THE IMPACT OF URBAN POLLUTION ON THE CONTAMINATION OF WATER, SEDIMENT AND ALGAE IN THE ZANDVLEI ESTUARY, CAPE TOWN, SOUTH AFRICA

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DECLARATION

I, **Luzuko Sidondi**, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/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.

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ABSTRACT

Estuaries are some of the most productive areas of the coastal environment as they provide essential ecosystem goods and services. However, as a result of rapid urbanisation coupled with population growth, these systems are under increasing pressure. The Zandvlei estuary in False Bay, Cape Town is an example of an estuarine system in the cool-temperate biogeographic region of South Africa that is affected by urban pollution emanating from various land uses (i.e. industrial activities). Metal contamination remains a concern in many parts of the world as it negatively affects aquatic ecosystems due to their persistent and toxic nature which could negatively affect human health. Little information is available on metal concentration levels in South African estuaries. As a result, there is a need for further investigation as metals have been identified as an emerging pollution pressure that requires attention. This study aimed to determine the concentration levels of six metals (i.e. Aluminium (Al), Manganese (Mn), Iron (Fe), Zinc (Zn), Lead (Pb), Copper (Cu)), including their spatial and temporal variations in water, sediment, and algae (i.e. *Enteromorpha* spp.) within the Zandvlei estuary. The information gathered in this study forms the basis for further metal pollution investigations within the study area. Samples were collected in the main body of the estuary on four separate occasions from five sampling sites. The collected water, sediment, and algae samples were acid digested and the concentrations determined by means of ICP-MS. Statistical analysis was conducted using the SigmaPlot (version 14.0) software by SYSTAT Software Inc. Statistical differences between the different sampling sites and sampling occasions for the subject metals were analysed and evaluated using the Kruskal-Wallis One-Way Analysis of Variance (ANOVA) on Ranks and Student Newman Kuels Method for post hoc tests. In cases where there were missing or unequal datasets, the Dunns Method was utilised for post hoc tests. The condition for the significant differences was set at $p<0.05$ for all statistical analyses. Spatial variations in the recorded metal concentrations showed significant differences at sites near stormwater drainage outlets as well as the influent rivers and this could be an indication of a potential source of contamination within these areas. Physicochemical parameters such as salinity, pH, and temperature also played an important role. Areas where there were standing waters due to the low gradient of the estuary (which consists of deeper channels as a result of dredging) and due to the closing of the estuary mouth also recorded metal concentrations that were relatively higher in some instances. Temporal variations in the recorded metal concentrations revealed a range of differences that were influenced by rainfall, salinity, and changes in anthropogenic inputs, among other factors. The highest mean concentration in water was recorded for Al (2.0438±2.7055 mg/ℓ). Fe recorded the highest mean concentration in both sediment (6744.90±5130.36 mg/kg) and macroalgae (4764.45±606.10 mg/kg). Metal concentrations were generally low in water samples, however, Al, Fe, Zn, Pb, and Cu exceeded one or more of the following water quality guidelines: South Africa’s Department of Water Affairs and Forestry (1996), Canadian Council of Resource and Environment Ministers (1987), Canadian Council of Ministers of the Environment (2001), and Australian and New Zealand Environment and Conservation Council (2000). Metal concentrations in water samples decreased in the following sequence: Al > Mn > Fