons of copper concentrations at GRB revealed that dry season copper concentration was significantly lower ($p<0.05$) than that for the wet season.

4.4.1.4.2 Hout Bay Harbour

Comparisons of copper concentrations in HB for the two seasons revealed that dry season copper concentrations were significantly higher ($p<0.05$) than that for the wet season.

4.4.1.4.3 Kalk Bay Harbour

No seasonal comparisons could be done in KB as copper was not detected in seawater for dry season sampling.

4.4.1.4.4 Gordon's Bay Harbour

Seasonal comparisons in GB revealed that copper concentrations for the dry season were significantly lower ($p<0.05$) than that for the wet season.

4.4.2 Sediment

Table 4. 6: Mean copper concentrations (mg/kg DW) (±SD) in sediment from sampling points in the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

		Sediment (mg/kg DW) (±SD)	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	ND	0.55 (±0.59)^{BCDE}
CGH:MPA	**CGH_{MPA}**	8.10 (±16.15)^{DE}	**289.87 (±614.30)**^{A}
GRB	**GRB1**	ND	2.03 (±1.73)^{ADE}
	GRB2	**52.14 (±78.11)*^{B}	8.59 (±3.50)^{AC}
	GRB3	*20.53 (±1.94)*^{B}	11.76 (±2.26)^{AC}
BB:MPA	**BB_{MPA}**	ND	0.55 (±0.59)^{BH}
CGH:MPA	**CGH_{MPA}**	8.10 (±16.15)	289.87 (±614.30)^{AH}
HB	**HB1**	0.97 (±2.17)	0.52 (±0.44)^{H}
	HB2	2.24 (±2.29)	1.10 (±0.55)^{H}
	HB3	ND	3.60 (±0.59)^{ABFG}
BB:MPA	**BB_{MPA}**	ND	0.55 (±0.59)^{BIJK}
CGH:MPA	**CGH_{MPA}**	8.10 (±16.15)^{IJ}	289.87 (±614.30)^{AI}
KB	**KB1**	**2145.39 (±843.60)**^{BJK}	**3432.16 (±2306.68)**^{ABJK}
	KB2	**19.55 (±10.00)*^{BIK}	8.52 (±2.00)^{AIK}
	KB3	*8.40(±2.21)^{IJ}	*22.24 (±5.23)*^{AIJ}
BB:MPA	**BB_{MPA}**	ND	0.55 (±0.59)^{BLMN}
CGH:MPA	**CGH_{MPA}**	8.10(±16.15)^{LN}	289.87 (±614.30)^{A}
GB	**GB1**	*55.15(±93.35)*^{BMN}	11.29 (±7.76)^{AN}
	GB2	13.65(±22.28)^{LN}	7.53 (±0.42)^{AN}
	GB3	***757.93(±531.95)**^{BLM}	**237.36 (±217.23**)^{ALM}

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference in mean copper concentrations between seasons per sampling point is indicated by an asterisk (*) on the left. ND=Not Detected; Numbers in italics= Exceed TEL (18.7mg/kg); Numbers in bold= Exceed PEL (108mg/kg); *n*= number of replicates.

4.4.2.1 Comparisons of copper concentrations in sediment from sampling points within each harbour and the two reference sites for the dry and wet seasons

4.4.2.1.1 ***Dry season***

4.4.2.1.1.1 Granger Bay Harbour

All pair multiple comparisons of copper concentrations from sediment in GRB revealed that GRB2 and GRB3 had significantly higher ($p<0.05$) copper concentrations than CGH_{MPA}. There was no statistically significant difference ($p>0.05$) between GRB2 and GRB3. It should be noted that copper was not detected at BBMPA and GRB1 and therefore were not included in the comparisons.

4.4.2.1.1.2 Hout Bay Harbour

The pairwise multiple comparisons of sediment copper concentrations in HB revealed no statistically significant differences ($p>0.05$) occurred between HB1 and HB2 and when compared to CGH_{MPA}.

4.4.2.1.1.3 Kalk Bay Harbour

Multiple comparisons of sediment copper concentrations at KB indicated that copper concentration at KB1 was significantly higher ($p<0.05$) than at KB2, KB3 and control CGH_{MPA}. Also, pairwise multiple comparisons revealed that KB2 had a significantly higher ($p<0.05$) copper concentration than KB3. Furthermore, the copper concentration at KB2 was significantly higher ($p<0.05$) than at CGH_{MPA}. No statistically significant difference ($p>0.05$) occurred between KB3 and CGH_{MPA}.

4.4.2.1.1.4 Gordon's Bay Harbour

The sediment copper concentrations in pairwise multiple comparisons revealed that GB3 had a significantly higher ($p<0.05$) copper concentration than GB1, GB2 and CGH_{MPA}. Also, comparisons showed that the copper concentration at GB1 was significantly higher ($p<0.05$) than at CGH_{MPA}. There was no significant difference ($p>0.05$) between GB2 and CGH_{MPA}.

4.4.2.1.2 ***Wet season***

4.4.2.1.2.1 Granger Bay Harbour

All pairwise multiple comparisons of sediment copper concentrations in GRB indicated that GRB1 had a significantly lower ($p<0.05$) copper concentration than GRB2, GRB3 and BB_{MPA}. Also, multiple comparisons revealed that the copper concentrations at GRB2 and GRB3 were significantly higher ($p<0.05$) than at BB_{MPA}. There was no significant difference ($p>0.05$)

between GRB2 and GRB3. Furthermore, all four harbours did not differ ($p>0.05$) significantly with CGH_{MPA}.

4.4.2.1.2.2 Hout Bay Harbour

The pairwise multiple comparisons of sediment copper concentrations in HB showed that HB3 had a significantly lower ($p<0.05$) copper concentration than HB1, HB2, and the two reference sites. There were no statistically significant differences ($p>0.05$) between HB1 and HB2, HB1 and the two reference sites, and between HB2 and the two reference sites.

4.4.2.1.2.3 Kalk Bay Harbour

Multiple comparisons of sediment copper concentrations at KB revealed that the copper concentration at KB1 was significantly higher ($p<0.05$) than at KB2, KB3 and the two reference sites. Also, comparisons revealed that KB2 had a significantly lower ($p<0.05$) copper concentration than KB3. Furthermore, comparisons showed that KB2 and KB3 had significantly lower ($p<0.05$) copper concentrations than BB_{MPA}. There were no statistically significant differences ($P>0.05$) between KB2 and CGH_{MPA}, and between KB3 and CGH_{MPA}.

4.4.2.1.2.4 Gordon's Bay Harbour

All pairwise multiple comparisons of sediment copper concentrations at GB indicated that GB3 had a significantly higher ($p<0.05$) copper concentration than GB1, GB2 and BB_{MPA}. Also, copper concentrations at GB2 and GB3 were significantly higher ($p<0.05$) than at BB_{MPA}. There were no statistically significant differences ($p>0.05$) between all four harbours and CGH_{MPA}.

4.4.2.2 Comparisons of copper concentrations in sediment between sampling seasons per sampling point

4.4.2.2.1 Granger Bay Harbour

Seasonal comparisons of sediment copper concentrations in GRB showed that GRB2 had a significantly higher ($p<0.05$) copper concentration in the dry season than in the wet season. There was no statistically significant seasonal difference ($p>0.05$) at GRB3. However, no comparisons were done for GRB1 as copper was not detected in the sediment from dry season sampling (Figure 4.3a).

4.4.2.2.2 Hout Bay Harbour

Comparisons of sediment copper concentrations at HB reveal no statistically significant seasonal differences ($p>0.05$) at HB1 and HB2. Copper was not detected in sediment from HB3 for dry season sampling (Figure 4.3b).

4.4.2.2.3 Kalk Bay Harbour

Seasonal comparisons of copper concentrations in sediment at KB indicated that KB2 had a significantly higher ($p<0.05$) copper concentration in the dry season than the wet season. Also, copper concentration at KB3 was significantly lower ($p<0.05$) for the dry season sampling than for the wet season. No significant seasonal difference ($p>0.05$) was found at KB1 (Figure 4.3c).

4.4.2.2.4 Gordon's Bay Harbour

Comparisons of copper concentrations in sediment at GB showed that GB3 had a significantly higher ($p<0.05$) copper concentration in the dry season than the wet season. No statistically significant seasonal differences ($p>0.05$) were found for GB1 and GB2 (Figure 4.3d).

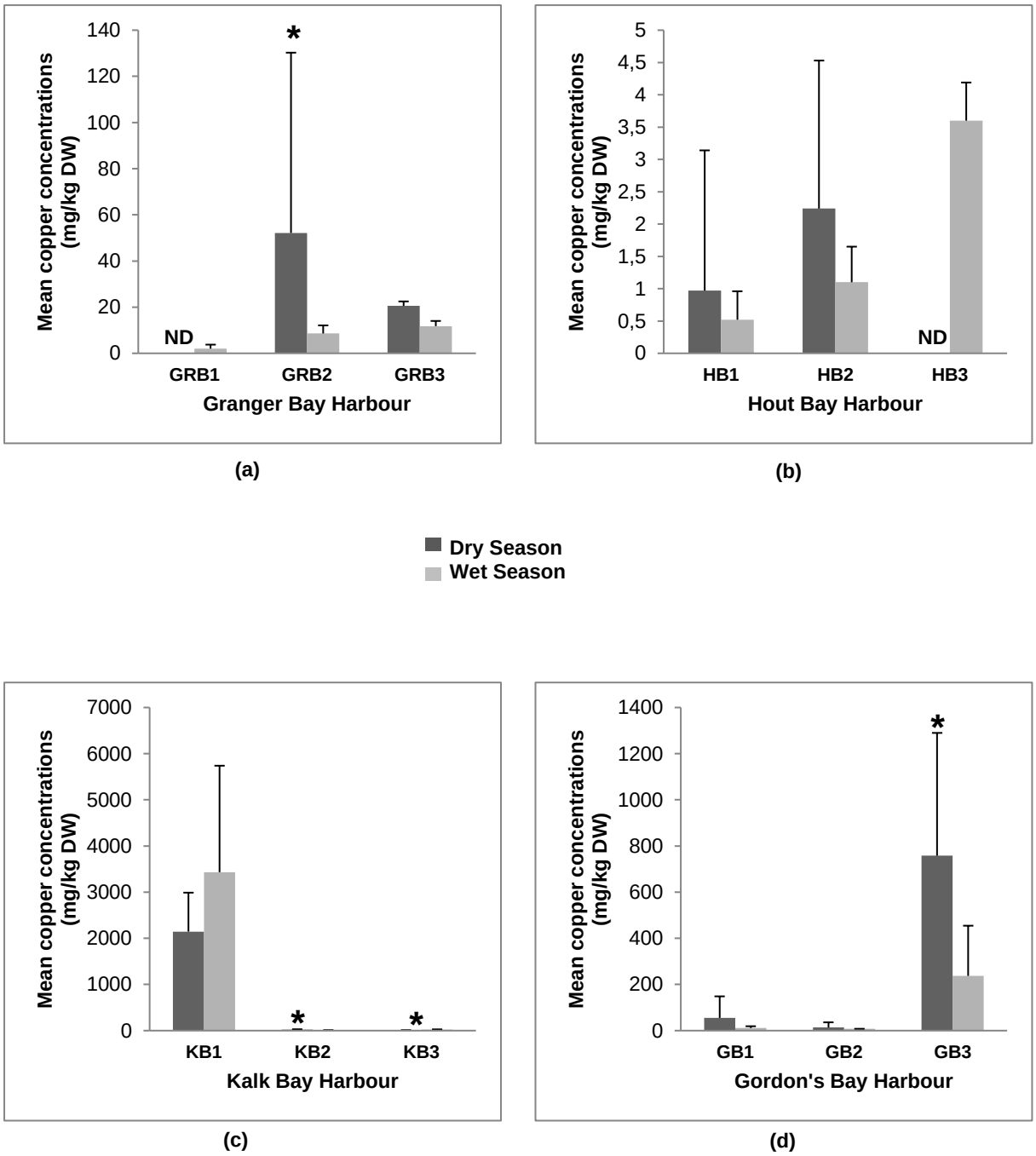


Figure 4. 3: Comparisons of mean copper concentration in sediment between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; (a): mean copper concentrations in GRB; (b): mean copper concentrations in HB; (c): mean copper concentrations in KB; (d): mean copper concentrations in GB.

4.4.2.3 Comparison of the pooled copper concentrations in sediment between the harbours and the two reference sites for the different sampling seasons

Table 4. 7: Pooled mean copper concentrations (mg/kg DW) (±SD) in sediment from the four harbours and the two reference sites for dry and wet season sampling occasions

	Sediment (mg/kg DW) (±SD)	
Harbours and reference sites	**Dry Season**	**Wet Season**
BB:MPA (*n*=5)	ND	0.55 (±0.59)^{EF}
CGH:MPA (*n*=5)	8.10 (±16.15)^{F}	**289.87 (±614.30)**
GRB (*n*=15)	*24.22 (±47.30)*^{D}	7.46 (±4.84)^{DE}
HB (*n*=15)	*1.07 (±1.93)^{CEF}	1.74 (±1.47)^{CEF}
KB (*n*=15)	**724.45 (±1133.59)**^{D}	**1154.31 (±2073.62)**^{CDB}
GB (*n*=15)	**275.58 (±401.50)**^{BD}	*85.39 (±160.85)*^{BD}

To compare copper concentrations in sediment between harbours, letters (A-F) were used to denote significant differences (i.e. significant difference from: BB:MPA=A; CGH:MPA=B; GRB=C; HB=D; KB=E and GB=F). Significant difference between seasons per harbour is indicated by an asterisk (*); ND=Not Detected; Numbers in italics= Exceed TEL (18.7mg/kg); Numbers in bold= Exceed PEL (108mg/kg); *n*= number of replicates.

*4.4.2.3.1 **Dry season***

All pairwise multiple comparisons of pooled sediment copper concentrations revealed that HB had a significantly lower ($p<0.05$) copper concentration than GRB, KB, and GB. Also, comparisons indicated that copper concentrations in GB was significantly higher ($p<0.05$) than in CGH: MPA (Table 4.7). There were no statistically significant differences ($p>0.05$) in copper concentrations between GRB and CGH: MPA, K and CGH: MPA, HB and CGH: MPA, and between KB and GB. BB: MPA was not included in the comparisons as no copper was detected for dry season sampling.

*4.4.2.3.2 **Wet season***

The pairwise multiple comparisons of pooled copper concentrations in sediment from all sampling sites indicated that HB had a significantly lower ($p<0.05$) copper concentration than GRB, KB and GB. Also, multiple comparisons revealed that KB had a significantly higher ($p<0.05$) copper concentration than GRB and BB: MPA. Likewise, copper concentration in GB was significantly higher ($p<0.05$) when compared to that of BB: MPA (Table 4.7). There were no statistically significant differences ($p>0.05$) between the four harbours and CGH: MPA. Furthermore, no significant differences ($p>0.05$) were found between GRB and BB: MPA, HB and BB: MPA, GRB and GB, and between KB and GB.

4.4.2.4 Comparisons of the pooled copper concentrations in sediment between sampling seasons per harbour.

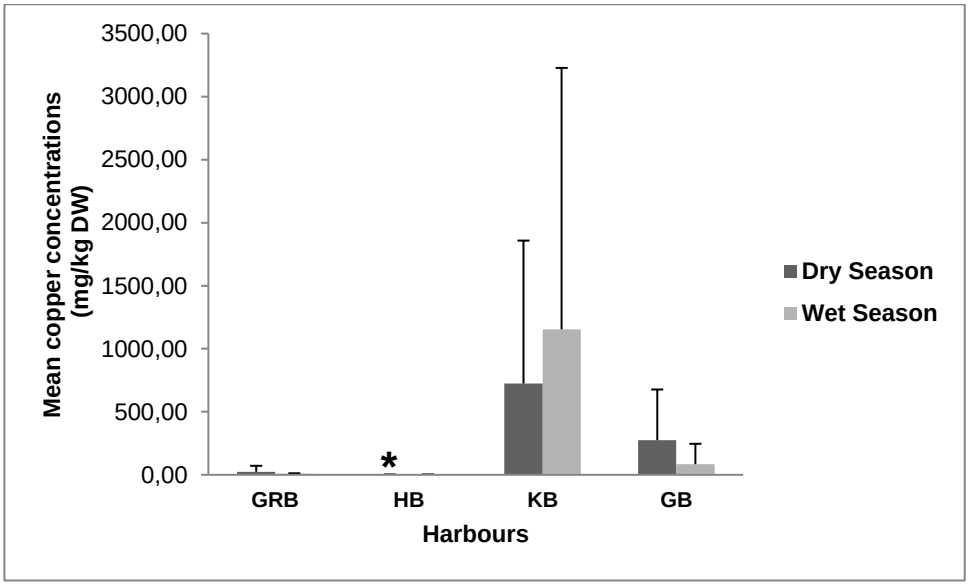


Figure 4. 4: Comparisons of pooled mean copper concentrations in sediment between seasons per harbour. An asterisk (*) above the bar showed a significant seasonal difference; Error bars = ±SD.

4.4.2.4.1 Granger Bay Harbour

Seasonal comparisons of copper concentrations in sediments from GRB indicated that no statistically significant differences ($p>0.05$) were found for the two seasons.

4.4.2.4.2 Hout Bay Harbour

Seasonal comparisons in HB revealed that copper concentration in the dry season was significantly lower ($p<0.05$) than in the wet season (Figure 4.4).

4.4.2.4.3 Kalk Bay Harbour

Comparisons of copper concentration in KB showed no statistically significant seasonal differences ($p>0.05$).

4.4.2.4.4 Gordon's Bay Harbour

No statistically significant seasonal difference ($p>0.05$) in copper concentration was found in GB for the two seasons.

4.4.3 *Burnupena* spp. soft tissue

Table 4. 8: Mean copper concentrations (mg/kg DW) (±SD) in *Burnupena* soft tissue from sampling points in the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

		***Burnupena* soft tissue (mg/kg DW) (±SD)**	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	*128.03 (±83.00)^{B}	46.64 (±8.76)^{B}
CGH:MPA	**CGH_{MPA}**	61.61 (±22.09)^{ACD}	81.69 (±23.65)^{A}
GRB	**GRB1**	147.49 (±26.63)^{B}	122.13 (±80.34)
	GRB2	*122.54 (±37.04)^{B}	75.76 (±27.44)
	GRB3	NF	NF
BB:MPA	**BB_{MPA}**	128.03 (±83.00)^{B}	46.64 (±8.76)^{BF}
CGH:MPA	**CGH_{MPA}**	61.61 (±22.09)^{A}	81.69 (±23.65)^{A}
HB	**HB1**	89.15 (±26.56)	85.15 (±12.15)^{A}
	HB2	NF	NF
	HB3	NF	NF
BB:MPA	**BB_{MPA}**	128.03 (±83.00)^{B}	46.64 (±8.76)^{BJ}
CGH:MPA	**CGH_{MPA}**	61.61 (±22.09)^{A}	81.69 (±23.65)^{A}
KB	**KB1**	NF	NF
	KB2	NF	53.87(±5.09)^{A}
	KB3	NF	NF
BB:MPA	**BB_{MPA}**	128.03 (±83.00)^{B}	46.64 (±8.76)^{BLMN}
CGH:MPA	**CGH_{MPA}**	61.61 (±22.09)^{ALM}	81.69 (±23.65)^{LA}
GB	**GB1**	*100.60 (±21.54)^{B}	57.16 (±14.39)^{ABMN}
	GB2	112.69 (±40.47)^{B}	107.50 (±101.66)^{AL}
	GB3	NF	101.95 (±40.11)^{AL}

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference in mean copper concentrations between seasons per sampling point is indicated by an asterisk (*) on the left. NF=Not Found; *n*= number of replicates.

4.4.3.1 Comparisons of copper concentrations in *Burnupena* soft tissue from sampling points within each harbour and the two reference sites for the dry and wet seasons

It should be noted that data were not available for some sampling points in the dry and wet season since no *Burnupena* spp*.* could be found at the time of sampling. Hence, these sampling points were not included in the statistical analyses.

4.4.3.1.1 ***Dry season***

4.4.3.1.1.1 Granger Bay Harbour

All pairwise multiple comparisons of *Burnupena* soft tissue copper concentrations in GRB revealed that the copper concentrations at GRB1 and GRB2 were significantly higher ($p<0.05$) than at CGH_{MPA} (Table 4.8). There were no statistical differences ($p>0.05$) between GRB1 and GRB2, GRB1 and BB_{MPA}, and between GRB2 and BB_{MPA}. No data were available for GRB3 as no *Burnupena* spp*.* were found at the time of sampling.

4.4.3.1.1.2 Hout Bay Harbour

Of the three sampling points in HB (i.e., HB1, HB2, and HB3), the *Burnupena* spp. were only found at HB1. However, the pairwise multiple comparisons for HB1 and the two reference sites reveal no statistically significant differences ($p>0.05$).

4.4.3.1.1.3 Kalk Bay Harbour

No *Burnupena* spp. were found in all three sampling points in KB and as such no data were available for analyses.

4.4.3.1.1.4 Gordon's Bay Harbour

All pairwise multiple comparisons of *Burnupena* soft tissue copper concentrations in GRB revealed that the copper concentrations at GB1 and GB2 were significantly higher ($p<0.05$) than at CGH_{MPA} (Table 4.8). There were no statistical differences ($p>0.05$) between GB1 and GB2, GB1 and BB_{MPA}, and between GB2 and BB_{MPA}. No data was available for GB3 as no *Burnupena* spp*.* were found at the time of sampling.

4.4.3.1.2 ***Wet season***

4.4.3.1.2.1 Granger Bay Harbour

No *Burnupena* spp*.* were found at GRB3 in GRB. All pairwise multiple comparisons between GRB1 and GRB2 and with the two reference sites showed no statistically significant differences ($p>0.05$).

4.4.3.1.2.2 Hout Bay Harbour

Of the three sampling points in HB (i.e., HB1, HB2, and HB3), the *Burnupena* spp. were only found at HB1 in the wet season sampling. Multiple comparisons revealed that HB1 had a significantly higher ($p<0.05$) copper concentration than BB_{MPA} (Table 4.8). No statistically significant difference ($p>0.05$) was found between CGH_{MPA}.

4.4.3.1.2.3 Kalk Bay Harbour

The *Burnupena* spp. were found only at KB2 in KB during the wet season. All pairwise multiple comparisons indicated that KB2 had a significantly higher ($p<0.05$) copper concentration than BB_{MPA} (Table 4.8). There was no significant difference ($p>0.05$) between KB2 and CGH_{MPA}. It is worth noting that KB1 and KB3 were not included in the comparisons as no data was available.

4.4.3.1.2.4 Gordon's Bay Harbour

All pairwise multiple comparisons of *Burnupena* soft tissue copper concentrations indicated that GB1 had a significantly lower ($p<0.05$) copper concentration than GB2, GB3, and CGH_{MPA}. Also, the copper concentration at GB1 was significantly higher ($p<0.05$) when compared to that of BB_{MPA}. Furthermore, multiple comparisons revealed that the copper concentrations at GB2 and GB3 were significantly higher ($p<0.05$) than for BB_{MPA} (Table 4.8). No statistically significant differences ($p>0.05$) occurred between GB2 and GB3, GB2 and CGH_{MPA}, and between GB3 and CGH_{MPA}.

4.4.3.2 Comparisons of copper concentrations in *Burnupena* soft tissue between sampling seasons per sampling point

4.4.3.2.1 Granger Bay Harbour

Seasonal comparisons of copper concentrations in *Burnupena* soft tissue in GRB showed that GRB2 had a significantly higher ($p<0.05$) copper concentration in the dry season than in the wet season sampling period (Figure 4.5a). There was no statistically significant seasonal difference ($p>0.05$) in copper concentrations at GRB1. At GRB3, no data was available in both sampling seasons for statistical analyses.

4.4.3.2.2 Hout Bay Harbour

Data were not available for statistical analyses for the three sampling points except HB1. However, seasonal comparisons of copper concentrations at HB1 revealed no statistically significant differences ($p>0.05$).

4.4.3.2.3 Kalk Bay Harbour

No seasonal comparisons were done for KB as data were only available for KB2 in the wet season.

4.4.3.2.4 Gordon's Bay Harbour

Comparisons of seasonal copper concentrations in *Burnupena* soft tissue in GB showed that GB1 had a significantly higher ($p<0.05$) copper concentration in the dry season than in the wet season sampling (Figure 4.5d). No statistically significant seasonal differences ($p>0.05$) were found for GB2. At GB3, data were not available for the dry season and therefore no seasonal comparisons could be performed.

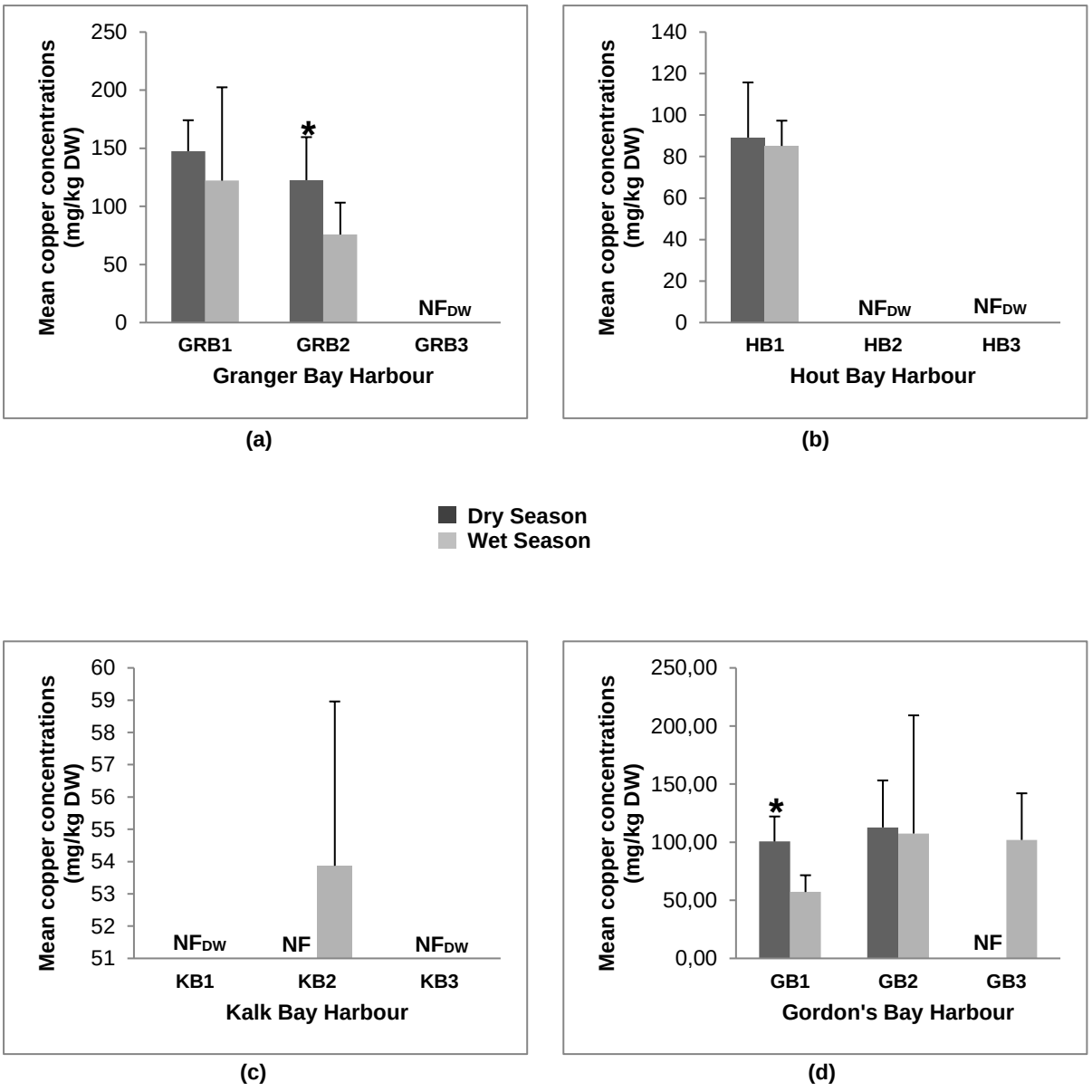


Figure 4. 5: Comparisons of mean copper concentration in *Burnupena* soft tissue between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; NA=Not Found; NF_{DW} =Not Found in Dry and Wet season; (a): mean copper concentrations in GRB; (b): mean copper concentrations in HB; (c): mean copper concentrations in KB (d): mean copper concentrations in GB.

4.4.4 *Burnupena* spp. shells

Table 4. 9: Mean copper concentrations (mg/kg DW) (±SD) in *Burnupena* shells from sampling points in the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

		***Burnupena* shells (mg/kg DW) (±SD)**	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	0.48 (±1.08)	0.51 (±0.34)^{BCD}
CGH:MPA	**CGH_{MPA}**	3.25 (±7.26)	2.00 (±0.57)^{A}
GRB	**GRB1**	3.05 (±2.22)	6.72 (±11.83)^{A}
	GRB2	5.61 (±4.43)	2.76 (±2.38)^{A}
	GRB3	NF	NF
BB:MPA	**BB_{MPA}**	0.48 (±1.08)	0.51 (±0.34)^{BF}
CGH:MPA	**CGH_{MPA}**	3.25 (±7.26)	2.00 (±0.57)^{A}
HB	**HB1**	6.54 (±4.62)	2.58 (±1.30)^{A}
	HB2	NF	NF
	HB3	NF	NF
BB:MPA	**BB_{MPA}**	0.48 (±1.08)	0.51 (±0.34)^{BJ}
CGH:MPA	**CGH_{MPA}**	3.25 (±7.26)	2.00 (±0.57)^{A}
KB	**KB1**	NF	NF
	KB2	NF	1.96 (±0.66)^{A}
	KB3	NF	NF
BB:MPA	**BB_{MPA}**	0.48 (±1.08)^{M}	0.51 (±0.34)^{BLMN}
CGH:MPA	**CGH_{MPA}**	3.25 (±7.26)^{M}	2.00 (±0.57)^{ALMN}
GB	**GB1**	3.59 (±1.03)^{M}	5.09 (±2.74)^{AB}
	GB2	*15.32 (±1.02)^{ABL}	5.56 (±1.69)^{AB}
	GB3	NF	6.23 (±2.19)^{AB}

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference in mean copper concentrations between seasons per sampling point is indicated by an asterisk (*) on the left. NF=Not Found; *n*= number of replicates.

4.4.4.1 Comparisons of copper concentrations in *Burnupena* shells from sampling points within each harbour and the two reference sites for the dry and wet seasons

4.4.4.1.1 ***Dry season***

4.4.4.1.1.1 Granger Bay Harbour

All pairwise multiple comparisons of *Burnupena* shells copper concentrations in GRB revealed that no statistically significant differences ($p>0.05$) occurred between GRB1 and GRB2 as well as when compared with the two reference sites. No data were available for GRB3 as no *Burnupena* spp. were found during the dry season sampling occasion.

4.4.4.1.1.2 Hout Bay Harbour

The pairwise multiple comparisons of *Burnupena* shells copper concentrations showed no significant differences ($p>0.05$) between HB1 and the two reference sites. HB2 and HB3 were not included in the analyses as there were no data available.

4.4.4.1.1.3 Kalk Bay Harbour

No statistical analyses could be performed in KB as data were not available for all three sampling points.

4.4.4.1.1.4 Gordon's Bay Harbour

All pairwise multiple comparisons of *Burnupena* shells copper concentrations in GB indicated that GB2 had a significantly higher ($p<0.05$) copper concentration than GB1 and the two reference sites. No data were available for comparisons at GB3 (Table 4.9).

4.4.4.1.2 ***Wet Season***

4.4.4.1.2.1 Granger Bay Harbour

All pairwise multiple comparisons of *Burnupena* shells copper concentrations in GRB revealed that the copper concentrations in GRB1 and GRB2 were significantly higher ($p<0.05$) than at CGH_{MPA} (Table 4.9). There was no statistically significant difference ($p>0.05$) between GRB1 and GRB2, GRB1 and BB_{MPA} and between GRB2 and BB_{MPA}. No data were available at GRB3 for statistical analyses.

4.4.4.1.2.2 Hout Bay Harbour

There were no data available for all the sampling points except at HB1. The pairwise multiple comparisons of *Burnupena* shells copper concentrations showed that HB1 had a significantly higher ($p<0.05$) copper concentration than BB_{MPA} (Table 4.9). No significant difference ($p>0.05$) was found between HB1 and CGH_{MPA}.

4.4.4.1.2.3 Kalk Bay Harbour

There were no data available for all the sampling points except at KB2. All pairwise multiple comparisons of *Burnupena* shells copper concentrations showed that KB2 had a significantly higher ($p<0.05$) copper concentration than BB_{MPA} (Table 4.9). No significant difference ($p>0.05$) was found between KB2 and CGH_{MPA}.

4.4.4.1.2.4 Gordon's Bay Harbour

All pairwise multiple comparisons of *Burnupena* shells copper concentrations in GB revealed that all three sampling points (GB1, GB2, and GB3) had significantly higher ($p<0.05$) copper concentrations than the two reference sites (Table 4.9). There were no statistically significant differences ($p>0.05$) between the sampling points.

4.4.4.2 Comparisons of copper concentrations in *Burnupena* shells between sampling seasons per sampling point

4.4.4.2.1 Granger Bay Harbour

Comparisons of copper concentrations in *Burnupena* shells between the two seasons in GRB show that there were no statistically significant seasonal differences ($p>0.05$) at GRB1 and GRB2. No comparison was done for GRB3 as data was not available for the dry and wet season.

4.4.4.2.2 Hout Bay Harbour

In HB, no data were available for the two sampling seasons at HB2 and HB3 for seasonal comparisons. However, a seasonal comparison of copper concentrations in *Burnupena* shells at HB1 revealed no statistically significant differences ($p>0.05$).

4.4.4.2.3 Kalk Bay Harbour

Statistical analyses could not be performed in KB as data were only available for KB2 in the wet season.

4.4.4.2.4 Gordon's Bay Harbour

Comparisons of *Burnupena* shells copper concentrations between seasons in GB indicated that GB2 had a significantly higher ($p<0.05$) copper concentration in the dry season than in the wet season (Figure 4.6d). There was no statistically significant seasonal difference ($p>0.05$) at GB1. Furthermore, no comparison could be done at GB3 as data were not available for the dry season.

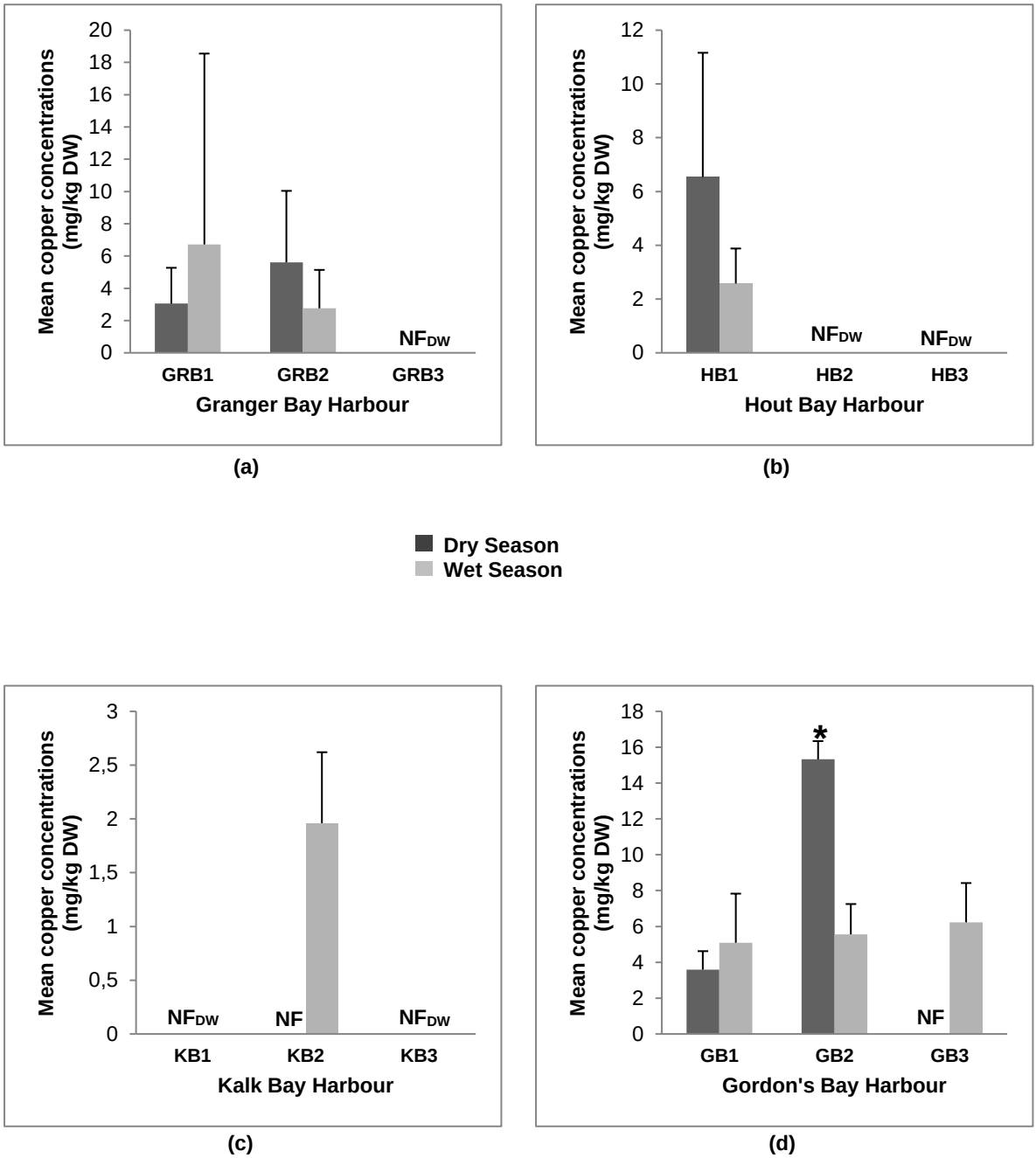


Figure 4. 6: Comparisons of mean copper concentration in *Burnupena* shells between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; NF=Not Found; NF_{DW} =Not Found in Dry and Wet season; (a): mean copper concentrations in GRB (b): mean copper concentrations in HB; (c): mean copper concentrations in KB; (d): mean copper concentrations GB.

4.4.4.3 Comparisons of copper concentrations between *Burnupena* soft tissue and shells per sampling point for the different sampling seasons

4.4.4.3.1 ***Dry season***

4.4.4.3.1.1 Granger Bay Harbour

Comparisons of copper concentrations between *Burnupena* soft tissue and shells revealed that the copper concentrations in *Burnupena* soft tissue at GRB1 and GRB2 were significantly higher ($p<0.05$) than that in their shells (Table 4.10). No data were available for comparison at GRB3.

4.4.4.3.1.2 Hout Bay Harbour

Comparisons at HB1 showed that the copper concentration in *Burnupena* soft tissue was significantly higher ($p>0.05$) than in the shells. Comparisons could not be done for HB2 and HB3 as no data were available.

4.4.4.3.1.3 Kalk Bay Harbour

No statistical analyses could be performed in KB as data were not available for all three sampling points.

4.4.4.3.1.4 Gordon's Bay Harbour

Comparisons revealed that the copper concentrations in *Burnupena* soft tissue at GB1 and GB2 were significantly higher ($p<0.05$) than that in the shells (Table 4.10). No data were available for comparison at GB3.

4.4.4.3.2 ***Wet season***

4.4.4.3.2.1 Granger Bay Harbour

Comparisons at GRB1 and GRB2 indicated that the copper concentrations in *Burnupena* soft tissue at were significantly higher ($p<0.05$) than that in the shells (Table 4.10). No data were available for comparison at GRB3.

4.4.4.3.2.2 Hout Bay Harbour

Comparisons revealed that the copper concentration in *Burnupena* soft tissue was significantly higher ($p>0.05$) than in the shells at HB1(Table 4.10). No comparisons could be done for HB2 and HB3 as no data were available.

4.4.4.3.2.3 Kalk Bay Harbour

Comparison at KB2 showed that the copper concentration in *Burnupena* soft tissue was significantly higher ($p>0.05$) than in the shells (Table 4.10). No comparisons could be done for KB1 and KB3 as no data were available.

4.4.4.3.2.4 Gordon's Bay Harbour

Comparisons revealed that the copper concentrations in *Burnupena* soft tissue for each of the three sampling points (GB1, GB2, and GB3) were significantly higher ($p<0.05$) than that in the shells (Table 4.11).

Table 4. 10: A comparison of mean copper concentrations in the soft tissue and shells of *Burnupena* spp. from the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

	***Burnupena* soft tissue and shells (mg/kg DW) (±SD)**			
	Dry season		**Wet season**	
Sampling Points	**Tissue**	**Shell**	**Tissue**	**Shell**
BB_{MPA}	128.03 (±83.00)*	0.48 (±1.08)	46.64 (±8.76)*	0.51 (±0.34)
CGH_{MPA}	61.61 (±22.09)*	3.25 (±7.26)	81.69 (±23.65)*	2.00 (±0.57)
GRB1	147.49 (±26.63)*	3.05 (±2.22)	122.13 (±80.34)*	6.72 (±11.83)
GRB2	122.54 (±37.04)*	5.61 (±4.43)	75.76 (±27.44)*	2.76 (±2.38)
GRB3	NF	NF	NF	NF
HB1	89.15 (±26.56)*	6.54 (±4.62)	85.15 (±12.15)*	2.58 (±1.30)
HB2	NF	NF	NF	NF
HB3	NF	NF	NF	NF
KB1	NF	NF	NF	NF
KB2	NF	NF	53.87(±5.09)*	1.96 (±0.66)
KB3	NF	NF	NF	NF
GB1	100.60 (±21.54)*	3.59 (±1.03)	57.16 (±14.39)*	5.09 (±2.74)
GB2	112.69 (±40.47)*	15.32 (±1.02)	107.50 (±101.66)*	5.56 (±1.69)
GB3	NF	NF	101.95 (±40.11)*	6.23 (±2.19)

A significant difference between copper concentrations in *Burnupena* soft tissue and shells per sampling point per season is indicated by an asterisk (*) on the right. NF=Not Found; *n*=number of replicates.

4.4.5 *Nucella* spp. soft tissue

Table 4. 11: Mean copper concentrations (mg/kg DW) (±SD) in *Nucella* soft tissue from sampling points in the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

		***Nucella* soft tissue (mg/kg DW) (±SD)**	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	21.87 (±7.74)^{CE}	19.84 (±6.43)^{E}
CGH:MPA	**CGH_{MPA}**	28.34 (±10.65)^{CE}	25.38 (±3.21)^{E}
GRB	**GRB1**	76.94 (±42.06)^{AB}	NF
	GRB2	NF	NF
	GRB3	*86.85 (±9.11)^{AB}	64.96 (±15.28)^{AB}
BB:MPA	**BB_{MPA}**	21.87 (±7.74)^{FGH}	19.84 (±6.43)^{FGH}
CGH:MPA	**CGH_{MPA}**	28.34 (±10.65)^{FGH}	25.38 (±3.21)^{FGH}
HB	**HB1**	54.62 (±8.66)^{ABG}	47.43 (±4.80)^{ABG}
	HB2	115.68 (±12.45)^{ABF}	129.75 (±16.28)^{ABFH}
	HB3	*119.23 (±11.50)^{AB}	50.46 (±6.14)^{ABG}
BB:MPA	**BB_{MPA}**	21.87 (±7.74)^{IJK}	19.84 (±6.43)^{IJK}
CGH:MPA	**CGH_{MPA}**	28.34 (±10.65)^{IJK}	25.38 (±3.21)^{IJK}
KB	**KB1**	466.72 (±36.89)^{ABJK}	508.20 (±71.24)^{ABJK}
	KB2	67.43 (±7.68)^{ABIK}	54.81 (±15.52)^{ABI}
	KB3	163.72 (±66.71)^{ABIJ}	156.50 (±148.86)^{ABI}
BB:MPA	**BB_{MPA}**	21.87 (±7.74)^{N}	19.84 (±6.43)^{N}
CGH:MPA	**CGH_{MPA}**	28.34 (±10.65)^{N}	25.38 (±3.21)^{N}
GB	**GB1**	NF	NF
	GB2	NF	NF
	GB3	*2211.61 (±3168.07)^{AB}	61.65 (±23.19)^{AB}

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference in mean copper concentrations between seasons per sampling point is indicated by an asterisk (*); NF=Not Found; *n*= number of replicates.

4.4.5.1 Comparisons of copper concentrations in *Nucella* soft tissue from sampling points within each harbour and the two reference sites for the dry and wet seasons

4.4.5.1.1 ***Dry season***

4.4.5.1.1.1 Granger Bay Harbour

All pairwise multiple comparisons of *Nucella* soft tissue copper concentrations in GRB revealed that GRB1 had a significantly higher ($p<0.05$) copper concentration than that of the two reference sites. Likewise, copper concentration at GRB3 was significantly higher ($p<0.05$) than that of the two reference sites (Table 4.11). There was no significant difference ($p>0.05$) between GRB1 and GRB3. However, GRB2 was not included in the analyses as no data was available for the dry season.

4.4.5.1.1.2 Hout Bay Harbour

The pairwise multiple comparisons of *Nucella* soft tissue copper concentrations in HB showed that the three sampling points had significantly higher ($p<0.05$) copper concentrations than that of the two reference sites. Also, copper concentration at HB2 was significantly higher ($p<0.05$) than at HB1 (Table 4.11). There were no statistically significant differences ($p>0.05$) between HB1 and HB3, and between HB2 and HB3.

4.4.5.1.1.3 Kalk Bay Harbour

All pairwise multiple comparisons of *Nucella* soft tissue copper concentrations in KB indicated that the three sampling points had significantly higher ($p<0.05$) copper concentrations than that of the two reference sites. Also, KB1 had a significantly higher ($p<0.05$) copper concentration than KB2 and KB3. Equally, copper concentration at KB3 was significantly higher ($p<0.05$) than at KB2 (Table 4.11).

4.4.5.1.1.4 Gordon's Bay Harbour

The pairwise multiple comparisons of *Nucella* soft tissue copper concentrations in GB showed a significantly higher ($p<0.05$) copper concentration at GB3 than in the two reference sites (Table 4.11). No data were available for comparisons at GB1 and GB2.

4.4.5.1.2 ***Wet season***

4.4.5.1.2.1 Granger Bay Harbour

All pairwise multiple comparisons of *Nucella* soft tissue copper concentrations in GRB revealed that GRB3 had significantly higher ($p<0.05$) copper concentration than the two reference sites (Table 4.11). No data were available for comparisons at GRB1 and GRB2.

4.4.5.1.2.2 Hout Bay Harbour

The pairwise multiple comparisons of *Nucella* soft tissue copper concentrations in HB indicated that the three sampling points had significantly higher ($p<0.05$) copper concentrations than that of the two reference sites. Also, HB2 had a significantly higher ($p<0.05$) copper concentration than HB1 and KB3 (Table 4.11). There was no statistically significant difference ($p>0.05$) between HB1 and HB3.

4.4.5.1.2.3 Kalk Bay Harbour

All pairwise multiple comparisons of *Nucella* soft tissue copper concentrations in KB showed that the three sampling points had significantly higher ($p<0.05$) copper concentrations than that of the two reference sites. Furthermore, the copper concentration at KB1 was significantly higher ($p<0.05$) than at KB2 and KB3 (Table 4.11). No statistically significant difference ($p>0.05$) was found between KB2 and KB3.

4.4.5.1.2.4 Gordon's Bay Harbour

The pairwise multiple comparisons of *Nucella* soft tissue copper concentrations in GB showed that GB3 had a significantly higher ($p<0.05$) copper concentrations than the two reference sites (Table 4.11). No data were available for statistical analyses at GB1 and GB2.

4.4.5.2 Comparisons of copper concentrations in *Nucella* soft tissue between sampling seasons per sampling point

4.4.5.2.1 Granger Bay Harbour

Comparisons of seasonal copper concentrations in *Nucella* soft tissue in GRB showed that GRB3 had a significantly higher ($p<0.05$) copper concentration in the dry season than in the wet season (Figure 4.7a). Comparisons were not done at GRB1 and GRB2 as data were not available at GRB1 in the wet season as well as in the latter for the dry and wet seasons.

4.4.5.2.2 Hout Bay Harbour

In HB, seasonal comparisons of *Nucella* soft tissue copper concentrations revealed that HB3 had a significantly higher ($p<0.05$) copper concentration in the dry season than in the wet season (Figure 4.7b). There were no statistically significant seasonal differences ($p>0.05$) for HB1 and HB2.

4.4.5.2.3 Kalk Bay Harbour

Comparisons of seasonal copper concentrations in *Nucella* soft tissue at all three sampling points (KB1, KB2, and KB3) in KB showed no statistically significant seasonal differences ($p>0.05$) (Figure 4.7c).

4.4.5.2.4 Gordon's Bay Harbour

Seasonal comparisons could not be performed at GB1 and GB2 as no data were available for the dry and wet seasons. However, comparisons at GB3 indicated that *Nucella* soft tissue copper concentration in the dry season was significantly higher ($p<0.05$) than that in the wet season (Figure 4.7d).

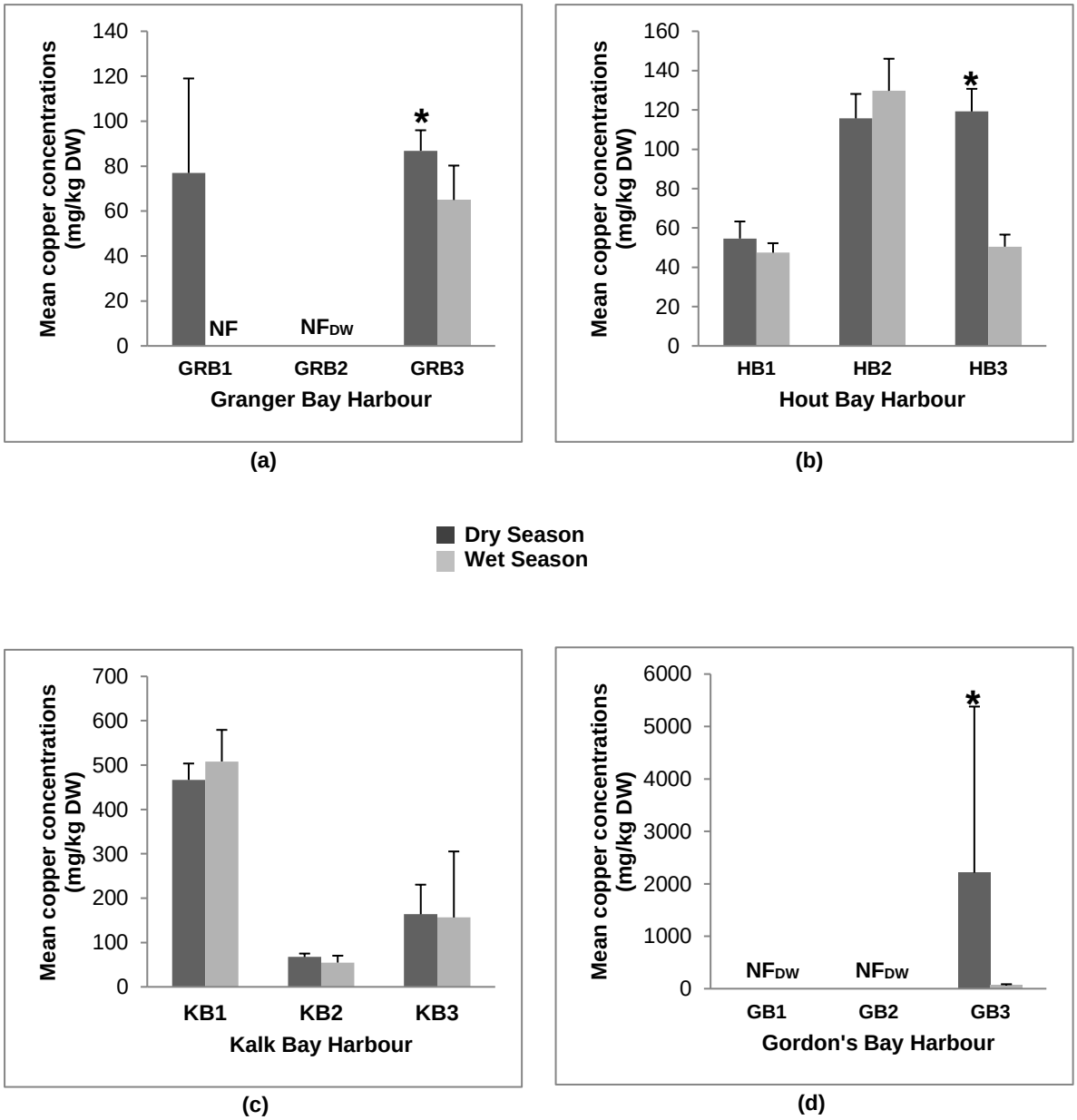


Figure 4. 7: Comparisons of mean copper concentration in *Nucella* soft tissue between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; NF_{DW} =Not Found in Dry and Wet season; (a): mean copper concentrations in GRB; (b): mean copper concentrations in HB; (c): mean copper concentrations in KB; (d): mean copper concentrations in GB.

4.4.6 *Nucella* spp. shells

Table 4. 12: Mean copper concentrations (mg/kg DW) (±SD) in *Nucella* shells from sampling points in the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

		***Nucella* Shells (mg/kg DW) (±SD)**	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB: MPA	**BB_{MPA}**	0.43 (±0.96)^{CE}	0.23 (±0.32)^{BE}
CGH: MPA	**CGH_{MPA}**	ND	3.84 (±1.95)^{AE}
GRB	**GRB1**	25.20 (±11.92)^{AE}	NF
	GRB2	NF	NF
	GRB3	53.77 (±16.20)^{AC}	20.17 (±22.34)^{AB}
BB: MPA	**BB_{MPA}**	0.43 (±0.96)^{FGH}	0.23 (±0.32)^{BFGH}
CGH: MPA	**CGH_{MPA}**	ND	3.84 (±1.95)^{AG}
HB	**HB1**	10.82 (±8.58)^{AGH}	5.34 (±4.19)^{A}
	HB2	*79.43 (±6.78)^{AF}	22.31 (±24.59)^{AB}
	HB3	*106.05 (±37.68)^{AF}	21.74 (±19.33)^{A}
BB:MPA	**BB_{MPA}**	0.43 (±0.96)^{IK}	0.23 (±0.32)^{IJK}
CGH: MPA	**CGH_{MPA}**	ND	3.84 (±1.95)^{IK}
KB	**KB1**	314.16 (±15.26)^{AJK}	246.74 (±190.94)^{ABJ}
	KB2	3.88 (±4.788)^{IK}	3.81 (±1.41)^{AIK}
	KB3	*200.93 (±18.46)^{AIJ}	101.37 (±32.75)^{ABJ}
BB:MPA	**BB_{MPA}**	0.43 (±0.96)^{N}	0.23 (±0.32)^{BN}
CGH: MPA	**CGH_{MPA}**	ND	3.84 (±1.95)^{A}
GB	**GB1**	NF	NF
	GB2	NF	NF
	GB3	*94.33 (±3.57)^{A}	6.42 (±3.01)^{A}

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference in mean copper concentrations between seasons per sampling point is indicated by an asterisk (*) on the left. NF=Not Found; ND= Not Detected; *n*= number of replicates.

4.4.6.1 Comparisons of copper concentrations in *Nucella* shells from sampling points within each harbour and the two reference sites for the dry and wet seasons

4.4.6.1.1 ***Dry season***

It is worth noting that CGH_{MPA} was not included in the comparisons as copper was not detected in the *Nucella* shell for dry season sampling.

4.4.6.1.1.1 Granger Bay Harbour

All pairwise multiple comparisons of *Nucella* shells copper concentrations in GRB showed that GRB1 and GRB3 had significantly higher ($p<0.05$) copper concentrations than BB_{MPA}. Also, the copper concentration at GRB3 was significantly higher ($p<0.05$) than at GRB1 (Table 4.12). GRB2 was not included in the comparisons as no data was available for the dry season.

4.4.6.1.1.2 Hout Bay Harbour

The pairwise multiple comparisons of *Nucella* shells copper concentrations in HB revealed that all three sampling points had significantly higher ($p>0.05$) copper concentrations than BB_{MPA}. Furthermore, comparisons indicated HB1 had a significantly lower ($p>0.05$) copper concentration than HB2 and HB3 (Table 4.12). No statistically significant difference ($p>0.05$) was found between HB2 and HB3.

4.4.6.1.1.3 Kalk Bay Harbour

All pairwise multiple comparisons of *Nucella* shells copper concentrations in KB indicated that KB1 and KB3 had significantly higher ($p<0.05$) copper concentration than BB_{MPA}. Also, comparisons revealed that copper concentration at KB1 was significantly higher ($p<0.05$) than at KB2 and KB3. Furthermore, the copper concentration recorded for KB3 was found to be significantly higher ($p<0.05$) than that for KB2 (Table 4.12). There was no statistically significant difference ($p>0.05$) between KB2 and BB_{MPA}.

4.4.6.1.1.4 Gordon's Bay Harbour

No data was available for comparisons at GB1 and GB2. However, comparisons between GB3 and BB_{MPA} revealed that GB3 had a significantly higher ($p<0.05$) copper concentration than the latter (Table 4.12).

4.4.6.1.2 ***Wet season***

4.4.6.1.2.1 Granger Bay Harbour

In GRB, all pairwise multiple comparisons of copper concentrations in *Nucella* shells indicated that GRB3 had a significantly higher ($p<0.05$) copper concentration than BB_{MPA} and CGH_{MPA} (Table 4.12). Data were not available for statistical analyses at GRB1 and GRB2.

4.4.6.1.2.2 Hout Bay Harbour

The pairwise multiple comparisons of *Nucella* shells copper concentrations showed that the copper concentrations found in all three sampling points in HB were significantly higher ($p<0.05$) than that for BB_{MPA}. Furthermore, comparisons revealed that at HB2 the copper concentration was significantly higher ($p<0.05$) than at CGH_{MPA} (Table 4.12). No statistically significant differences ($p>0.05$) were found between HB1 and CGH_{MPA}, HB2 and CGH_{MPA}, as well as among the three sampling points.

4.4.6.1.2.3 Kalk Bay Harbour

In KB, pairwise multiple comparisons indicated that copper concentrations at KB1 and KB3 were significantly higher than that of the two reference sites. Similarly, the copper concentrations at KB1 and KB3 were significantly higher ($p<0.05$) than at KB2. Furthermore, KB2 had a significantly higher ($p<0.05$) copper concentration than BB_{MPA} (Table 4.12). No significant differences ($p>0.05$) in copper concentrations were found between KB2 and CGH_{MPA} and as well as between KB1 and KB3.

4.4.6.1.2.4 Gordon's Bay Harbour

Comparisons could not be executed at GB1 and GB2 as data was not available for the wet season. However, comparisons between GB3 and BB_{MPA} indicated that GB3 had a significantly higher ($p<0.05$) copper concentration than the latter (Table 4.12). No statistically significant difference ($p>0.05$) was found between GB3 and CGH_{MPA}.

4.4.6.2 Comparisons of copper concentrations in *Nucella* shells between seasons per sampling point

4.4.6.2.1 Granger Bay Harbour

Statistical analyses could not be performed for GRB1 and GRB2 as data was not available for the dry season at GRB1 and for both dry and wet season for the latter. However, comparisons of seasonal copper concentrations at GRB3 revealed no significantly significant difference ($p>0.05$).

4.4.6.2.2 Hout Bay Harbour

In HB, comparisons of seasonal copper concentrations in *Nucella* shells showed that HB2 and HB3 had significantly higher ($p<0.05$) copper concentrations in the dry season than in wet season (Figure 4.8b). No statistically significant seasonal difference ($p>0.05$) was found at HB1.

4.4.6.2.3 Kalk Bay Harbour

Comparisons of seasonal copper concentrations in *Nucella* shell indicated that KB3 had a significantly higher ($p<0.05$) copper concentration in the dry season than in the wet season (Figure 4.8c). There were no statistically significant seasonal differences ($p>0.05$) at KB1 and KB2.

4.4.6.2.4 Gordon's Bay Harbour

Seasonal comparisons could not be executed at GB1 and GB2 as no data were available for the dry and wet season. Nevertheless, comparisons at GB3 revealed that copper concentration in *Nucella* shells for the dry season was significantly higher ($p<0.05$) than that for the wet season (Figure 4.8d).

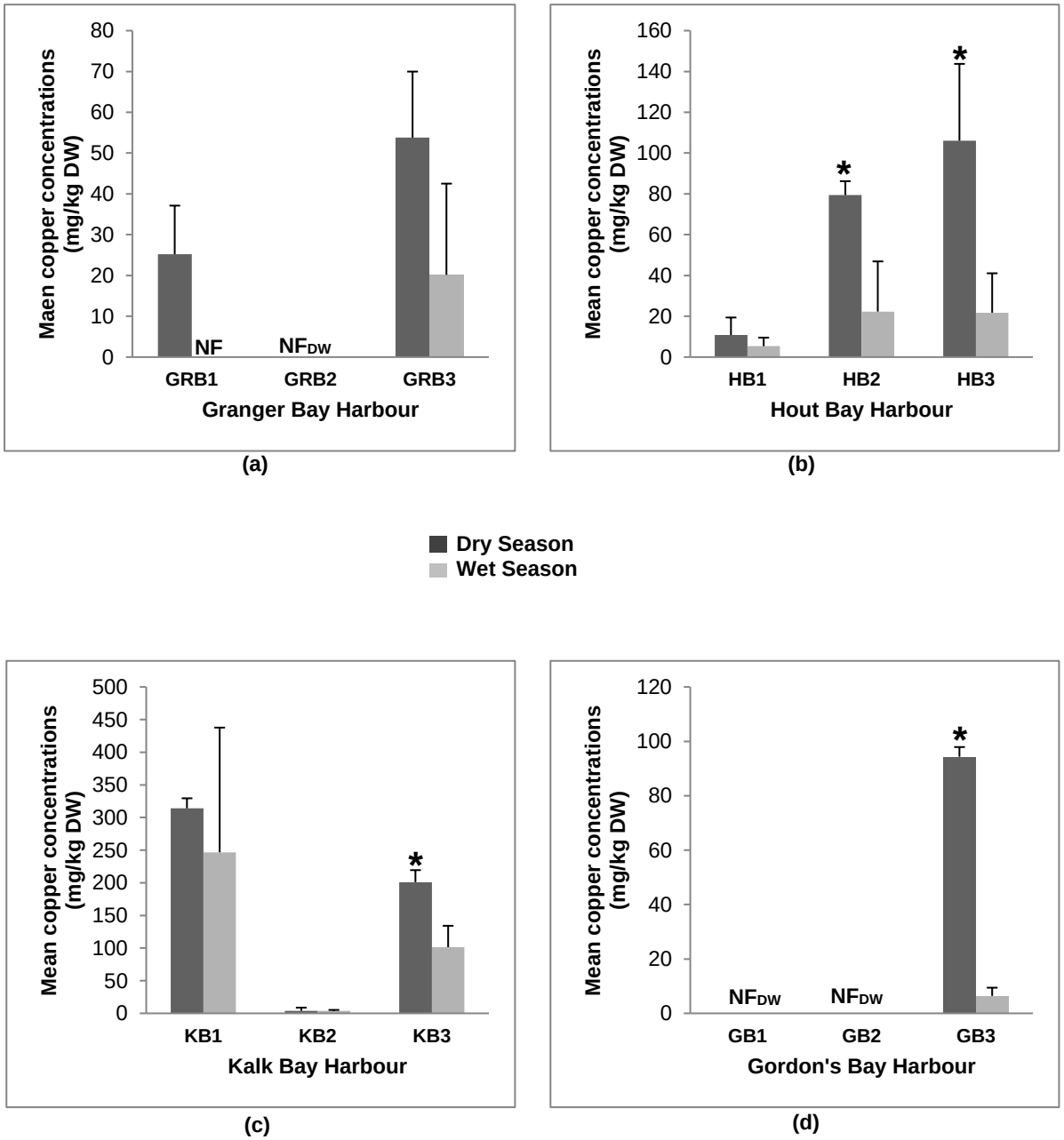


Figure 4. 8: Comparisons of mean copper concentration in *Nucella* shells between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; NF=Not Found; NF_{DW} =Not Found in Dry and Wet season; (a): mean copper concentrations in GRB (b): mean copper concentrations in HB; (c): mean copper concentrations in KB; (d): mean copper concentrations in GB.

4.4.6.3 Comparisons of copper concentrations between *Nucella* soft tissue and shells per sampling point for the different sampling seasons

4.4.6.3.1 ***Dry season***

4.4.6.3.1.1 Granger Bay Harbour

Comparisons of copper concentrations between *Nucella* soft tissue and shells in GRB revealed that the copper concentration in the soft tissue at GRB1 and GRB3 were significantly higher than that for the shells (Table 4.13). Comparisons could not be done at GRB2 as data was not available.

4.4.6.3.1.2 Hout Bay Harbour

Comparisons in HB indicated that the copper concentrations in the soft tissue at HB1 and HB2 were significantly higher ($p<0.05$) than for the shells (Table 4.13). There was no significant difference ($p>0.05$) between *Nucella* soft tissue and shells at HB3.

4.4.6.3.1.3 Kalk Bay Harbour

In KB, comparisons revealed that the concentrations of copper in the soft tissue at KB1 and KB2 were significantly higher ($p<0.05$) than that in the shells (Table 4.13). There was no significant difference ($p>0.05$) between *Nucella* soft tissue and shells at KB3.

4.4.6.3.1.4 Gordon's Bay Harbour

Comparisons in GB indicated that the concentration of copper in the soft tissue at GB3 was significantly higher ($p<0.05$) than that in the shell (Table 4.13). No comparisons were done for GB1 and GB2 as no data were available.

4.4.6.3.2 ***Wet season***

4.4.6.3.2.1 Granger Bay Harbour

Comparisons in GRB showed that the concentration of copper in the soft tissue at GRB3 was significantly higher ($p<0.05$) than that in the shell (Table 4.13). No comparisons were done for GB1 and GB2 as no data were available.

4.4.6.3.2.2 Hout Bay Harbour

In HB, comparisons indicated that the concentrations of copper in the soft tissue at HB1, HB2 and HB3 were significantly higher ($p<0.05$) than that in the shells (Table 4.13).

4.4.6.3.2.3 Kalk Bay Harbour

Comparisons in KB revealed that the concentration of copper in the soft tissue at KB2 was significantly higher ($p<0.05$) than that for the shell (Table 4.13). No statistically significant differences ($p>0.05$) were found between *Nucella* soft tissue and shells at KB1 and KB3.

4.4.6.3.2.4 Gordon's Bay Harbour

Comparisons in GRB showed that copper concentration in the soft tissue at GRB3 was significantly higher ($p<0.05$) than that for the shell (Table 4.13). No comparisons were done for GB1 and GB2 as no data were available.

Table 4. 13: A comparison of mean copper concentrations in the soft tissue and shells of *Nucella* spp. from the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

	***Nucella* soft tissue and shells (mg/kg DW) (±SD)**			
	Dry season		**Wet season**	
Sampling points	**Tissue**	**Shell**	**Tissue**	**Shell**
BB_{MPA}	21.87 (±7.74)*	0.43 (±0.96)	19.84 (±6.43)*	0.23 (±0.32)
CGH_{MPA}	28.34 (±10.65)	ND	25.38 (±3.21)*	3.84 (±1.95)
GRB1	76.94 (±42.06)*	25.20 (±11.92)	NF	NF
GRB2	NF	NF	NF	NF
GRB3	86.85 (±9.11)*	53.77 (±16.20)	64.96 (±15.28)*	20.17 (±22.34)
HB1	54.62 (±8.66)*	10.82 (±8.58)	47.43 (±4.80)*	5.34 (±4.19)
HB2	115.68 (±12.45)*	79.43 (±6.78)	129.75 (±16.28)*	22.31 (±24.59)
HB3	119.23 (±11.50)	106.05 (±37.68)	50.46 (±6.14)*	21.74 (±19.33)
KB1	466.72 (±36.89)*	314.16 (±15.26)	508.20 (±71.24)	246.74 (±190.94)
KB2	67.43 (±7.68)*	3.88 (±4.788)	54.81 (±15.52)*	3.81 (±1.41)
KB3	163.72 (±66.71)	200.93 (±18.46)	156.50 (±148.86)	101.37 (±32.75)
GB1	NF	NF	NF	NF
GB2	NF	NF	NF	NF
GB3	2211.61 (±3168.07)*	94.33 (±3.57)	61.65 (±23.19)*	6.42 (±3.01)

A significant difference between copper concentrations in *Nucella* soft tissue and shells per sampling point per season is indicated by an asterisk (*) on the right; ND = Not Detected; NF= Not Found; *n*= number of replicates.

4.4.6.4 The relationship between copper concentrations in ambient samples and the gastropod soft tissue in the harbours and reference sites for the dry and wet seasons

The correlation (Spearman Rank Order) between copper concentrations in the ambient samples (seawater and sediment) and the gastropod soft tissue were computed, and the results displayed in Table 4.14. It is worth noting that only the datasets of the *Nucella* spp. were used in the correlation analyses. This is because the datasets for the *Burnupena* spp. were incomplete (see Table 4.8 and 4.11).

Table 4. 14: The Spearman Rank Order Correlation coefficients (r_s) between mean copper concentrations in ambient samples and *Nucella* soft tissue in the harbours and reference sites

		Dry season	Wet season	Dry season	Wet season
Copper		Seawater vs Soft tissue (*Nucella* spp.)	Seawater vs Soft tissue (*Nucella* spp.)	Sediment vs Soft tissue (*Nucella* spp.)	Sediment vs Soft tissue *(Nucella* spp.)
Harbours and reference sites	**BB: MPA** r_s *p* *n*	 -0.600 0.350 5	 -0.1000 0.950 5	NA	 0.300 0.683 5
	CGH: MPA r_s *p* *n*	 0.0513 0.950 5	 0.300 0.683 5	 -0.894 0.0833 5	 0.600 0.350 5
	GRB r_s *p* *n*	 -0.182 0.607 10	 -0.900 0.0833 5	 0.239 0.490 10	 -0.700 0.233 5
	HB r_s *p* *n*	 0.213 0.433 15	 -0.0828 0.763 15	 0.110 0.686 15	 0.0143 0.954 15
	KB r_s *p* *n*	NA	 0.644* 0.00934 15	 0.379 0.158 15	 0.843* 0.0000002 15
	GB r_s *p* *n*	 0.000 1.000 5	 -0.1000 0.950 5	 -1.000* 0.0167 5	 -0.600 0.350 5

r_s = correlation coefficient; *p*= P Value; *n*= number of samples; NA = Not Analysed; * Correlation significant at p<0.05; vs= versus.

4.4.6.4.1 ***Dry season***

The results showed that no significant correlations (p>0.05) were found in copper concentrations between seawater and the soft tissue of *Nucella* spp. in GRB, HB, GB and the reference sites (CGH: MPA and BB: MPA) during the dry season. No correlation analyses were done for KB as copper was not detected in seawater samples during the dry season.

A significant negative correlation (r_s=-1.000; p<0.05) was found in copper concentrations between sediment and the soft tissue of *Nucella* spp. in GB during the dry season (Table 4.14). No significant correlation (p>0.05) were found in copper concentrations between sediment and the soft tissue of *Nucella* spp. in GRB, HB, HB and CGH: MPA. No correlation analyses were done for BB: MPA as copper was not detected in sediment samples during the dry season.

4.4.6.4.2 ***Wet season***

The results showed that a positive significant correlation (r_s=0.644, p<0.05) was found between copper concentrations in seawater samples and the soft tissue of *Nucella* spp. in KB during the wet season (Table 4.14). No significant correlations (p>0.05) were found in copper concentrations between seawater and the soft tissue of *Nucella* spp. in GRB, HB, GB and the reference sites.

A significant positive correlation (r_s=0.843; p<0.05) was found in copper concentrations between sediment and the soft tissue of *Nucella* spp. in KB during the wet season (Table 4.14). No significant correlations (p>0.05) were found in copper concentrations between seawater and the soft tissue of *Nucella* spp. in GRB, HB, GB and the reference sites.

4.4.6.5 The relationship between copper concentrations in ambient samples and the gastropod shells in the harbours and reference sites for the dry and wet seasons

The Spearman's rank order correlation analyses between copper concentrations in the ambient samples (seawater and sediment) and the gastropods shells were computed, and the results displayed in Table 4.15. As already mentioned, (section 4.4.6.4), the correlation analyses were restricted only to the *Nucella* spp. due to the incomplete datasets for the *Burnupena* spp.

Table 4. 15: The Spearman Rank Order Correlation coefficients (r_s) between mean copper concentrations in ambient samples and *Nucella* shells in the harbours and reference sites

		Dry season	**Wet season**	**Dry season**	**Wet season**
Copper		Seawater vs shell (*Nucella* spp.)	Seawater vs shell (*Nucella* spp.)	Sediment vs shell (*Nucella* spp.)	Sediment vs shell (*Nucella* spp.)
Harbours and reference sites	**BB: MPA** r_s *p* *n*	0.000 1.000 5	-0.224 0.683 5	NA	0.894 0.0833 5
	CGH: MPA r_s *p* *n*	NA	-0.1000 0.950 5	NA	-0.300 0.683 5
	GRB r_s *p* *n*	0.588 0.0665 10	0.300 0.683 5	0.562 0.0812 10	-0.1000 0.950 5
	HB r_s *p* *n*	0.147 0.593 15	-0.151 0.584 15	-0.266 0.332 15	0.736* 0.00130 15
	KB r_s *p* *n*	NA	0.404 0.131 15	0.456 0.0834 15	0.918* 0.000000200 15
	GB r_s *p* *n*	0.000 1.000 5	0.600 0.350 5	0.1000 0.950 5	-0.600 0.350 5

r_s = correlation coefficient; *p*= P Value; *n*= number of samples; NA = Not Analysed; * Correlation significant at p<0.05; vs= versus.

4.4.6.5.1 ***Dry season***

The results showed that no significant correlations ($p>0.05$) were found in copper concentrations between the seawater samples and the shells of *Nucella* spp. in GRB, HB, GB and BB: MPA during the dry season. No correlation analyses were performed for KB and CGH: MPA as copper was not detected in the seawater samples and shells of *Nucella* spp. during the dry season.

There were no significant correlations ($p>0.05$) between copper concentrations in the sediment samples and the shells of *Nucella* spp. in the four harbours. No correlation analyses were performed for BB: MPA and CGH: MPA as copper was not detected in the sediment samples and the shells of *Nucella* spp. during the dry season.

4.4.6.5.2 ***Wet season***

The results showed that no significant correlations ($p>0.05$) were found in copper concentrations between the seawater samples and the shells of *Nucella* spp. in the harbours and reference sites during the wet season.

A significant positive correlation ($r_s=0.736$ and $r_s=0.918$; $p<0.05$) was found in copper concentrations between the sediment samples and the shells of *Nucella* spp. in HB and KB during the wet season (Table 4.15). The results revealed that no significant correlations ($p>0.05$) were found in copper concentrations between the sediment samples and the shells of *Nucella* spp. in GRB, HB and the reference site during the wet season.

4.5 ZINC CONCENTRATIONS

4.5.1 Seawater

The mean zinc concentrations (mg/L) (±SD) measured in the seawater samples from the four harbours and two reference sites for the study period is displayed in Table 4.16.

Table 4. 16: Mean zinc concentrations (mg/L) (±SD) in seawater from sampling points in the four harbours and the reference sites for dry and wet season sampling occasions (*n*=5)

		Seawater (mg/L) (±SD)	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	1.7679 (±0.6393)^{BC}	0.0343 (±0.0362)^{CDE}
CGH:MPA	**CGH_{MPA}**	0.8876 (±0.4354)^{AC}	0.0124 (±0.0227)^{CDE}
GRB	**GRB1**	0.1430 (±0.2013)^{AB}	0.4201 (± 0.1452)^{ABD}
	GRB2	ND	0.1667 (±0.1136)^{ABCE}
	GRB3	ND	0.3823 (±0.1583)^{ABD}
BB:MPA	**BB_{MPA}**	1.7679 (±0.6393)^{BFGH}	0.0343 (±0.0362)^{FGH}
CGH:MPA	**CGH_{MPA}**	0.8876 (±0.4354)^{A}	0.0124 (±0.0227)^{FGH}
HB	**HB1**	0.3238 (±0.3789)^{A}	0.1674 (±0.0791)^{AB}
	HB2	0.7403 (±0.4992)^{A}	0.1665 (±0.0537)^{AB}
	HB3	0.4868 (±0.5481)^{A}	0.0981 (±0.0662)^{AB}
BB:MPA	**BB_{MPA}**	1.7679 (±0.6393)^{B}	0.0343 (±0.0362)^{B}
CGH:MPA	**CGH_{MPA}**	0.8876 (±0.4354)^{A}	0.0124 (±0.0227)^{A}
KB	**KB1**	ND	0.0630 (±0.0676)
	KB2	ND	0.1240 (±0.1189)
	KB3	ND	0.0047 (±0.0106)
BB:MPA	**BB_{MPA}**	1.7679 (±0.6393)^{LMN}	0.0343 (±0.0362)^{B}
CGH:MPA	**CGH_{MPA}**	0.8876 (±0.4354)^{LMN}	0.0124 (±0.0227)^{A}
GB	**GB1**	0.3408 (±0.4786)^{AB}	0.0033 (±0.0073)
	GB2	0.1314 (±0.2128)^{AB}	0.0047 (±0.0106)
	GB3	*0.4888 (±0.4998)^{AB}	0.0087 (±0.0194)

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference between seasons per sampling point is indicated by an asterisk (*). ND=Not Detected; Dotted underline numbers =Exceed SAWQGs (0.02mg/L); *n*= number of replicates.

4.5.1.1 Comparisons of zinc concentrations in seawater from sampling points within each harbour and the two reference sites for the dry and wet seasons

4.5.1.1.1 ***Dry season***

4.5.1.1.1.1 Granger Bay Harbour

The pairwise multiple comparisons of zinc concentrations in seawater revealed that GRB1 had a significantly lower ($p<0.05$) zinc concentration than the two reference sites (BB_{MPA} and CGH_{MPA}) (Table 4.16). At GRB2 and GRB3, zinc was not detected in the seawater samples

4.5.1.1.1.2 Hout Bay Harbour

Comparisons of seawater zinc concentrations at HB showed that all the three sampling points (HB1, HB2 and HB3) and CGH differed significantly ($p<0.05$) from BB_{MPA} (Table 4.16). However, no significant differences ($p>0.05$) were observed between all three sampling points and as well with CGH_{MPA}.

4.5.1.1.1.3 Kalk Bay Harbour

In KB, zinc was not detected in the seawater samples collected at the three sampling points.

4.5.1.1.1.4 Gordon's Bay Harbour

All pairwise multiple comparisons of zinc concentrations in seawater indicated that all three sampling points (GB1, GB2 and GB3) were significantly different ($p<0.05$) from the two reference sites (Table 4.16). However, there were no significant differences ($p>0.05$) among the sampling points.

4.5.1.1.2 ***Wet season***

4.5.1.1.2.1 Granger Bay Harbour

Pairwise multiple comparisons of seawater zinc concentrations revealed that GRB1 had a significantly higher ($p<0.05$) zinc concentration than GRB2 and the two reference sites. Also, seawater zinc concentration at GRB3 was significantly higher ($p<0.05$) than that of GRB2 and the two reference sites (Table 4.16). No significant difference ($p>0.05$) was found between GRB1 and GRB3.

4.5.1.1.2.2 Hout Bay Harbour

Comparisons of zinc concentrations in seawater indicated that all three sampling points (HB1, HB2 and HB3) were significantly different ($p<0.05$) from the two reference sites (Table 4.16). However, no significant differences ($p>0.05$) were found among the sampling points.

4.5.1.1.2.3 Kalk Bay Harbour

Seawater zinc concentrations in pairwise multiple comparisons revealed no statistically significant differences ($p>0.05$) between all three sampling points (KB1, KB2, and KB3) and the two reference sites.

4.5.1.1.2.4 Gordon's Bay Harbour

Comparisons of zinc concentrations in seawater showed no significant differences ($p>0.05$) in concentrations among all three sampling points (GB1, GB2, and GB3) and the two reference sites.

4.5.1.2 Comparisons of zinc concentrations found in seawater between sampling seasons per sampling point

The Mann-Whitney Rank Sum Test performed for seawater zinc concentrations between sampling seasons per sampling points in the four harbours revealed that there were no statistically significant seasonal differences ($p>0.05$) in each of the three sampling points in HB, KB, and GB (Figure 4.9a, 4.9b and 4.9c). However, in GRB, seawater zinc concentration at GRB3 was significantly higher ($p<0.05$) in the dry season than in the wet season (Figure 4.9d).

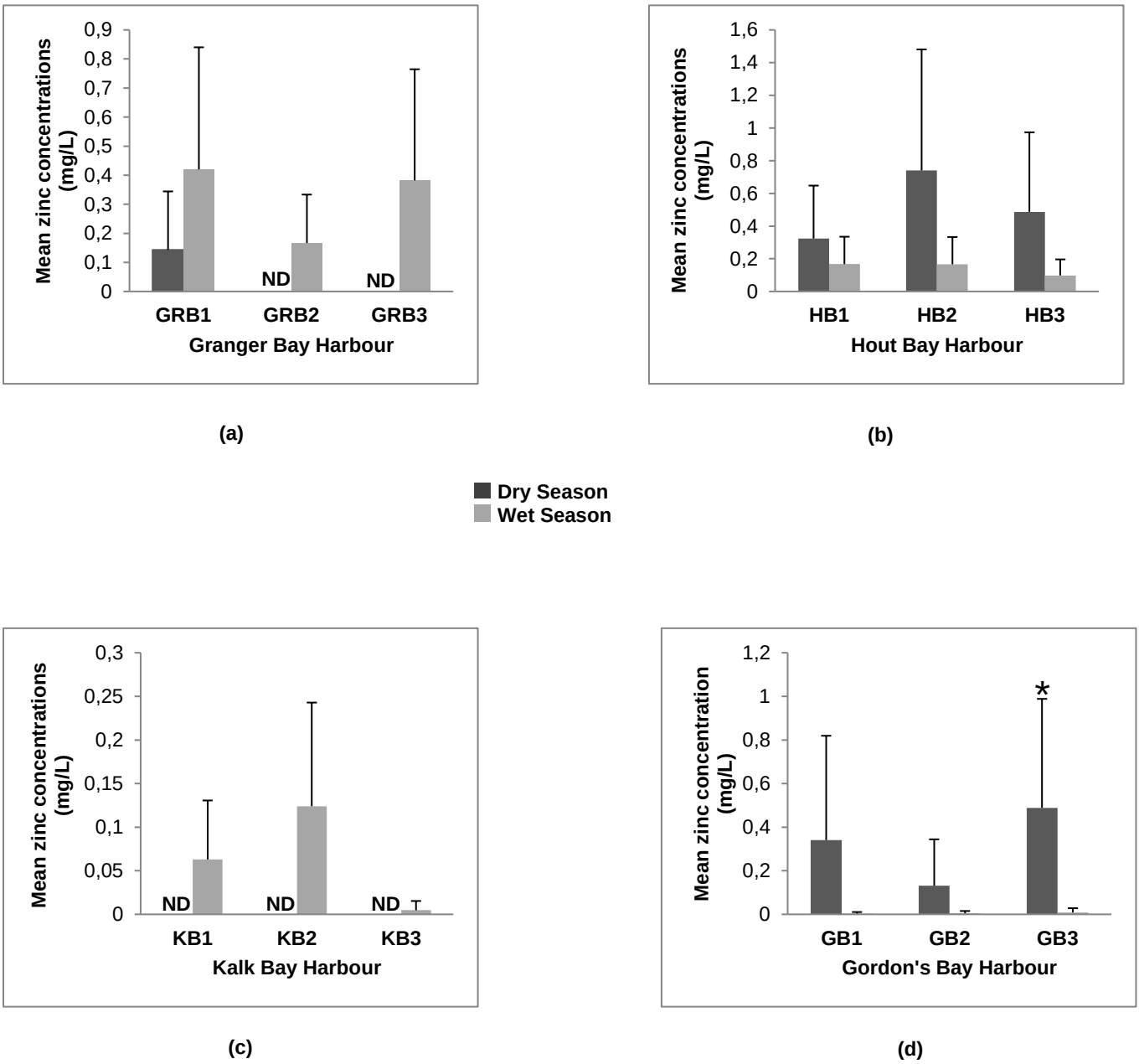


Figure 4. 9: Comparisons of mean zinc concentration in seawater between between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; ND=Not Detected; (a): mean zinc concentrations in GRB; (b): mean zinc concentrations in HB; (c): mean zinc concentrations in KB; (d): mean zinc concentrations in GB.

4.5.1.3 Comparison of the pooled zinc concentrations in seawater between the harbours and the two reference sites for the different sampling seasons

Table 4. 17: Pooled mean zinc concentrations (mg/L) (±SD) in seawater from the four harbours and the two reference sites for dry and wet season sampling occasions

	Seawater (mg/L) (±SD)	
Harbours and reference sites	**Dry season**	**Wet season**
BB:MPA (*n*=5)	1.7679 (±0.6393)^{BCD}	0.0343 (±0.0362)^{DF}
CGH:MPA (*n*=5)	0.8876 (±0.4354)^{ACF}	0.0124 (±0.0227)^{C}
GRB (*n*=15)	*0.0477 (±0.1282)^{ABF}	0.3230 (±0.1680)^{BDEF}
HB (*n*=15)	*0.5170 (±0.4791)^{A}	0.1440 (±0.0707)^{ACEF}
KB (*n*=15)	ND	0.0639 (±0.0890)^{CD}
GB (*n*=15)	*0.3203 (±0.4157)^{BC}	0.0055 (±0.0126)^{ACD}

To compare zinc concentrations in seawater between harbours letters (A-F) were used to denote significant differences (i.e. significant difference from: BB:MPA=A; CGH:MPA=B; GRB=C; HB=D; KB=E and GB=F). Significant differences between seasons per sampling site are indicated by asterisks (*). ND= Not Detected; Dotted underline numbers =Exceed SAWQGs (0.02mg/L); *n*= number of replicates.

*4.5.1.3.1 **Dry season***

All pairwise multiple comparisons of seawater zinc concentrations revealed that GRB had a significantly lower ($p<0.05$) zinc concentration than in GB and the reference sites. Similarly, there were significant differences ($p<0.05$) between HB and BB: MPA as well as between GB and CGH: MPA (Table 4.17). No other significant differences ($p>0.05$) were found for zinc concentrations between the harbours and the two reference sites. It should be noted that KB was not included in the statistical analyses as zinc was not detected in the seawater samples collected during the dry season.

*4.5.1.3.2 **Wet season***

The pairwise multiple comparisons for zinc concentration in seawater indicated that GRB had a significantly higher ($p<0.05$) zinc concentration than in the three harbours (HB, KB and GB) and the reference site (CGH: MPA). Also, there were statistically significant differences ($p<0.05$) in zinc concentrations between GB and BB: MPA, as well as when zinc concentration in HB was compared to that in KB, GB and BB: MPA (Table 4.17). However, no significant differences ($p>0.05$) were found between GRB and BB: MPA, HB and CGH: MPA, GB and CGH: MPA, KB and GB and as well as between KB and the two reference sites.

4.5.1.4 Comparisons of the pooled zinc concentrations in seawater between the sampling seasons per harbour

Seasonal comparisons of the pooled zinc concentrations in seawater per harbour are displayed in Figure 4.10. The seasonal comparison for KB was not done as zinc was not detected in the seawater samples collected during the dry season.

4.5.1.4.1 Granger Bay Harbour

Comparisons of seawater zinc concentrations at GRB showed that it was significantly higher ($p<0.05$) in the wet season than during the dry season (Figure 4.10).

4.5.1.4.2 Hout Bay Harbour

Comparisons of zinc concentrations in seawater at HB indicated a significantly higher ($p<0.05$) concentration in the dry season than the wet season (Figure 4.10).

4.5.1.4.3 Kalk Bay Harbour

No seasonal comparison for zinc concentrations in seawater was performed at KB as no zinc was detected in the seawater samples collected in the dry season. However, zinc was not detected in the wet season sampling.

4.5.1.4.4 Gordon's Bay Harbour

Comparisons of seawater zinc concentration at GB revealed a significantly higher ($p<0.05$) concentration in the dry season than the wet season (Figure 4.10).

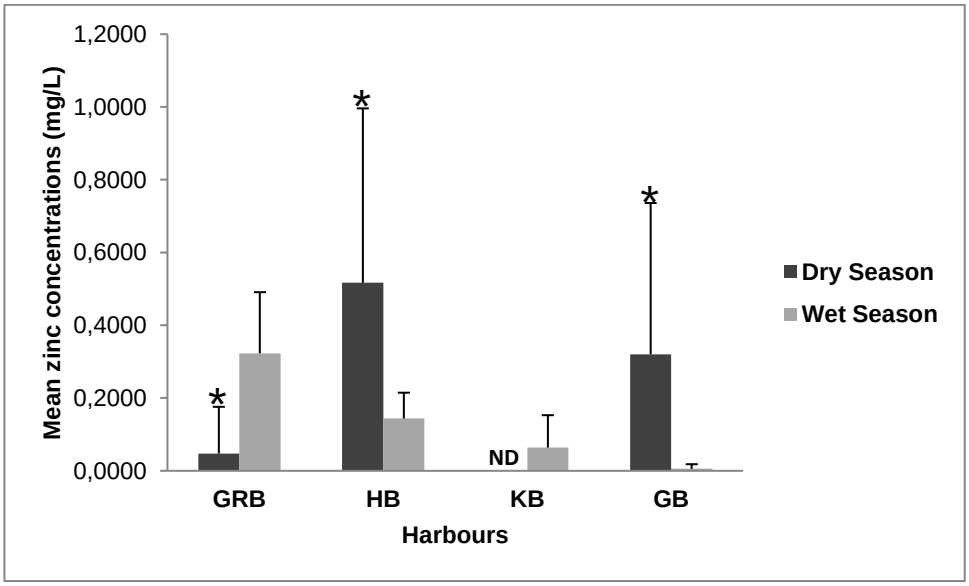


Figure 4. 10: Comparisons of pooled mean zinc concentrations in seawater between seasons per harbour. An asterisk (*) above the bar showed a significant seasonal difference; Error bars = ±SD; ND=Not Detected

4.5.2 Sediment

The mean zinc concentrations (mg/kg DW) (±SD) measured in the sediment samples from the four harbours and the two reference sites for the study period are displayed in Table 4.20.

Table 4. 18: Mean zinc concentrations (mg/kg DW) (±SD) in sediment from sampling points in the four sampling sites and the two reference sites for dry and wet season sampling occasions (*n*=5)

		Sediment (mg/kg DW) (±SD)	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	25.09 (± 35.75)^{C}	1.20 (±1.53)^{BCDE}
CGH:MPA	**CGH_{MPA}**	39.45 (± 24.96)^{C}	*203.77(± 423.14)*^{ACDE}
GRB	**GRB1**	2.35 (± 3.38)^{ABDE}	5.79 (±3.79)^{ABDE}
	GRB2	*192.45 (±334.73)*^{C}	41.6 (±15.11)^{ABC}
	GRB3	40.92 (±10.65)^{C}	50.98 (±16.82)^{ABC}
BB:MPA	**BB_{MPA}**	25.09 (± 35.75)	1.20 (±1.53)^{BG}
CGH:MPA	**CGH_{MPA}**	39.45 (± 24.96)	*203.77(± 423.14)*^{A}
HB	**HB1**	*23.18 (±12.14)	2.13 (± 2.31)^{H}
	HB2	*24.91 (± 20.37)	5.08 (± 3.46)^{A}
	HB3	6.97 (±13.07)	9.55 (± 4.425)^{F}
BB:MPA	**BB_{MPA}**	25.09 (± 35.75)^{I}	1.20 (±1.53)^{BIJK}
CGH:MPA	**CGH_{MPA}**	39.45 (± 24.96)^{I}	203.77(± 423.14)^{ADI}
KB	**KB1**	**1807.13 (±608.55)**^{ABJK}	**2380.43 (±1456.79)**^{ABJK}
	KB2	16.82 (±12.69)^{I}	27.05 (±10.59)^{AI}
	KB3	10.26 (±22.93)^{I}	29.17 (± 3.17)^{AI}
BB:MPA	**BB_{MPA}**	25.09 (± 35.75)^{LMN}	1.20 (±1.53)^{B}***^{LMN}***
CGH:MPA	**CGH_{MPA}**	39.45 (± 24.96)^{LMN}	203.77(± 423.14)^{A}
GB	**GB1**	**1340.78 (±1585.74)**^{AB}	*129.61(±33.17)*^{A}
	GB2	*167.12 (±45.32)*^{AB}	*193.23 (±105.58)*^{A}
	GB3	***486.68 (±115.87)**^{AB}	*136.28 (±110.50)*^{A}

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=*A*; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. Significant differences between seasons per sampling point is indicated by an asterisk (*); Numbers in italics= Exceed TEL (124mg/kg); Numbers in bold= Exceed PEL (271mg/kg); *n*=number of replicates.

4.5.2.1 Comparisons of zinc concentrations in sediment from sampling points within each harbour and the two reference sites for dry and wet seasons

4.5.2.1.1 ***Dry season***

4.5.2.1.1.1 Granger Bay Harbour

The pairwise multiple comparisons of zinc concentrations in sediment from the three sampling points (GRB1, GRB2 and GRB3) and the two reference sites (BB_{MPA} and CGH_{MPA}) during the dry season indicated that GRB1 had a significantly lower ($p<0.05$) zinc concentration than BB_{MPA}, CGH_{MPA}, GRB2 and GRB3 (Table 4.18). No other sampling points differed significantly from each other ($p>0.05$).

4.5.2.1.1.2 Hout Bay Harbour

The pairwise multiple comparisons of zinc concentrations in sediment between the three sampling points (HB1, HB2 and HB3) with the two reference sites reveal no significant differences ($p>0.05$).

4.5.2.1.1.3 Kalk Bay Harbour

In KB, pairwise comparisons of zinc concentrations in sediment showed that KB1 had a significantly higher ($p<0.05$) zinc concentration than BB_{MPA}, CGH_{MPA}, KB2, and KB3 (Table 4.18). There were no other statistically significant differences ($p>0.05$) in sediment zinc concentrations between the other sampling points.

4.5.2.1.1.4 Gordon's Bay Harbour

In GB, the pairwise multiple comparisons of zinc sediment concentrations reveal no statistically significant differences ($p<0.05$) between all the three points (GB1, GB2 and GB3). However, zinc sediment concentrations for all three points differ ($p<0,05$) significantly from the two reference sites (Table 4.18).

4.5.2.1.2 ***Wet season***

4.5.2.1.2.1 Granger Bay Harbour

The pairwise multiple comparisons of sediment zinc concentrations showed that the sampling points in GRB differed ($p<0.05$) significantly from each other and the two reference sites except for GRB2 and GRB3 which reveal no significant difference ($p>0.05$) from each other. Zinc concentration in sediment from CGH_{MPA} was significantly higher ($p<0.05$) than at GRB1, GRB2 and GRB3. Zinc concentration recorded in sediment from BB_{MPA} was significantly lower ($p<0.05$) than at GRB1, GRB3 and GRB3 (Table 4.18).

4.5.2.1.2.2 Hout Bay Harbour

In HB, pairwise comparisons of zinc concentrations for sediments from all sampling points reveal that the zinc sediment concentrations differed significantly ($p<0.05$) between HB1 and HB3, and between HB2 and BB_{MPA} (Table 4.18). No significant differences ($p>0.05$) in pairwise multiple comparisons for zinc sediment concentrations were observed for the rest of the sampling points.

4.5.2.1.2.3 Kalk Bay Harbour

All pairwise multiple comparisons of sediment zinc concentrations in KB indicted that KB1 zinc concentration differed ($p<0.05$) significantly from the other sampling points (KB2 and KB3) and the two reference sites. Furthermore, KB2 and KB3 were significantly different ($p<0.05$) from BB_{MPA} (Table 4.18). No significant differences ($p>0.05$) in pairwise comparisons of sediment zinc concentrations were observed between KB2 and KB3 as well as between CGH_{MPA}.

4.5.2.1.2.4 Gordon's Bay Harbour

Pairwise multiple comparisons of sediment zinc concentrations from all sampling points showed that GB1, GB2 and GB3 differed ($p<0.05$) significantly with BB_{MPA} (Table 4.18). However, no significant differences ($p>0.05$) in pairwise multiple comparisons were observed amongst the three sampling points (GB1, GB2, and GB3) as well as between CGH_{MPA}.

4.5.2.2 Comparisons of zinc concentrations in sediment between sampling seasons per sampling point

4.5.2.2.1 Granger Bay Harbour

In GRB, no statistically significant differences ($p>0.05$) were found when comparing the zinc concentrations for sediment between sampling seasons (dry season and wet season) per sampling point.

4.5.2.2.2 Hout Bay Harbour

The Mann-Whitney Rank Sum Test performed for zinc concentrations for sediment in HB, showed that the zinc concentrations at HB1 and HB2 were significantly higher ($p<0.05$) in the dry season than in the wet season (Figure 4.11b). No significant seasonal difference ($p>0.05$) was found for HB3.

4.5.2.2.3 Kalk Bay Harbour

Comparisons of sediment zinc concentrations in HB from the three sampling points (KB1, KB2 and KB3) showed no statistically significant seasonal differences ($p>0.05$) when compared.

4.5.2.2.4 Gordon's Bay Harbour

Comparisons of sediment zinc concentrations in GB between seasons per sampling point, revealed that zinc concentrations were significantly higher ($p<0.05$) at GB3 in the dry season than in the wet season (Figure 4.11d). However, sediment zinc concentrations at GB1, and GB2 in the dry season showed no significant seasonal differences ($p>0.05$) when compared with zinc concentrations in the wet season.

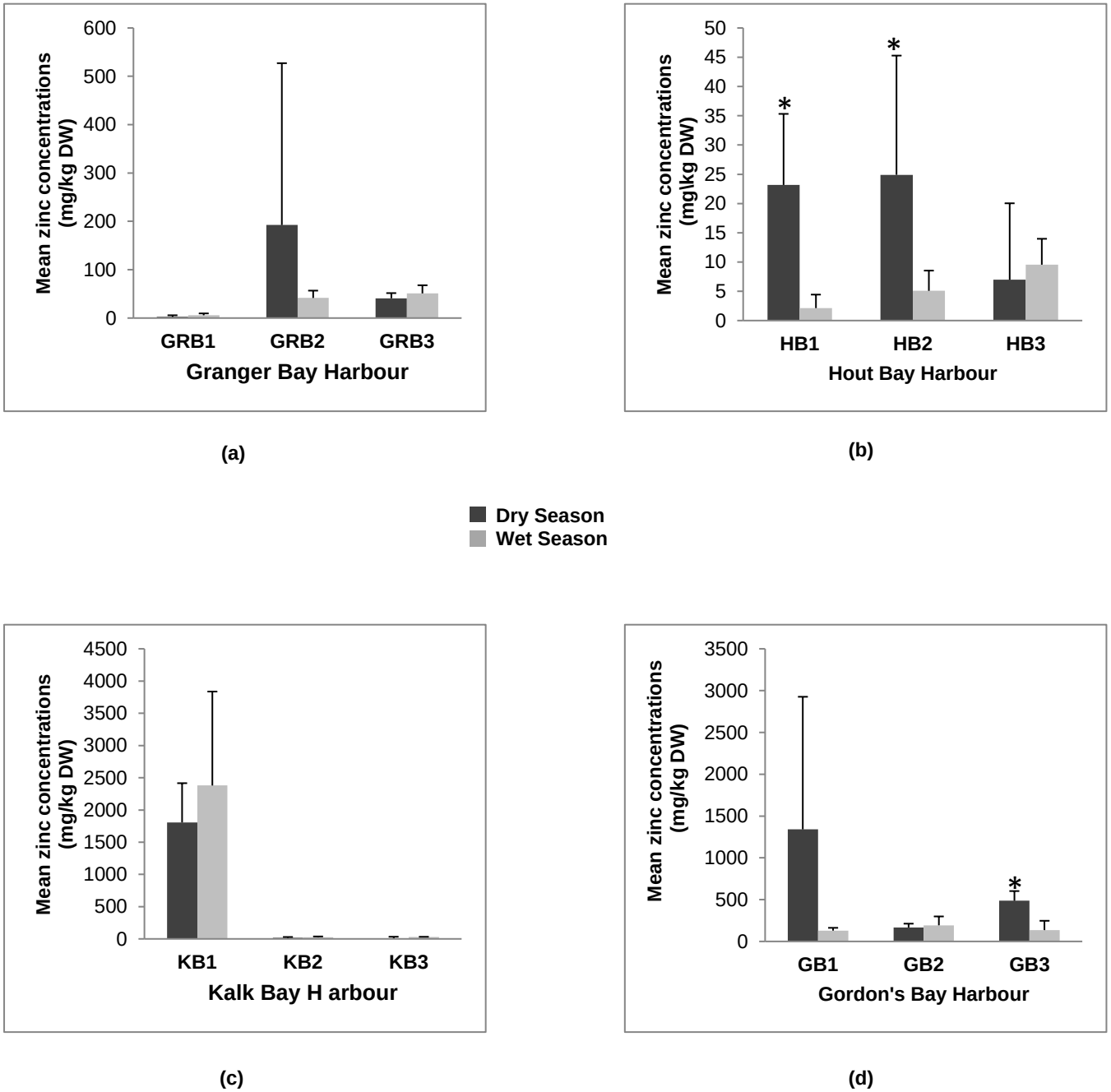


Figure 4. 11: Comparisons of mean zinc concentrations in sediment between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; (a): mean zinc concentrations in GRB; (b): mean zinc concentrations in HB; (c): mean zinc concentrations in KB; (d): mean zinc concentrations in GB.

4.5.2.3 Comparison of the pooled zinc concentrations in sediment between the sampling sites and the two reference sites for the different sampling seasons

To compare zinc concentrations in sediment between the harbours and the two reference sites, datasets of the three sampling points per harbour were pooled for different sampling seasons. The pooled datasets were used for statistical analysis (Table 4.19).

Table 4. 19: Pooled mean zinc concentrations (mg/kg DW) (±SD) in sediment from the four harbours and two reference sites for dry and wet season sampling occasions

	Sediment (mg/kg DW) (±SD)	
Harbours and reference sites	**Dry season**	**Wet season**
BB:MPA (*n*=5)	25.09 (±35.75)^{F}	1.20 (±1.53)^{DFE}
CGH:MPA (*n*=5)	39.45 (±24.96)	*203.77 (±423.14)*
GRB (*n*=15)	78.58 (±198.15)^{F}	32.82 (±23.60)^{DF}
HB (*n*=15)	*18.36 (±16.72)^{F}	5.59 (±4.53)^{ACFE}
KB (*n*=15)	**611.40 (±933.79)^{F}**	**812.22 (±1387.03)^{AD}**
GB (*n*=15)	***664.86 (±992.90)^{ACDE}**	*153.04 (±88.66)*^{ACD}

To compare zinc concentrations in sediment between sampling sites, letters (A-F) were used to denote significant differences from (i.e. significant difference from: BB:MPA=A; CGH:MPA=B; GRB=C; HB=D; KB=E and GB=F). Significant difference between seasons per sampling site is indicated by asterisk (*); Numbers in italics= Exceed TEL (124mg/kg); Numbers in bold= Exceed PEL (271mg/kg); *n*= number of replicates.

*4.5.2.3.1 **Dry season***

All pairwise multiple comparisons for sediment zinc concentrations showed that GB had a significantly higher ($p<0.05$) zinc concentration than the other three harbours (GRB, HB and KB) and the reference site (BB: MPA) (Table 4.19). No significant difference ($p>0.05$) was found between GB and reference site CGH: MPA. Similarly, there were no statistically significant differences ($p>0.05$) among the three sites (GRB, HB and KB) and the two reference sites.

*4.5.2.3.2 **Wet season***

Comparisons of zinc concentrations in sediment revealed that HB had a significantly lower ($p<0.05$) zinc concentration than GRB, KB, and GB but was significantly higher ($p<0.05$) than zinc concentration at BB: MPA. Furthermore, there were significant differences ($p<0.05$) in zinc concentrations between GRB and GB, KB and BB: MPA, and between GB and BB (Table 4.19). There were no significant differences ($p>0.05$) between GRB and KB, KB and GB, and between GRB and BB: MPA. Likewise, no statistically significant differences ($p>0.05$) were found between CGH: MPA and the four harbours.

4.5.2.4 Comparisons of the pooled zinc concentrations in sediment between sampling seasons per harbour

The mean zinc concentrations in sediment from pooled datasets of the three sampling points in each harbour during the two seasons are presented graphically to facilitate comparisons (Figure 4.12).

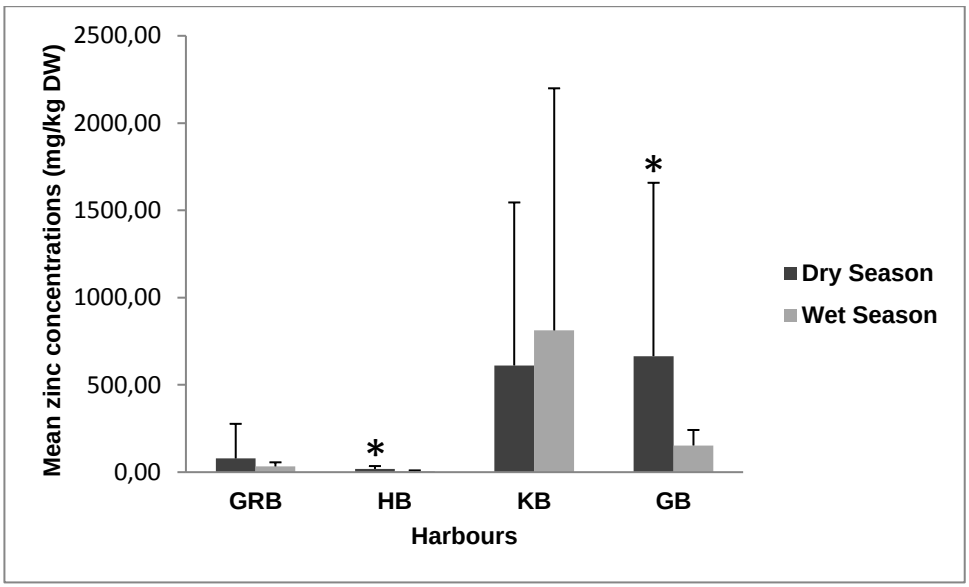


Figure 4. 12: Comparisons of pooled mean zinc concentrations found in sediment between seasons per harbour. An asterisk (*) above the bar showed a significant seasonal difference; Error bars = ±SD.

4.5.2.4.1 Granger Bay Harbour

The Mann-Whitney Rank Sum Test performed for sediment zinc concentrations in GB between sampling seasons showed no statistically significant seasonal difference ($p>0.05$).

4.5.2.4.2 Hout Bay Harbour

Sediment zinc concentrations comparisons in HB between the two sampling seasons reveal a significant seasonal difference ($p<0.05$) (Figure 4.12).

4.5.2.4.3 Kalk Bay Harbour

There was no statistically significant seasonal difference ($p>0.05$) for sediment zinc concentrations in KB between the two sampling seasons when compared.

Gordon's Bay Harbour

In GB, comparisons of sediment zinc concentrations between the two sampling seasons indicated a significant seasonal difference ($p<0.05$) (Figure 4.12).

4.5.3 *Burnupena* spp. soft tissue

Table 4. 20: Mean zinc concentrations (mg/kg DW) (±SD) in *Burnupena* soft tissue from sampling points in the four sampling sites and the two reference sites for dry and wet season sampling occasions (*n*=5)

		***Burnupena* soft tissue (mg/kg DW) (±SD)**	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	424.28 (±311.01)^{C}	144.17 (±87.86)^{C}
CGH:MPA	**CGH_{MPA}**	287.61 (±190.63)^{C}	220.61 (±75.11)^{C}
GRB	**GRB1**	1010.79 (±93.79)^{ABD}	852.16 (±577.06)^{ABD}
	GRB2	*516.84(±164.17)^{C}	124.18 (±52.62)^{C}
	GRB3	NF	NF
BB:MPA	**BB_{MPA}**	424.28 (±311.01)	144.17 (±87.86)
CGH:MPA	**CGH_{MPA}**	287.61 (±190.63)	220.61 (±75.11)
HB	**HB1**	554.47 (±478.75)	331.90 (±250.14)
	HB2	NF	NF
	HB3	NF	NF
BB:MPA	**BB_{MPA}**	424.28 (±311.01)	144.17 (±87.86)
CGH:MPA	**CGH_{MPA}**	287.61 (±190.63)	220.61 (±75.11)
KB	**KB1**	NF	NF
	KB2	NF	159.75 (±107.43)
	KB3	NF	NF
BB:MPA	**BB_{MPA}**	424.28 (±311.01)	144.17 (±87.86)
CGH:MPA	**CGH_{MPA}**	287.61 (±190.63)	220.61 (±75.11)
GB	**GB1**	*267.91 (±35.15)	94.54 (±30.95)
	GB2	223.81 (±34.55)	190.96 (±209.26)
	GB3	NF	119.89 (±92.91)

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference between seasons per sampling point is indicated by an asterisk (*); NF= Not Found; *n*= number of replicates.

4.5.3.1 Comparisons of zinc concentrations in *Burnupena* soft tissue collected from sampling points within each harbour and the two reference sites for dry and wet seasons

4.5.3.1.1 ***Dry season***

4.5.3.1.1.1 Granger Bay Harbour

All pairwise multiple comparisons procedures for zinc concentrations in *Burnupena* soft tissue indicated that GRB1 had a significantly higher ($p<0.05$) zinc concentration than GRB2 and the two reference sites (Table 4.20). There were no significant differences ($p>0.05$) between GRB2 and the two reference sites. GRB3 was not included in the analyses as *Burnupena* spp. were not found at this point during the dry season sampling period.

4.5.3.1.1.2 Hout Bay Harbour

Comparisons of zinc concentrations in *Burnupena* soft tissue revealed no significant differences between two of the three sampling points (i.e., HB1 and HB2) and the reference sites. However, HB3 was not included in the analyses due to the absence of the species during sampling.

4.5.3.1.1.3 Kalk Bay Harbour

No statistical analyses were performed for all three sampling points in KB as the *Burnupena* spp. were not found during the time of sampling.

4.5.3.1.1.4 Gordon's Bay Harbour

Comparisons of zinc concentrations in *Burnupena* soft tissue showed no significant differences between GB1, GB2 and the two reference sites. GB3 was not included in the statistical analyses due to the absence of the species during the sampling period

4.5.3.1.2 ***Wet season***

4.5.3.1.2.1 Granger Bay Harbour

The pairwise multiple comparisons for zinc concentrations in *Burnupena* soft tissue revealed that GRB1 had a significantly higher ($p<0.05$) zinc concentration than GRB2 and the two reference sites (Table 4.20). There were no significant differences ($p>0.05$) between GRB2 and the two reference sites. GRB3 was not included in the analyses as the *Burnupena* spp. were not found at this point during the dry season sampling period.

4.5.3.1.2.2 Hout Bay Harbour

Comparisons of zinc concentrations in *Burnupena* soft tissue revealed no statistically significant differences ($p>0.05$) between HB1, HB2 and the two reference sites. The sampling point, HB3 was not included in the analyses due to the absence of the species during sampling.

4.5.3.1.2.3 Kalk Bay Harbour

The *Burnupena* spp. were not found in two (KB1 and KB3 of the three sampling points (KB1, KB2, and KB3) in KB. However, KB3 did not reveal any statistically significant differences ($p>0.05$) when compared with the two reference sites.

4.5.3.1.2.4 Gordon's Bay Harbour

The three sampling points at GB did not differ ($p>0.05$) significantly among each other and with the two reference sites.

4.5.3.2 Comparisons of zinc concentrations in *Burnupena* soft tissue between sampling seasons per sampling point

4.5.3.2.1 Granger Bay Harbour

Seasonal comparisons of zinc concentrations in *Burnupena* soft tissue showed that GRB2 had a significantly higher ($p<0.05$) zinc concentration in the dry season than during the wet season (Figure 4.13a). Furthermore, zinc concentrations found in *Burnupena* soft tissue at GRB1 did not differ ($p>0.05$) significantly for the dry and wet season. The absence of *Burnupena* spp. at GRB3 implied no data were available for statistical analyses

4.5.3.2.2 Hout Bay Harbour

In HB, the *Burnupena* spp. were found only at HB1 of the three sampling points. Nevertheless, comparisons of zinc concentration in *Burnupena* soft tissue at HB1 for the dry season and wet season revealed no significant differences ($p>0.05$).

4.5.3.2.3 Kalk Bay Harbour

In KB, no *Burnupena* spp. were found in the three sampling points during the dry season. Although these gastropods were found at KB2 during the wet season sampling, seasonal comparisons for zinc concentrations in the soft tissue were not performed as there was no data available for the dry season.

4.5.3.2.4 Gordon's Bay Harbour

Comparisons of seasonal zinc concentrations in *Burnupena* soft tissue from GB showed that GB1 had a significantly higher ($p<0.05$) zinc concentration in the dry season than during the wet season (Figure 4.13d). There were no significant seasonal differences ($p>0.05$) for GB2. However, the absence of the gastropods in GB3 during the dry season sampling period implied

no data was available. Hence, no statistical analyses were performed although the gastropods were found during the wet season.

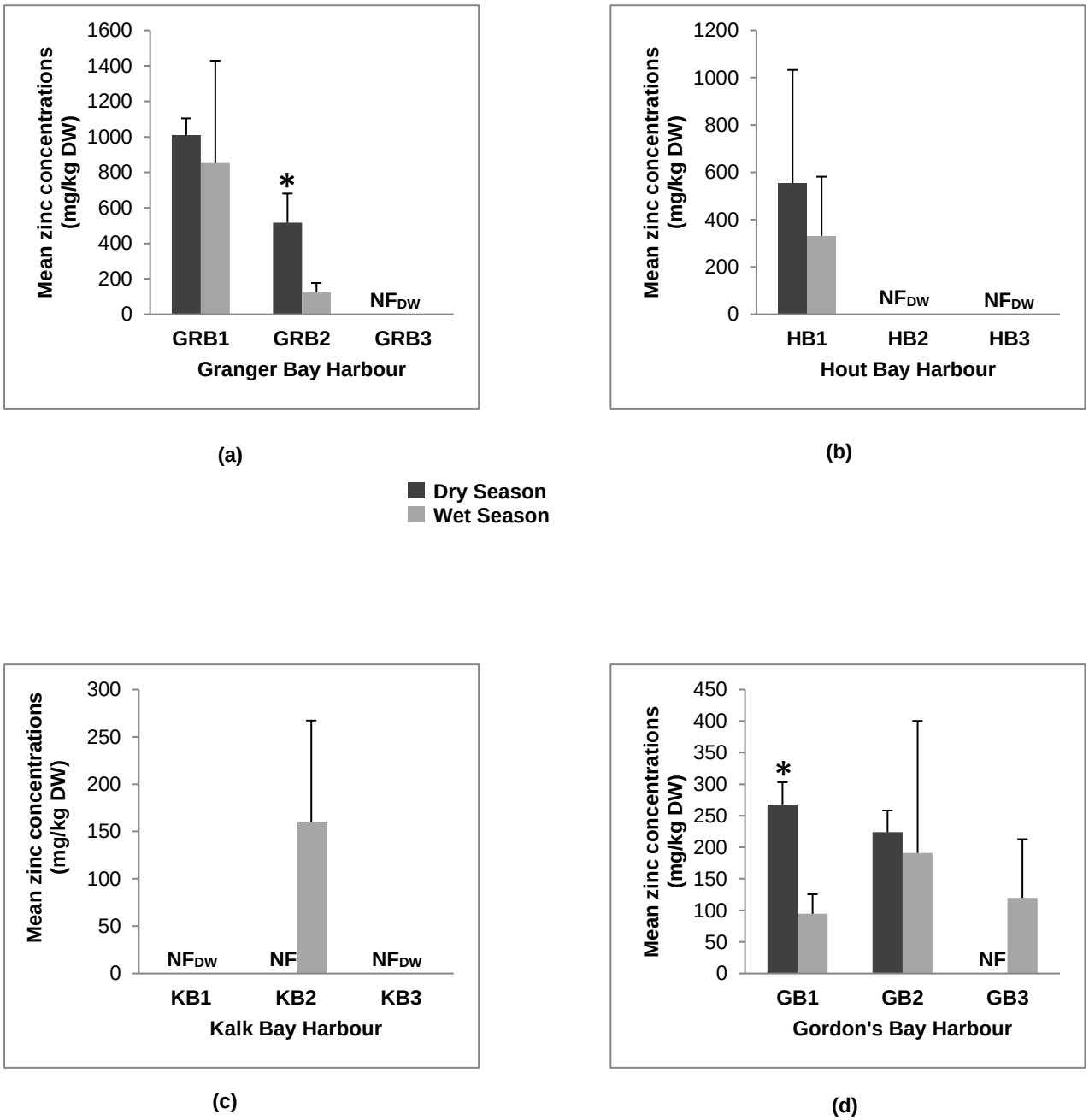


Figure 4. 13: Comparisons of mean zinc concentrations in *Burnupena* soft tissue between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; NF=Not Found; NF_{DW} =Not Found for Dry and Wet season; (a): mean zinc concentrations in GRB (b): mean zinc concentrations in HB; (c): mean zinc concentrations in KB; (d): mean zinc concentrations in GB.

4.5.4 *Burnupena* spp. shells

Table 4. 21: Mean zinc concentrations (mg/kg DW) (±SD) in *Burnupena* shells from sampling points in the four sampling sites and the two reference sites for dry and wet season sampling occasions(*n*=5)

		***Burnupena* shells (mg/kg DW) (±SD)**	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	ND	37.09 (±55.72)^{D}
CGH:MPA	**CGH_{MPA}**	3.04 (±6.79)	12.17 (±3.10)^{D}
GRB	**GRB1**	10.80 (±16.75)	51.18 (±101.74)^{D}
	GRB2	16.31 (±17.07)	0.67 (±1.06)^{ABC}
	GRB3	NF	NF
BB:MPA	**BB_{MPA}**	ND	37.09 (±55.72)^{F}
CGH:MPA	**CGH_{MPA}**	3.04 (±6.79)	12.17 (±3.10)^{F}
HB	**HB1**	0.31 (±0.69)	3.31 (±3.42)^{AB}
	HB2	NF	NF
	HB3	NF	NF
BB:MPA	**BB_{MPA}**	ND	37.09 (±55.72)^{J}
CGH:MPA	**CGH_{MPA}**	3.04 (±6.79)	12.17 (±3.10)^{J}
KB	**KB1**	NF	NF
	KB2	NF	5.27 (±2.40)^{AB}
	KB3	NF	NF
BB:MPA	**BB_{MPA}**	ND	37.09 (±55.72)
CGH:MPA	**CGH_{MPA}**	3.04 (±6.79)^{M}	12.17 (±3.10)
GB	**GB1**	ND	18.37 (±15.66)
	GB2	*57.99 (±5.79)^{B}	9.24 (±3.32)
	GB3	NF	7.96 (±9.59)

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference between seasons per sampling point is indicated by an asterisk (*). ND= Not Detected; NF= Not Found; *n*= number of replicates.

4.5.4.1 Comparisons of zinc concentrations in *Burnupena* shells collected from sampling points within each harbour and the two reference sites for dry and wet seasons

4.5.4.1.1 ***Dry season***

Comparisons of zinc concentrations in *Burnupena* shells collected from sampling points within each sampling site were dependent on the availability of data. The *Burnupena* spp. were either not found or zinc was not detected when analysed in some of the sampling points in the harbours. Hence these sampling points were therefore not included in the statistical analyses in their respective sampling sites. It should also be noted that zinc was not detected in *Burnupena* shells from BB: MPA and as such, it was excluded when statistical analyses were performed for sampling points in each harbour.

4.5.4.1.1.1 Granger Bay Harbour

All pairwise multiple comparisons of zinc concentrations in *Burnupena* shells from GRB1, GRB2 and CGH_{MPA} revealed no statistically significant differences ($p>0.05$). At GRB3, no *Burnupena* spp. were found during sampling. Therefore, GRB3 was excluded from the statistical analyses as data was not available.

4.5.4.1.1.2 Hout Bay Harbour

The *Burnupena* spp. were found only at HB1 in HB during sampling. However, no statistically significant difference ($p>0.05$) was found between HB1 and CGH_{MPA}.

4.5.4.1.1.3 Kalk Bay Harbour

The *Burnupena* spp. were not found in all three sampling points in KB at the time of sampling. Therefore, no statistical analyses were performed as no data was available.

4.5.4.1.1.4 Gordon's Bay Harbour

Of the three sampling points at GB, statistical analyses were performed only between GB2 and CGH_{MPA}. No *Burnupena* spp. were found at GB3 at the time of sampling which implied no data availability. Although the *Burnupena* spp. were found at GB1, zinc was not detected in the shells when analysed. Therefore, GB1 and GB3 were excluded from the statistical analyses. A comparison between GB2 and CGH_{MPA} revealed a statistically significant difference ($p<0.05$) (Table 4.21).

4.5.4.1.2 ***Wet season***

As already mentioned for the dry season, sampling points where *Burnupena* spp. were not found or where zinc was not detected in the shells were excluded from statistical analyses in the respective sampling sites.

4.5.4.1.2.1 Granger bay Harbour

The pairwise multiple comparisons of zinc concentrations in shells indicated that GRB2 had a significantly lower ($p<0.05$) zinc concentration than GRB1 and the two reference sites (Table 4.21). No statistically significant differences ($p>0.05$) were found between GRB1 and the two reference sites. GRB3 was not included in the statistical analyses as no data was available.

4.5.4.1.2.2 Hout Bay Harbour

Comparisons of *Burnupena* shells zinc concentrations revealed that HB1 had a significantly lower ($p<0.05$) zinc concentration than the two reference sites (Table 4.21). No data was available for HB2 and HB3 as the species were not found at the time of sampling.

4.5.4.1.2.3 Kalk Bay Harbour

Burnupena shells zinc concentrations in pairwise multiple comparisons indicated that KB2 had a significantly lower ($p<0.05$) zinc concentration than the two reference sites. No data were available for statistical analyses for KB1 and KB3 as the species were not found at the time of sampling.

4.5.4.1.2.4 Gordon's Bay Harbour

Pairwise multiple comparisons of zinc concentrations in shells showed no statistically significant differences ($p>0.05$) among the three sampling points (GB1, GB2, and GB3) and the two reference sites.

4.5.4.2 Comparisons of zinc concentrations found in *Burnupena* shells between sampling seasons per sampling point

Seasonal comparisons were not executed at sampling points within each harbour where the *Burnupena* spp. were not found or where zinc was not detected in the shells in either of the two seasons.

4.5.4.2.1 Granger Bay Harbour

Comparisons of zinc concentrations in the *Burnupena* shells at GRB1 and GRB2 revealed no statistically significant seasonal differences ($p>0.05$). No data was available for GRB3, as the gastropods were not found at the time of sampling for the two seasons.

4.5.4.2.2 Hout Bay Harbour

Comparison of zinc concentrations in the shells at HB1 showed no statistically significant seasonal differences ($p>0.05$). The *Burnupena* spp. were not found at HB2 and HB3 during the two sampling seasons. Hence no data were available for statistical analyses for these two sampling points.

4.5.4.2.3 Kalk Bay Harbour

The *Burnupena* spp. were not found in all three sampling points (KB1, KB2 and KB3) during the dry season but were present at KB2 during the wet season sampling. However, because the gastropods could only be found in one sampling season for the same sampling point or not found entirely for both sampling seasons, no statistical analyses could be performed in KB.

4.5.4.2.4 Gordon's Bay Harbour

Seasonal comparisons of zinc concentrations in shells indicted that GB2 had a significantly higher ($p<0.05$) zinc concentration in the dry season than during the wet season (Figure 4.14d). No comparisons were performed for sampling points GB1 and GB3 as data were only available for the wet season.

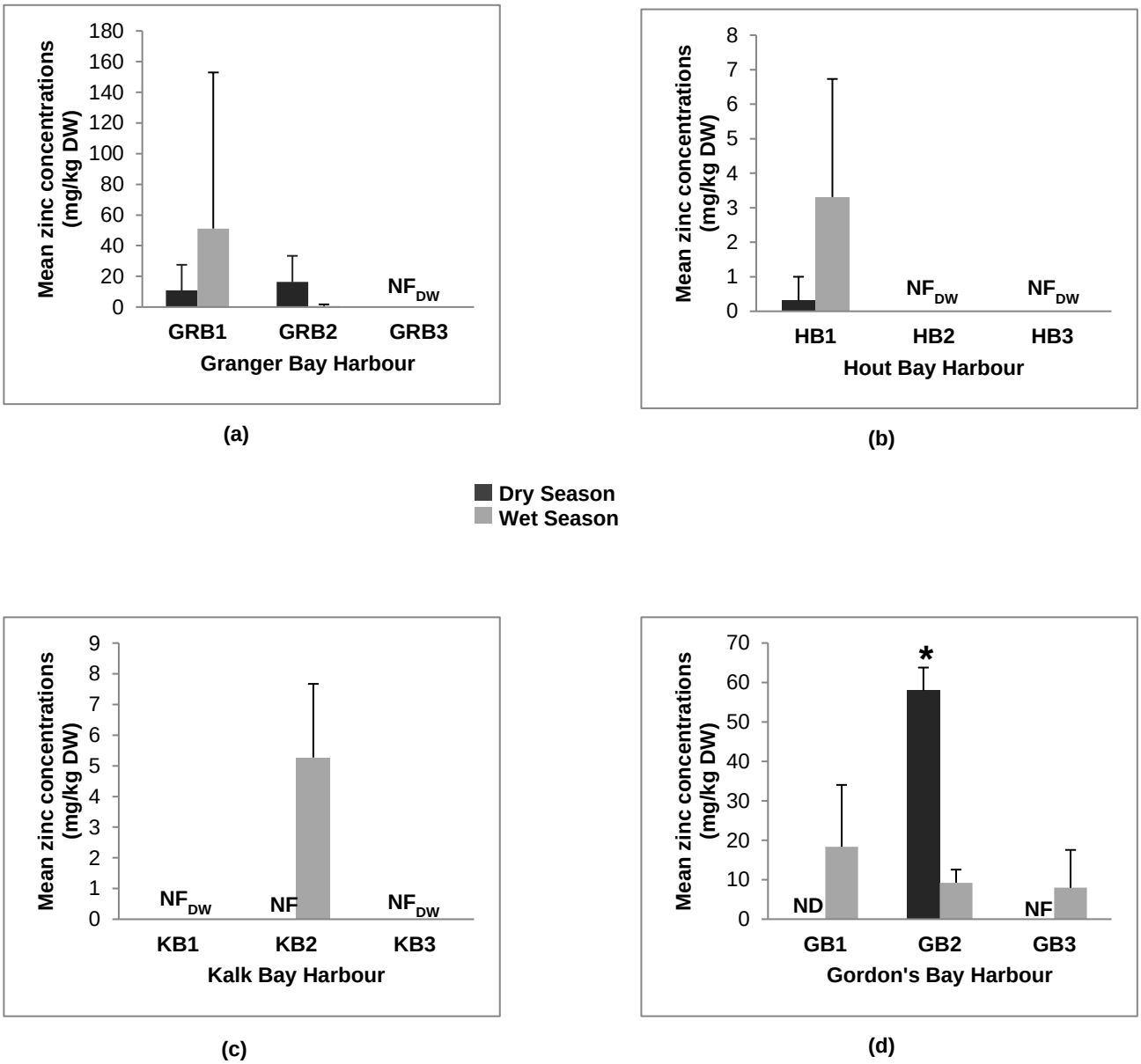


Figure 4. 14: comparisons of mean zinc concentrations in *Burnupena* shells between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; NF=Not Found; ND=Not Detected; NF_{DW} =Not Found for Dry and Wet season; (a): mean zinc concentrations in GRB (b): mean zinc concentrations in HB; (c): mean zinc concentrations in KB; (d): mean zinc concentrations in GB.

4.5.4.3 Comparisons of zinc concentrations between *Burnupena* soft tissue and shells per sampling point for the different sampling seasons

4.5.4.3.1 ***Dry season***

4.5.4.3.1.1 Granger Bay Harbour

Comparisons of zinc concentrations at GRB revealed that zinc concentrations found in the soft tissue at GRB1 and GRB2 were significantly higher ($p<0.05$) than that in the shells (Table 4.22). No comparisons were performed for GRB3 as the species were not found during the dry season sampling.

4.5.4.3.1.2 Hout Bay Harbour

Zinc concentration in the soft tissue at HB1 was significantly higher ($p<0.05$) when compared to that in the shell (Table 4.22). The gastropods were not found at the time of sampling at HB2 and HB3, therefore no data was available for comparisons.

4.5.4.3.1.3 Kalk Bay Harbour

The gastropods were not found in all three sampling points; hence, there were no data available for statistical analyses.

4.5.4.3.1.4 Gordon's Bay Harbour

Comparisons of zinc concentration at GB2 revealed that zinc concentration in the soft tissue was significantly higher ($p<0.05$) than in the shell (Table 4.22). At GB1, zinc was not detected in the shell; therefore, no comparisons could be performed. Similarly, no statistical analyses were carried out at GB3 as the gastropods were not found during sampling.

4.5.4.3.2 ***Wet season***

4.5.4.3.2.1 Granger Bay Harbour

Comparisons of zinc concentrations at GRB revealed that zinc concentrations found in the soft tissue at GRB1 and GRB2 were significantly higher ($p<0.05$) than that in the shells (Table 4.22). No comparisons were performed for GRB3 as the species were not found during the wet season sampling.

4.5.4.3.2.2 Hout Bay Harbour

Zinc concentration in the soft tissue at HB1 was significantly higher ($p<0.05$) when compared to that in the shell (Table 4.22). The gastropods were not found during the wet season sampling at HB2 and HB3, therefore no data was available for comparisons.

4.5.4.3.2.3 Kalk Bay Harbour

Comparisons of zinc concentration at KB2 revealed that zinc concentration in the soft tissue was significantly higher ($p<0.05$) than in the shell (Table 4.22). No data was available for comparisons at KB1 and KB3 as the species were not found at the time of sampling

4.5.4.3.2.4 Gordon's Bay Harbour

Comparisons of zinc concentrations at GB revealed that zinc concentrations found in the soft tissue at all three sampling points (GB1, GB2, and GB3) were significantly higher ($p<0.05$) than that found in the shells (Table 4.22).

Table 4. 22: A comparison of mean zinc concentrations in the soft tissue and shells of *Burnupena* spp. from the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

	***Burnupena* soft tissue and shells (mg/kg DW) (±SD)**			
	Dry season		**Wet season**	
Sampling points	**Tissue**	**Shell**	**Tissue**	**Shell**
BB_{MPA}	424.28(±311.01)	ND	144.17 (±87.86)	37.09(±55.72)
CGH_{MPA}	287.61(±190.63)*	3.04 (±6.79)	220.61 (±75.11)*	12.17(±3.10)
GRB1	1010.79(±93.79)*	10.80(±16.75)	852.16 (±577.06)*	51.18(±101.74)
GRB2	516.84(±164.17)*	16.31(±17.07)	124.18 (±52.62)*	0.67(±1.06)
GRB3	NF	NF	NF	NF
HB1	554.47(±478.75)*	0.31(±0.69)	331.90 (±250.14)*	3.31(±3.42)
HB2	NF	NF	NF	NF
HB3	NF	NF	NF	NF
KB1	NF	NF	NF	NF
KB2	NF	NF	159.75(±107.43)*	5.27(±2.40)
KB3	NF	NF	NF	NF
GB1	267.91(±35.15)	ND	94.54(±30.95)*	18.37(±15.66)
GB2	223.81(±34.55)*	57.99(±5.79)	190.96(±209.26)*	9.24(±3.32)
GB3	NF	NF	119.89(±92.91)*	7.96(±9.59)

Significant difference between zinc concentrations in *Burnupena* soft tissue and shells per sampling point per season is indicated by two asterisks (*). NF=Not Found; ND= Not Detected; *n*= number of replicates.

4.5.5 *Nucella* spp. soft tissue

Table 4. 23: Mean zinc concentrations (mg/kg DW) (±SD) in *Nucella* soft tissue from sampling points in the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

		***Nucella* soft tissue (mg/kg DW) (±SD)**	
Harbours and reference sites	**Sampling points**	**Dry season**	**Wet Season**
BB:MPA	**BB_{MPA}**	193.65 (±150.10)^{E}	164.21 (±74.91)^{BE}
CGH:MPA	**CGH_{MPA}**	130.25 (±82.84)^{E}	302.68 (±72.02)^{AE}
GRB	**GRB1**	262.27 (±77.87)^{E}	NF
	GRB2	NF	NF
	GRB3	1263.02 (±233.12)^{ABC}	1058.07 (±368.35)^{AB}
BB:MPA	**BB_{MPA}**	193.65 (±150.10)^{FGH}	164.21 (±74.91)^{BFGH}
CGH:MPA	**CGH_{MPA}**	130.25 (±82.84)^{FGH}	302.68 (±72.02)^{AFGH}
HB	**HB1**	488.50 (±48.32)^{ABGH}	554.18 (±229.19)^{ABGH}
	HB2	*1297.75 (±182.76)^{ABF}	1882.87 (±372.22)^{ABFH}
	HB3	1138.88 (±83.36)^{ABF}	1002.67 (±157.16)^{ABFG}
BB:MPA	**BB_{MPA}**	193.65 (±150.10)^{IJK}	164.21 (±74.91)^{BI}
CGH:MPA	**CGH_{MPA}**	130.25 (±82.84)^{IJK}	302.68 (±72.02)^{AI}
KB	**KB1**	*2229.36 (±279.14)^{ABJK}	1654.53 (±63.20)^{ABJK}
	KB2	503.70 (±92.64)^{ABIK}	693.82 (±386.14)^{AI}
	KB3	*1802.16 (±97.22)^{ABIJ}	797.14 (±433.94)^{AI}
BB:MPA	**BB_{MPA}**	193.65 (±150.10)^{N}	164.21 (±74.91)^{BN}
CGH:MPA	**CGH_{MPA}**	130.25 (±82.84)^{N}	302.68 (±72.02)^{AN}
GB	**GB1**	NF	NF
	GB2	NF	NF
	GB3	*5045.44 (±2447.15)^{AB}	77.20 (±15.14)^{AB}

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference between seasons per sampling point is indicated by an asterisk (*). NF=Not Found; *n*= number of replicates.

4.5.5.1 Comparisons of zinc concentrations in *Nucella* soft tissue from sampling points within each harbour and the two reference sites for dry and wet seasons

4.5.5.1.1 ***Dry season***

4.5.5.1.1.1 Granger Bay Harbour

All pairwise multiple comparisons procedures for zinc concentrations in *Nucella* soft tissue indicated that GRB3 had a significantly higher ($p<0.05$) zinc concentration than GRB2 and the two reference sites. No comparisons were performed for GRB2 as the gastropods were not found at the time sampling.

4.5.5.1.1.2 Hout Bay Harbour

Comparisons of zinc concentrations in *Nucella* soft tissue revealed that HB1 had a significantly lower ($p<0.05$) zinc concentration than HB2 and HB3 but significantly higher ($p<0.05$) than the two reference sites. Also, zinc concentrations at HB2 and HB3 were significantly higher ($p<0.05$) than for BB_{MPA} and CGH_{MPA}. There was no statistically significant difference ($p>0.05$) between HB2 and HB3.

4.5.5.1.1.3 Kalk Bay Harbour

The pairwise multiple comparisons for zinc concentrations in *Nucella* soft tissue showed that KB1 had a significantly higher ($p<0.05$) zinc concentration than KB2, KB3 and the two reference sites. All pairwise multiple comparisons also revealed that the zinc concentrations at KB2 and KB3 were significantly higher ($p<0.05$) than for BB_{MPA} and CGH_{MPA}. Furthermore, the zinc concentration recorded at KB3 was also found to be significantly higher ($p<0.05$) than that at KB2.

4.5.5.1.1.4 Gordon's Bay Harbour

Pairwise multiple comparisons for *Nucella* soft tissue zinc concentrations at GB revealed that GB3 had a significantly higher ($p<0.05$) zinc concentration than the two reference sites. No comparisons were done for GB1 and GB2 as the gastropods were not found at the time of sampling.

4.5.5.1.2 ***Wet season***

4.5.5.1.2.1 Granger Bay Harbour

All pairwise multiple comparisons procedures for zinc concentrations in *Nucella* soft tissue indicated that GRB3 had a significantly higher ($p<0.05$) zinc concentration than BB_{MPA} and

CGH_{MPA}. No data were available for comparative purposes at GRB1 and GRB2 as gastropods were during wet season sampling.

4.5.5.1.2.2 Hout Bay Harbour

The pairwise multiple comparisons for zinc concentrations in *Nucella* soft tissue showed that HB2 had a significantly higher ($p<0.05$) zinc concentration than HB1, HB3 and the two reference sites. Also, HB1 and HB3 zinc concentrations were significantly higher ($p<0.05$) when compared to the two reference sites. The pairwise multiple comparisons also reveal that HB1 had a significantly higher ($p<0.05$) zinc concentration than HB3.

4.5.5.1.2.3 Kalk Bay Harbour

Pairwise multiple comparisons for zinc concentrations in *Nucella* soft tissue revealed that KB1 had a significantly higher ($p<0.05$) zinc concentration than KB2, KB3, and the two reference sites. Also, zinc concentrations at KB2 and KB3 were significantly higher ($p<0.05$) than for reference site BB. There were no statistically significant differences ($p>0.05$) between KB2 and KB3, and when compared with reference site CGH_{MPA}.

4.5.5.1.2.4 Gordon's Bay Harbour

Comparisons for *Nucella* soft tissue zinc concentrations at GB revealed that GB3 had a significantly lower ($p<0.05$) zinc concentration than the two reference sites. No comparisons could be performed for GB1 and GB2 as the gastropods were not found during the wet season sampling.

4.5.5.2 Comparisons of zinc concentrations found in *Nucella* soft tissue between sampling seasons per sampling point

It should be noted that seasonal comparisons were not performed at sampling points within each harbour where the *Nucella* spp. were not found or where zinc was not detected in the shells in either of the two seasons.

4.5.5.2.1 Granger Bay Harbour

Comparisons of zinc concentrations revealed no statistically significant seasonal difference ($p>0.05$) at GRB3. The gastropods were not found during the wet season sampling for GRB1 and during both seasons for GRB2.

4.5.5.2.2 Hout Bay Harbour

Comparisons of zinc concentrations indicated that HB2 had a significantly lower ($p<0.05$) zinc concentration in the dry season than during the wet season sampling (Figure 4.15b). No statistically significant seasonal differences ($p>0.05$) were found for HB1 and HB3.

4.5.5.2.3 Kalk Bay Harbour

Comparisons revealed that the zinc concentrations at KB1 and KB3 were significantly higher ($p<0.05$) in the dry season than in the wet season sampling (Figure 4.15c). There was no statistically significant seasonal difference ($p>0.05$) at KB2

4.5.5.2.4 Gordon's Bay Harbour

Comparisons of zinc concentrations revealed that GB3 had a significantly higher ($p<0.05$) zinc concentration in the dry season than in the wet season (Figure 4.15d). The *Nucella* spp. were not found during the dry and wet seasons for both GB1 and GB2.

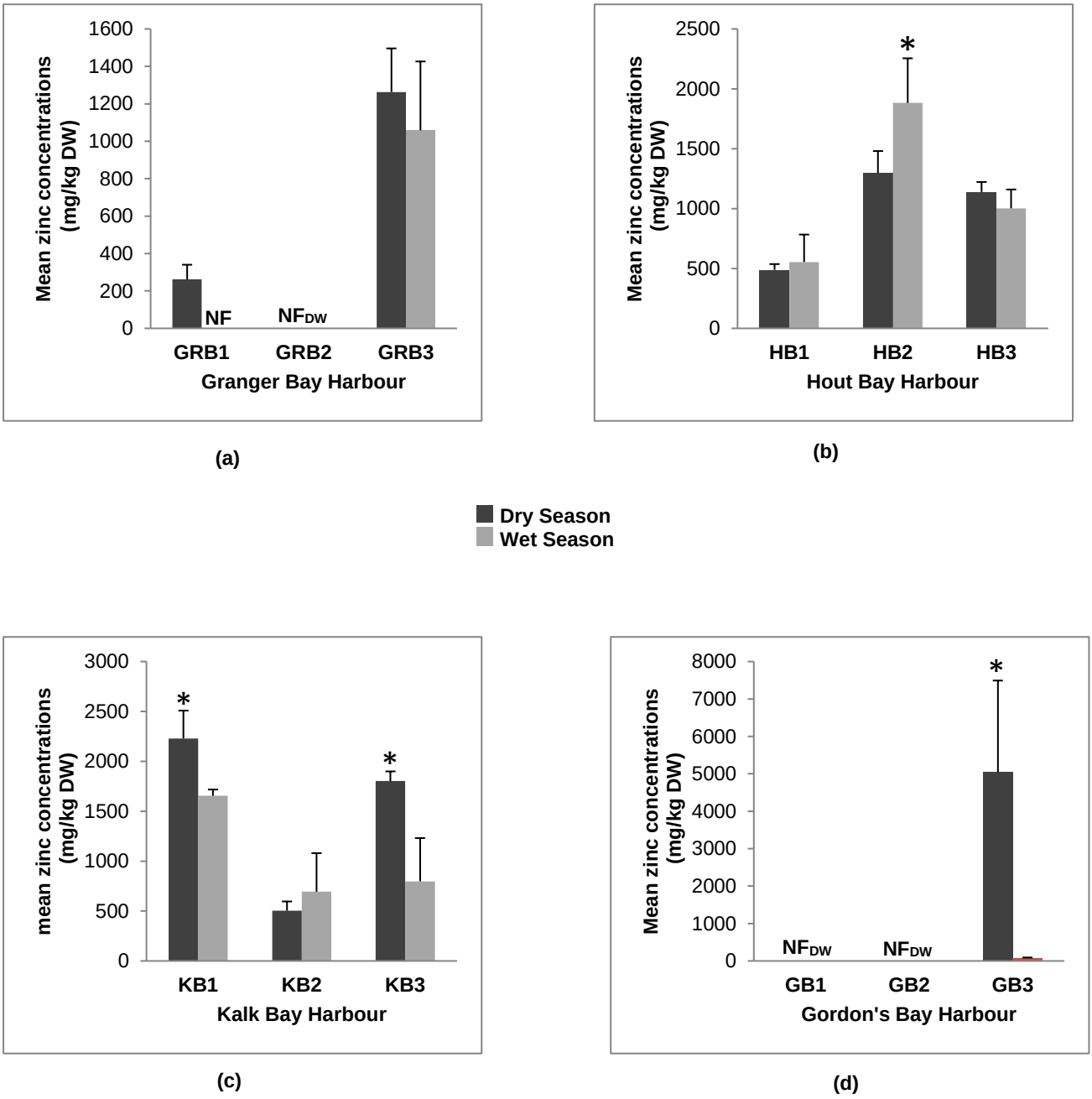


Figure 4. 15: Comparisons of mean zinc concentrations in *Nucella* soft tissue between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; NF=Not Found; NF_{DW} =Not Found for Dry and Wet season; (a): mean zinc concentrations in GRB; (b): mean zinc concentrations in HB; (c): mean zinc concentrations in KB; (d): mean zinc concentrations in GB.

4.5.6 *Nucella* spp. shells

Table 4. 24: Mean zinc concentrations (mg/kg DW) (±SD) in *Nucella* shells from sampling points in the four sampling sites and the two reference sites for dry and wet season sampling occasions (*n*=5)

		***Nucella* shells (mg/kg DW) (±SD)**	
Harbours and reference sites	**Sampling Points**	**Dry season**	**Wet season**
BB:MPA	**BB_{MPA}**	ND	4.13 (±2.97)^{B}
CGH: MPA	**CGH_{MPA}**	ND	8.99 (±2.10)^{A}
GRB	**GRB1**	ND	NF
	GRB2	NF	NF
	GRB3	116.52 (±83.01)	18.39 (±17.30)
BB:MPA	**BB_{MPA}**	ND	4.13 (±2.97)^{BFGH}
CGH: MPA	**CGH_{MPA}**	ND	8.99 (±2.10)^{AFGH}
HB	**HB1**	20.23 (±19.42)^{GH}	21.34 (±8.45)^{AB}
	HB2	*120.91 (±13.98)^{F}	50.42 (±33.16)^{ABH}
	HB3	*122.46 (±65.00)^{F}	16.91 (±10.52)^{ABG}
BB:MPA	**BB_{MPA}**	ND	4.13 (±2.97)^{BIK}
CGH: MPA	**CGH_{MPA}**	ND	8.99 (±2.10)^{AIK}
KB	**KB1**	*102.41 (±5.54)	182.65 (±89.72)^{ABJK}
	KB2	ND	6.09 (±4.34)^{IK}
	KB3	ND	31.89 (±27.82)^{ABIJ}
BB:MPA	**BB_{MPA}**	ND	4.13 (±2.97)^{B}
CGH: MPA	**CGH_{MPA}**	ND	8.99 (±2.10)^{A}
GB	**GB1**	NF	NF
	GB2	NF	NF
	GB3	ND	11.12 (±6.94)

Sampling points within a harbour were compared to each other and the two reference sites per season. Significant difference from: BB_{MPA}=A; CGH_{MPA}=B; GRB1=C; GRB2=D; GRB3=E; HB1=F; HB2=G; HB3=H; KB1=I; KB2=J; KB3=K; GB1=L; GB2=M; GB3=N. A significant difference between seasons per sampling point is indicated by an asterisk (*). ND= Not Detected; NF= Not Found; *n*= number of replicates.

4.5.6.1 Comparisons of zinc concentrations in *Nucella* shells from sampling points within each harbour and the two reference sites for dry and wet seasons

4.5.6.1.1 ***Dry season***

The two reference sites were not included in the statistical analyses for all four harbours as zinc was not detected in the shells. It should also be noted that in some of the sampling points in the harbours zinc was not detected (Table 4.24).

4.5.6.1.1.1 Granger Bay Harbour

No comparisons could be done as data was only available for GRB3. The gastropods were not found at GRB2, while zinc was not detected at GRB1.

4.5.6.1.1.2 Hout Bay Harbour

Pairwise multiple comparisons of zinc concentrations in *Nucella* shells revealed that HB1 had a significantly lower ($p<0.05$) zinc concentration than HB2 and HB3 (Table 4.24). There was no statistically significant difference ($p>0.05$) between HB2 and HB3.

4.5.6.1.1.3 Kalk Bay Harbour

Zinc was not detected in all but one (i.e., KB1) of the three sampling points in KB. Hence, no statistical analyses could be done.

4.5.6.1.1.4 Gordon's Bay Harbour

The *Nucella* spp. were not found at GB1 and GB2 and zinc were not also detected at GB3, as a result, no comparisons could be done.

4.5.6.1.2 ***Wet season***

4.5.6.1.2.1 Granger Bay Harbour

The *Nucella* spp. were not found in all but one sampling point (i.e., GRB3). However, comparisons between GRB3 and the two reference sites revealed no statistically significant differences ($p>0.05$).

4.5.6.1.2.2 Hout Bay Harbour

All pairwise multiple comparison procedures of zinc concentrations in *Nucella* shells indicated that HB2 had a significantly higher ($p<0.05$) zinc concentration than HB3 and the two reference sites (Table 4.24). Comparisons also revealed that zinc concentrations at HB1 and HB3 were significantly higher ($p<0.05$) than for the two reference sites. There were no statistically significant differences ($p>0.05$) between HB1 and HB2, and HB1 and HB3.

4.5.6.1.2.3 Kalk Bay Harbour

Multiple comparisons of zinc concentrations in *Nucella* shells revealed that KB1 had a significantly higher ($p<0.05$) zinc concentration than KB2, KB3, and the two reference sites. Comparisons also indicated that KB3 had a significantly higher ($p<0.05$) zinc concentration than KB2 and the two reference sites (Table 4.24). No statistically significant difference ($p>0.05$) was found between KB2 and the two reference sites.

4.5.6.1.2.4 Gordon's Bay Harbour

Of the three sampling points at GB, the *Nucella* spp. were only found at GB3. However, comparison of zinc concentration at GB3 to that of the two reference sites showed no statistically significant differences ($p>0.05$).

4.5.6.2 Comparisons of zinc concentrations found in *Nucella* shells between sampling seasons per sampling point

As already mentioned previously, seasonal comparisons could not be performed at sampling points where the gastropods were not found or where zinc was not detected in the samples in either one or both seasons.

4.5.6.2.1 Granger Bay Harbour

Seasonal comparisons could not be done for GRB1 and GRB2. However, comparisons of zinc concentration in *Nucella* shells at GRB3 did not show a statistically significant seasonal difference ($p>0.05$).

4.5.6.2.2 Hout Bay Harbour

Comparisons of zinc concentrations in *Nucella* shells at HB2 and HB3 revealed a significantly higher ($p<0.05$) zinc concentration in the dry season than in the wet season (Figure 4.16b). No statistically significant seasonal difference ($p>0.05$) was found at HB1.

4.5.6.2.3 Kalk Bay Harbour

Comparison of zinc concentration in *Nucella* shell at KB1 indicated a significantly lower ($p<0.05$) zinc concentration in the dry season than in the wet season (Figure 4.16c). No seasonal comparisons could be performed at KB2 and KB3 as zinc was not detected in the shells for the dry season sampling.

4.5.6.2.4 Gordon's Bay Harbour

The *Nucella* spp. were not found at GB1 and GB2 for both seasons. Zinc was also not detected in the shells at GB3 for the dry season sampling; therefore, seasonal comparisons could not be done for GB.

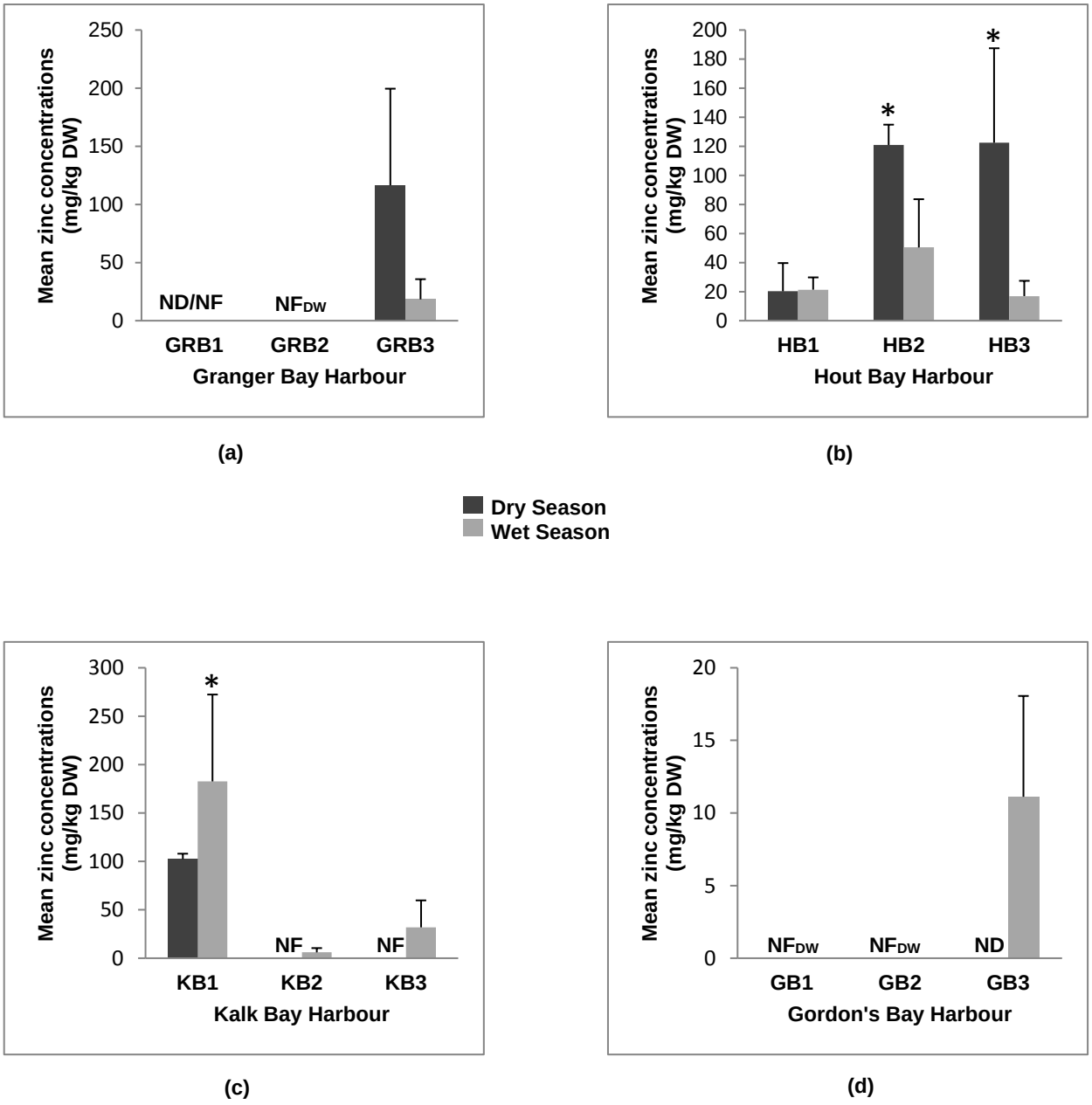


Figure 4. 16: Comparisons of mean zinc concentrations in *Nucella* shells between the dry and wet season per sampling point in the four harbours. Asterisk (*) above the bar showed significant seasonal difference; Error bars = ±SD; NF=Not Found; NF_{DW} =Not Found for Dry and Wet season; ND/NF= Not Detected for Dry season and Not Found for Wet season; (a): mean zinc concentrations in GRB; (b): mean zinc concentrations in HB; (c): mean zinc concentrations in KB; (d): mean zinc concentrations in GB.

4.5.6.3 Comparisons of zinc concentrations between *Nucella* soft tissue and shells per sampling point for the different sampling seasons

4.5.6.3.1 ***Dry season***

4.5.6.3.1.1 Granger Bay Harbour

Comparisons of zinc concentrations in GRB revealed that zinc concentration in the *Nucella* soft tissue at GRB3 was significantly higher ($p<0.05$) than that in the shells (Table 4.25). Zinc was not detected in shells at GRB1 while the *Nucella* spp. were not found at GRB2 during the sampling occasion. Therefore, no comparisons could be performed for GRB1 and GRB2.

4.5.6.3.1.2 Hout Bay Harbour

Comparisons in HB indicated that zinc concentrations in the *Nucella* soft tissue from all three sampling points (HB1, HB2, and HB3) were significantly higher ($p<0.05$) than in the shells (Table 4.24).

4.5.6.3.1.3 Kalk Bay Harbour

Comparisons of zinc concentrations in KB showed that zinc concentration in *Nucella* soft tissue at KB1 was significantly higher ($p<0.05$) than that in the shells (Table 4.24). No comparisons were done for KB2 and KB3 as zinc was not detected in the shells.

4.5.6.3.1.4 Gordon's Bay Harbour

At GB3, zinc was only detected in the soft tissue and not in the shells while at GB1 and GB2, the *Nucella* spp. were not found during sampling. Therefore, no comparisons could be done in GB.

4.5.6.3.2 ***Wet season***

Comparisons in the two reference sites revealed that zinc concentrations were significantly higher ($p<0.05$) in the soft tissue than in the shells for both controls.

4.5.6.3.2.1 Granger Bay Harbour

Comparisons in GRB showed that the zinc concentration in *Nucella* soft tissue at GRB3 was significantly higher ($p<0.05$) than in the shells. No comparisons were done for GRB1 and GRB2 as the *Nucella* spp. were not found during the sampling occasion.

4.5.6.3.2.2 Hout Bay Harbour

Comparisons in HB showed that the zinc concentrations recorded in *Nucella* soft tissue from all three sampling points (HB1, HB2, and HB3) were significantly higher ($p<0.05$) than in the shells.

4.5.6.3.2.3 Kalk Bay Harbour

In KB, comparisons revealed that zinc concentrations in the *Nucella* soft tissue from all three sampling points (KB1, KB2 and KB3) were significantly higher ($p<0.05$) than in the shells (Table 4.25).

4.5.6.3.2.4 Gordon's Bay Harbour

Comparisons in GB revealed that the zinc concentration in the *Nucella* soft tissue at GB3 was significantly higher ($p<0.05$) than in the shells (Table 4.25). No comparisons were done for GB1 and GB2 as the gastropods were not found during the sampling occasion.

Table 4. 25: A comparison of mean zinc concentrations in the soft tissue and shells of *Nucella* spp. from the four harbours and the two reference sites for dry and wet season sampling occasions (*n*=5)

	***Nucella* soft tissue and shells (mg/kg DW) (±SD)**			
	Dry season		**Wet season**	
Sampling points	**Tissue**	**Shell**	**Tissue**	**Shell**
BB_{MPA}	193.65 (±150.10)	ND	164.21 (±74.91)*	4.13 (±2.97)
CGH_{MPA}	130.25 (±82.84)	ND	302.68 (±72.02)*	8.99 (±2.10)
GRB1	262.27 (±77.87)	ND	NF	NF
GRB2	NF	NF	NF	NF
GRB3	1263.02 (±233.12)*	116.52 (±83.01)	1058.07 (±368.35)*	18.39 (±17.30)
HB1	488.50 (±48.32)*	20.23 (±19.42)	554.18 (±229.19)*	21.34 (±8.45)
HB2	1297.75 (±182.76)*	120.91 (±13.98)	1882.87 (±372.22)*	50.42 (±33.16)
HB3	1138.88 (±83.36)*	116.52 (±83.01)	1002.67 (±157.16)*	16.91 (±10.52)
KB1	2229.36 (±279.14)*	102.41 (±5.54)	1654.53 (±63.20)*	182.65 (±89.72)
KB2	503.70 (±92.64)	ND	693.82 (±386.14)*	6.09 (±4.34)
KB3	1802.16 (±97.22)	ND	797.14 (±433.94)*	31.89 (±27.82)
GB1	NF	NF	NF	NF
GB2	NF	NF	NF	NF
GB3	5045.44 (±2447.15)	ND	77.20 (±15.14)*	11.12 (±6.94)

Significant difference between zinc concentrations in *Nucella* soft tissue and shells per sampling point per season is indicated by an asterisk (*). NF=Not Found; ND= Not Detected; *n*= number of replicates.

4.5.6.4 The relationship between zinc concentrations in ambient samples (seawater and sediment) and the gastropod soft tissue in the harbours and reference sites for the dry and wet seasons

The correlations between zinc concentrations in the ambient samples (seawater and sediment) and the gastropod soft tissue were computed, and the results displayed in Table 4.26. As already mentioned, (section 4.4.6.4), the correlation analyses were restricted only to the *Nucella* spp. due to the incomplete datasets for the *Burnupena* spp.

Table 4. 26: The Spearman Rank Order Correlation coefficients (r_s) between mean zinc concentrations in ambient samples and *Nucella* soft tissue in the harbours and reference sites

		Dry season	Wet season	Dry season	Wet season
Zinc		Seawater vs Soft tissue (*Nucella* spp.)	Seawater vs Soft tissue (*Nucella* spp.)	Sediment vs Soft tissue (*Nucella* spp.)	Sediment vs Soft tissue (*Nucella* spp.)
Harbours and reference sites	**BB: MPA** r_s *p* *n*	-0.103 0.783 5	-0.1000 0.950 5	0.700 0.233 5	-0.410 0.450 5
	CGH: MPA r_s *p* *n*	0.200 0.783 5	0.224 0.683 5	0.200 0.783 5	0.800 0.133 5
	GRB r_s *p* *n*	-0.365 0.275 10	-0.1000 0.950 5	0.912* 0.0000002 10	-0.800 0.133 5
	HB r_s *p* *n*	0.250 0.359 15	0.0893 0.743 15	-0.186 0.498 15	0.492 0.0597 15
	KB r_s *p* *n*	NA	-0.0400 0.883 15	0.560* 0.0287 15	0.539* 0.0367 15
	GB r_s *p* *n*	0.0513 0.950 5	-0.707 0.133 5	0.000 1.000 5	-0.800 0.133 5

r_s= correlation coefficient; *p*= P Value; *n*= number of replicates; NA = Not Analysed; * Correlation significant at p<0.05; vs= versus

4.5.6.4.1 ***Dry season***

The results showed that no significant correlations (p>0.05) were found in zinc concentrations between seawater and *Nucella* soft tissue in GRB, HB, GB and the reference sites during the dry season. The relationship in zinc concentrations between seawater and *Nucella* soft tissue in KB could not be determined due to that fact that zinc was not detected in seawater samples during the dry season.

The results revealed that significant positive correlations (r_s=0.912 and r_s=0.560, p<0.05) were found in zinc concentrations between sediment and *Nucella* soft tissue in GRB and KB (Table 4.26). No significant correlations (p>0.05) were found in zinc concentrations between the sediment samples and the *Nucella* soft tissue in HB, GB and the reference sites.

4.5.6.4.2 ***Wet season***

The results showed that no significant correlations (p>0.05) were found in zinc concentrations between the seawater samples and *Nucella* soft tissue in the harbours and the reference sites during the wet season.

A significant positive correlation (r_s=0.539, p<0.05) was found in zinc concentrations between sediment and *Nucella* soft tissue in KB during the wet season (Table 4.26). The results showed that no significant correlations (p>0.05) were found in zinc concentrations between sediment and *Nucella* soft tissue in GRB, HB, GB and the reference sites.

4.5.6.5 The relationship between zinc concentrations in ambient samples (seawater and sediment) and the gastropod shells in the harbours and reference sites for the dry and wet seasons

The correlations between zinc concentrations in the ambient samples (seawater and sediment) and the gastropod shell were computed, and the results displayed in Table 4.27. only datasets for the *Nucella* spp. were used.

Table 4. 27: The Spearman Rank Order Correlation coefficients (r_s) between mean zinc concentrations in ambient samples and *Nucella* shells in the harbours and reference sites

		Dry season	Wet season	Dry season	Wet season
Zinc		Seawater vs shell (*Nucella* spp.)	Seawater vs shell (*Nucella* spp.)	Sediment vs shell (*Nucella* spp.)	Sediment vs shell (*Nucella* spp.)
Harbours and reference sites	**BB: MPA** r_s *p* *n*	NA	 0.800 0.133 5	NA	 -0.872 0.0833 5
	CGH: MPA r_s *p* *n*	NA	 -0.447 0.450 5	NA	 0.1000 0.950 5
	GRB r_s *p* *n*	 -0.597 0.0599 10	 0.700 0.233 5	 0.772* 0.00686 10	 0.1000 0.950 5
	HB r_s *p* *n*	 0.175 0.523 15	 0.00714 0.974 15	 -0.147 0.593 15	 0.105 0.695 15
	KB r_s *p* *n*	NA	 -0.276 0.312 15	 0.773* 0.000231 15	 0.668* 0.00614 15
	GB r_s *p* *n*	NA	 0.707 0.133 5	NA	 0.400 0.517 5

r_s = correlation coefficient; *p*= P Value; *n*= number of samples; na = Not analysed; * Correlation significant at p<0.05; vs= versus.

4.5.6.5.1 ***Dry season***

The results showed that no significant correlations (p>0.05) were found in zinc concentrations between the seawater samples and the shells of *Nucella* spp. in GRB and HB, during the dry season. No correlation analyses were done for KB, GB and the reference sites as zinc was not detected in the seawater samples or *Nucella* shells or the *Nucella* spp. were not found during the dry season (Table 4.16 and 4.24).

The results revealed that significant positive correlations (r_S=0.772 and r_S=0.773, p<0.05) were found in zinc concentrations between the sediment samples and the *Nucella* shell in GRB and KB (Table 4.27). No significant correlation (p>0.05) was found between zinc concentrations in the sediment sample and the *Nucella* shells in HB. No correlation analyses were performed for GB and the reference sites as zinc were not detected in the sediment samples during the dry season.

4.5.6.5.2 ***Wet season***

The results showed that no significant correlations (p>0.05) were found in zinc concentrations between the seawater samples and *Nucella* shells in the harbours and the reference sites during the wet season.

A significant positive correlation (r_S=0.668, p<0.05) was found in zinc concentrations between sediment and *Nucella* shells in KB during the wet season (Table 4.27). No significant correlations (p>0.05) were found in zinc concentrations between the sediment samples and the *Nucella* shells in GRB, HB, GB and the reference sites.

CHAPTER FIVE

DISCUSSION: COPPER AND ZINC

5.1 Metal concentrations in seawater within and between harbours

Copper occurs naturally in seawater with background concentrations found within estuarine and coastal seawater ranging between 5 x 10^{-4} and 3 x10^{-3} mg/L (Thomas & Brooks, 2010). Bruland (1983) reported a range between 3 x 10^{-5} and 3.8 x 10^{-4} mg/L, with an average of 2.5 x 10^{-4} mg/L in seawater. According to Thomas & Brooks (2010), copper can potentially accumulate in the marine environment around enclosed harbours with restricted water exchange and high boat densities. For instance, Schiff et al. (2007) and Biggs & D'Anna (2012) reported high concentrations of copper up to 0.021mg/L and 0.022mg/L, respectively, in the surface waters of San Diego Bay, California. In the UK, dissolved copper concentrations up to 0.0048 and 0.0067 mg/L have been reported from the vessel and recreational vessel harbours (Jones and Bolam 2007). In South Africa, Lusher (1984) reported the average copper concentrations in marine surface waters to be 8.99 x 10^{-4} mg/L. Also, a recent study by Sparks et al. (2017), reported an average copper concentration of 1x10^{-5}mg/L in intertidal waters from the west coast of the Cape Peninsula.

Like copper, zinc is a ubiquitous element in nature, making up between 0.0005% and 0.02% of the Earth's crust (Irwin et al., 1997). The concentrations of zinc in oceans are less than 0.001mg/L (Bruland et al., 1979), but concentrations in coastal areas and estuaries are frequently much higher. For example, Morse et al. (1993) observed 3 x 10^{-5} to 0.0045mg/L of dissolved zinc in the water column of Galveston Bay and Law et al. (1994) reported concentrations of 0.00043 to 0.022mg/L in subsurface seawater from British estuaries. The WHO (2001), reported low baseline concentrations of dissolved zinc between 2 x 10^{-6} – 1x10^{-4}mg/L in surface ocean waters. According to Riley & Chester (1976), the average concentration of zinc in unpolluted seawater is 0.005mg/L. Zinc concentration in coastal waters lies in the range of 0.0003 to 0.07mg/L (Bryan & Langston, 1992; Sadiq, 1992; UNEP, 1993). The average zinc concentration in South African surface marine waters has been reported as 6.59 x 10^{-3}mg/L (Lusher, 1984). In a more recent study, Sparks et al. (2017) reported mean zinc concentrations in surface waters of 1.1 x 10^{-4}mg/L. In the waters of open, well-mixed recreational vessel harbours, zinc concentrations may be 0.002 to 0.004mg/L higher than open coastal waters and may be as high as 0.02mg/L in the waters of enclosed harbours due to leaching from sacrificial anodes (Bird et al., 1996). Elevated concentrations of zinc have been

reported in several recreational vessel harbours worldwide (e.g., Matthiessen et al., 1999; Boxall et al., 2000).

5.1.1 Comparisons in the dry season

Mean copper concentrations (mg/L) found in seawater at sampling points within the four harbours in the dry season ranged from not detected (ND) to 0.0818±0.0494 (Table 4.4). The results showed that the mean copper concentration recorded at the reference site (BB_{MPA}) was significantly higher than at GRB2, GB1 and GB3. The oceanographic regime of BB_{MPA} is influenced by both the strong-flowing Agulhas current that moves down the east coast and the cold Benguela upwelling system of the west coast which extends as far as Cape Agulhas (Figure 2.1) (Lutjeharms et al., 2001; Lutjeharms, 2006). Therefore, it could be suggested that the Benguela upwelling which is driven by the predominantly south-easterly winds, associated with the summer wind pattern and the strong-flowing Agulhas current which might transport contaminants (such as copper) from the east coast may have accounted for the higher mean copper concentration in seawater at BB_{MPA}. The mean concentration of copper recorded in seawater at HB1, HB2, HB3 and GB3 exceeded the 0.003mg/L threshold for copper recommended by the South African Water Quality Guidelines (SAWQGs) for coastal marine waters (DEA, 2018). Although there were insignificant differences in the mean copper concentrations found between the sampling points in each harbour, observations revealed that copper variability between the sampling points in the harbours may be influenced by proximity to contamination sources as well as the potential for water circulation and dilution. The sampling points, GRB2, HB2 and GB3 which recorded the highest mean copper concentrations in seawater within their respective harbours were in areas inside the harbour protected against strong water movements (tidal currents) and of intense harbour activities such as boat repair and maintenance (e.g. scouring of boat hulls), vessel launching as well as vessel moorings. This may suggest that the higher mean copper concentrations recorded at these sampling points could be due to the leaching of copper from copper-based antifouling coatings used on the vessel hulls into the surrounding waters. This assertion is consistent with other findings worldwide. For example, Biggs & D'Anna (2012) found a rapid increase in copper concentrations in water in a new recreational boat harbour in San Diego Bay, with an increase in boat activities. Also, Matthiessen et al. (1999) and Hall & Anderson (1999) found that harbour areas in Europe with intense vessel activities had higher surface seawater copper concentrations than open coastal areas. Likewise, Hall et al. (1992) found a decrease in copper concentrations in water with distance away from a recreational boat harbour in Chesapeake Bay, Maryland.

The mean zinc concentrations (mg/L) recorded in seawater at sampling points within the four harbours during the dry season ranged from ND to 0.4888 ± 0.4998 (Table 4.16). The results indicated that there were no significant differences in the mean zinc concentrations recorded in seawater between the sampling points in each harbour. The mean zinc concentrations recorded in seawater from the reference sites (BB_{MPA} and CGH_{MPA}) were unexpectedly significantly higher than at GRB1, GB1, GB2 and GB3. Likewise, the mean zinc concentrations in seawater from BB_{MPA} was significantly higher than at HB1, HB2 and HB3. These higher mean zinc concentrations could be attributed to stormwater runoff from the surrounding area that drains into the reference sites, particularly at BB_{MPA} where rainfall recorded (152mm) during the dry season sampling period was the highest (Table 4.3). Furthermore, the higher mean zinc concentrations may be attributed to the hydrological regime of the reference sites. The reference sites (BB_{MPA} and CGH_{MPA}) were in open waters with strong tidal currents and wave motion resulting to high flow rate while the sampling points except GRB1 were located inside the harbours with limited flow rate. Therefore, the high flow rate of the overlying waters at the reference sites associated with strong tidal currents and wave motion may expose anoxic surface sediment to oxic conditions thereby increasing the oxidation rate of the organic compound and sulphide fraction resulting to the release of metals. This might have accounted for the higher mean zinc concentrations recorded in seawater from the reference sites. Additionally, the high flow rate may contribute to the physical disturbance of surface sediment which could change the physiochemical properties of the environment, such as pH and dissolved oxygen (DO). It should be noted that DO measurements in seawater at sampling points were not recorded during this study. This information could also have been valuable to elucidate the variation in zinc concentrations. The process of upwelling during the dry season brings nutrient-rich deep waters to the surface which enhances phytoplankton growth followed by increasing of suspended organic matter (Bazzi, 2014). The sampling points (GRB1, HB1, HB2, HB3, GB1, GB2 and GB3) had a low exchange with the waters of the open sea and longer residence time coupled with direct anthropogenic influence providing favourable conditions for phytoplankton to bloom especially during upwelling. In contrast, the reference sites were in open waters with high tidal circulation and low residence time hence limited phytoplankton growth. It could be suggested that the variation in the mean zinc concentrations recorded in seawater between the sampling points (GRB1, HB1, HB2, HB3, GB1, GB2 and GB3) and the reference sites may be attributed to the consumption of zinc by phytoplankton. Therefore, one might assume that more zinc will be removed from the waters in these sampling points with higher phytoplankton growth than from the reference sites. Furthermore, constant vessel traffic may result in high concentrations of suspended particles, which on their surface may rapidly adsorb ionic zinc. Due to absorption onto particles, zinc sedimentation is enhanced resulting in lower concentrations in seawater (Ravera et al., 2003). The relatively higher zinc

concentrations in the reference sites demonstrate that even MPA are influenced by metal pollution due to coastal dynamics, long-distance transport and persistence of metals in the marine environment. The mean concentration of zinc recorded in seawater at the sampling points (GRB1, HB1, HB2, HB3, GB1, GB2 and GB3) and the reference sites exceeded the 0.02mg/L target value for zinc prescribed by the SAWQGs for coastal marine waters (DEA, 2018). This could have deleterious effects on the marine organism and associated non-aquatic life.

The pooled mean copper concentrations (mg/L) recorded in seawater from the four harbours in the dry season ranged from ND to 0.0480±0.0408 (Table 4.5). This was higher when compared to the mean copper concentrations in coastal waters from the northern Gulf of Suez in Egypt, with a range of 0.002mg/l to 0.003mg/l in the summer (EL-Moselhy et al., 1999). The pooled mean copper concentrations in seawater for the harbours are in the decreasing order: HB > GB > GRB > KB. The pooled mean concentrations of copper in seawater recorded in HB and GB exceeded the SAWQGs for copper in marine waters set at 0.003mg/L and could adversely affect all marine life and associated non-aquatic organisms. The pooled mean copper concentration in seawater recorded in GB (0.0054±0.0156mg/L) was also found to be higher than the copper concentration of $9.5 \times 10^{-5} \pm 4.0 \times 10^{-5}$mg/L previously reported by Mdzeke (2004) in the same harbour. Also, the mean copper concentration in seawater at GB (0.0054±0.0156mg/L) was higher when compared to the highest mean copper concentration of 3.37×10^{-3}mg/L in the Gulf of Chabahar, Iran during the summer. This higher pooled mean copper concentration in GB may be attributed to copper-based antifouling leachates from hulls of vessels residing at berths (mostly recreational boats and a few fishing boats) and the boat repair and maintenance facility in the harbour precinct. According to Bighiu et al. (2017), leisure vessels are moored 90% of the time in harbours. Therefore, more biocides (e.g. copper) are leached into the surrounding waters and contributing to increasing pollution in harbours. The pooled mean copper concentration recorded in HB was significantly higher than for GRB and GB. The significantly higher pooled mean copper concentration in HB could largely be attributed to copper-based antifouling leachates stemming from the constant vessel repair and maintenance activities taking place in the harbour (e.g. the scouring and re-application of copper-based antifouling paints on vessel hulls) as could be seen during the sampling occasion. Also, the longer mooring time of recreational and leisure vessels inside the harbour may contribute to the high mean copper concentration in seawater at HB. These findings are in agreement with those of Young et al. (1979), Jones & Bolam (2007), Schiff et al. (2007), Bazzi (2014) and Sparks et al. (2017). The pooled mean copper concentration recorded in HB was higher than the concentration of 0.0002±0.0002mg/L previously reported by Sparks et al. (2017) in the same harbour. Also, the mean copper concentration recorded in HB was

found to be higher when compared to copper concentrations in seawater reported in other studies elsewhere. For example, Mirzaei et al. (2016) reported a copper concentration of 0.0034mg/L in seawater during the summer in the North Coast of Oman Sea. Bazzi, (2014) reported a highest mean copper concentration in seawater of 3.37 x 10^{-3}mg/L in the Gulf of Chabahar, Iran during the summer. Also, Li et al. (2009) reported a mean concentration of copper in seawater of 0.0007±0.00014mg/L in the summer from Chongming Island, Yangtze Estuary in China. It is worth noting that HB which is a mixed-use harbour is the largest and the busiest of the four harbours with respect to vessel-related activities. It is a large industrial fishing harbour with processing facilities, traditional fishing vessels, a yacht basin with many berthed yachts and recreational motor fishing boats. Therefore, the higher concentration of copper recorded in seawater in the harbour may be concomitant with its shared uses. Also, the sewage outfall in HB could be a possible source of elevated copper concentration in the harbour. Furthermore, it may be suggested that inputs from the Disa River Hout Bay (known as the most polluted river in South Peninsula) that receives stormwater and effluents from faulty or poor sewage reticulation systems serving an ever-expanding informal settlement may add to the high copper burden.

The pooled mean zinc concentrations (mg/L) recorded in seawater from the four harbours during the dry season ranged from ND to 0.5170±0.4791 (Table 4.17). This was higher when compared to the range of 0.001 to 0.0044mg/L reported by El-Moselhy et al. (1999) in coastal waters from the northern Gulf of Suez in Egypt. The pooled mean zinc concentrations in seawater for the harbours are in the decreasing order: HB > GB > GRB > KB. The results revealed a similar pattern with the results recorded for copper. The pooled mean concentration in seawater from GB was significantly higher than for GRB. This could be attributed to the intense vessel-related activities (e.g., moorings; vessel launching; vessel repair and maintenance) taking place in GB. Like copper, zinc is also commonly included in AF paints as a binder and/or pigment (Yebra et al. 2004), as an anticorrosion additive (Lahbib et al., 2013), and by itself a principal biocide in AF paints (Watermann et al., 2005; Turner, 2010). Zinc is also used as sacrificial anodes (Matthiessen et al., 1999; Warnken et al., 2004; Costa et al., 2013) which are attached to vessel hulls and other submerged metal surfaces in marine waters. Bird et al. (1996), estimated zinc inputs to an enclosed recreational harbour to be 1728kg/yr. from steel superstructure and 74kg/yr. from moored vessels. Therefore, the higher mean zinc concentration in GB could be due to the release of zinc into the surrounding water from AF paints, oil waste from recreational vessels, recreational harbour superstructures and zinc-based sacrificial anodes (Bird et al., 1996; Comber et al., 2002; Singhasemanon et al., 2009; Ytreberg et al., 2010; Costa & Wallner-Kersanach, 2013; Daehne et al., 2017). The pooled mean zinc concentrations recorded in GRB, HB and GB were higher when compared

with the highest mean zinc concentrations of 0.0180mg/L and 0.0044mg/L reported by Bazzi (2014) and El-Moselhy et al. (1999), respectively. When compared with the guidelines, the pooled mean zinc concentrations recorded in GRB, HB and GB exceeded the 0.02mg/L threshold for zinc recommended by the SAWQGs for coastal marine waters.

5.1.2 Comparisons in the wet season

The mean copper concentrations (mg/L) found in seawater at sampling points within the fours harbours during the wet season ranged from 0.0009±0.0016 to 0.0520±0.0934 (Table 4.4). The results showed no significant differences in mean copper concentrations between sampling points in each harbour. Unexpectedly, the mean copper concentration at BB_{MPA} was significantly higher than at HB1, HB2 and HB3. This could be attributed to the increased surface and stormwater runoff from the surrounding area due to high rainfall in the wet season. The mean copper values recorded at the sampling points in GRB (GRB1, GRB2 and GRB3) and GB (GB1, GB2 and GB3) as well as at KB1 and KB3 exceeded the target value for copper (0.003mg/L) in marine waters recommended by the SAWQGs. These higher mean copper concentrations may reflect different input scenarios under factors such as proximity to point sources, urban stormwater and riverine influxes, water movement, temperature and pH as well as vessel traffic. GRB is situated on the Table Bay coastline approximately 4km from Cape Town CBD and adjacent to Green Point. These areas are highly urbanized with residential and commercial development which may contribute to the elevated copper load in the harbour. The shoreline to the south of GRB from Green Point is used intensively for stormwater discharge and has two offshore deep ocean wastewater outfalls (Quick & Roberts, 1993). Therefore, urban stormwater runoff which may be contaminated with copper from electrical wiring, pesticides, plumbing and air conditioning tubing and roofing as well as from vehicle brake pads (Brinkmann, 1985; Prestes et al., 2006; McCarthy et al., 2008; Mills & Williamson, 2008; McKenzie et al., 2009; Tiefenthaler et al., 2008; Pennington & Webster-Brown, 2008; Larsen & Rob, 2016) may add to the existing copper burden in the harbour. The results indicated that GRB1 recorded the highest mean copper concentration in seawater for the wet season. This higher mean copper concentration may be attributed to the increased surface and urban stormwater runoff that drains into the harbour because of high precipitation during the wet season (Table 4.3). It should be noted that GRB1 was in open waters and adjacent to the Metropolitan Golf course and the main road (Beach Road) as well as in proximity to the Granger Bay Marina. Therefore, it could be suggested that the predominant sources of copper at GRB1 may come from stormwater and surface run-off contaminated with copper that might have come from copper-based pesticides used for the prevention of algae growth in the Golf Club ponds as well as from automobile brake-pad wear from the Beach Road (Seabrook, 2012). GRB2 was located close to a public slipway at the Oceana Power Boat Club (OPBC)

and adjacent to the Granger Bay Marina. The slipway is one of the few launching sites for leisure and recreational fishing boats, commercial line fishing fleets as well as other users (e.g. dive charter operators, research groups, the Two Oceans Aquarium, the police and emergency services). The high vessel traffic associated with the frequent use of the slipway may account for the high mean concentration of copper in seawater at GRB2. GRB3 was located on the left side of the East Pier of the Victoria Basin which caters for a wide range of commercial vessels such as fishing and recreational vessels as well as cruise ships. Therefore, the higher mean copper concentration at GRB3 could be associated with copper-based antifouling leachates from the high vessel traffic at the Victoria Basin which may be transported by tidal currents and wave action. GRB is approximately 500m away from the Port of Cape Town which is a busy container port, second only to Durban in South Africa and positioned on one of the world's busiest route. The port has two dry docks, a ship repair facility and the Victoria and Alfred Basins used by smaller commercial vessels including fishing and pleasure boats. Therefore, the intense vessel activities (e.g. repair and maintenance) within the port precinct and the high vessel traffic in the harbour may be a potential source of copper in seawater. The dissolved copper in seawater may be dispersed to surrounding waters by tidal currents and wave action. This may, therefore, account for the higher mean copper values recorded at the sampling points in GRB. The mean copper concentrations in the sampling points in HB were below the SAWQGs threshold value of 0.003mg/L for copper. In GB, the high mean copper concentrations recorded in seawater at the sampling points could be attributed to copper-based antifouling leachates from vessel-related activities as well as riverine inputs. GB3 was located within the Old Harbour and recorded the highest mean copper concentration for this harbour. The Old Harbour which still functions as a landing facility for West Coast Rock Lobster (WCRL) is predominantly used by the yacht club for recreational vessel moorings. Other users include the South African Navy, a few fishing vessels, the ski boat launch and the National Sea Rescue Institute. The harbour is also used as a launching site for deep-sea fishing charters, scenic cruises and shark-viewing trips to Seal Island and has an active vessel repair and maintenance facility located within the precinct. The vessel activities (e.g. lengthy berthing time of leisure vessels, high vessel traffic as well as vessel repair and maintenance activities) within the harbour may have accounted for the higher seawater mean copper concentration at GB3. GB1 and GB2 were located at the Harbour Island which has a recreational vessel harbour that berths leisure vessels and motor launches and has a slipway used for vessel launching. In recreational vessel harbours, vessels are likely to reside in their berths for longer periods and are regularly cleaned (Jones & Bolam, 2007). Copper leaches slowly from copper-based AF paints into the immediate water surroundings of a vessel's hull as intended and also is released into the water when vessel hulls are cleaned (Carson et al., 2009). Therefore, longer berthing periods and regular cleaning of vessel hulls may result to increase copper

concentrations in the surrounding waters which are further compounded by restricted water circulation. This may provide an explanation for the higher mean copper values recorded at GB1 and GB2. The mean copper concentrations at GB1, GB2 and GB3 exceeded the SAWQGs target value of 0.003mg/L for copper in coastal marine waters. GB2 which was located a distance away from a potential copper source (at the mouth of the harbour) and closer to open waters had the lowest mean copper concentration of the three sampling points. This decrease in mean copper concentration with increasing distance from a potential source (i.e. berthed vessels) is in agreement with Hall et al. (1992). According to Pineda et al. (2012), the level of copper is also dependent on the rate of water exchange, making copper concentrations higher in areas with restricted water movement and tidal flushing. This is true for the sampling points in GB, as GB1 and GB3 were in the inner harbour with minimal water circulation while GB2 was closer to the harbour outlet (close to open waters) with increase water movement. In KB, KB1 and KB3 were also located inside the harbour area which is sheltered from strong water movements. KB1 which was in proximity to a slipway and a vessel repair facility recorded the highest mean copper concentration in the harbour. It was observed during sampling occasions that intensive vessel repair and maintenance activities were taking place in the harbour. At the repair facility, vessels were being sanded or power washed to remove old paint before a new copper-based antifouling coating was applied. During this process, antifouling paint flakes and dust are generated and could be seen on hard standings and the slipway of the repair facility. This observation has also been reported by other studies elsewhere such as Weinstein (1996), Axiak et al. (2000), Prasad & Schafran (2006), Links et al. (2007), Kotrikla (2009) and Turner (2010). Through runoff and wash-down or as windblown dust, the copper enriched paint flakes and dust enter the surrounding waters (Jones & Turner, 2010) which may account for the higher mean copper concentration at KB1. This explanation may also have accounted for the high mean copper concentration at KB3. Again, as previously mentioned, there is a decrease in copper levels with increasing distance from a potential source and this is evident with the variation in mean copper concentrations at KB1 and KB3. It should be noted that KB is predominantly used by fishing vessels all year round. These fishing vessels have limited residing periods at berths (Jones & Bolam, 2007) in contrast to recreational vessels. This may suggest that the higher mean copper concentrations at KB1 and KB3 may have resulted predominantly from vessel repair and maintenance activities in the harbour. This explanation has also been suggested in other studies worldwide such as Turner (2013) in his study to investigate metals in two UK leisure boatyards and Eklund et al. (2014) in a study to determine the degree of contamination in a vessel maintenance facility in Sweden.

The mean zinc concentrations (mg/L) recorded in seawater at sampling points within the four harbours during the wet season ranged from 0.0033±0.0073 to 0.1674±0.0791 (Table 4.16). The results showed no significant differences in the mean zinc concentrations in seawater between sampling points in the harbours except in GRB. The mean zinc concentration recorded in GRB2 was significantly higher than at GRB1 and GRB3. This could be attributed to the leaching of zinc from AF paints applied to the hulls of leisure crafts that are frequently launched to the sea at the slipway close to GRB2. Also, surface and stormwater runoff from the surrounding area (residential areas, parking lot, road surfaces) may have contributed to the higher mean zinc concentration at GRB2. As expected, the mean concentrations of zinc recorded in seawater at all three sampling points in GRB and HB were significantly higher than at the reference sites (BB_{MPA} and CGH_{MPA}). This could be ascribed to surface and stormwater runoff from the surrounding areas (with extensive residential and commercial development) as well as from the immediate vicinity of the harbours into the harbours (Greenfield et al., 2011). Also, the leaching of zinc from vessel-related activities (particularly vessel moorings, repair and maintenance of vessels, and vessel traffic) may have contributed significantly to the higher mean zinc concentrations at the sampling points in GRB and HB. Additionally, riverine inflow, particularly in HB, may have also accounted for the higher mean zinc concentrations. When compared with the SAWQGs for coastal marine waters, the mean zinc concentrations in seawater at all three sampling points in GRB and HB, were above the recommended threshold of 0.02mg/L for zinc. Also, the mean zinc concentrations in KB1 and KB2 exceeded the SAWQGs. Remarkably, the mean zinc concentration at the reference site BB_{MPA} exceeded the recommended limit for zinc. This could be attributed to increased stormwater runoff from the surrounding area that drains into BB_{MPA} since rainfall recorded during the sampling period was high (98.0mm). It is worth noting that rainfall recorded in BB_{MPA} in the three months prior to the sampling period (i.e., June: 204.7mm; July: 157.0mm and August: 129.0mm) were the highest when compared with that of the harbours and the other reference site (Table 4.3).

When comparing between harbours during the wet season, the pooled mean copper concentrations (mg/L) in seawater from the four harbours ranged from 0.0013±0.0019 to 0.0261±0.0555. This was higher when compared to mean copper concentrations in coastal waters from the northern Gulf of Suez in Egypt, with a range of 0.0005mg/l to 0.0089mg/l in the winter (EL-Moselhy et al., 1999). The pooled mean copper concentrations for the four harbours are in the decreasing order: GRB > GB > KB > HB. The results showed that the pooled mean copper concentrations in seawater from GRB, GB and KB were well above the SAWQGs recommended target value of 0.003mg/L for copper in seawater which could result to serious environmental problems. This higher mean copper values recorded in GRB, GB and KB may be associated with a number of anthropogenic sources such as vessel-related

activities (Young et al., 1979; US EPA, 1985), surface and urban stormwater runoff (Dickson & Hunter, 1981; Bartlett, 1986; Valkirs et al., 1994; Fatoki & Mathabatha, 2001; Lee & Jones-lee, 2003; Pennington & Webster-Brown, 2008) as well as riverine inputs (Weideborg et al., 2003; Khan et al., 2014). GRB is located within a highly developed urban environment and supports a range of water-based recreational activities. It is adjacent to the Port of Cape Town and is used by a variety of small leisure vessels, which constantly move in and out of the launching and mooring facilities at the OPBC and Granger Bay Marina. Vessel traffic within GRB is high as there are few alternative launching sites in the area. Therefore, it can be suggested that the higher pooled mean copper concentration recorded in GRB might be due to the leaching of copper from AF paints. Also, the increase in surface and urban stormwater runoff that drains into the harbour in the wet season may add to the existing copper load from vessel-related activities. Another alternative explanation for the higher mean copper concentration in GRB might be attributed to the sewage outfalls in Green Point which are close to GRB. According to Warnken et al. (2004), difficulties often arise when trying to separate copper inputs from vessel related sources (predominantly antifouling paint hull coating) and inputs from other sources such as urban stormwater runoff. For example, studies by Young et al. (1979), Lee & Jones-Lee (2003) and Pennington & Webster-Brown (2008) have actually suggested that urban runoff, rather than recreational vessels, are the most predominant sources of metals such as copper in harbours and berthing areas. However, suggestions that vessel-related activities constitute an important source of copper in harbours (Young et al., 1979; Schiff et al., 2004) cannot be discounted. It is worth noting that while inputs from urban stormwater runoff are intermittent (mostly in the wet season); the inputs from vessel-related activities (e.g. leaching of copper-based antifouling paints from vessel hulls and vessel repair and maintenance) are frequent. The local oceanographic regime of GRB may also have contributed to the elevated copper level in the harbour. The harbour is situated at the most south-westerly edge of Table Bay and currents within the Bay are wind-driven and generally weak (average of 0.2m/s) with limited influence. The outside shelf currents (such as the weak northward-flowing Benguela Current and weak inner-shelf currents) also have minimal influence in the Bay. Depending on the wind direction in the Bay, the water circulation pattern is either in a clockwise (southerly) or anti-clockwise (northerly) direction. The northerly current which is driven by the south-easterly wind is predominant (69-80% of the time) with a surface flow of between 0.2 and 0.3 m/s. The speed of wind-induced currents decreases rapidly with depth, so much so that in the bottom currents flow faster than 0.05m/s for only 5% of the time in the Bay. According to Van Ieperen (1971) approximately 80% of the time there is no visible bottom currents and the residence time of water in the Bay varies from 15 to more than 190 hours with an average of four days. Consequently, the bottom waters are poorly flushed and thus favouring the trapping of contaminants such as copper within the harbour. Additionally,

the two large rivers that open into Table Bay (the Diep River and the Salt River) may potentially expose the Bay to many pollutants such as metals. These pollutants may be dispersed by currents into GRB, thereby increasing the metal load. In GB, the higher mean copper concentration recorded in seawater may be attributed to the leaching of copper from moored leisure crafts in the harbour. Also, inputs from adjacent catchments (riverine) and stormwater runoff may have added to the existing copper load. For example, the Sir Lowry's Pass River which receives surface and stormwater runoff from agricultural land uses (e.g. vineyards), hardened roads and residential areas as well as treated effluent from the Gordon's Bay Wastewater Treatment Works (Hutchings et al., 2016) eventually enters the ocean at GB approximately 1km to the north of the harbour. In KB, the higher mean copper concentration could be attributed to the frequent repair and maintenance of vessels which releases copper-based AF paint residues that are discharged into the surrounding water in the harbour. Grounded and abandoned vessels in the harbour vicinity (Tolhurst et al., 2007; Turner et al., 2008) may have also contributed to the elevated copper levels. Also, surface runoff which may be contaminated with copper from the adjacent railway line (cables) and the undeveloped land used as a parking lot for visitors may partly contribute to the high copper concentration in KB. Copper-based AF paints are often used in preserving railway trestles (Mdzeke, 2004) and may be the source of copper in surface runoff. Also, the railway cables, as well as car tyres (brake linings), may be sources of copper in surface runoff (Boller & Steiner, 2002), that eventually enter the harbour. When compared with other studies, the pooled mean copper concentrations in seawater from GRB, GB and KB were higher than the highest mean copper concentration of 0.0069±0.00009mg/L and 0.00574mg/L reported in the winter by Li et al. (2009) and Bazzi (2014), respectively. Also, this values (GRB, GB and KB) were higher when compared with the highest mean copper concentration in seawater of 0.00002±0.00003mg/L reported by Sparks et al. (2017) in winter from the west coast of the Cape Peninsula. The pooled mean copper concentration in GB was higher when compared with the copper concentration reported by Mdzeke (2004) from the same the harbour which was below the detection limit. The pooled mean copper concentration in HB was significantly lower when compared to GRB and GB. This may be associated with the hydrodynamics of HB. The enclosed nature of the harbour makes it an area of poor tidal flushing and water circulation with long residence time. This may favour the removal of copper from water by particulate matter and subsequent deposition to bed sediment, thus lowering its concentration in the water column (Yi et al., 2011). Furthermore, it may also be suggested that freshwater inflow from the Disa River during the wet season when precipitation was high (72.6mm) could have a diluting effect in the harbour waters, thus lowering the concentration of copper in the harbour. Another possible explanation for the lower seawater mean copper concentration in HB may have been due to the biological uptake of metals from surface waters by phytoplankton and microplankton which may lower

the metal concentrations in the surface waters (Orr et al., 2008). The pooled mean copper concentration in HB was found to be lower when compared to the SAWQGs target value for copper in marine waters. On the contrary, this value was higher when compared to the mean copper concentration of 0.00002±0.00003mg/L reported by Sparks et al. (2017) from the same harbour in the winter.

The pooled mean zinc concentrations (mg/L) in seawater from the four harbours ranged from 0.0055±0.0126 to 0.0639±0.0890 (Figure 4.17). This was higher when compared with the range of 0.0054 to 0.0255mg/L and 0.0067 to 0.0226mg/L reported elsewhere by El-Moselhy et al. (1999) and Bazzi (2014), respectively. The pooled mean zinc concentrations for the four harbours are in the decreasing order: GRB > HB > KB > GB. The results show that there were significant differences between the harbours. The pooled mean zinc concentration in GRB was significantly higher than that in HB, KB and GB. This could be attributed to urban surface and stormwater runoff that drains into the harbour in the wet season. Also, the presence of a recreational vessel harbour (Granger Bay Marina) and two vessels launch sites within GRB as well as a nearby shipping port (Port of Cape Town) may have contributed to the higher mean zinc concentration (leaching of zinc from AF paint on vessel hulls and zinc-based sacrificial anodes). Furthermore, the sewer outfall in Green Point which is located near GRB could have been a possible source of elevated zinc concentration in GRB. When compared with other studies done elsewhere, the mean zinc concentration in GRB was higher than the highest mean zinc concentrations of 0.0204mg/L and 0.0255mg/L reported by Mirzaei et al. (2016) and El-Moselhy et al. (1999), respectively, in the winter. The pooled mean zinc concentration in seawater from HB was significantly higher than that in KB and GB. This concentration was also higher when compared to the mean zinc concentration reported by Sparks et al. (2017) in the same harbour in the winter which was below the detection limit. As previously mentioned, HB was the busiest harbour with respect to vessel traffic (mostly commercial fishing vessels and recreational crafts) and vessel activities when compared with the other harbours. The harbour also supports several industrial activities such as the fish processing and fishmeal factories as well as a marine outfall. Therefore, it could be suggested that the intense vessel traffic and high vessel density (increased moorings) in the harbour which in turn may result to an increase in the release of zinc from AF paints applied on vessel hulls as well as from zinc-based sacrificial anodes attached on vessel bottoms may have significantly contributed to the higher mean zinc concentration in HB. Also, the waste discharged from industrial activities into the harbour and from the sewage outfall could provide a possible source of the higher zinc concentration in HB. Another possible explanation for the higher pooled mean zinc concentration in HB could be attributed to surface and stormwater runoff in the wet season from the adjacent suburb and the immediate vicinity of the harbour that drains

into the harbour. Furthermore, the increase in freshwater inflow into the harbour from the Disa River during the wet season (which may carry along contaminants) may also provide a possible source of the higher zinc concentration. The pooled mean zinc concentrations in seawater at GRB, HB and KB were higher than the maximum permissible level of 0.02mg/L specified by SAWQGs.

5.1.3 Comparisons between the dry and wet season

When comparing the mean copper concentrations in seawater from sampling points within harbours between the two seasons, the results showed that there were insignificant seasonal differences between sampling points in the harbours except at HB2 and GB2 (Figure 4.1). Generally, a similar seasonal pattern was observed whereby the mean copper concentrations recorded in seawater at sampling points in the harbours were higher in the wet season than the dry season, except in HB. The mean concentration of copper in seawater at HB2 was significantly higher in the dry season than in the wet season (Figure 4.1b). This could be attributed to intense vessel traffic and high vessel density (predominantly leisure crafts) at HB2 during the dry season resulting in an increase in copper leachates from AF paints used on vessels hulls. Another possible explanation could be due to upwelling in the summer. Alternatively, dilution (mixing and dispersion) of seawater by freshwater inflow from adjacent catchment (Disa River) during the wet season when rainfall was higher (72.6mm) may have resulted to lower mean copper concentration in the wet season (Hall et al., 1992; Adamu et al., 2015). It is worth noting that the mean concentrations of copper were also higher in the dry season than the wet season at HB1 and HB3 although the seasonal differences were not significant. The mean concentration of copper recorded in seawater at GB2 was significantly higher in the wet season than in the dry season (Figure 4.1d). This could be ascribed to increase surface and stormwater runoff in the wet season. Also, GB2 was located inside a recreational vessel harbour (Harbour Island) close to a mooring area with the frequent mooring of leisure crafts especially during the wet season when vessel activities were least. This may result in an increase in copper leachates from moored leisure crafts (with longer residing time) thus contributing to the elevated copper concentration in the wet season. The mean concentration of copper at GB1 and GB3, were higher in the wet season than in the dry season, although the differences were not significant.

When comparing the mean zinc concentrations in seawater from sampling points within harbours between the two seasons, the results showed that there were no significant seasonal differences between sampling points in the harbours, except at GB3 (Figure 4.9). The results revealed a similar seasonal pattern with the mean zinc concentrations in seawater at sampling points in the harbours being higher in the dry season than the wet season, except in GRB and

KB. The mean zinc concentration at GB3 was significantly higher in the dry season than the wet season (Figure 4.9d). This could be attributed to an increase in the release of zinc from AF paints into the surrounding waters associated with the increase in vessel traffic/ density as well as vessel-related activities (e.g., repair and maintenance) in the dry season. Also, the increase in vessel traffic/density in the dry season may in turn result to an increase in the release of zinc from zinc-based sacrificial anodes attached to vessel bottoms, thus contributing to the elevated zinc concentration at GB3 (Byers, 1993; Bird et al., 1996).

When comparing the pooled mean copper concentrations in the two seasons between the harbours, the pooled mean copper concentrations recorded in the wet season were higher than that in the dry season in the harbours, except in HB (Figure 4.2). The pooled mean copper concentrations in GRB and GB were significantly higher in the wet season than in the dry season (Figure 4.2). This increase could be attributed to the leaching of copper from copper-based antifouling coatings on moored vessel hulls (particularly in GB with high leisure craft density); stormwater runoff that drain into the harbours as well as riverine inputs from adjacent catchments (e.g. Sir Lowry's Pass River that opens into GB). Although the pooled mean copper concentration in KB was higher in the wet season than in the dry season, the difference was not significant. Unlike the other harbours, the pooled mean concentration of copper in HB was significantly higher in the dry season than in the wet season (Figure 4.2). This could be attributed to the high vessel density (mostly leisure crafts) and vessel traffic (mostly commercial fishing vessels) in the harbour during the dry season resulting to more copper from AF paints used on vessel hulls being leached into the surrounding waters. Also, copper-based AF paint residues from frequent vessel repair and maintenance during the dry season may have accounted for the higher mean copper concentration. Another possible explanation for the higher mean copper concentration in the dry season could be credited to the upwelling process that sweeps through the south-west coastline in the summer. This process may remobilise metals such as copper from bed sediment to surface water. It is worth noting that the high variability in copper concentrations in seawater samples with respect to seasonal differences, time of day, the extent of freshwater inputs, hydrological factors such as tides and currents and physicochemical parameters such as pH and salinity makes it difficult to compare different locations for their magnitude of copper contamination.

When comparing the pooled mean zinc concentrations in the two seasons between the harbours, the results showed that there were significant seasonal differences in HB, GB and GRB (Figure 4.10). The pooled mean zinc concentrations in HB and GB were significantly higher in the dry season than the wet season. This could be attributed to an increase in the leaching of zinc from AF paints and zinc-based sacrificial anodes due to the increase in vessel traffic as well as vessel-related activities (such vessel repair and maintenance, effluent

discharge from fishing activities, bilge waters and oil waste from recreation vessels) in the dry season. Another possible explanation for the higher mean zinc concentration in the dry season could be attributed to localized upwelling at HB and GB. Also, the circulation pattern of the surface currents in GB may have contributed to the elevated zinc concentration. In the dry season, the predominant surface currents in False Bay follow a clockwise circulation pattern under the prevailing south-easterly winds (Atkins, 1970). During this current regime, anti-clockwise retentive gyres tend to develop in Gordon's Bay, thus resulting in the entrapment of water. Because of the sheltered nature of Gordon's Bay, there is evidence of accumulation of contaminants in this area (Atkins, 1970; Taljaard et al., 2000), which may have accounted for the higher pooled mean concentration in GB during the dry season. Conversely, the pooled mean zinc concentration in GRB was significantly higher in the wet season than the dry season. This might be attributed to the increase in urban surface and stormwater runoff (from residential areas, golf course, road surfaces) in the wet season when rainfall was higher in the months leading to the sampling period (i.e., June: 78.4mm; July: 136.6mm; August: 53.2mm) (Table 4.3).

5.2 Metal concentrations in sediment within and between harbours

Coastal marine sediment are a major repository of metals as a result of adsorption, precipitation, diffusion processes, chemical reactions, biological activity as wells as the combined effect of these processes (Ramirez et al., 2005). The role of sediment as both a source and sink of dissolved contaminants has been recognized for some time (Fowler, 1982). Sediment represent a long-term source of contamination to the food web (Burton, 2002), and can become a potential source of metals such as copper and zinc, releasing them into the overlying water column (Jones & Turki, 1997; Wauhob et al., 2007; Soliman et al., 2015). Therefore, sediment can pose a major risk as a source of metal pollution in the aquatic environment (Burton, 2002; Wepener & Vermeulen, 2006). High concentrations of copper and zinc in sediment from harbours in the west coast of Sweden, including natural harbours in pristine areas have been linked to the use of AF paints (Eklund et al., 2016).

Natural background copper concentrations in marine sediment are in the range of 10 to 50mg/kg DW (Salomons & Förstner, 1984; Ridgway & Price, 1987). Boyden (1975) reported a range of 1 to 60mg/kg DW in Poole Harbour, UK. Copper concentrations in unpolluted sediment have been reported to be in the order of 10mg/kg DW or less and in excess of 2000mg/kg DW in polluted sediment (Legórburu & Cantón, 1992; Bryan & Langston, 1992). According to Hennig (1985), copper levels in sediment around South Africa ranged from 0.5 to 74mg/kg DW.

Natural background total zinc concentrations are usually up to 100mgk/kg DW in sediment (WHO, 2001) and in marine and estuarine sediment, zinc concentrations vary widely (Neff, 2002). For instance, in the UK estuaries, zinc concentrations in sediment range from baseline levels of less 100mg/kg to 3000mg/kg (Bryan & Langston, 1992). Luoma & Phillips (1988) reported sediment zinc concentrations from San Francisco Bay in the range of 140 to 1890mg/kg. The seasonal mean concentrations of zinc found in sediment from Bilbao Estuary (Spain), ranged from 536 to 5261mg/kg DW (Ruiz & Saiz-Salinas, 2000). Zinc levels found in sediment around South Africa were reported to range between 0.41 to 287mg/kg DW (Hening, 1985). In a recent study along the west coast of the Cape Peninsula, Sparks et al. (2017) reported seasonal mean zinc concentrations of 14.30 ± 50.55mg/kg DW in sediment. According to Campbell & Tessier (1996) the strong affinity of zinc for aquatic particles, mainly, iron and manganese oxides, and organic matter result in its deposition in bottom sediments in association with these materials.

In this study, the metal concentrations found in sediment were compared to the TEL and PEL of the BCLME-SQGs (Table 3.2) as no SQGs exist for South Africa. The TEL is the concentration below which adverse effects are not expected on sediment-dwelling organisms, while PEL is concentration above which adverse effects are expected to frequently occur (Macdonald et al., 1996). Metal concentrations lower than TEL values are indicative of rare pollution with little to no biotoxicity. Therefore, such pollution rarely induces negative ecological effects. In contrast, metal concentrations that are between TEL and PEL are indicative of moderate pollution that may pose negative ecological risks. When metal concentrations exceed PEL, serious pollution may occur and may be associated with considerable ecotoxicity (Wang et al., 2014).

5.2.1 Comparisons in the dry season

The mean copper concentrations (mg/kg DW) in sediment collected at sampling points within the four harbours ranged from not detected (ND) to 2145.39 ± 843.60 (Table 4.6). The results showed that KB1 recorded the highest mean copper concentration for the dry season, while the lowest concentrations (not detected) were recorded at GRB1 and HB3. The mean copper concentrations recorded at GRB2 and GRB3 were significantly higher than at CGH_{MPA} (reference site) (Table 4.6). These higher mean copper concentrations could be attributed to different vessel-related activities taking place at GRB2 and GRB3. CGH_{MPA} was in a restricted zone MPA with no vessel-related activities permitted which may explain the lower mean copper concentration. Copper was not detected in sediment collected at BB_{MPA} (reference site) during the dry season. When compared to the BCLME-SQGs, the mean concentration of copper recorded at GRB2 and GRB3, exceeded the recommended TEL value for copper (BCLME,

2006), indicating that the copper toxicity could cause infrequent adverse effects to marine organisms. Also, the mean copper concentration in sediment at GRB2 was higher than that at GRB3, although it was not statistically significant. Interestingly, there is a similar trend with respect to the variation of copper concentrations with distance from contamination sources. The mean copper concentrations tend to decrease with increasing distance from potential sources of contamination. This pattern was observed in the results recorded at GRB2 and GRB3. The mean concentration of copper in sediment at HB1 and HB2, were lower when compared to the mean sediment copper concentration at CGH_{MPA}. Also, the result indicated that the mean copper concentrations in sediment at HB1 and HB2 were below the BCLME-SQGs TEL value for copper. The mean copper concentration in sediment at HB2 was higher than at HB1; however, this difference was not statistically significant. HB2 was located inside the harbour with less mixing of the waters and close to an area of high vessel density (leisure vessels mooring area) while HB1 was located outside the secondary breakwater (northern section of the harbour) in open waters (frequent mixing of water). This might have contributed to the higher mean copper concentration at HB2 than at HB1. The results show that the mean concentration of copper recorded in sediment at KB1 (highest copper concentration for the dry season) was significantly higher than at KB2, KB3 and CGH_{MPA} (Table 4.6). KB1 was near the vessel repair and maintenance facility in the harbour and this could explain for the significantly higher mean copper concentration. It should be noted that during the dry season sampling occasion, it was observed that the cleaning of vessel hulls (by scraping and sanding) for refurbishment at the vessel maintenance facility, resulted in the shedding of paint fragments. These fragments/particles which could be seen on the hard-standings and the slipway of the vessel repair facility may find their way into the surrounding waters either by wind-blown or wash down and could eventually be deposited in the sediment (Lagerström et al., 2016). Metals in sediment are derived predominantly from deposition of suspended particles that have received their metal content from both the source particles and from adsorption of dissolved metals (Huanxin et al., 2000). Several studies have reported that spent antifouling paint fragments typically contain high concentrations of copper (Singh & Turner, 2009; Turner, 2010; Parks et al., 2010; Rees et al., 2014; Lagerström et al., 2016). For instance, Turner (2010) analysed paint fragments and found concentrations of copper above 35% equivalent to cuprous oxide (Cu_2O) of about 40%. Studies (Turner et al., 2008; Jones & Turner, 2010) have shown that metals such as copper contained in paint residues that end up in the sediment become bioavailable upon ingestion by organisms that derive nutrition from organic matter in sedimentary deposits. The fact that copper was not detected in seawater at the sampling points in KB, particularly at KB1 which recorded the highest mean copper concentration in the sediment for the dry season, could support the fact that poor tidal flushing and water circulation of semi enclosed harbours may favour the removal of copper from water by particulate matter

and subsequent deposition to bed sediment, thus lowering its concentration in the water column. When compared with the BCLME-SQGs, the mean concentration of copper at KB1 exceeded the PEL value for copper (Table 3.2). As already mentioned, the metal concentrations above PEL are indicative of serious pollution which may be deleterious to the sediment-dwelling organism. At KB2, the level of copper was significantly higher than at KB3 and CGH_{MPA}. Although KB2 was located outside the harbour area away from vessel-related activities, the higher copper content in the sediment could be ascribed to stormwater runoff and surface runoff from the vehicle main roadway (Drapper et al., 2000), the railway line (Mdzeke, 2004), the parking lot, as well as the residential area located approximately 40m from the coastline. The concentration of copper recorded at KB2 was between the TEL and PEL value for copper which is indicative of moderate pollution and may pose a threat to aquatic organisms. KB3 which was located inside the harbour further away from vessel-related activities (e.g. vessel launching, mooring and repairs) in an area of a relatively high rate of water exchange recorded the lowest copper concentration in KB. The fact that KB3 was located a distance away from the presumed source of contamination (vessel-related activities) and the relatively high rate of water exchange rate associated with a lower retention time of contaminants such as copper may account for the lower copper concentration. In GB, the highest mean concentration of copper in sediment was recorded at GB3 while the lowest was at GB2. The mean concentrations of copper recorded in sediment at GB1 and GB3 were significantly higher when compared with CGH_{MPA}. This could be attributed largely to the leaching of copper from copper-based AF paints used on vessel hulls into the surrounding waters which may then be adsorbed to suspended particulates (after oxidation) and subsequently deposited on the harbour bed (Young et al., 1979; Schiff et al., 2004). It should be noted that GB1 and GB3 were located close to areas of shared uses in the harbour with high recreational vessel density and longer berthing time (recreational vessel mooring areas). Lengthier residing times implies more leaching of copper biocide into surrounding waters (Jones & Bolam, 2007), and consequently increase copper in sediment. Additional significant copper inputs may occur from vessel maintenance and repair activities within the harbour precinct (Young & Alexander, 1974; Strong, 2005; Huntingford & Turner, 2011). A similar explanation may apply to GB3 which recorded the highest mean copper concentration in the harbour. GB2 was located at the mouth of the harbour a distance away from potential copper sources (i.e. areas of concentrated vessel-related activities) and close to open waters and this may explain for the low copper concentration at this point. Several studies have reported low metal concentrations (e.g. copper) in sediment with increasing distance from the source of contamination (Eklund et al., 2008; Waltham et al., 2011), as well as in open water areas (Jones-lee et al., 1994; USEPA, 2002). The mean copper concentration recorded in sediment

from GB1 was between TEL and PEL. GB3 exceeded the PEL value which is indicative of serious pollution and may likely cause harm or adverse biological effects to marine organisms.

The mean zinc concentrations (mg/kg DW) in sediment from sampling points within the four harbours ranged from 2.35± 3.38 to 1807.13±608.55 (Table 4.18). The highest mean zinc concentration in sediment was recorded in KB1 while the lowest was recorded at GRB1. The results showed that, like for seawater, the mean zinc concentrations recorded in sediment were also significantly higher at the reference sites (BB_{MPA} and GCH_{MPA}) compared to GRB1. This higher mean zinc concentration could be attributed to the difference in sediment characteristics such as grain size. The percentage of clay in the sediment from the reference sites (7%) was more than that from GRB (5%) (Table 4.2). The suggestion that sediment grain size may have an effect on sediment metal concentrations is in agreement with the finding of other studies that found that small particle-size accumulate the highest concentrations of metals such as zinc (Thomson et al., 1984; Horowitz, 1991; Chakrapani & Subramanian, 1993; Lakhan et al., 2003). The general consensus is that metals are predominantly concentrated in the clay and silt sediment fraction with a particle size of less than 0.063mm (Abdolhossein, 2008). The enrichment of the clay and silt fraction by anthropogenic metals such zinc is due to increase surface area, higher clay/silt mineral and organic content, and presence of Iron-Manganese phases (Förstner et al., 1982). It should be noted that physiochemical characteristics such as the concentration of organic carbon as well as iron/manganese concentration which may control metal concentrations were not measured. This information could be useful in providing further explanation for the significantly higher mean zinc concentrations in sediment at the reference sites. The mean zinc concentrations in sediment at GRB2 and GRB3 were significantly higher than that at GRB1 (Table 4.18). This could be attributed to the vessel-related activities taking place near these sampling points. The mean zinc concentration in sediment at GRB2 was between the recommended TEL and PEL for zinc. This might be indicative of moderate pollution that may pose negative ecological risks. The mean zinc concentration in sediment at KB1 was significantly higher than at the reference sites (BB_{MPA}, GCH_{MPA}). Also, KB1 was significantly higher than KB2 and KB3. These results showed a similar trend to that of copper at the sampling points in KB. The significantly higher mean zinc concentration in sediment at KB1 could be ascribed to the intense vessel repair and maintenance activity taking place close KB1 which is associated with the leaching of zinc from AF paint residues from hull cleaning. An analysis of paint fragments by Turner (2010) in recreational UK boatyard revealed zinc concentrations of above 15%, corresponding to approximately 20% of zinc oxide (ZnO). The mean zinc concentration in sediment at KB1 was 6 times above the recommended PEL value for zinc. This might be suggestive of serious pollution and may likely cause harm or adverse effects to marine organisms. The mean zinc

concentrations in sediment at GB1, GB2 and GB3 were significantly higher than the concentrations at the reference sites. This could be attributed to the leaching of zinc from vessel-related activities (e.g., vessel launching, mooring of leisure crafts as well as vessel repair and maintenance) taking place in proximity to these sampling points. Also, the leaching of zinc from sacrificial zinc anodes attached to vessel bottoms may contribute to the higher mean zinc concentration (Rousseau et al., 2009; Rees et al., 2017). There were no significant differences in the mean zinc concentrations recorded in sediment between the sampling points in GB. However, it is worth noting that GB1 recorded the highest sediment mean zinc concentration in the harbour. This could be attributed to the release of zinc from AF paint flakes from vessel hulls resulting from the frequent launching of vessels at the slipway close to GB1. Also, the constant leaching of AF paints predominantly from the hulls of leisure vessels that have relatively longer mooring time as well as from visiting vessels may contribute to the elevated mean zinc concentration at GB1. The mean zinc concentrations in sediment at GB1 and GB3 exceeded the PEL value for zinc, while GB2 was between TEL and PEL.

For comparisons in the dry season between harbours, the pooled mean copper concentrations (mg/kg DW) in sediment from the four harbours ranged between 1.07±1.93 to 724.45±1133.59 (Table 4.7). This was higher when compared to the mean copper concentrations in a range of 5.810 to 28.05mg/kg recorded by El-Moselhy et al. (1999) in coastal sediment from the northern Gulf of Suez in Egypt, during the summer. The pooled mean sediment copper concentrations recorded in the harbours are in the decreasing order: KB > GB > GRB > HB. The results showed that KB recorded the highest sediment pooled mean copper concentration. Interestingly, the lowest pooled mean copper concentration in sediment was recorded in HB, which from observation during the sampling occasions was the busiest (with respect to vessel traffic and density) of the four harbours. The pooled mean copper concentration in sediment at HB was significantly lower than in GRB, KB and GB. This lower pooled mean copper concentration could be attributed to natural and anthropogenic disturbances in the harbour which may result in the remobilization of metals as a result of sediment resuspension (Eggleton & Thomas, 2004). For example, a natural disturbance such as the coastal upwelling process that is predominant in south-west coastline during the dry season may result to the release of metals (e.g. copper) back to the overlying water column, most likely due to the bed sediment resuspension. Also, when physical and biological processes such as bioturbation, storms, remedial dredging and unremitting vessel traffic (vessel propellers generating waves) occur, resuspension of sediment may potentially lead to the release of metals from sediment into the water column (Eggleton & Thomas, 2004; Newman & Watling, 2007; Huang et al., 2012). These suggestions are supported by the copper concentration pattern in seawater compared to sediment in HB during the dry season. The results showed that HB recorded the highest

pooled mean copper concentration in seawater (Table 4.5) and the lowest pooled mean copper concentration in sediment (Table 4.7) during the dry season. Changes in the physicochemical parameters such dissolved oxygen concentration, salinity and pH, of the overlying water may also lead to the release of metals from sediment (Jolley et al., 2005; Atkinson et al., 2007; Hong et al., 2011; Li et al., 2013). This may have also accounted for the lower pooled mean copper concentration in sediment recorded in HB. The partitioning of metals is governed by pH, where a decrease in pH increases the solubility of metal complexes, and as such metals will be released more rapidly from the sediment. At low pH and dissolved oxygen in the overlying waters, the oxidation rate of the dissolved iron (II)/manganese (II) released from sediments will decrease. This will lead to a decrease in the rate of metal precipitation from overlying waters as oxides of iron and manganese (e.g. iron (III) hydroxide and manganese dioxide), resulting in a net increase in dissolved metals in the overlying water. This statement concurs with the results of this study for copper in seawater from HB during the dry season. The mean pH value in HB (pH 7.2) was the lowest of the four harbours (Table 4.1) and may, therefore, have contributed to the lower pooled mean copper concentration in sediment in HB. It should be noted that other physicochemical parameters of the seawater samples such as dissolved oxygen, salinity, organic particles and total suspended solids were not measured and could be valuable in interpreting the results. The pooled mean copper concentration recorded in HB (1.07±1.93mg/kg DW) for the dry season in this study was higher than the 0.22±0.24mg/kg DW recorded in the summer by Sparks et al. (2017) in a recent study carried out in the same site. This may likely be indicative of on-going pollution taking place in HB. The pooled mean copper concentration in sediment from KB was higher than that recorded in GRB, GB and CGH_{MPA} although no significant differences were recorded. This is could be attributed largely to the vessel repair and maintenance activities which were taking place in KB at the time of sampling. It should be noted that the pooled mean copper concentration in sediment from GB was higher than that from GRB although there was no significant difference. GB has shared uses (e.g. recreation crafts, commercial fishing vessels as well as a repair and maintenance facility) which may have accounted for the higher pooled mean copper concentration. Also, during the dry season, the occurrence of anti-clockwise retentive gyres off the waters of Gordon's Bay results in the entrapment of water in the north-eastern corner of the Bay (Atkins, 1970). This may also provide an explanation for the higher pooled mean copper concentration in sediment from GB, because of the entrapment of contaminants such as copper in the waters which are able to bind to suspended particles that are eventually deposited to the bottom sediment. By comparing the pooled mean copper concentrations in sediment from the harbours to the BCLME-SQGs for copper, it can be observed that KB and GB exceeded the PEL value by 6 and 2 times, respectively. This may imply that there is severe pollution in KB and GB and which may be deleterious to sediment-dwelling organisms. The

pooled mean copper concentration in GRB was between TEL and PEL and may pose a threat to aquatic organisms. It is interesting to note that vessel repair and maintenance operations were ongoing during the dry season sampling occasion in KB and GB. This may have significantly contributed to the higher pooled mean copper concentrations in sediment from these two harbours.

For comparisons in the dry season between harbours, the pooled mean zinc concentrations (mg/kg DW) in sediment from the four harbours ranged between 18.36±16.72 to 666.86±999.90 (Figure 4.19). This was higher when compared to zinc concentrations in the range of 18.52 to 54.6mg/kg recorded by El-Moselhy et al. (2016) in sediment collected from the northern part of the Gulf of Suez during summer. The pooled mean sediment zinc concentrations recorded in the harbours are in the decreasing order: GB > KB > GRB > HB. The result showed that the pooled mean zinc concentration recorded in sediment from GB was significantly higher than that from GRB, HB and KB (Table 4.19). A similar pattern was observed between GB and GRB for water. This higher pooled mean zinc concentration in sediment may be attributed to the intense vessel-related activities taking place in GB associated with its shared uses. As already mentioned for copper, the occurrence of the anti-clockwise retentive gyres off the coast of Gordon's Bay during the dry season may have also contributed to the higher sediment mean zinc concentration in GB. Although HB recorded the lowest sediment mean zinc concentration between the harbours in the dry season, it was higher when compared with the result (0.40±0.47mg/kg) reported by Sparks et al. (2017) in a previous study in the same harbour. The pooled mean zinc concentration recorded in sediment from KB was higher when compared to GRB and HB, although the differences were statistically insignificant. The pooled mean zinc concentrations in sediment at GB and KB were well above the recommended PEL value for zinc. This might be suggestive of serious pollution associated with an increased rate of adverse effect on benthic organisms.

5.2.2 Comparisons in the wet season

The mean copper concentrations (mg/kg DW) found in sediment at sampling points within the fours harbours for the wet season ranged from 0.52±0.44 to 3432.16±2306.68 (Table 4.6). The results showed that the highest mean concentration of copper was recorded at KB1 as was the case for the dry season, while the lowest was at HB1. When comparing copper concentrations between the sampling points at GRB and the two reference sites, the results indicated that the three sampling points (GRB1, GRB2 and GRB3) were significantly higher than BB_{MPA}. These high copper concentrations could be attributed to several vessel-related activities taking place within GRB as compared to BB_{MPA} which is a marine protected area with no vessel-related activities. GRB1 was in open waters approximately 100m from the Water

Club Marina yacht basin which has a slipway used for vessel surveys, repair and maintenance. GRB2 was near the OPBC slipway which is the only launching facility on the Table Bay coastline open to the public, some governmental, institutional, law enforcement and rescue services. GRB3 was located on the left side of the East Pier of the Victoria Basin which is used for a wide range of commercial vessels. The mean copper concentration in sediment at GRB2 and GRB3 were significantly higher than that of GRB1. This is expected, as GRB1 was in open waters with high tidal flushing (low retention time) and a distance away from vessel-related activities (e.g. vessel repair and maintenance, recreational vessel density and vessel launching facilities). The mean copper concentrations recorded in sediment at GRB1, GRB2 and GRB3 were below the TEL value for copper. These results suggest that there are likely to be no adverse effects on the marine ecosystem at these sampling points (Macdonald et al., 1996). The mean copper concentration in sediment recorded at HB3 was significantly higher than that recorded at HB1, HB2 and BB_{MPA}. This may be ascribed to the release of copper from vessel repair and maintenance as well as to surface and stormwater runoff from the surrounding area and hard surfaces (roads and parking lots near the harbour). It should be noted that HB3 was located close to the breakwater in the southern section of the harbour and adjacent to the slipway and a vessel repair and maintenance facility. This may likely have accounted for the higher mean copper concentration in sediment from HB3. Surprisingly, the mean copper concentration recorded in sediment from CGH_{MPA} was significantly higher than that from HB3. Although CGH: MPA is a restricted zone MPA, the higher mean copper concentration in sediment at CGH_{MPA} may likely be associated with the high maritime traffic around the CGH: MPA. The CGH: MPA is located within a major global trade route and a significant transit point for oil tanker shipments worldwide. An estimated 4.9 million barrels a day (bbl/d) of maritime crude oil moved around the CGH: MPA in both directions in 2013 and accounted for approximately 9% of maritime oil trade (EIA, 2014). According to Wepener & Degger (2012), about 28% of the oil exports from the Middle East go through the CGH: MPA. Therefore, it could be suggested that sources of copper at CGH_{MPA} are from the leaching of copper-based antifouling paints from moving vessels (OSPAR, 2010); accidental spillages and operational discharges such as bilge and ballast water (Tornero & Hanke, 2016). Also, CGH: MPA is in Cape Point which is one of the most popular tourist attractions in Cape Town. According to CTT (2017), about 1.2 million tourists visited Cape Point between 2016 and 2017. These touristic activities may be associated with increased vehicle traffic (tour and private vehicles). Therefore, surface and stormwater runoff from impervious surfaces such as roads and parking lots located near the CGH: MPA may provide an alternative explanation for the higher mean copper concentration recorded in sediment from CGH_{MPA}. Another possible explanation is that the higher mean zinc concentration in sediment at CGH_{MPA} may come from lithogenic sources. The results showed that no sampling point in HB exceeded the TEL value

for copper. This suggests that adverse effects are unlikely to occur on benthic organisms. The mean copper concentration in sediment at KB1 was significantly higher than at KB2, KB3, BB_{MPA} and CGH_{MPA}. This result is concomitant with the dry season results and may affirm the intense nature of the vessel repair and maintenance activity taking place at KB1. The mean copper concentration in sediment at KB3 was significantly higher than that at KB2. This higher mean copper concentration may be attributed to the fact that KB3 was near the sources of copper contamination in the harbour than KB2. Again, there is a similar trend, with a decrease in copper concentrations away from the potential source of contamination (Hall et al., 1992). The mean copper concentration in sediment at KB2 and KB3 were significantly higher than at $BB_{MPA.}$ This may be ascribed to the release of copper from vessel hulls (associated with the use of copper-based AF paints) into surrounding waters and subsequent precipitation to bottom sediment. Also, the increased surface and stormwater runoff in the wet season from the main road, the railway line, the parking lot and the residential areas which are adjacent to KB may have contributed to the higher mean copper concentrations, particularly at KB2. The mean copper concentration in sediment at KB1 far exceeded the PEL concentration which may increase the possibility of biological effect to aquatic organisms. Also, the mean copper concentration in sediment at KB3 was between TEL and PEL and of which toxic effects to aquatic organisms may occasionally occur. The mean copper concentration in sediment at GB3 was significantly higher than at GB1, GB2 and BB_{MPA}. GB3 was in the Old Harbour, which has shared uses such as commercial fishing and recreational vessels as well as a repair and maintenance facility. Recreational vessels tend to reside longer in their berth and would generally have the antifouling fouling coating cleaned more often particularly during the wet season. The cleaning process may release copper into the surrounding environment which eventually is incorporated into sediment while the longer residence time implies more copper is likely to be leached into the surrounding waters. Therefore, it could be suggested that the higher mean copper concentration in sediment at GB3 may have resulted from the frequent cleaning and longer residence time of recreational vessels in the harbour during the wet season. The mean copper concentrations in sediment at GB1 and GB2 were significantly higher than at BB_{MPA}. GB1 was located close to a slipway which is used for the launching of vessels, while GB2 was at the mouth of the harbour and close to a mooring site. Therefore, the higher mean copper concentrations in sediment at GB1 and GB2 may be attributed to the leaching of copper from copper-based AF paints used on vessel hulls into the surrounding waters. The dissolved copper is absorbed onto suspended particles and subsequently accumulates in sediment. The mean copper concentration in sediment at GB1 was higher than at GB2 although the difference was not significant. However, the fact that GB1 was located inside the harbour area with low water exchange rate while GB2 was at the mouth of the harbour and close to open waters, where the rate of water exchange is high, may have

accounted for the gradient. It is worth noting that the mean copper concentration in sediment at CGH_{MPA} was higher than at GRB1, GRB2, GRB3, HB1, HB2, KB2, KB3, GB1, GB2 and GB3 although there were no significant differences. As already discussed earlier, the higher mean copper concentration in sediment at CGH_{MPA} may be ascribed to the high vessel traffic around CGH: MPA (shipping lane), surface and stormwater runoff coming from impervious surfaces such as roads and parking lots as well as lithogenic sources. The mean copper concentrations in sediment at GB1 and GB2 were below the TEL concentration. The mean copper concentrations in sediment at GB3 and CGH_{MPA} were above the PEL concentration and is indicative of serious pollution which is associated with considerable ecotoxicity (Wang et al., 2014).

The mean zinc concentrations (mg/kg DW) recorded in sediment at sampling points within the fours harbours for the wet season ranged from 2.13±2.31 to 2380.43±1456.79 (Table 4.18). The results showed a similar pattern to that of copper with the highest sediment zinc concentration recorded at KB1 while the lowest was at HB1. Surprisingly, the mean zinc concentration recorded in sediment at CGH_{MPA} was significantly higher than at GRB1, GRB2 and GRB3. This could be attributed to the difference in sediment characteristics such as grain size as already mentioned for zinc in the dry season. Another possible explanation for the significantly higher mean zinc concentration in sediment at CGH_{MPA} could be ascribed to surface runoff from roads and parking lots associated with high vehicle traffic in Cape Point as well as lithogenic sources. Furthermore, the fact that CGH: MPA is located along a major shipping lane in the southern tip Africa may have contributed to the higher mean zinc concentration recorded at this reference site. The mean zinc concentrations in sediment at GRB1, GRB2 and GRB3 were significantly higher than that at BB_{MPA}. This could be attributed to vessel-related activities as well as surface and stormwater runoff from the vicinity of the sampling points (GRB, GRB2 and GRB3). The mean zinc concentrations in sediment at GRB2 and GRB3 were significantly higher than at GRB1. This could be associated with vessel-related activities (e.g., vessel launching and mooring) taking place near GRB2 and GRB3. The results revealed that the mean zinc concentration recorded in sediment at HB2 was significantly higher than at BB_{MPA}. This could likely be attributed to vessel activities taking place at HB2. The mean zinc concentration in sediment at HB3 was significantly higher than that at HB1. This could be associated with vessel repair and maintenance activities taking place close to HB3. The results showed that the mean zinc concentrations recorded in sediment from KB1, KB2 and KB3 were significantly higher than that at BB_{MPA}. This could be ascribed to vessel-related activities taking place at these sampling points. The mean zinc concentration recorded in sediment at KB1 (highest mean zinc concentration) was significantly higher than that at KB2 and KB3. This could largely be attributed to the leaching of zinc from AF paint

fragments associated with vessel repair and maintenance taking place adjacent to KB1. According to Boxall et al. (2000), a significant proportion of biocidal metals such as zinc released during vessel repair and maintenance are probably associated with particulate material (e.g. paint fragments/particles, etc.). When these fragments/particles are washed down into receiving waters it is likely to accumulate in the sediment. According to the BCLME-SQGs, the mean zinc concentration recorded in sediment at KB1 exceeded the recommended PEL value for zinc by 9 times in the order of magnitude. In GB, the mean zinc concentrations in GB1, GB2 and GB were significantly higher than that at BB_{MPA}. This could also be associated with the vessel-related activities taking place near these sampling points (GB1, GB2 and GB3). The pooled mean zinc concentrations recorded in sediment at GB1, GB2 and GB3 exceeded the TEL value but was below the PEL value.

For comparisons in the wet season between harbours, the pooled mean copper concentrations (mg/kg DW) in sediment from the four harbours ranged between 1.74±1.47 to 1154±2073.62 (Figure 4.7). This was higher when compared to the mean copper concentrations (mg/kg DW) recorded in coastal sediment from the northern Gulf of Suez in Egypt, with a range of 4.690 to 32.77 in the winter (EL-Moselhy et al., 1999). The pooled mean copper concentrations recorded in sediment from the harbours are in the decreasing order: KB > GB > GRB > HB. The results showed that KB recorded the highest pooled mean copper concentration in sediment while HB recorded the lowest. It should be noted that the results followed a similar trend as was observed in the dry season. The pooled mean copper concentration in sediment from KB was significantly higher than that from GRB and HB. As already mentioned, vessel repair and maintenance activities were predominant in KB during the two sampling periods. This may probably have accounted for the higher pooled copper concentration in sediment from KB. Furthermore, surface and stormwater runoff from the adjoining residential area, the railway line, the main road and the parking lot may have contributed to the higher copper burden in the KB (Figure 3.6). It should be noted that the pooled mean copper concentration in sediment from KB was higher than that in GB, although no significant differences were found. Also, the mean copper concentration in sediment at CGH_{MPA} was surprisingly higher than that from GRB, HB and GB although there were no significant differences. This may likely be attributed to the high shipping traffic around CGH: MPA, surface and stormwater runoff coming from impermeable surfaces such as roads and parking lots which are near the sampling point (Figure 3.10) as well as lithogenic sources. The results revealed that, like for seawater, the pooled mean copper concentration in sediment from GRB was significantly higher than that from HB. This higher mean copper concentration in sediment from GRB may be attributed to increased surface and stormwater runoff from the Golf Club as well as from the main road (Beach Road) in the wet season (Figure 3.4). Also, GRB's proximity to the Port

of Cape Town where shipping activities are intensive may have accounted for the higher mean copper concentration due to circulation and dispersion by waves or tidal currents. Like for seawater, the pooled mean copper concentration in sediment from GB was significantly higher than that from HB. This result shows a similar pattern to the result recorded for the dry season between the two harbours and may suggest that physicochemical parameters (e.g. dissolved oxygen concentration, salinity and pH, etc.), as well as physical and biological processes (e.g. storms, remedial dredging, unremitting vessel traffic, and bioturbation, etc.), may affect copper sorption-desorption between sediment and the overlying water. Although HB recorded the lowest sediment mean copper concentration between harbours in the wet season, it was higher than that (below detection limit) reported by Sparks et al. (2017) in the same harbour. The pooled mean copper concentration in sediment from GB was higher than that from GRB although there was no statistically significant difference. As already mentioned, the shared use nature of GB may account for the higher pooled mean copper concentration associated with vessel repair and maintenance as well as leaching from moored vessels. Another possible explanation for the higher pooled mean copper concentration in sediment from GB may be because of increase in surface and stormwater runoff from surrounding areas during the wet season as well as inputs from the Sir Lowry's Pass River which enters the ocean from the north-eastern corner of the bay. It should be noted that this result followed a similar pattern as observed in the dry season when comparing the two harbours. The results showed that KB exceeded the PEL concentration 11 times in the order of magnitude while GB was higher than the TEL concentration but below the PEL threshold. The pooled mean copper concentrations in sediment from GRB and HB were below the TEL concentration. These results, therefore, suggest that adverse biological effects caused by copper may: (i) frequently occur in KB, (ii) occasionally occur in GB and (iii) rarely occur in GRB and HB.

For comparisons in the wet season between harbours, the pooled mean sediment zinc concentrations (mg/kg DW) from the four harbours ranged between 5.59±4.53 and 812.22±1387.03 (Figure 4.18). This was higher than the mean zinc concentrations (mg/kg DW) in the range of 25.33±2.87 to 151.00±36.48 recorded by Abouhend & El-moselhy (2015) in sediment collected from the northern Red Sea coast during summer. The pooled mean sediment zinc concentrations recorded in the harbours are in the decreasing order: KB > GB > GRB > HB. The result showed that the highest pooled mean zinc concentration in sediment was recorded at KB. This could be associated with the presence of a slipway as well as a vessel repair and maintenance facility close to KB. The pooled mean zinc concentration in sediment from HB was significantly lower than that in GRB, KB, GB and BB: MPA. A similar pattern was observed in water between HB and GRB. This lower pooled mean zinc concentration in sediment from HB could be attributed to natural and anthropogenic

disturbances in the harbour which may result in the remobilization of metals as a result of sediment resuspension (Eggleton & Thomas, 2004). For example, when natural disturbances (e.g. upwelling, tidal movements, wave actions, etc.) as well as physical and biological processes such as bioturbation, storms, remedial dredging and unremitting vessel traffic (vessel propellers generating waves) occur, resuspension of sediment may potentially lead to the release of metals from sediment into the water column (Eggleton & Thomas, 2004; Newman & Watling, 2007; Huang et al., 2012). Another possible explanation for the lower mean zinc concentration in sediment from HB could be attributed to the removal of zinc from the sediment by sediment-dwelling organisms, as well as phytoplankton. The pooled mean zinc concentration recorded in sediment from GB was significantly higher than that at GRB. This could be attributed to the leaching of zinc from predominantly recreational vessels moored in the harbour. The pooled mean zinc concentration recorded in sediment from KB exceeded the PEL value by 3 times in the order of magnitude, while that from GB exceeded the TEL.

5.2.3 Comparisons between the dry and wet seasons

When comparing the mean copper concentrations in sediment from sampling points within harbours between the two seasons, GRB2, KB2 and GB3 were significantly higher in the dry season as compared to the wet season (Figure 4.3a, 4.3c and 4.3d, respectively), unlike with seawater, where there were general insignificant differences. GRB2 was located close to a slipway at OPBC which provides the only public access to the sea on the Table Bay coastline. KB2 was in open waters and close to a vessel repair and maintenance facility and GB3 was also located near a repair and maintenance facility as well close to a recreational vessel mooring area. Therefore, the higher mean copper concentrations in sediment recorded at these three respective sampling points in the dry season could be attributed to the increase in vessel-related activities such as vessel traffic, vessel mooring as well as vessel repair and maintenance. KB3 recorded a significantly higher mean copper concentration in the wet season compared to the dry season (Figure 4.3c). This could be ascribed to the increase in rainfall during the wet season (Table 4.2) resulting to an increase in surface and stormwater runoff from hard surfaces such as the main road, the parking lot and the railway line which are adjacent to KB3 (Figure 3.6). It is worth noting that the mean copper concentration recorded in sediment at KB1 was higher in the wet season compared to the dry season although the difference was not significant (Figure 4.3c). This could be attributed to additional inputs from surface runoff because of the increase in rainfall which may add to the existing copper load from vessel-related activities. Also, the mean copper concentrations in sediment at GRB3, HB1, HB2, GB1 and GB2, were higher in the dry season compared to the wet season although there were no statistically significant differences (Figure 4.3a, 4.3b and 4.3d, respectively). As previously explained, the seasonal variation in mean copper concentrations at sampling points

could be attributed to the changes in vessel usage during the two seasons. Based on observation during sampling periods, there was an increase in vessel-related activities in the dry season than in the wet season in the harbours. This may have accounted for the higher mean copper concentrations recorded in sediment in the dry season than the wet season.

When comparing the mean zinc concentrations in sediment from sampling points within harbours in the two seasons, HB1, HB2 and GB3 were significantly higher in the dry season than the wet season (Figure 4.11b and 4.11d). This could be attributed to an increase in vessel-related activities in the dry season resulting in an increase in the release of zinc from AF paints as well as from zinc-based sacrificial anodes used on vessel bottoms. The mean zinc concentration recorded in sediment from KB1 in wet season was higher than that for the dry season (Figure 4.15c), although there were no significant differences. This result showed a similar trend to that of copper at KB1. Therefore, it could be suggested that increase in surface and stormwater runoff from hard surfaces (e.g. main road, the parking lots, railway line, etc.) and the surrounding suburb in wet season may have contributed to the higher mean zinc concentration.

When comparing the pooled mean copper concentrations in sediment for the two seasons between harbours, HB was significantly higher in the wet season than in the dry season (Figure 4.4). This may be attributed to increase in stormwater runoff from the adjacent suburb and factories as well as influx of contaminants (such as copper) from the Disa River during the wet season when rainfall was high. This may have been compounded by the existing copper burden from vessel-related activities in the harbour. Furthermore, the pooled mean copper concentration in sediment recorded in GRB and GB were higher in the dry season compared to the wet season although there were no significant differences. This could be ascribed to the increase in vessel-related activities (e.g. increase in vessel traffic/density and vessel repair and maintenance activities) within the two harbours during the dry season. In KB, the pooled mean copper concentration in sediment recorded in the wet season was higher when compared to the dry season although the difference was not significant. It could be suggested that increase in surface and stormwater runoff from the adjacent suburb, the railway line and impervious surfaces (the main road and the parking lots) during the wet season when rainfall was high may have contributed to the higher pooled mean copper concentration (Figure 3.6; Table 4.2). Another possible explanation could be that during the wet season when vessel usage is low, the residing time of vessels in the harbour increases and there is also an increase in vessel repair and maintenance activities which may result to more copper being leached into the surrounding environment.

When comparing the pooled mean zinc concentrations in sediment for the two seasons between harbours, like for seawater, HB and GB had significantly higher sediment mean zinc concentration in the dry season than in the wet season (Figure 4.12). This could be attributed to the increase in vessel-related activities in the dry season. Like the results recorded for copper in sediment from KB, the mean zinc concentration in sediment from KB was also higher in the wet season than the dry season, although there was no significant difference. This could be attributed to the increase in surface and stormwater runoff from the vicinity of the harbour during the wet season when rainfall was high which is compounded by the intense vessel related activity taking place all year round.

5.3 Metal concentrations in gastropod soft tissue within harbours

The approach based on the bioaccumulation capacity of some chemical substances such as metals is among the most important methodological approaches of biological monitoring of a marine ecosystem (Conti & Iacobucci, 2008). This capacity is present in many marine organisms such as molluscs, which can be proposed as possible biomonitors. One of the advantages of employing biomonitors in assessing metal pollution in marine ecosystems is that the element levels found in their tissue reflect the amount of bioavailable metal present in the environment (Coughtrey & Martin, 1977; Bryan et al., 1984; Balogh, 1988; Lyngby, 1987). The bioaccumulated concentrations in a biomonitor are a direct reflection of the total integrated bioavailability and contamination of the areas under investigation (Rainbow et al., 2002).The chemical analysis of contaminants in water or sediments does not provide such a measure of bioavailability, as not much is known of the relative or absolute availability of metals in different forms to biota (Phillips, 1980). The use of molluscs to monitor metal pollution in the coastal environment is well established (Goldberg et al. 1978, Phillips, 1979, 1985, Rainbow, 1978; Phillips & Rainbow, 1988). Although gastropods have been used less extensively for biomonitoring than other molluscs such as bivalves (Berger & Dallinger, 1993), many have the required attributes to be effective biomonitors (Phillips, 1977; Phillips & Rainbow, 1993; Langston & Spence, 1995). Few studies worldwide have used gastropods in biomonitoring studies (Ireland & Wootton, 1977; Peerzad et al., 1990; Kang et al., 2000); Blackmore, 2001; Campanella et al., 2001; Taylor & Maher, 2006; Maher et al., 2016; Krupnova et al., 2017).

Burnupena spp. and *Nucella* spp. are the two gastropod molluscs that were employed in this study. They are widely distributed ranging from the west coast to the east coast of Southern Africa (Wickens & Griffiths, 1985; Dempster & Branch, 1999). *Burnupena* spp. are opportunistic scavengers (may quickly detect and prey on any injured living animal) which are abundant in the lower intertidal zone and subtidally (Branch, 1978; Branch et al., 2010).

Nucella spp. are predatory whelks that live in the intertidal and subtidal zones of the rocky shores and will prey on mussels, limpets, barnacles and periwinkles (Wickens & Griffiths, 1985; Branch et al., 2010). A number of studies have revealed that gastropods can accumulate in their soft tissue substantial amount of metals through the body wall (permeable membranes) and diet (Blackmore & Morton, 2001; Wang, 2002; Proum et al., 2016). Therefore the possible route of exposure to metals for these two gastropods will be through dietary uptake and to a lesser extent direct absorption from the water column (Boyden & Phillips, 1981; Taylor & Maher, 2003; Boucetta et al., 2016; Bighiu, 2017). According to Blackmore (2000) and Wang & Ke (2002), metal accumulation in gastropods seems to be dominated by food chain transfer. It is important, therefore, to consider the feeding habit of the organism under investigation as it could have an effect on the metal concentrations (Bat et al., 1994; Conti & Iacobucci, 2008). *Burnupena* spp. and *Nucella* spp. are carnivorous and second level consumers. Therefore, it can be supposed that the metal concentrations in their soft tissue may reflect the metal concentrations found in their diet (which may be mussels or barnacles). Unfortunately, there is a lack of enough existing literature on the use of *Burnupena* spp. and *Nucella* spp. as biomonitors of metal pollution. Therefore, comparison of the results obtained herein with the results of other similar studies was not possible. It is worth noting that there is substantial evidence of the variability in the ability of molluscs to accumulate metals (Campanella et al., 2001; Cubadda et al., 2001; Rabaoui et al., 2014), as even conspecifics may display different accumulation strategies. As already mentioned, (Chapter 4), *Burnupena* spp. and *Nucella* spp. were not available at all sampling points in each harbour and at all sampling seasons. Hence, comparisons of mean copper and zinc concentrations in the gastropods between harbours were not possible. Copper and zinc are essential elements for aquatic organisms. Copper is essential for the synthesis of hemocyanin, a blood pigment in marine gastropods (Yap & Cheng, 2013) and also a cofactor for regulating the activity of copper-dependent enzymes. Zinc is of major importance in enzymatic and metabolic processes. It is known to act as an enzyme cofactor in over 200 enzymes (Vallee & Auld, 1990) and respiratory pigments of marine invertebrates (Cubadda et al., 2001). However, copper and zinc can become toxic depending on their concentration and speciation in the aquatic environment (Sunda, 1989). These metals may also bioaccumulate in organisms and thereby pose a potential threat to the food chain.

Elevated concentrations of copper and zinc in other gastropods from polluted coastal areas have been reported worldwide. For example, in coastal waters around Wales, mean copper and zinc concentrations in the range of 166.4±5.8 to 458.1±53.7mg/kg DW and 492.1±27.8 to 2354.6±289.0mg/kg DW, respectively, were recorded in the gastropod, *Thais lapillus*. In Hong Kong coastal waters, copper and zinc concentrations as high as 1860mg/kg DW and 1850 to

2050mg/kg DW, respectively, have been measured in carnivorous gastropods (Blackmore & Morton, 2001). Gastropods samples from the Northern Adriatic Sea in Italy revealed high copper and zinc concentrations of 800±318mg/kg and 576±129mg/kg DW, respectively (Berto et al., 2012). Also, high mean copper and zinc concentrations of 215.9±86.1mg/kg DW and 868.8±262.3mg/kg DW were found in *Thais clavigera* soft tissue from East Johor coastal waters in Malaysia (Rahman et al., 2016). The results of this study revealed the bioaccumulation of copper and zinc in the *Burnupena* and *Nucella* soft tissue and the variations within and between the harbours.

5.3.1 Comparisons in the dry season

The mean copper concentrations (mg/kg DW) in *Burnupena* soft tissue at sampling points within the four harbours in the dry season ranged from 89.15±26.56 to 147.49±26.63 (Table 4.8). The results showed that the mean copper concentrations in *Burnupena* soft tissue at GRB1, GRB2, GB1 and GB2 were significantly higher than at CGH_{MPA} (Table 4.8). A similar pattern was observed in sediment for GRB2, GRB3 and GB. These sampling points were in areas close to vessel-related activities (e.g. vessel mooring; vessel launching; vessel repair and maintenance, etc.) within their respective harbours and as such may likely have accounted for the higher mean copper concentrations in the *Burnupena* soft tissue. Copper is the main biocide used in present-day AF paints and is regarded as an important anthropogenic source of copper in the aquatic environment. Therefore, it could be suggested that the leached copper from the AF paints used on vessel hulls may become bioavailable for uptake by the organism through dietary transfer as well as through the dissolved phase. Also, the mean copper concentration in *Burnupena* soft tissue at HB1 was higher than that at CGH_{MPA} although no statistically significant difference was found. This could be attributed to the intense vessel traffic and vessel repair and maintenance activities as well as the discharge of waste from the fish processing facilities and fishmeal factory in the harbour precinct. The mean copper concentration in *Burnupena* soft tissue at BB_{MPA} was remarkably higher than at GRB2, HB1, GB1 and GB2 although there were no statistically significant differences. A similar pattern was observed when compared with the results recorded for the mean copper concentration in seawater at these sampling points. This higher mean copper concentration in *Burnupena* soft tissue at BB_{MPA} could be attributed to the local wind-induced coastal upwelling during the summer months (Jackelman et al., 1991) which is associated with the diverse current systems of the area (Lutjeharms et al., 2001; Lutjeharms, 2006). Another possible explanation could be changes in physicochemical properties of the different environments and the biology of the gastropods. Many environmental and biological factors can combine to influence metal concentrations and bioaccumulation in marine molluscs (Gay & Maher, 2003). Intrinsic factors

such as size, sex, reproductive state, individual variability in metal uptake, changes in tissue composition and diet and extrinsic factors such as hydrodynamics of the environment, temperature, pH, salinity, dissolved oxygen, availability of food and metal-metal relationships can influence metal concentrations (Boyden & Phillips, 1981; Li et al., 2009). It should be noted that *Burnupena* spp*.* could not be found in some of the sampling points at the time of sampling during the dry season (Table 3.1 and 4.8).

The mean zinc concentrations (mg/kg DW) in *Burnupena* soft tissue at sampling points within the four harbours in the dry season ranged from 223.81±34.55 to 1010.79±93.79 (Table 4.20). The results showed that there were no significant differences between sampling points and the reference sites in the harbours, except in GRB. The mean zinc concentration recorded in *Burnupena* soft tissue at GRB1 was significantly higher than the reference sites (BB_{MPA} and CGH_{MPA}). This could be ascribed to vessel-related activities, stormwater runoff as well as wastewater discharge (Green point and Camps Bay outfalls). Also, the mean zinc concentration in *Burnupena* soft tissue at GRB1 was significantly higher than at GBR2 (Table 4.20). This could be attributed to road and stormwater runoff at GRB1 as well as the metal handling strategies of the individual gastropods. It should be noted that GRB1 was located adjacent to the main road and near to stormwater outfalls in GRB.

The mean copper concentrations (mg/kg DW) in *Nucella* soft tissue at sampling points within the four harbours in the dry season ranged from 54.62±8.66 to 2211.61±3168.07 (Table 4.11). The mean copper concentrations in *Nucella* soft tissue at sampling points in GRB (GRB1 and GRB3), HB (HB1, HB2 and HB3), KB (KB1, KB2 and KB3) and GB (GB3) were significantly higher than at BB_{MPA} and CGH_{MPA} (Table 4.11). This could likely be ascribed to several vessel-related activities taking place near to these sampling points in the respective harbours. A similar pattern was observed in sediment at GRB1, GRB3, KB1, KB2, GB1 and GB3 with CGH_{MPA}. The mean copper concentration in *Nucella* soft tissue at HB2 was significantly higher when compared to HB1. HB1 was in open waters outside the harbour with high tidal circulation of water and no vessel mooring while HB2 was located inside the harbour where there was high vessel density (artisanal fishing vessels, commercial fishing vessels, recreation and leisure vessels) and longer mooring (especially for recreational and leisure vessels) as well as limited water circulation. The leaching of copper from the hulls of moored vessels a well as from the vessel traffic which may be exacerbated by the poor flushing rate could have accounted for the higher mean copper concentration in *Nucella* soft tissue at HB2. Like the results for mean copper concentration in sediment, the mean copper concentration in *Nucella* soft tissue at KB1 was significantly higher than that at KB2 and KB3 (Table 4.11). KB1 was located close to a slipway and a vessel repair and maintenance facility in the harbour. Therefore, it could be suggested that copper-based AF paint fragments and dust generated

from vessel repair and maintenance activities may likely have accounted for the higher mean copper concentration found in *Nucella* soft tissue at KB1 (Sarkar et al., 2008; Gadd & Cameron, 2012; Eklund & Eklund, 2014). Paint flakes and dust particles generated during vessel maintenance are a direct source of copper contamination in harbours (Huntingford & Turner, 2011). It should be noted that there were ongoing vessel maintenance activities close to KB1 at the time of sampling. Also, the mean copper concentration in *Nucella* soft tissue at KB3 was significantly higher than at KB2. KB3 was located inside the harbour and closer to the vessel repair and maintenance facility while KB2 was located outside the harbour in open waters with a higher tidal circulation rate. Hence, the vessel repair and maintenance activities (e.g., the scraping and re-application of copper-based AF paint on vessel hulls) may likely have accounted for the higher mean copper concentration in *Nucella* soft tissue at KB3. The significant variation in mean copper concentrations in *Nucella* soft tissue between the sampling points at KB (KB1, KB2 and KB3) are indicative of considerable variability in bioavailability. It could be observed that the mean copper concentrations in *Nucella* soft tissue at KB1, KB2 and KB3 decrease with increasing distance from potential sources of contamination. *Nucella* spp. could not be found at GRB2, GB1 and GB2 in the dry season sampling occasion (Table 3.1 and 4.11).

The mean zinc concentrations (mg/kg DW) in *Nucella* soft tissue at sampling points within the four harbours in the dry season ranged from 262.27±77.87 to 5045.44±2447.15 (Table 4.23). The results revealed that the highest mean zinc concentration in *Nucella* soft tissue was recorded at GB3. This could largely be attributed to vessel repair and maintenance activity taking place at GB3 during the dry season. Also, the increase in vessel usage which is associated with high vessel traffic and density in the dry season, particularly recreational vessels may have also accounted for the highest mean zinc concentration at GB3. The mean zinc concentrations in *Nucella* soft tissue at GRB3, HB1, HB2, HB3, KB1, KB2, KB3 and GB3 were significantly higher than the reference sites (Table 4.23). This could be attributed to several vessel-related activities (e.g. vessel launching, maintenance and repair, mooring, etc.) taking place close to these sampling points in the harbours during the dry season. Similar patterns were observed for mean zinc concentrations in sediment at KB1, GB1, GB2 and GB3. The mean zinc concentration recorded in *Nucella* soft tissue at GRB3 was significantly higher than at GRB1. This could be attributed to the fact that GRB3 was closer to the Port of Cape Town (Victoria Basin) with intense vessel traffic and high vessel density (both shipping and recreational). The results showed that the mean zinc concentrations recorded in *Nucella* soft tissue at HB2 and HB3 were significantly higher than at HB1. HB2 was located inside the harbour with high vessel density while HB3 was close to a vessel repair and maintenance facility. Therefore, this may have accounted for the higher mean zinc concentrations in HB2

and HB3. The mean zinc concentrations recorded in *Nucella* soft tissue at KB1 and KB3 were significantly higher than at KB2. This could be attributed to vessel repair and maintenance activities taking place close to these two sampling points. Also, the mean zinc concentration recorded in *Nucella* soft tissue at KB1 was significantly higher than at KB3. KB1 was closer to the slipway as well as the vessel repair and maintenance facility which might provide an explanation for the higher mean zinc concentration at KB1. The mean zinc concentration in *Nucella* soft tissue decreased linearly with increasing distance from the source of contamination. This pattern was also observed for mean copper concentrations in *Nucella* soft tissue at sampling points in KB.

5.3.2 Comparisons in the wet season

The mean copper concentrations (mg/kg DW) in *Burnupena* soft tissue at sampling points within the four harbours in the wet season ranged from 53.87±5.09 to 122.13±80.34 (Table 4.8). The mean copper concentration in *Burnupena* soft tissue at HB1, KB2, GB1, GB2 and GB3 were significantly higher than at BB_{MPA}. A similar pattern for the mean copper concentration in sediment were observed at KB2, GB1, GB2 and GB3. These higher mean copper concentrations in *Burnupena* soft tissue could be attributed to vessel-related activities, surface and stormwater runoff as well as riverine inputs. The mean copper concentration in *Burnupena* soft tissue at GRB1 and GRB2 were higher when compared to BB_{MPA}, although there were no significant differences. This may also be ascribed to vessel-related activities as well as surface and stormwater runoff in the wet season. It should be noted that GRB1 and GRB2 were located adjacent to the Port of Cape Town (intense vessel traffic) and urban development (residential and commercial development). The mean copper concentration in *Burnupena* soft tissue at GRB1 was higher than at GRB2, although no significant difference was found. GRB1 was located adjacent to the main road (Beach Road) with high vehicle traffic and in proximity to the marine sewage outfall in Green point. Therefore, it could be suggested that the sewage outfall and the increased surface and stormwater runoff in the wet season from the main road (brake and tyre wears) as well as from the residential and commercial areas may have accounted for the higher mean copper concentration in *Burnupena* soft tissue at GRB1. The mean copper concentration in *Burnupena* soft tissue at CGH_{MPA} (reference site) was significantly higher than at GB1. The *Burnupena* soft tissue mean copper concentration at CGH_{MPA} was also higher than at GRB2 and KB2 although there were no significant differences. As previously mentioned, CGH: MPA is located along a major global trade route and a significant transit point for oil tanker shipments. Therefore, the leaching of copper-based AF paints from moving vessels; accidental spillages as well as operational discharges such as bilge and ballast water may likely have accounted for the higher mean copper concentration

in *Burnupena* soft tissue at CGH_{MPA}. Another possible explanation for the higher mean copper concentration is the increased surface runoff from impervious surfaces such as roads and car parks in the wet season, as well as from lithogenic sources. The *Burnupena* soft tissue mean copper concentrations at GB2 and GB3 were significantly higher than that at GB1. A similar pattern for mean copper concentration in sediment was observed at GB2 and GB3. The higher mean concentration of copper recorded in *Burnupena* soft tissue at GB2 could have been influenced by environmental (e.g., temperature, pH, salinity, total suspended solids, dissolved oxygen, metal concentrations in prey, etc.) and biological (e.g., reproductive state, sex, age and size, etc.) factors (Catsiki et al., 1994; Boening, 1999; Blackmore, 2000). It should be noted that differences coming from factors such as temperature, pH and size of the organism were taken into consideration. GB3 was located inside the harbour (Old Harbour) with mixed use (leisure crafts, commercial fishing and naval vessels) and intense vessel-related activities such as vessel traffic as well as repair and maintenance. This may have accounted for the higher mean copper concentration in *Burnupena* soft tissue. The *Burnupena* spp. were not found at GRB3, HB2, HB3, KB1 and KB3 in the wet season sampling occasion (Table 3.1 and 4.8).

The mean zinc concentrations (mg/kg DW) in *Burnupena* soft tissue at sampling points within the four harbours in the wet season ranged from 119.89±92.91 to 852.16±577.06 (Figure 4.20). The results showed that there were no significant differences between the sampling points and the reference sites in the harbours, except in GRB. The mean zinc concentration in *Burnupena* soft tissue at GRB1 was significantly higher than the reference sites (Table 4.20). A similar pattern was observed for mean zinc concentration in sediment at GBR1. This higher mean zinc concentration in *Burnupena* soft tissue could largely be attributed to vessel-related activities taking place close to GRB1. Also, the mean zinc concentration in *Burnupena* soft tissue at GRB1 was significantly higher than at GBR2. This could be ascribed to surface and stormwater runoff (particularly from the main road, the golf course and the residential areas). Another possible explanation for the higher mean zinc concentration in *Burnupena* soft tissue in GRB1 could be attributed to wastewater discharges from the Green Point and Camps Bay outfalls.

The mean copper concentrations (mg/kg DW) in *Nucella* soft tissue at sampling points within the four harbours in the wet season ranged from 47.43±4.80 to 508.20±71.24 (Table 4.11). The lowest mean copper concentration in *Nucella* soft tissue was at HB1 while the highest was at KB1. The results revealed that the *Nucella* soft tissue mean copper concentrations at GRB3, HB1, HB2, HB3, KB1, KB2; KB3 and GB3 were significantly higher than at BB_{MPA} and CGH_{MPA} (Table 4.11). This could largely be attributed to the vessel related activities taking

place within these sampling points. Another possible explanation could be the increase in surface and stormwater runoff as well as riverine inputs in the wet season. *Nucella* soft tissue mean copper concentration at HB2 was significantly higher than at HB1 and HB3. This could be ascribed to the leaching of copper-based AF paints from moored vessel hulls (predominantly leisure vessels) as well as from vessel traffic (commercial fishing vessels) at this sampling point which may become bioavailable for uptake by the gastropods. This may also be compounded by the vast area of impervious surfaces in the harbour which may direct stormwater runoff and contaminants into the harbour waters. The results showed that, like for sediment, the mean concentration of copper in *Nucella* soft tissue at KB1 was significantly higher than that at KB2 and KB3. This could be attributed to the leaching of copper from AF paint fragments and dust particles which are released from the vessel repair and maintenance facility. The mean copper concentration in *Nucella* soft tissue at KB3 was 3 times higher than at KB2, although the difference was not statistically significant. KB3 was in proximity to the vessel repair and maintenance facility than KB2 and this may have accounted for the higher mean concentration of copper in *Nucella* soft tissue. It was observed that results for *Nucella* soft tissue at KB1, KB2 and KB3 exhibited a similar trend with the results for sediment in the wet season. Once again, the mean concentration of copper in *Nucella* soft tissue at KB1, KB2 and KB3 showed a decrease with increasing distance from potential sources of contamination. *Nucella* spp. were not found at GRB1, GRB2, GB1 and GB2 at the time of sampling in the wet season (Table 3.1 and 4.11).

The mean zinc concentrations (mg/kg DW) in *Nucella* soft tissue at sampling points within the four harbours in the wet season ranged from 77.20±15.14 to 1654.53±63.20 (Table 4.23). The results revealed that the highest mean zinc concentration in *Nucella* soft tissue during the wet season was recorded at KB1. This could mainly be attributed to the intense vessel repair and maintenance activity taking place close to KB1 throughout the year. Also, surface and stormwater runoff from hard surfaces (e.g. main road, parking lots, etc.), the railway line and the suburb during the wet season when rainfall was high may have added to the existing zinc load from vessel-related activities. The mean zinc concentrations in *Nucella* soft tissue at GRB3, HB1, HB2, HB3 and KB1 were significantly higher than that at CGH_{MPA} and BB_{MPA}. A similar pattern was observed for mean zinc concentrations in sediment between KB1, GRB3 and the reference sites, and between HB2 and BB_{MPA}. This higher mean concentration in *Nucella* soft tissue could largely be attributed to vessel-related activities taking place close to these sampling points as well as to surface and stormwater runoff in the wet season. Also, the results showed that, similar to sediment, the mean zinc concentrations in *Nucella* soft tissue at KB2 and KB3 were significantly higher than at BB_{MPA}. Vessel-related activities, as well as surface and stormwater runoff, may also have accounted for the significantly higher mean zinc

concentrations in *Nucella* soft tissue at KB2 and KB3. Surprisingly, the mean zinc concentrations in *Nucella* soft tissue at the reference sites were significantly higher than at GB3 (Table 4.23). This could be attributed to the difference in metal handling strategies of the individual gastropods resulting from the net difference between rates of uptake and excretion of metals caused by changes in body tissue associated with environmental variables (e.g. pH, salinity, temperature, organic carbon, etc.). The mean zinc concentration in *Nucella* soft tissue at HB2 was significantly higher than at HB1 and HB3. This could be ascribed to the increased leaching of zinc from AF paint and zinc-based sacrificial anode used on vessel bottoms associated with high vessel traffic and density as well as poor water circulation. Also, the results showed that, like for sediment, the mean zinc concentration in *Nucella* soft tissue at HB3 was significantly higher than at HB1. This could be attributed to the vessel repair and maintenance activity taking place close to HB3. The results revealed that, like for sediment, the mean zinc concentration in *Nucella* soft tissue at KB1 was significantly higher than at KB2 and KB3. This could be attributed to the intense vessel repair and maintenance activity (e.g. scraping and sanding of old AF paints, repainting, etc.) taking place close to KB1.

5.3.3 Comparisons between the dry and wet seasons

Temporal changes in organism's soft tissue metal concentrations can be influenced by variation of metal inputs, exposure to bioavailable metals, physicochemical conditions and changes in organism's physiology (Luoma & Rainbow, 2008). The effect of season on copper and zinc concentrations in *Burnupena* and *Nucella* soft tissue showed similar seasonal patterns at sampling points in the harbours. The results showed that mean copper and zinc concentrations in the *Burnupena* and *Nucella* soft tissue were generally higher in the dry season than in the wet season.

When comparing the mean copper concentrations in *Burnupena* soft tissue from sampling points within the harbours between the sampling seasons, GRB2 and GB1 were significantly higher in the dry season than in the wet season (Figure 4.5a and 4.5d). It was observed during the sampling occasions that vessels related activities (e.g., vessel repair and maintenance, vessel-based whale watching, etc.) were predominant in the dry season than in the wet season. This could, therefore, provide an explanation for the higher mean copper concentrations in *Burnupena* soft tissue in the dry season than the wet season. It may also be suggested that extensive upwelling which occurs along the west coast in the summer may remobilize metals such as copper from bottom sediment to surface waters which may become bioavailable for uptake by organisms. It should be noted that comparisons could not be done

for some of the sampling points as the *Burnupena* spp. were not found in either one or both seasons (Figure 4.5).

When comparing the mean zinc concentrations in *Burnupena* soft tissue from sampling points within the harbours between the sampling seasons, GRB2 and GB1 were significantly higher in the dry season than in the wet season (Figure 4.13a and 4.13d). This could be attributed to an increase in vessel-related activities in the dry season than the wet season at GRB2 and GB1. It should be noted that the mean zinc concentrations in *Burnupena* soft tissue at GBR1, HB1 and GB2 were higher in the dry season than the wet season although there were no significant differences (Figure 4.13a, 4.13b and 4.13d, respectively).

When comparing the mean copper concentrations in *Nucella* soft tissue from sampling points within the harbours between the sampling seasons, GRB3, HB3 and GB3 were significantly higher in the dry season than in the wet season (Figure 4.7a, 4.7b and 4.7d). GRB3 was located adjacent to the Port of Cape Town with intense shipping traffic; GB3 was located near to a vessel repair and maintenance facility inside the harbour (Old Harbour) and HB3 was located inside the harbour and adjacent to a vessel repair and maintenance facility. Therefore, increase vessel traffic as well as vessel repair and maintenance activities in the dry season may have accounted for the higher mean copper concentrations in *Nucella* soft tissue. An alternative explanation could be variations in water temperature. For example, the seawater temperature recorded at HB3 was 15.9°C in the dry season and decreased to14.3°C in the wet season (Table 4.1). Environmental factor such as water temperature has been suggested to influence metal accumulation in marine organisms (Orren et al., 1980). Temperature affects metal chemistry in seawater by changing chemical speciation (Byrne et al., 1988; Blust et al., 1994). According to Mubiana & Blust (2007) and Rouane-Hacene et al. (2015), chemical speciation indicates that an increase in temperature generally results in the increase in the concentrations and activities of bioavailable metal forms, hence, enhances uptake. Comparisons could not be done for some of the sampling points as *Nucella* spp. were not found in either one or both seasons (Figure 4.7).

When comparing the mean zinc concentrations in *Nucella* soft tissue from sampling points within the harbours between the sampling seasons, KB1, KB3 and GB3 were significantly higher in the dry season than in the wet season (Figure 4.15c and 4.15d). As already explained, this could be attributed to an increase in vessel repair and maintenance activities taking place at these sampling points in the dry season. Unlike the other sampling points, the mean zinc concentration in *Nucella* soft tissue at HB2 was significantly higher in the wet season than the dry season. As already mentioned, HB2 was located close to a vessel mooring area with predominantly recreational crafts. Therefore, the long residing time of recreational

crafts especially in the wet season (low vessel usage) may result to an increase in the leaching of zinc from AF paints and a zinc-based sacrificial anode into the surrounding environment. This may have accounted for the higher mean zinc concentration in the wet season. Also, high phytoplankton density in the dry season may result to decrease zinc concentration in the water and a low concentration of zinc in the algal cells due to biological dilution (Ravera et al., 2003). This may result in the low concentration of zinc in the diet of *Nucella* spp. (which constitute either barnacles or mussels that feed on phytoplankton), hence lower mean zinc concentration in the dry season. Furthermore, environmental variables such as salinity and temperature which have been recognized as factors that influence metal uptake by molluscs (Frazier, 1976), may contribute to seasonal mean zinc variation at HB2. Higher temperatures may result in an increase in metal uptake, while the increase in salinity may result in a decrease in metal uptake (Maher et al., 2016; Phillips, 1976). However, there was not a considerable difference in the ambient seawater temperatures recorded at HB2 in the dry season and wet season to significantly influence the uptake of zinc by the gastropod (Table 4.1). Also, the salinity of the ambient seawater was not measured which could have been useful in providing further explanation for the higher mean zinc concentration in *Nucella* soft tissue at HB2 in the wet season.

5.4 Metal concentrations in gastropods shells within harbours

The concentrations of metals in the soft tissue of organism reflects current level of contamination of the medium under investigation, while those in the shell may reflect the time-integrated metal contamination of the environment (Huanxin et al., 1999). According to Ravera et al. (2003), this difference is as a result of the metabolic turnover time, which is very slow for the shell and relatively rapid for the soft tissue. To ascertain the potentiality of mollusc's shells as a suitable biomonitor of metal pollution in the marine environment, it is necessary to understand the process of shell secretion and associated metal incorporation. This process starts with the mantle secreting an organic substance, known as the periostracum, which forms an external protective coating on the shell. A crystalline calcium carbonate layer is then deposited against this protein-rich layer. As the mollusc grows, epithelial cells within the mantle cavity accumulate calcium and bicarbonate ions which are then transported through the organism to the extrapallial fluid. The mantle also secretes periostracum material into the extrapallial fluid, which forms the organic matrix for the nucleation of additional calcium carbonate crystals, in the form of either calcite or aragonite or a mixture of both polymorphs (Langston & Bebianno, 1998; Westbroek & de Jong, 1983). The shells of marine molluscs are predominantly composed of this carbonate material (approximately 98%) (Cravo et al., 2004; Palpandi et al., 2010). Metals such as copper and zinc can substitute for the calcium ions in

the calcite or aragonite and thus become incorporated in the calcium carbonate crystals (Tynan et al., 2005). It can be presumed that through this process, any metals found incorporated into the calcium carbonate structure have been taken up from the environment and were actively metabolised by the organism (Tynan et al., 2005). Furthermore, metals can also be absorbed into the skeletal organic matrix or entrapped as a separate mineral phase. According to Bertine & Goldberg (1972), mollusc's shells may also act as a biodeposition site for unwanted chemical species such as metals. It appears likely that molluscs might utilize the deposition process of new shell material to relocate bioaccumulated contaminants (e.g., metals) from the metabolically active soft tissue to the relatively inert shell material (Walsh et al., 1995).

The soft tissue of marine molluscs has been largely accepted as a more efficient accumulators of metals than shells (Rainbow, 1990; Brown & Depledge, 1998). Most studies have reported much higher concentrations of metals such as copper and zinc in gastropods soft tissue than in their shells (Ireland & Wootton, 1977; De Wolf et al., 2001; Cravo & Bebianno, 2005; Edward et al., 2010; Manavi, 2013; Kesavan et al., 2013; Kupekar & Kulkarni, 2014). However, some studies have made use of the calcified shell of molluscs and suggested that shells can provide a more accurate indication of environmental change and pollution; show less variability than an organism's soft tissue and provide a historical record of metal content throughout the organism's lifetime, with this record still preserved after death (Badran, 1998; Palpandi et al., 2010; El-Sorogy et al., 2013). The mollusc's shell composition is strongly related to the chemical mineralogy which includes metals accumulated from the environment. Therefore metal concentrations in the shells follow metal concentrations in the environment (Carell et al., 1987), and could be valuable in monitoring metal pollution in the marine environment.

The results of this study revealed copper and zinc bioaccumulation in the shells of the two gastropods (*Burnupena* spp. and *Nucella* spp.) collected at different sampling points in the harbours and the two reference sites.

5.4.1 Comparisons in the dry season

The mean copper concentrations (mg/kg DW) in *Burnupena* shells at sampling points within the four harbours in the dry season ranged from 3.05±2.22 to 15.32±1.02 (Table 4.9). There were no significant differences in the mean copper concentrations in the *Burnupena* shells between the sampling points and the reference sites in GRB and HB. The results showed that the mean copper concentration in *Burnupena* shells at GB2 was significantly higher than at GB1, BB_{MPA} and CGH_{MPA} (Table 4.9). This could be ascribed to the leaching of copper from moored leisure vessels which were close to GB2. Another possible reason could be the

difference in metal handling strategies of the individual gastropods (Edward et al., 2010). These strategies result from the net difference between rates of uptake and excretion of metals which is caused by the change in body tissue associated with environmental parameters. no data were available for GRB3, HB2, HB3, GB3 and KB as the *Burnupena* spp. were not found at the time of sampling in the dry season (Table 3.1 and 4.9).

The mean zinc concentrations (mg/kg DW in *Burnupena* shells at sampling points within the four harbours in the dry season ranged from 0.31±0.69 to 57.99±5.79 (Table 4.21). The results showed that there were no significant differences in the mean zinc concentrations in *Burnupena* shells between sampling points and the reference sites in the harbours, except in GB. Like for sediment, the mean zinc concentration in *Burnupena* shells at GB2 was significantly higher than at CGH_{MPA} (Table 4.21). This could be ascribed to the leaching of zinc from AF paints and zinc-based sacrificial anodes associated with moored leisure vessels which were close to GB2.

The mean copper concentrations (mg/kg DW) in *Nucella* shells at sampling points within the four harbours in the dry season ranged from 10.82±8.58 to 314.16±15.26 (Table 4.12). The results revealed that, like for sediment (at GRB3) and *Nucella* soft tissue, the mean copper concentrations in *Nucella* shells at GRB1 and GRB3 were significantly higher than that at BB_{MPA}. This could be attributed to vessel-related activities taking place at Granger Bay Marina yacht basin and the Port of Cape Town as well as the Green Point sewage outfall. The mean copper concentration in *Nucella* shells at GRB3 was significantly higher than at GRB1. GRB3 was closer to the Port of Cape Town (intense vessel traffic) than GRB1 and this could have accounted for the higher mean copper concentration in the *Nucella* shells at GRB3. The mean copper concentrations in *Nucella* shells at HB1, HB2 and HB3 were significantly higher than at BB_{MPA}. These results show a similar pattern with the results recorded for *Nucella* soft tissue. These higher mean copper concentrations in *Nucella* shells could be attributed to the intense vessel-related activities taking place close to these sampling points. The mean concentration of copper in the *Nucella* shells at HB2 and HB3 were significantly higher than at HB1. A similar pattern was observed for *Nucella* soft tissue between HB2 and HB1. HB2 was located inside the harbour with intense vessel traffic as well as high vessel mooring time. This may have accounted for the higher mean copper concentration found in *Nucella* shells at HB2. HB3 was located inside the harbour and near to a vessel repair and maintenance facility and this could explain the higher mean copper concentration found in the *Nucella* shells. Like for *Nucella* soft tissue, the mean copper concentrations in *Nucella* shells at KB1 and KB3 were significantly higher than at KB2 and BB_{MPA}. The presence of a vessel repair and maintenance facility in the harbour (close to KB1 and KB3) may have accounted for the higher mean copper

concentration found in *Nucella* shells at KB1 and KB3. The results showed that, like for sediment and *Nucella* soft tissue, the mean copper concentration in *Nucella* shells at KB1 was significantly higher than that at KB2 and KB3. KB1 was closer to the slipway as well as the vessel repair and maintenance facility than KB2 and KB3 and this might have accounted for higher mean copper concentration in *Nucella* shells. It is interesting to note that there was also a pattern of decreasing mean copper concentration in *Nucella* shells with increasing distance from the potential contamination source. Like for *Nucella* soft tissue, the mean copper concentration in *Nucella* shells at GB3 was significantly higher than at BB_{MPA}. Copper leachates from residing and moving vessels as well as from antifouling paints fragments resulting from vessel repair and maintenance may have accounted for the higher mean copper concentration in *Nucella* shells at GB3. *Nucella* spp. were not found at GRB2, GB21 and GB2 at time of sampling in the dry season (Table 3.1 and 4.12). Copper was not detected in *Nucella* shells from CGH_{MPA}.

The mean zinc concentrations (mg/kg DW) in *Nucella* shells at sampling points within the four harbours in the dry season ranged from ND to 122.46±65.00 (Table 4.24). The results revealed that zinc was undetectable in *Nucella* shells in some of the sampling points in the harbours and the reference sites, except at GRB3, KB1, HB1, HB2 and HB3. The results revealed that, like for *Nucella* soft tissue, the mean zinc concentration in *Nucella* shells at HB2 and HB3 were significantly higher than that at HB1. HB2 and HB3 were located close to areas of high vessel density (vessel mooring area) and vessel repair and maintenance activities, respectively. This might have accounted for the significantly higher mean zinc concentrations in *Nucella* shells at HB2 and HB3.

5.4.2 Comparisons in the wet season

The mean copper concentrations (mg/kg DW) in *Burnupena* shells at sampling points within the four harbours in the wet season ranged from 1.96±0.66 to 6.23±2.19 (Table 4.9). There were no significant differences between sampling points in each harbour for the wet season. The mean copper concentrations at GRB1, GRB2, HB1, and KB2 were significantly higher than at BB_{MPA}. Similar patterns were observed in sediment (at GRB1, GRB2, KB2) and *Burnupena* soft tissue (at HB1 and KB2). These higher mean copper concentrations in *Burnupena* shells could mainly be attributed to the increase in surface and stormwater runoff from impervious surfaces (roads and parking lots) as well as from the residential areas due to the increase in rainfall during the wet season (Table 4.3). Like for *Burnupena* soft tissue (at GB), the mean copper concentrations in *Burnupena* shells at GB1, GB2 and GB3 were significantly higher than that at BB_{MPA} and CGH_{MPA}. These higher mean copper concentrations

in *Burnupena* shells could be due to the increase in surface and stormwater runoff (from hard surfaces and the residential areas) in the wet season when rainfall was high. Another possible explanation could be the increase in copper leachates from vessel hulls in the wet season when there is an increase in vessel density due to increased mooring time of some vessels (e.g., recreational vessels).

The mean zinc concentrations (mg/kg DW) in *Burnupena* shells at sampling points within the four harbours in the wet season ranged from 0.67±1.06 to 51.18±101.74 (Table 4.21). Remarkably, the mean zinc concentrations in *Burnupena* shells at the reference sites were significantly higher than that at GRB2, HB1 and KB2. This could be attributed to surface and stormwater runoff from the surrounding areas as well as from lithogenic sources. It should be noted that although there is little or no vessel-related activities taking place at the reference sites (MPA which are presumed to be pristine), other anthropogenic activities such as residential development and ecotourism in these areas may contribute to the influx contaminants such as zinc. Alternatively, the difference in metal handling strategies of the *Burnupena* spp. at different sampling points may also have contributed to the variation in the mean zinc concentrations. The results showed that, like for *Burnupena* soft tissue, the mean zinc concentration in *Burnupena* shell at GRB1 was significantly higher than that at GRB2. This could be ascribed to surface and stormwater runoff as well as wastewater discharges from the Green Point and Camps Bay outfalls. GRB1 was closer to stormwater outlets as well as the outfalls than GRB2.

The mean copper concentrations (mg/kg DW) in *Nucella* shells at sampling points within the four harbours in the wet season ranged from 3.81±1.41 to 246.74±190.94 (Table 4.12). The results revealed that, like for *Nucella* soft tissue, the mean copper concentrations at GRB3, HB2, KB1 and KB3 were significantly higher than at BB_{MPA} and CGH_{MPA}. This could largely be attributed to vessel-related activities (e.g., vessel repair and maintenance, vessel traffic, moorings) taking place at these sampling points. Like for *Nucella* soft tissue, the mean copper concentrations in *Nucella* shells at HB1, HB3, KB2 and GB3 were significantly higher than at BB_{MPA}. This could be ascribed to riverine inputs particularly at HB1 and GB3 as well as surface and stormwater runoff that drains into the harbours. KB1 and KB3 had significantly higher mean copper concentrations than KB2. A similar pattern was observed for *Nucella* soft tissue between KB1 and KB2. These results also showed a similar pattern with the results for the dry season and may suggest that the sources of contamination are the same. Again, it is interesting to note that a similar pattern of decreasing mean copper concentrations in *Nucella* shell with increasing distance from potential sources of contamination was observed.

The mean zinc concentrations (mg/kg DW) in *Nucella* shells at sampling points within the four harbours in the wet season ranged from 6.09±4.34 to 182.65±89.72 (Table 4.24). The results showed that the mean zinc concentrations in *Nucella* shells at HB1, HB2, HB3, KB1 and KB3 were significantly higher than at the reference sites (Table 4.24). Similar patterns were observed for *Nucella* soft tissue between HB1, HB2, HB2, KB1 and the reference sites. These higher mean zinc concentrations in *Nucella* shells could be attributed to vessel-related activities taking place near these sampling points. The results showed that, like for *Nucella* soft tissue, the mean zinc concentration in *Nucella* shells at HB2 was significantly higher than that at HB3. This could be ascribed to increased leaching of zinc in AF paints and zinc-based sacrificial anodes from moored vessel hulls in the wet season when vessel usage is low. the mean zinc concentrations in *Nucella* shells at KB1 and KB3 were significantly higher than that at KB2. A similar pattern was observed for *Nucella* soft tissue between KB1 and KB2. These higher mean zinc concentrations in *Nucella* shells could be attributed to vessel repair and maintenance activities taking place close to KB1 and KB2. Again, there was a gradient in mean zinc concentrations in *Nucella* shells at sampling points in KB with increasing distance from potential contamination sources.

5.4.3 Comparisons between the dry and wet season

The results showed no significant seasonal differences in the mean copper concentrations recorded in *Burnupena* shells at sampling points in the four harbours, except at GB2 (Figure 4.6). The mean concentration of copper in *Burnupena* shells at GB2 was significantly higher in the dry season than the wet season (Figure 4.6d). This could be attributed to the increase in the leaching of copper from vessel hulls during the dry season when vessel-related activities are higher in the harbour. It is worth noting that during the two sampling seasons, it was observed that the harbours were busier in the dry season than wet season in terms of vessel-related activities.

The mean zinc concentrations recorded in *Burnupena* shells at sampling points in the four harbours, except at GB2 showed no significant seasonal differences (Figure 4.14). The mean zinc concentration in *Burnupena* shells at GB2 was significantly higher in the dry season than the wet season (Figure 4.14d). This could be attributed to the increase in the release of zinc from AF paints and zinc-based sacrificial anodes on vessel bottoms in the dry season associated with an increase in vessel-related activities.

The mean concentration of copper in *Nucella* shells at HB2, HB3, KB3 and GB3 were significantly higher in the dry season than the wet season (Figure 4.8b, 4.8c, and 4.8d). These sampling points were near to areas with vessel-related activities such as moorings, vessel

repair and maintenance facilities as well as slipways in their respective harbours. Therefore, it may be suggested that the higher mean copper concentrations found in *Nucella* shells in the dry season might have resulted from the increase in vessel-related activities.

The mean zinc concentrations in *Nucella* shells at HB2 and HB3 were significantly higher in the dry season than the wet season (Figure 4.16b). As already mentioned, the increase in vessel-related activities near these sampling points in the dry season may account for the significantly higher mean zinc concentrations. Remarkably, the mean zinc concentration in *Nucella* shells at KB1 was significantly higher in the wet season than the dry season (Figure 4.16c). This could be attributed to the surface and stormwater runoff from the surrounding areas (e.g. parking lots, main road, railway line, residential area, etc.) in the wet season associated with an increase in rainfall (Table 4.3). This may have been exacerbated by the constant vessel repair and maintenance activities taking place close to this sampling point.

5.4.4 Comparisons between soft tissue and shells

Comparisons between the mean concentrations of copper and zinc in soft tissue and shells of the *Burnupena* spp. and *Nucella* spp. at sampling points in the harbours for the dry and wet season showed a similar trend. As mentioned previously, it has been recognized that mollusc soft tissue bioaccumulate higher concentrations of copper and zinc than the shells. In this study, the results indicated that the soft tissue of the two gastropods had higher mean concentrations of copper and zinc than their shells. The higher mean concentration of copper and zinc found in soft tissue rather than shells was in agreement with other studies elsewhere (e.g., Bertine & Goldberg, 1972; Ireland & Wootton, 1977; Amin et al., 2006; Yap & Cheng, 2009; Kupekar et al., 2012; Palpandi & Kessavan, 2012; Yap & Cheng, 2013; Kupekar & Kulkarni, 2014). The differential accumulation of copper and zinc in the soft tissue and shells showed that the metal accumulation and the metal binding capabilities of soft tissue and shells vary.

The mean copper concentrations found in the soft tissue of *Burnupena* spp. at sampling points (GRB1, GRB2, HB1, GB1 and GB2) and *Nucella* spp. at sampling points (GRB1, GRB3, HB1, HB2, KB1, KB2 and GB3) were significantly higher than in the shells during the dry season (Table 4.10 and 4.13). Similarly, the mean copper concentrations in the soft tissue of *Burnupena* spp. at sampling points (GRB1, GRB2, HB1, KB2, GB1, GB2 and GB3) and *Nucella* spp. at sampling points (GRB3, HB1, HB2, HB3, KB1 and GB3) were significantly higher than in the shells during the wet season (Table 4.10 and Table 4.13). These results could be related to the differences in the accumulation of the soft tissue and shells of the molluscs (Yap et al., 2008). In the soft tissue of molluscs such as gastropods, copper may be

bound to metallothioneins (Canli et al., 1997; Dallinger et al., 1997; Anna et al., 2011) which play an important role in copper detoxification while in the shells copper may be fixed in the crystalline lattices of the carbonate structures of the shells (Watson et al., 1995). Copper is an essential and potentially toxic metal. It plays important roles in the growth and cell metabolism of most aquatic organisms, including molluscs. Gastropods need copper as an essential constituent for their respiratory pigment hemocyanin (Gundacker, 2000). Therefore, the higher mean copper concentrations found in the soft tissue than in shells of the two gastropods could be attributed to the role of copper as a component of metabolically essential biomolecules including enzymes, metalloenzymes and respiratory pigments. (Catsiki et al., 1994; Langston et al., 1998; Rainbow, 1997).

The mean zinc concentrations in the soft tissue of *Burnupena* spp. at sampling points (GRB1, GRB2, HB1, GB1 and GB2) and *Nucella* spp. at sampling points (GRB3, HB1, HB2, HB3 and KB1) were significantly higher than in the shells during the dry season (Table 4.22 and 4.25). Likewise, the mean zinc concentrations in the soft tissue of *Burnupena* spp. at sampling points (GRB1, GRB2, HB1, KB2, GB1, GB2 and GB3) and *Nucella* spp. at sampling points (GRB3, HB1, HB, HB3, KB1, KB2 KB3 and GB3) were significantly higher than in the shells during the wet season (Table 4.22 and 4.25). The significantly higher mean zinc concentrations found in soft tissue than in the shells of the two gastropods was in agreement with the findings of Cravo et al. (2004) in the south coast of Portugal, who reported that zinc levels were consistently higher in the soft tissue than shells of the gastropod *Patella aspera*. Similar results were also reported for the gastropods *Nerita lineata* (Amin et al., 2006) and *Nerita oryzarum* (Ambekar et al., 2016). Like copper, higher concentrations of zinc in the gastropods soft tissue than the shells could be attributed to its metabolic requirement where it acts as a cofactor in over 200 enzymatic processes with important biological functions (Li et al., 2009; Nashaat et al., 2016).

5.5 Relationship between metal concentrations in seawater and the *Nucella* spp.

To determine if there was a causal relationship between the mean copper and zinc concentrations in seawater and the subsequent accumulation in the *Nucella* spp., the datasets from sampling points in each harbour and reference sites for each season were subjected to the Spearman's rank order correlation analyses (Table 4.14, 4.15, 4.26 and 4.27).

5.5.1 Correlations in the dry season

The results revealed that there were no significant correlations between the mean copper and zinc concentrations in seawater and those in the *Nucella* spp. (soft tissue and shells) in the harbours and reference sites during the dry season (Table 4.14, 4.15, 4.26 and 4.27). It was shown that copper and zinc contents of soft tissue and shell of *Nucella* spp. may not be directly influenced by those of the ambient seawater. This may be indicative of the fact that changes in copper and zinc loading in seawater are not the only factors that influence the bioavailability of these metals to the *Nucella* spp. This is in line with Lau et al. (1998) who suggested the factors which may affect bioaccumulation of metals in aquatic organisms are biological factors such as mode of feeding, age, sex, size and growth rate of the organism (Pentreath, 1976; Mance, 1987; Abdel Gawad, 2018), as well as physicochemical parameters such as pH, salinity, oxygen concentrations and temperature (Besser et al., 1996). As already mentioned in the literature, the *Nucella* spp. are carnivorous gastropods, therefore it could be suggested the accumulated copper and zinc in the *Nucella* spp. (soft tissue and shell) may come from their diet (mostly bivalves or barnacles attached on rocks).

5.5.2 Correlations in the wet season

The results showed that there was a significant positive correlation between the mean copper concentrations in seawater and those in the soft tissue of *Nucella* spp. in KB during the wet season (Table 4.14). The positive correlation between the mean copper concentration in the seawater and that in the soft tissue of the *Nucella* spp. in KB may reflect the high level of copper contamination in the seawater or in the trophic chain. This is evident, as vessel-related activities were predominant in KB which might have been exacerbated by the inflow of surface and stormwater runoff during the wet season. There were no significant correlations in mean copper concentrations between seawater and soft tissue of *Nucella* spp. in GRB, HB, GB and the reference sites, as well as between seawater and shells of *Nucella* spp. in the four harbours and reference sites (Table 4.14 and 4.15). Similarly, there were no significant correlations between mean zinc concentrations in seawater and those in the *Nucella* spp. (soft tissue and shell) in the harbours and reference sites during the wet season (Table 4.26 and 4.27). As

previously explained, copper and zinc loading in the ambient seawater may not directly influence the bioaccumulation in the soft tissue or shell of the *Nucella* spp.

5.6 Relationship between metal concentrations in sediment and the *Nucella* spp.

To establish the relationship between mean copper and zinc concentrations in ambient sediment and the bioaccumulation in *Nucella* spp., a correlation matrix was calculated for the concentration of these metals in the sediment samples and those in the soft tissue and shell of *Nucella* spp (Table 4.14, 4.15, 4.26 and 4.27).

5.6.1 Correlations in the dry season

The results showed that there was a significant negative correlation between mean copper concentrations in the sediment and those in the soft tissue of *Nucella* spp. in GB, whereas significant positive correlations were found between zinc concentrations in the sediment and those in the soft tissue and shell of *Nucella* spp in GRB and KB during the dry season (Table 4.14, 4.15, 4.26 and 4.27). The negative correlation in mean copper concentrations between the sediment and those in the soft tissue of *Nucella* spp. in GB could be due to the complexity of the processes and factors such as pH, redox potential, temperature, hardness, nutrients concentration and total organic content that controls the scavenging or release of metals by the ambient sediment (Tessier & Campbell, 1987; Boudou & Ribeyre, 1997). Nevertheless, it may be suggested that natural (e.g., upwelling) and anthropogenic (e.g., vessel traffic) disturbances in the harbour may result in the remobilization of sediment-bound copper to the overlying seawater due to changes in physicochemical conditions (Eggleton & Thomas, 2004; Newman & Watling, 2007; Huang et al., 2012). This may lead to a decrease in sediment-bound copper and an increase in dissolved copper in seawater which may become bioavailable to the *Nucella* spp through the food chain and to a lesser extent by direct absorption from seawater. As already mentioned, *Nucella* spp. feeds on bivalves such as mussels which are filter feeders and can uptake copper from the re-suspended sediments in seawater. It may, therefore, be suggested that *Nucella* spp which mainly feeds on bivalves may accumulate the metals from the bivalves. To this effect, the relationship in metal concentrations between the predator *Nucella* spp. and the prey organism (e.g., barnacles and mussels) could be an interesting aspect to be considered in the future. The positive correlations between mean zinc concentrations in the sediment and those in the soft tissue and shells of the *Nucella* spp. most likely reflect the constant inputs of zinc to the organism's surroundings. As already mentioned in the literature, GRB and KB were harbours located close to coastal development with several anthropogenic inputs which may represent the main sources of zinc into these harbours.

5.6.2 Correlations in the wet season

The results showed that there were significant positive correlations between mean copper and zinc concentrations in the sediment and those of the soft tissue and shell of *Nucella* spp. in KB during the wet season (Table 4.14, 4.15, 4.26 and 4.27). Also, a significant positive correlation was found between mean copper concentrations in sediment and those of the shell of *Nucella* spp. in HB (Table 4.15). The results agree with that of other studies that also found a relationship between metal concentrations in sediment and those in molluscs (Phillips & Yim, 1981; Langston, 1986). As previously explained, the positive correlations in mean copper and zinc concentrations between the sediment and those of the soft tissue and/or shell of *Nucella* spp. may indicate that metal concentrations (copper and zinc) in the *Nucella* spp. (soft tissue and/or shell) generally followed the level of contamination of their immediate environment (particularly that found in the sediment) (Rzymski et al., 2014). The positive correlation between mean zinc concentrations in the sediment and those of the shell of *Nucella* spp in HB could be explained by the fact that the shell may be an important part of the organism's detoxification mechanism (Jantataeme et al., 1996). At first, metals may bioaccumulate in the soft tissue of the gastropod before part of the metabolized metals are biodeposited in the shell (Yap et al., 2003). On the other hand, there were no significant correlations in the mean copper and zinc concentrations between the sediment and those of the soft tissue of *Nucella* spp. in GRB, HB, GB and the reference sites (Table 4.14 and 4.26). Also, no significant correlations were found between mean copper concentrations in the sediment and the concentrations in the shells of *Nucella* spp in GRB, GB and the reference sites (Table 4.15). The mean zinc concentrations between sediment and those of the shell of *Nucella* spp. in GRB, HB, GB and the reference sites, revealed no significant correlations (Table 4.27). The insignificant correlations in the mean copper and zinc concentrations between the sediment and those of the soft tissue and/or shell of *Nucella* spp. indicated that copper and zinc concentrations in the sediment may not be directly or solely reflected in the tissue or the shell of *Nucella* spp. It may, therefore, be suggested that changes in the sediment metal (copper and zinc) loading in these sampling sites was not the only factor that influenced metal concentration in the *Nucella* spp. The release of metal such as copper and zinc from sediment and the subsequent bioaccumulation in organisms is controlled by different processes (e.g., metal speciation, metal-metal interaction, the control exerted by oxides of iron/manganese and organics to which metals are preferentially bound, etc.) and the many physicochemical (e.g., pH, salinity, oxygen concentrations, temperature, etc.) and biological (e.g., diet, sex, size, reproductive cycle, etc.) factors (Rainbow, 1997; Hamed & Emara, 2006; Azizi et al., 2018) .

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The following section summarises the key findings of this study with respect to the research objectives along with recommendations for future research.

1. ***To determine copper and zinc concentrations in surface water and sediment in selected harbours within the Cape Town Metropole***

The results showed that there was a generally insignificant difference in mean copper and zinc concentrations in seawater between sampling points in the harbours during the dry season and wet season. Remarkably, BB_{MPA} which was a reference site recorded the highest mean copper and zinc concentrations in seawater. This could be attributed to (i) the oceanographic regime of BB: MPA which is influenced by both the strong-flowing Agulhas and the cold Benguela upwelling system of the west coast (ii) increase surface and stormwater runoff in the wet season. It is noteworthy that copper and zinc concentrations in seawater were generally not lower in the reference sites as presumed. It may, therefore, be suggested that coastal dynamics, long-distance transport and the persistent nature of metals in the marine environment may account for higher copper and zinc concentrations in the reference sites. Although there were insignificant differences in the mean copper and zinc concentrations between sampling points in the harbours, observations revealed that mean copper and zinc concentrations were generally higher at sampling points close to areas of restricted water exchange, high vessel-related activities, as well as urban development. The mean copper and zinc concentrations in seawater generally tended to decrease with increasing distance from contamination sources.

The comparisons of the pooled mean copper and zinc concentrations in seawater between harbours in the dry season and wet season revealed some significant differences. This could be attributed to the intensity of vessel-related activities in the harbours. It should be noted that there are no existing data on vessel usage for the harbours under study; hence, the distinction in vessel usage is based on observations during sampling occasions. When comparing between the harbours, the highest mean copper and zinc concentrations in seawater was recorded at HB in the dry season. The mean copper and zinc concentrations in seawater at some of the sampling points were well above the SAWQGs for copper and zinc in coastal marine waters. Also, the pooled mean copper and zinc concentrations in seawater recorded in some of the harbours exceeded the guidelines. This could have deleterious effects on the

marine organism and associated non-aquatic life. On a seasonal scale, the mean copper and zinc concentrations in seawater from the harbours were generally higher in the dry season than the wet season. This could be associated to the increase in the leaching of AF paints due to intense vessel-related activities (vessel traffic, moorings, vessel repair and maintenance, etc.) in the dry season (Matthiessen et al.,1999).

Sediments represent the most important reservoir of metals in coastal waters due to their strong metal-adsorbing capacity (Pan & Wang, 2012). The significantly higher mean copper and zinc concentrations in sediment at some sampling points in the harbours could be attributed to the presence of antifouling particles (which were clearly visible during sample digestion) associated with vessel repair and maintenance activities as well as the poor rate of water circulation at these sampling points. It is becoming more and more evident that AF paint residues generated during vessel hull maintenance are subject to considerable dispersion in the aquatic and terrestrial environments. Several studies have reported that spent AF paint fragments contain high concentrations of copper and zinc (e.g., Singh & Turner, 2009; Turner, 2010; Parks et al., 2010; Rees et al., 2014; Lagerström et al., 2016). KB1 recorded the highest mean copper and zinc concentrations in sediment which could be attributed to high vessel traffic as well as vessel repair and maintenance activity taking place at this sampling point. The mean copper and zinc concentrations in the sediment samples assessed were compared with the BCLME-SQGs in southern Africa. According to the BCLME-SQGs, mean copper and zinc concentrations in sediment between TEL and PEL are indicative of moderate pollution that may pose negative ecological risks. The mean copper and zinc concentrations above PEL are suggestive of serious pollution and may be associated with considerable ecotoxicity. The generally higher mean copper and zinc concentrations in the dry season than the wet season could also be attributed to the increase in AF paint leachates due to intense vessel-related activities during the dry season.

It can, therefore, be established that this study revealed concentration levels of copper and zinc in seawater and sediment with some sampling points in the harbours experiencing elevated concentrations which appear to exceed water and sediment quality guidelines for marine life.

2. ***To determine the levels of copper and zinc bioaccumulation in the two gastropods from the harbours under study.***

The multifaceted interrelationship between organisms and ecosystem makes the results of ecotoxicological studies difficult to evaluate the impact of metals in situ; however, it aids the assessment of long-term effects of metals on organisms. In this study soft tissue and shells of

Burnupena spp. and *Nucella* spp. were analysed for copper and zinc contamination in the harbours.

Several studies have revealed that gastropods can accumulate in their soft tissue substantial amount of metals through the body wall and diet (Blackmore & Morton, 2001; Wang, 2002; Proum et al., 2016). The mean copper and zinc concentrations recorded in *Burnupena* and *Nucella* soft tissue showed significant variations between some sampling points in the harbours and the reference sites. This may be attributed to proximity to sources of contamination, rate of water exchange, metal handling strategies (storage and detoxification strategies) of the gastropods (Edward et al., 2010), as well as environmental factors (e.g., pH, temperature, salinity, nutrition, etc.). The highest mean copper and zinc concentrations in the soft tissue in this study were recorded in the *Nucella* spp. at GB3. This may likely be attributed to the leaching of AF paints associated with intense vessel traffic, high vessel density (mooring) as well as vessel repair and maintenance. On a seasonal scale, the results showed that the mean copper and zinc concentrations in the two gastropods soft tissue were generally higher in the dry season than the wet season. This could be attributed to the increase in vessel-related activities (e.g., high vessel traffic, vessel mooring, vessel repair and maintenance activities, etc.) during the dry season.

Mantle tissue of gastropods is the site of shell deposition (Walsh et al., 1995). Metal ions (such as copper and zinc ions) may become incorporated in the calcium carbonate crystals of the shell by substituting calcium ions or by association with the organic component of the shell (Al-Dabbas et al., 1984; Tynan et al., 2005). Shells may also act as a biodeposition site of unwanted chemical species (such as metals) from the metabolically active soft tissue to the inactive shell (Walsh et al., 1995; Yap et al., 2003). Some studies have made use of the calcified shell of molluscs and suggested that shells can provide a more accurate indication of environmental change and pollution. As already mentioned, mollusc's shell composition is strongly associated with the chemical mineralogy which comprises of metals accumulated from the environment. Therefore, metal concentrations in the shells follow the metal concentrations in their environment. The result of this study showed that there were generally no significant variations in the mean metal concentrations recorded in the *Burnepena* and *Nucella* shells between the sampling points in the harbours for the two seasons, particularly for copper. However, there were some significant variations in mean copper and zinc concentrations recorded in the two gastropod shells between the sampling points and the reference sites. The highest mean copper and zinc concentrations in the shells in this study were recorded in the *Nucella* spp. at KB1 in the dry season and wet season. This could be ascribed to the intense vessel repair and maintenance activity taking place at KB1. With respect to seasonal variation, the results revealed significant seasonal variations in the mean

copper and zinc concentrations recorded in the *Burnupena* and *Nucella* shells. The mean copper and zinc concentrations recorded in shells of these gastropods were generally higher in the dry season than the wet season. This could be attributed to the intense vessel-related activities in the dry season.

The results of this study revealed that there were significant variations in metal concentrations between the soft tissue and shell. The mean copper and zinc concentrations recorded in the soft tissue of the *Burnepena* spp. and *Nucella* spp. were significantly higher than that in the shell. This differential accumulation may be due to both the metabolism and the pathway of metals in the soft tissue, which is very different from those in the shell. This finding is in agreement with the results of many studies that showed that soft tissue of molluscs accumulate higher concentrations of copper and zinc than shells (e.g., Bertine & Goldberg, 1972; Ireland & Wootton, 1977; Amin et al., 2006; Yap & Cheng, 2009; Kupekar et al., 2012; Yap & Cheng 2013; Kupekar & Kulkarni, 2014).

In conclusion, the results of this study showed that the *Burnupena* spp. and *Nucella* spp. differentially bioaccumulate copper and zinc in the soft tissue and shell.

3. ***To determine the suitability of the two gastropods for use as biomonitors of metal contamination***

The accumulated metal concentrations in a biomonitor reflect directly the time-integrated metal bioavailability and contamination of the area under investigation. This can be explained by the fact that biomonitors such as molluscs bioaccumulate metals in their tissue which is directly proportional to the degree of environmental contamination from water, suspended particles, sediment and through food chains (Luoma, 1983; Blackmore, 2001). It should be noted that the two gastropods (*Burnupena* spp. and *Nucella* spp.) used in this study are carnivorous (scavenging and predatory, respectively) and the exposure route to metals (copper and zinc) is through their diet (e.g. barnacles, mussels, etc.) and to a limited extent through absorption from the water column. The results of this study revealed that *Burnupena* spp. and *Nucella* spp. recorded higher mean copper and zinc concentrations at supposed contaminated sampling points in response to exposure to these metals, indicating that they are net accumulators of metals. Both gastropods are therefore capable of reflecting differences in copper and zinc concentrations between environments. Therefore, adding to having the general characteristics of a good biomonitor as outlined in section 2.4.3.1, this study has shown that the two gastropods can be considered as possible biomonitors of metal contamination. However, further ecotoxicological studies with the aim of fully understanding

the accumulation patterns of these gastropods and to assess their robustness for use in routine biomonitoring are required.

4. ***To determine whether there is a causal relationship between copper and zinc contents in the gastropods and the concentrations in water and sediment collected from the harbours.***

The correlation analyses revealed that there was generally no causal relationship between the concentrations of copper and zinc in the *Nucella* spp. (soft tissue and shell) and the concentrations in seawater and sediment in the harbours and reference sites although some distinct trends were observed. It was shown that the concentrations of copper and zinc in the soft tissue or shell of *Nucella* spp. may not be directly affected by those of the ambient seawater and sediment. It may, therefore, be presumed that the changes in copper and zinc loading in the ambient seawater and sediment were not the only factors that influenced the level of bioavailability of these metals to the *Nucella* spp. Therefore, it is possible that the bioaccumulation of copper and zinc in the soft tissue or shell of *Nucella* spp. may have been influenced by many physicochemical and biological parameters. Dietary uptake of metals has been increasingly recognized as an important exposure route of metal bioaccumulation in gastropods (Wang & Ke, 2002; Wang, 2002; Blackmore, 2000; Khan et al., 2013; Bordbar et al., 2015), and it has been proven that gastropods accumulate metals mainly from their diet (bivalves and barnacles). Since the results of this study reveal that there were generally no direct relationships between copper and zinc contents in the *Nucella* spp. and the concentrations in seawater and sediment, it could, therefore, be suggested that the *Nucella* spp. are exposed to these metals through their diet. However, this suggestion needs to be further investigated.

In conclusion, it is often difficult to clearly separate sources of metals (Cu and Zn) in the marine environment as these sources are multifarious. However, based on the findings of this study, it is conceivable that the predominant sources of copper and zinc contamination in the harbours are from vessel-related activities (such as vessel traffic, vessel moorings and vessel repair and maintenance procedures) associated with the use of metal-based antifouling paints on vessel hulls. This assertion is consistent with other findings worldwide (e.g., Young et al., 1979; Matthiessen et al.,1999; Hall & Anderson, 1999; Biggs & D'Anna, 2012). A further possible source of zinc in harbour waters is sacrificial anodes that are attached to vessel bottoms and other submerged metal structures (Bird et al., 1996). Other anthropogenic diffuse sources of copper and zinc include agricultural land run-off (through the rivers that discharge

into the harbours), untreated and treated sewage (from informal settlements and Wastewater Treatment Works, respectively), surface and stormwater runoff, marine outfalls and industrial discharges (Mdzeke, 2004). It should be noted that the semi-enclosed nature of these harbours makes it vulnerable to the effects of these metal sources which would disperse extremely slowly due to restricted tidal exchange. Hence, a significant part of the contaminant load remains in the harbour area.

6.2 LIMITATIONS AND RECOMMENDATIONS

6.2.1 Limitations

- Additional environmental parameters such as salinity, dissolved oxygen concentration, and suspended particle matter, should be taken into consideration in assessing the metal levels in the abiotic and biotic matrices. These physicochemical parameters may provide useful data for the elucidation of metal concentrations in seawater, sediment as well as metal bioavailability and uptake in the organisms.
- The concentration of metals in molluscs depends on the accumulation strategies adopted by each species for each metal. Therefore, knowledge of the biology/physiology (e.g., sex, reproductive cycles, diet, etc.) of the organisms, an essential requirement for a biomonitor must be known so that causes of variation other than metal exposure can be considered.
- A prey organism (e.g., mussels or barnacles) should be included in future studies to ascertain the route of metal uptake by the gastropods.
- The identification of the precise species for use as a potential biomonitor is vital, not only for robustness within a biomonitoring programme but also for comparisons with other studies worldwide.

6.2.2 Recommendations

- Further studies on the bioavailability and toxicity of copper and zinc by means of, for example, metallothionein measurements as biomarker in the two gastropods should be done, to ascertain their suitability as biomonitors of metal contamination. Metallothionein is a cysteine-rich, metal-binding protein induced in direct response to metal contamination. It is involved in sequestering and in so doing detoxifies metals such as copper and zinc, and levels of this protein thus increase in organisms which are exposed to a surplus of metals (like copper and zinc).

- Based on the findings in this study, it is evident that copper and zinc inputs (predominantly from AF paints) at some of the sampling points exceeded water and sediment quality guidelines; therefore, management strategies are needed to monitor copper and zinc levels and their impact on biota in the harbours. Drawing from the TBT experience, policies monitoring studies should be developed to regulate biocides to avoid harmful effects to the marine environment.
- The impact of residual AF paints at vessel repair and maintenance sites should be recognised as a significant, long-term environmental problem.
- Although the data obtained in this study showed that the predominant sources of copper and zinc in the harbours may be attributed to vessel-related activities, other diffuse sources such as urban stormwater runoff, riverine inputs, sewage and industrial effluents may also have contributed sizable amounts and cannot be ignored. Therefore, further research should be carried out to better distinguish the sources of metals contamination in the harbours.

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APPENDICES

Appendix A

Figure A 1: Granger Bay Harbour (GRB)

Figure A 2: Hout Bay Harbour (HB)

Figure A 18: Kalk Bay Harbour

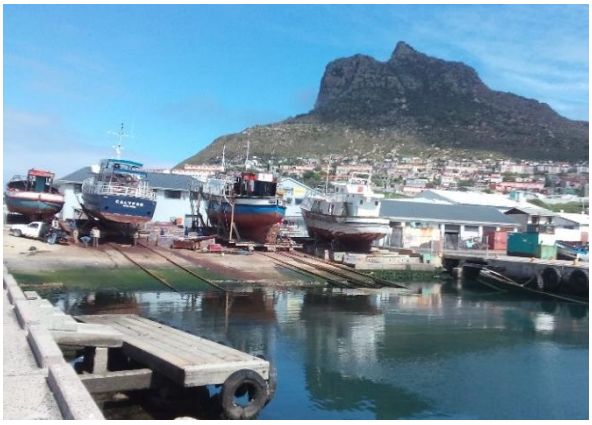
Figure A 34: Gordon's Bay Harbour (GB) [(i) Harbour Island and (ii) Old Harbour]

Figure A 50: Betty's Bay Marine Protected Area (BB:MPA)

Figure A 66: Cape of Good Hope Restricted Zone (CGH:MPA)

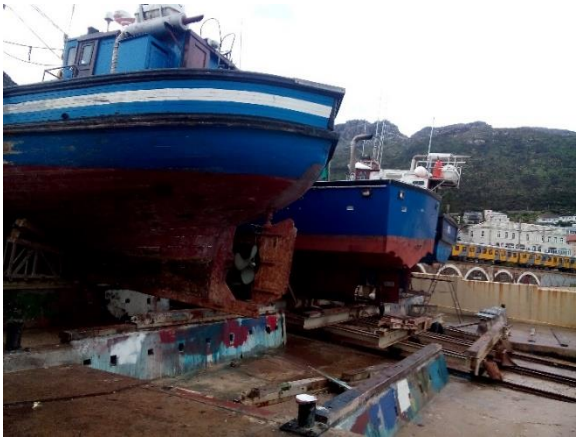
Appendix B

Moored Leisure Vessels in GRB



Vessel Repair and Maintenance in HB

Moored Fishing Vessels in KB



Vessel Repair and Maintenance in KB

AF paint used on vessels at KB

Moored Recreational and Fishing Vessels in GB

Figure B 1: Vessel-related in the harbours

Appendix C

Ethics letter and Data Collection Permits

Cape Peninsula University of Technology

P.O. Box 1906 • Bellville 7535 South Africa •Tel: +27 21 953 8677 (Bellville), +27 21 460 4213 (Cape Town)

Office of the Chairperson Research Ethics Committee	Faculty of Applied Sciences

The Faculty Research Committee, in consultation with the Chair of the Faculty Ethics Committee, have determined that the research proposal of **Winston Fru** for research activities related to the **MTech: Environmental Health** at the Cape Peninsula University of Technology requires / **does not** require ethical clearance.

Title of dissertation/ thesis:	Copper in water, sediment and gastropods in the harbours of the City of Cape Town, South Africa

Comments: (Add any further comments deemed necessary, eg permission required)
Research activities are restricted to those detailed in the research proposal.

Signed: Chairperson: Research Ethics Committee	30/Sept/2015 Date

To develop and manage a system of national parks that represents the biodiversity, landscapes, and associated heritage assets of South Africa for the sustainable use and benefit of all.

South African NATIONAL PARKS

Permit number: CRC/2015/025--2015/V1

CAPE RESEARCH CENTRE
P.O. Box 216, STEENBERG, 7947
Tel: +27 (0)21 713 7511; Fax: +27 (0)21 712 0131

Research Permit: TABLE MOUNTAIN NATIONAL PARK 08 October 2015

Mr. Winston Fru
Cape Peninsula University of Technology, Department of environmental Health

Herewith the permit for your research project titled: "**Copper in water, sediment and gastropods in the harbours of the city of Cape Town, South Africa**", valid from **08 October 2015** until **30 September 2016**.

The approval is subject to the following special conditions. The Park Management staff must be contacted prior to entry into the park (see list of staff members below). Use may only be made of accepted roads and pathways, unless otherwise agreed with the Park Management staff. You are allowed access to the **TMNP South Area, specifically Cape of Good Hope Section (see map below for restricted areas)** of the parks. The permit must be retained in the possession of researchers.

Standard Conditions:
- The use of non-demarcated areas will lead to the disturbance of animals and eco-systems, trampling of vegetation and soil erosion and only the use of accepted pathways and areas is therefore permitted, UNLESS BY SPECIAL ARRANGEMENTS. PLEASE CONTACT THE PARK MANAGEMENT STAFF IF RESTRICTED AREAS NEED TO BE ACCESSED.
- No damage shall be permitted to any natural vegetation, environment or property.
- Animals may not be disturbed in any way.
- Uncontrolled vehicle access and parking could cause damage to vegetation and soil erosion and therefore only the use of approved vehicles routes and parking areas is allowed.
- Fires can cause loss of vegetation, soil erosion and life and therefore fires, and braai's are not permitted unless in dedicated braai areas.
- Remove all rubbish and waste as it has an effect on the health of visitors, animals and plants.
- Other visitors to the area and or neighbours may not be hindered in any way.
- Pollution affects the health and safety of animals, plants, visitors and neighbours and is not permitted.
- Excessive noise affects animals (e.g. birds nesting in the areas), visitors and or neighbours and is not permitted.
- Your permit must be retained and kept on your person at all times, and produced on request.
- The areas under the control of SANParks are used entirely at your own risk. South African National Parks, its Board, directors, employees and agents are not liable for any loss or damage to the property or possession of any guest or participant (or accompanying minor) whether such damage is caused by the negligent act or omission of South African National Parks; arising from death or any bodily injuries of whatsoever nature sustained by a guest or participant (or accompanying minor) whether such injuries are caused by the negligent act or omission by South African National Parks, and/or by the defective functioning of any apparatus. The guest or participant and/or his/her/their estate hereby indemnifies South African National Parks against any claim, action, judgment, costs and/or expenses which may be made against South African National Parks and as may in any way be related to the above. The onus lies with the company or applicant to ensure that they are adequately insured.
- Please note that you (your staff etc) are subject to the conditions of Section 86 of the National Environmental Management Act (107 of 1998) and the National Environmental Act: Protected Areas Act (Act 57 of 2003) and any other relevant legislation for the duration of your stay in the National Park (e.g. adherence to the Registrar's requirements for guides).

- SANParks staff's instructions, notices, regulations and signs must be complied with.
- The activity shall be restricted to the area applied for.
- Gate and operating times to be complied with.
- NO PETS ALLOWED

Special Conditions:

- The researcher is allowed to collect the following samples from the Cape of Good Hope Restricted MPA Zone;
  - 100 ml of sea water will be collected (5 x 20ml replicates) using 1L plastic containers.
  - 5g of sediment will be collected (5 x 1g replicates) using a hand held plastic scoop.
  - 5 Samples of gastropod species (Burnupena sp.) and 5 samples of gastropod species (Nucella cingulata) will be collected by hand.
  - The samples will be collected once in the summer (January or February or March) and once in the winter (June or July or August).

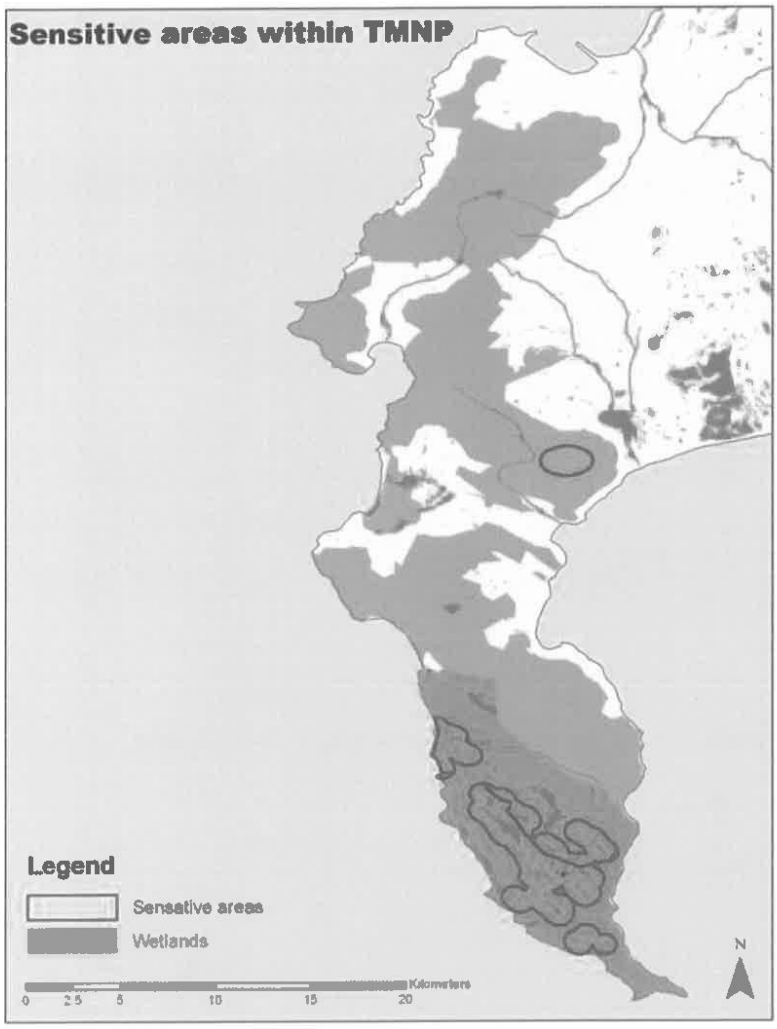
Park and Area	Park management staff	Telephone Number:
TMNP-South	Justin Buchman Justin.Buchman@sanparks.org	021 780 9100

Yours faithfully,

Debbi Winterton, Science Liaison Officer; E-mail: deborah.winterton@sanparks.org

To develop and manage a system of national parks that represents the biodiversity, landscapes, and associated heritage assets of South Africa for the sustainable use and benefit of all.

South African NATIONAL PARKS



643 Leyds Street
MUCKLENEUK
0002

P.O. Box 787
PRETORIA
0001

Tel: 012 426-5000

central reservations: 012 426 9111
reservations@sanparks.org
www.sanparks.org

CapeNature

HEAD OFFICE

postal	Private Bag X29
physical	PGWC Shared Services Center, Cnr Bosduif & Volstruis Streets, Bridgetown 7764
website	www.capenature.co.za
enquiries	Carlo Arendorf
telephone	+27 21 483 0122
fax	+27 86 556 7734
email	caarendorf@capenature.co.za
reference	1/2/2/1/2/F
date	22 December 2015

Mr. W Fru
Cape Peninsula University of Technology
Department of Environment and Occupational Studies
P O Box 652
CAPE TOWN
8000

Dear Mr Fru

APPLICATION TO ENTER AND REMAIN IN A NATURE RESERVE FOR SCIENTIFIC PURPOSES

I refer to your application to collect specimens for research purposes in the Western Cape Province.

Attached is permit No. **0052-AAA008-00029** dated **22 December 2015** to collect specimens in the Western Cape Province. Please take special note of the standard conditions attached to the permits. I specifically draw your attention to permit condition (i). **It is imperative that you make contact with the Reserve Manager BEFORE you intend collecting on any nature reserve, conservation area, wilderness area and / or state forest**. No deviation is allowed from the fore-mentioned conditions without the prior written approval of the Chief Executive Officer: Western Cape Nature Conservation Board.

Please also take note of the *pro forma* (copy attached), which must please be used when submitting your collection / distribution records to CapeNature as per the conditions to your permit. Please feel free to add columns for extra data to the *pro forma* but no columns should be deleted. This pro forma is also available electronically from CapeNature.

Should you have any queries please do not hesitate to contact this office.

Yours faithfully,

CHIEF EXECUTIVE OFFICER

Western Cape Province

Telephone No: (027) 021 483 0000
EMail: permits.fax@capenature.co.z
PGWC Shared Services Centre
cnr Bosduif and Volstruis Streets
Bridgetown
7764

CapeNature

Facsimile No: (027)0865567734
Internet: www.capenature.co.za
Private Bag X29
Gatesville
7766

PERMIT TO ENTER OR TO ENTER AND REMAIN IN A NATURE RESERVE FOR SCIENTIFIC PURPOSES

(Issued in terms of the provisions of the Nature Conservation Regulations no: 955 of 1975)

Not Transferable

Holder			
Full Name	Mr. W Fru	**Identity No.**	01382418
Trade Name	Cape Peninsula University of Technology	**Registration No.**	AAA008-01629
Postal Address	Department of Environment and Occupational Studies P O Box 652	**Physical Address**	NA
Suburb\Town	Cape Town	**Suburb\Town**	NA
Province\State	Western Cape	**Province\State**	
Country	South Africa	**Country**	
Postal\Zip Code	8000	**Longitude**	.0000
		Latitude	.0000

In terms of and to the provisions of the abovementioned Regulations framed thereunder, the holder of this permit and people specified on the attached addendum is authorised to enter or to enter and remain in a nature reserve or any portion thereof for scientific purposes. See conditions on last page.

Details			
Permit/Licence No	**0052-AAA008-00029**	*Stamp:*	CapeNature FAUNA + FLORA + HUNTING + CITES
Expiry Date	30/09/2016		
Date Issued	22/12/2015		
Amount Paid	R 0.00		
Reference			
File Code	1/2/2/1/2/F		

Description	Property
Organization	Cape Peninsula University of Technology
Person	Fru W Mr.
ID	01382418
Properties	Bettys Bay Marine Protected Area
Physical Address	Bettys Bay Marine Protected Area
District	Bettys Bay
Province/State	Western Cape
Country	South Africa
Longitude	.0000
Latitude	.0000

Species(Scientific Name)	Qty	Note
Gridled dogwhelk(Nucella cingulata)	0	5 x 5 points
Numupena spp.(Burnupena spp.)	0	5 x 5 points

C. Arendorf

22/12/2015

Issued by:	Approved on Behalf CEO	Effective Date	Signature of Holder
Carlo Arendorf	Western Cape Nature Conservation Board		I acknowledge, accept and understand fully the permit conditions as described

22/12/2015 10:49:00

software by: www.u-rad.com

Standard Conditions

1. The holder of this permit shall return it together with a full report of all activities, findings and observations made while on the nature reserve to the Chief Executive Officer: Western Cape Nature Conservation Board, Private Bag X29, Gatesville, 7766, within fourteen days from the date of expiry thereof.

2. THIS PERMIT IS SUBJECT TO SPECIAL CONDITIONS:

Special Conditions

NUMBER OF PERSONS ENGAGED IN THIS PROJECT:

Mr. W Fru - 01362418 (Passport)
Prof. J P Odendal - 7208085274084
Prof. R G Snyman - 7003010129085

- 100ml seawater will be collected (5 x 20ml replicates) using IL t plastic containers;
- 5g of sediment will be collected (5 x 1g replicates) using a hand held plastic scoop.

CONDITIONS APPLICABLE TO RESEARCHERS UNDERTAKING RESEARCH OR OTHER COLLECTING WORKS ON PROVINCIAL CONSERVATION AREAS AND / OR PRIVATELY OWNED LAND IN THE PROVINCE OF WESTERN CAPE:

1. THE MANAGER OF THE RELEVANT CONSERVATION AREA(S) (IF ANY) MUST BE INFORMED TIMEOUSLY BEFORE ANY CONSERVATION AREA IS ENTERED FOR COLLECTING OR RESEARCH PURPOSES AND THE MANAGER'S WRITTEN PERMISSION TO ENTER SUCH RESERVE MUST BE ACQUIRED BEFOREHAND. THIS PERMIT DOES NOT GRANT THE PERMIT HOLDER AUTOMATIC ACCESS TO ANY NATURE RESERVE, CONSERVATION AREA, WILDERNESS AREA AND / OR STATE FOREST. ANY OTHER / FURTHER CONDITIONS OR RESTRICTIONS THAT THE MANAGER MAY STIPULATE AT HIS / HER DISCRETION MUST ALSO BE ADHERED TO. THIS PERMIT MUST BE AVAILABLE TO BE SHOWN ON DEMAND.

2. For projects of more than one year's duration a progress report must be submitted to The Chief Executive Officer: Western Cape Nature Conservation Board before 31 December of each year.

3. One copy of all completed reports, publications, or articles (including books, videos, CDs, DVDs etc.) resulting from the project/collection must be submitted to The Chief Executive Officer: Western Cape Nature Conservation Board free of charge.

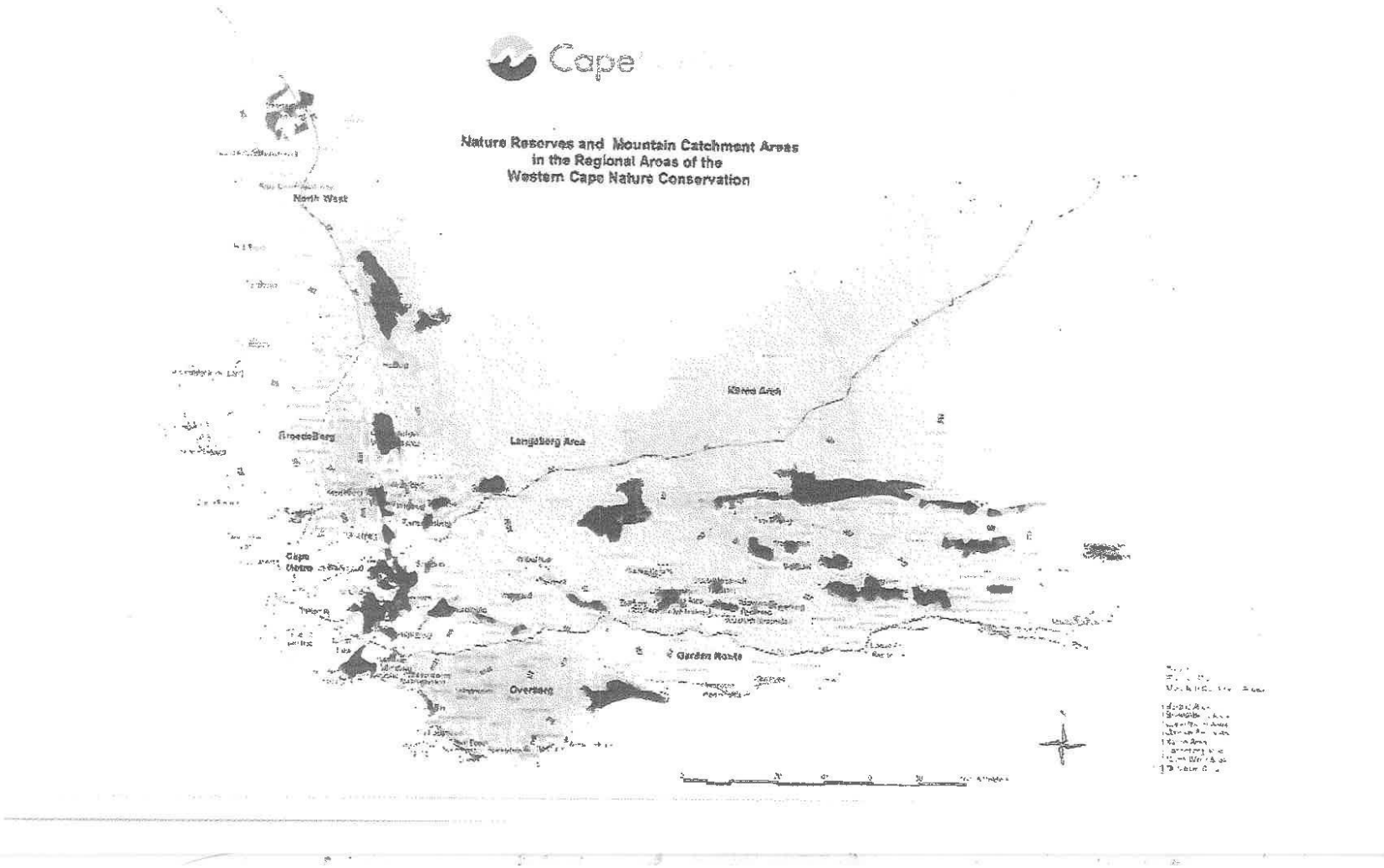
4. Should a report, publication, article or thesis arise from this project/collection, an acknowledgement to Western Cape Nature Conservation Board must be included.

5. The Forest Act 1984 (Act 122 of 1984) and regulations, the Nature Conservation Ordinance, 1974 (Ordinance 19 of 1974) and all regulations in terms of the Ordinance must be adhered to.

6. No material (fauna or flora) may be collected, disturbed or removed without the express WRITTEN permission from CapeNature.

pp CHIEF EXECUTIVE OFFICER
WCNCB

CapeNature
FAUNA + FLORA + HUNTING + CITES


Cape
Nature Reserves and Mountain Catchment Areas
in the Regional Areas of the
Western Cape Nature Conservation
North West
Klein Karoo
Langeberg Area
Overberg

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Name, Department of Agriculture, Forestry and Fisheries
Private Bag X250, Pretoria 0001

Tel: number 021-7901440. Fax: number. E-mail: address LeviB@daff.gov.za

03 November 2015

To whom it may concern

I hereby give Mr Winston Fru (st no 214274047) permission to collect samples of water, sediment and gastropods in the Hout Bay harbour area as part of his research project titled "**copper in water, sediment and gastropods in the harbours of the City of Cape Town, South Africa**"

Yours faithfully

Mr Bezuidenhout

Acting harbour manager (Houtbay harbour

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Name, Department of Agriculture, Forestry and Fisheries
Branch: FISHERIES. KALK BAY HARBOUR OFFICE, Kalk Bay

Tel. number. 021 788 8313 Fax: number. 021 788 1038 E-mail: address MariannaJ@daff.govt.za
Kalkbay@daff.gov.za

Mr. Winston Fru
Student No. 214274047
Cape Peninsula University of Technology
Department of Environmental Health

Dear Sir

RE: PERMISSION TO DO THE RELEVANT RESEARCH IN KALK BAY HARBOUR.

In my capacity as Acting Harbour Master, I hereby give you permission to enter Kalk Bay Harbour to do your research samples for Copper, sediment and gastropods of the water inside the Harbour.

Please keep your allocated permits with you when entering Kalk Bay Harbour.

Yours sincerely

Marianna Jordaan: Acting Harbour Master
Department of Agriculture, Forestry and Fisheries
Branch: SED: Proclaimed Fishing Harbour
Kalk Bay Harbour
Kalk Bay
0837600054
Email: Kalkbay@daff.gov.za
MariannaJ@daff.gov.za

agriculture,
forestry & fisheries
Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Chief Directorate, Monitoring, Control and Surveillance

Gordons bay Old harbour, Old Beach road, Gordons Bay 7140. Tel: number.021 856 0356
Fax: 086 556 1230 Enquires: NombonisoJ@daff.gov.za

To:

Mr Winston Fru

Student No: 214274047

Cape Peninsula University of technology

Dear Winston Fru

PERMISSION TO CONDUCT RELEVANT RESEARCH IN GORDONS BAY AREA.

Permission is granted to conduct your research in Gordons bay area, however a Fishery Control Officer must be informed by sms prior any activities to be conducted. In the following number 0732645953/0789750272.

Please ensure that you have your allocated permit from the Department during any activities conducted.

Yours Faithfully

Nomboniso Jozi 2016/01/25.

Department of Agriculture, Forestry and Fisheries

Branch: Fisheries

Directorate: Monitoring, Control and Surveillance

TRANSNET

national ports authority

5 February 2016

Mr Winston Fru
(Student)
Department of Environmental Health
Cape Peninsula University of Technology
P.O Box 652
Cape Town
8000

Attention: Dear Mr Fru (fruw@cput.ac.za)

PERMISSION FOR RESEARCH INVESTIGATION

Subject: Submission to grant a student (Mr. Winston Fru) approval to conduct a research investigation in the Port of Cape Town.

Purpose: To grant approval to the student an opportunity to conduct his research programme in the Port of Cape Town: The study entitled Copper in water, sediment and gastropods in the harbours of the City of Cape Town, South Africa.

Discussion:

Worldwide anthropogenic activities have degraded the coastal system. The coastal zones of the City of Cape Town are not excluded due to increasing urbanization and industrialization that poses a threat to the ecosystem. The City's harbours are significant hub for development and commercial activities which includes shipping, fishing, construction, dredging and tourism (recreational). Harbours are being affected of contaminants for pollutants where metals can be traced. Worldwide, metals have been identified as one of the most pervasive classes of contaminants in harbours. Metals may occur in water and sediments and bioaccumulation in the tissues of many organisms. They pose a severe risk to human and ecosystem health because of their toxicity, persistence and bioaccumulation characteristics.

The tributyltin (TBT) antifouling paint (AFP) was banned in 2003, and its phasing out by 2008 introduced the renewed use of copper-based antifouling paints. Most contemporary marine antifouling paints (AFPs) contain a copper-based biocide (e.g. cuprous oxide or, less commonly, cuprous thiocyanate) (Turner, 2010), which leaches into the environment where it may exert unintended toxic effects on non-target organisms. Concerns have been raised over the effect of these copper ions on the marine environment with high levels being detected in areas of heavy boat activities. Unfortunately, these levels are likely going to worsen with increased harbour activities coupled with other influxes such as industrial and wastewater discharge, surface run-off and atmospheric deposition, pollution received from the City of Cape Town via the stormwater etc. therefore, the study is critical for the environmental health.

Transnet SOC Ltd
Registration Number
1990/000900/30

TNPA Head Office
30 Wellington Road
Parktown
Johannesburg
2193

P.O. Box 32696
Braamfontein, Johannesburg
South Africa, 2012
T +27 11 355-9001
F +27 11 355-9262

Directors: LC Mabaso (Chairperson) SI Gama* (Acting Group Chief Executive) Y Forbes GJ Mahlatela PEB Mathekga ZA Nagdee VM Nkonyane SD Shane BG Stagman PG Williams GJ Pita* (Group Chief Financial Officer)
*Executive

Group Company Secretary: ANC Ceba

www.transnet.net

Benefit to TNPA:
The candidate shall share the findings of the investigation with the National Ports Authority, Port of Cape Town. It is expected by law that TNPA to phase out the use of tributyltin (TBT) antifouling paint in the activities within the Port. The study will therefore, strengthen the results of the current studies that are undertaken in the Port of Cape Town and the explored opportunities will further assist in decision making.

Confidential:

The researcher to ensure that the information is responsible for is classified according to the Transnet policy. The information cannot be used for any commercial purposes but for academic benefit in scientific field.

Specific conditions:-

(i) The permission to conduct the research investigation is valid for a period of one year (15 February 2016-15 February 2017). It is the responsibility of the candidate for renewal. It is advised that the candidate to submit his/her renewal two months (60 days) before the 15th February 2017 to SHE Department.

(ii) Allow for the collection, possession and transportation for bona fide research projects of the Department of Environmental Health, Cape Peninsula University of Technology.

(iii) The certified copy of this approval shall be produced at the entrance security gate of the Port together with the customer entrance card/ letter that will be issued when the samples with be collected.

(iv) The Port induction is compulsory before collection of the samples onsite. SHE Department is responsible for inducting the candidate. If the candidate will be accompanied by his/her assistant researchers for field work, the candidate should disclose such information of the team members so that they may all be inducted.

(v) The candidate must have obtained a permit from Department of Environmental Affairs and the Department of Agriculture and, Forestry and Fisheries before collecting any samples in the Port premises.

(vi) The following amounts of specimens per species may be collected, as detailed below:
- Gastropods , maximum of 50 per species
- Sediment, maximum of 500 grams
- Seawater, any amount

(vii) Personal Protective equipment is compulsory in all the areas of Transnet National Ports Authority.

(viii) Any installation must be removed on the termination of the project (s).

(ix) The candidate will be under the supervision of Port Environmental Specialist, **AM Melato** (021 449 2152), michael.melato@transnet.net, division of Environmental Department.

(x) An application for a future permit may be refused if the conditions above and the Port Acts are not adhered to.

Recommendation:

It is hereby recommended that the candidate to carry this permit at all times when collecting the samples within the Port and to adhere with all the requirements of the Port.

.....

Michael Melato, *Ph. D Candidate*

Port Environmental Specialist

michael.melato@transnet.net

05/02/2016

Date

environmental affairs
Department:
Environmental Affairs
REPUBLIC OF SOUTH AFRICA

agriculture, forestry & fisheries
Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Enquiries: Dr Alan Boyd
Tel.: 021-819 5006

Enquiries: Dr Kim Prochazka
Tel.: 021 402 3546
Fax:021 402 3639

E-mail : researchpermits@daff.gov.za

Mr Winston Fru
Department of Environmental Health
Cape Peninsula University of Technology
P O Box 652
Cape Town
8000

Attention: Dear Mr Fru (fruw@cput.ac.za)

INTEGRATED ENVIRONMENTAL AND FISHERIES RESEARCH AND DEVELOPMENT PERMIT FOR THE PURPOSES OF A SCIENTIFIC INVESTIGATION OR PRACTICAL EXPERIMENT IN TERMS OF SECTION 83 OF THE MARINE LIVING RESOURCES ACT, 1998 (ACT NO. 18 OF 1998).

This permit is an integrated research permit issued jointly by the Department of Environmental Affairs and the Department of Agriculture, Forestry and Fisheries authorising a scientific investigation or practical experiment in terms of section 83 of the Marine Living Resources Act of 1998 (Act No. 18 of 1998) ("the Act"), which experiment or investigation includes both environmental and fisheries components as part of the project.

I, the undersigned, acting Chief Director: Fisheries Research and Development, Branch: Fisheries Management, Department of Agriculture, Forestry and Fisheries (the Chief Director) acting in pursuance of the delegated authority conferred upon me by the Honourable Minister of Agriculture, Forestry and Fisheries as contemplated in terms of Section 79 of the Marine Living Resources Act of 1998 (Act No. 18 of 1998) ("the Act") hereby jointly issue this permit, in terms of Section 83 of the Act, to the following person(s)/institution to engage in the scientific investigation or practical experiment referred to below:

I, the undersigned, Director: Biodiversity and Coastal Research, Branch Oceans and Coasts, Department of Environmental Affairs (the Director) acting in pursuance of the delegated authority conferred upon me by the Honourable Minister of Environmental Affairs as contemplated in terms of Section 79 of the Marine Living Resources Act of 1998 (Act No. 18 of 1998) ("the Act") hereby jointly issue this permit, in terms of Section 83 of the Act, to the following person(s)/institution to engage in the scientific investigation or practical experiment referred to below:

PERMIT REFERENCE NUMBER: RES2016/87
PERSON(S) INSTITUTION: Mr Winston Fru, Department of Environmental Health, Cape Peninsula University of Technology,
SCIENTIFIC INVESTIGATION OR PRACTICAL EXPERIMENT: Collection, possession and transportation of marine organisms for the purposes of research,

subject to the following conditions:

1. SPECIFIC CONDITIONS FOR THE DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES

1.1. This permit allows for the collection, possession and transportation for *bona fide* research projects of the Department of Environmental Health, Cape Peninsula University of Technology as authorised by the Head of the Department.

1.2. A certified copy of this permit shall be carried by staff during collections and must be shown to a Fishery Control Officer or any other authorized person on demand. Staff undertaking collections shall identify themselves, if requested to do so, by means of an identification document issued by the Cape Town University of Technology.

1.3. The following amounts of specimens per species may be collected, as detailed below:

Species/ Group	Quantity
Gastropods (such as *Burnupena* spp., *Nucella* spp., and others)	Maximum 50 per species
Sediment	Maximum 500 grams
Seawater	Any amount

1.4. No abalone may be collected with this permit

1.5. No specimens should be used in any way in a bioprospecting context.

1.6. No harmful chemicals are to be used when collecting marine species. Limited use of fish anaesthetics (including rotenone) is permitted if no other suitable technique is available to

collect fishes, and should be kept to a minimum. Local authorities should be advised when rotenone is to be used to collect fish.

1.7. Any installations must be removed on termination of the project(s).

1.7. The report, as required under Condition 3.15, should provide details of the dates, locations, species and quantities collected.

2. SPECIFIC CONDITIONS FOR THE DEPARTMENT OF ENVIRONMENTAL AFFAIRS

2.1. No marine mammals, turtles or marine birds may be collected or disturbed.

CONDITIONS PERTAINING TO ACTIVITIES WITHIN MARINE PROTECTED AREAS

2.2. The permit holder may undertake research within the Table Bay National Park and Betty's Bay MPAs. A permit from CapeNature is required before sampling may take place in the Betty's Bay MPA. Any research within the Table Bay National Park MPA may only be undertaken in terms of the permit obtained from SANParks attached to the application. Activities are restricted to the areas of the MPA as indicated in the permit from SANParks.

2.3 The holder of this permit shall inform the relevant management authority responsible for the management of Betty's Bay MPA (CapeNature) and Table Mountain National Park MPA (SANParks) of the sampling date(s) and area(s) prior to each collection and must lodge a copy of this permit with the MPA Managing Authority.

3. GENERAL CONDITIONS

3.1. This permit is issued subject to the provisions and regulations of the following laws:
(a) The Marine Living Resources Act, 1998 (Act No. 18 of 1998) ("the Act"), and all regulations published in terms thereof;
(b) The National Environmental Management Act, 1998 (Act No. 107 of 1998) ("NEMA"), and in particular, the regulations that control vehicle use in the coastal zone (as amended);
(c) The National Environmental Management Biodiversity Act, 2004 (Act No. 10 of 2004);
(d) The National Environmental Management Protected Areas Act, 2003 (Act No. 57 of 2003);
(e) The Sea Birds and Seals Protection Act, 1973 (Act No. 46 of 1973); and
(f) Prevention of Pollution from Ships Act (Act No. 2 of 1986).
(g) National Environmental Management Integrated Coastal Management Act, 2008 (Act No. 24 of 2008)
(h) Any other relevant law

3.2. If, in the opinion of the Chief Director or Director there are sound reasons for doing so, the Chief Director or Director may amend the relevant conditions of the permit.

3.3. Any reference to the Permit Holder in these permit conditions includes the entity or person, his/her or its employees (whether permanent, full-time or part-time), his/her or its contractors, agents or advisers, being cognisant of the course and scope of their contractual relationship.

3.4. A breach of the provisions of the Act, regulations or these permit conditions by the Permit Holder may result in the initiation of legal proceedings (civil or criminal). A breach includes:

(a) furnishing information to which the Department of Environmental Affairs (the Department) is entitled, which is not true or complete;

(b) contravening or failing to comply with a permit condition or with the provisions of the Act;

(c) being convicted of an offence in terms of the Act; or

3.5. The Permit Holder shall store at their registered place of business/residence the original permit issued. The Permit Holder shall at all times, have available a true certified copy of this permit which should be produced on demand by any law enforcement official.

3.6. This permit shall only be utilized by the individual/organisation whose name appears on the permit.

3.7. If the permit is in the name of an institution/company/close corporation the individual utilizing the permit shall in addition to a certified copy of this permit, be in possession of identification and/or letter which identifies the individual as an authorized person or employee of the permit holder.

3.8. Any individual utilizing this permit shall in addition to the above conditions have a certified copy of any other permit/exemption required in terms of other legislation including any permit or exemption in terms of the Marine Living Resources Act, 1998 (Act No. 18 of 1998).

3.9. An application for a future permit may be refused if the conditions of this permit are not adhered to.

3.10. In terms of the Act, the permit holder is obliged to report to the Minister any contravention of the provisions of the Act by any other person.

3.11. The Permit Holder must safely store all inorganic waste material, garbage and pollutants on board the vessel or at the site of research activities if on land. Should the Permit Holder discard any inorganic waste material, garbage or pollutants into the sea or coastal environment, the Department may institute legal proceedings (civil or criminal) which may include, suspension of the permit for a period determined by the Department and the Permit Holder shall take those steps considered necessary in terms of relevant legislation

to remedy any pollution caused.

3.12. This permit does not in any way absolve the holder from the obligations of and adhering to the remainder of the provisions and conditions of the Act, regulations or any other law.

3.13. Specimens collected in terms of this permit shall not be sold or offered for sale.

3.14. No vehicle may be used in the coastal zone in terms of this permit unless the permit holder is in possession of a valid permit to use a vehicle in the coastal zone in terms of the Regulations for the Control of Use of Vehicles in the Coastal Zone (GNR 1426 of 7 December 2004).

3.15. Reports as stipulated in the 'Specific Conditions' must be submitted to the Chief Director: Fisheries Research and Development, Department of Agriculture, Forestry and Fisheries, Branch: Fisheries Management (Attention: Dr Kim Prochazka), Private Bag X2, Roggebaai, 8012 and to The Director: Dr Alan Boyd, Biodiversity and Coastal Research, Department of Environmental Affairs, Branch: Oceans and Coasts, by email to ajboyd@environment.gov.za. Such reports must be submitted within the timeframes provided, before any renewal of this permit or application for any other permit will be considered.

3.16. This permit may not be utilised within a marine protected area for collecting, monitoring or any other activity which is prohibited in terms of section 43 of the Act and regulations unless such activity is specifically authorised in the Specific Conditions section of this permit.

4. PERMIT VALIDITY PERIOD

This permit is valid from **1 January 2016** until **31 December 2016.**

MR JUSTICE MATSHILI
ACTING CHIEF DIRECTOR: FISHERIES RESEARCH AND DEVELOPMENT
DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES
DATE: 06/01/2016

DR ALAN BOYD
DIRECTOR: BIODIVERSITY AND COASTAL RESEARCH
DEPARTMENT OF ENVIRONMENTAL AFFAIRS
DATE: 6 Jan 2016

Appendix D

SAWS disclosure statement for rainfall data provision

South African Weather Service

ISO 9001 Certified Organisation

DISCLOSURE STATEMENT

The provision of the data is subject to the User providing the South African Weather Service (SAWS) with a detailed and complete disclosure, in writing and in line with the requirements of clauses 1.1 to 2.4 (below), of the purpose for which the specified data is to be used. The statement is to be attached to this document as Schedule 1.

1 **Should the User intend using the specified data for commercial gain then the disclosure should include the following**:

1.1 the commercial nature of the project/funded research project in connection with which the User intends to use the specified data;

1.2 the names and fields of expertise of any participants in the project/funded research project for which the specified data is intended; and

1.3 the projected commercial gains to the User as a result of the intended use of the specified data for the project/funded research project.

2 **Should the User intend using the specified data for the purposes of conducting research, then the disclosure should include the following**:

2.1 the title of the research paper or project for which the specified data is to be used;

2.2 the details of the institution and supervisory body or person(s) under the auspices of which the research is to be undertaken;

2.3 an undertaking to supply SAWS with a copy of the final results of the research in printed and/or electronic format; and

2.4 the assurance that no commercial gain will be received from the outcome from the research.

If the specified data is used in research with disclosure being provided in accordance with paragraph 2 and the User is given the opportunity to receive financial benefit from the research following the publication of the results, then additional disclosure in terms of paragraph 1 is required.

The condition of this disclosure statement is applicable to the purpose and data requirements of the transaction recorded in Schedule 1 on page 2. This statement is effective from October 2017.

Bolepi House, 442 Rigel Avenue South, Erasmusrand, 0181 Private Bag X097, Pretoria, 0001 Tel: + 27 (0) 12 367 6000

www.weathersa.co.za USSD: *120*7297#

South African Weather Service
ISO 9001 Certified Organisation

Disclosure Statement

SCHEDULE 1

Please note: The South African Weather Service will only act upon customer requirements noted on this disclosure statement and not from any other correspondence.

FULL PERSONAL DETAILS OF USER

Full Names	Winston Fru
University/school/organisation	Cape Peninsula University of Technology
Student Number (if applicable)	214274047
Email address	fru1701@gmail.com
Cellphone	0730349348
Supervisor	Prof J.P. Odendaal
Project/Thesis Title	Copper and zinc in water, sediment and gastropods in the harbours of the Cape Town Metrople, South Africa
Current registered degree (e.g. BSc)	Master of Environmental Health
Expected finalization date (MMYYYY)	December 2018

The South African Weather Service reserves the right to request, at any time, from the student proof of registration for the Degree at the University.

THE PURPOSE (*Please indicate a detailed description of the purpose for which the data will be used)*

The data will be used to better explain the findings of my research.

DATA REQUIRED *(Please include the weather elements (e.g. rain, temperature), place/s and time period)*

Monthly Rainfall Data

2015; 2016 and 2017

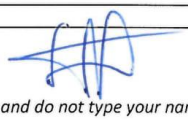
Cape of Good Hope (Cape point), Betty's Bay Marine Protected Area (Betty's Bay), Granger Bay Harbour (Cape Town), Hout Bay Harbour (Hout Bay), Kalk Bay Harbour (Kalk Bay) and Gordon's Bay Harbour (Gordon's Bay)

I hereby accept that:

- SAWS will be acknowledged in the resulting thesis/project or when published, for the data it provided.
- SAWS will be provided with a copy of the final results in printed or electronic format.
- The data received shall not be provided to any third party.

South African Weather Service
ISO 9001 Certified Organisation

Disclosure Statement

Signature of the User:  Date: 13-10-2017

(*Please sign the document and do not type your name in as this is a legal document and requires a signature.*)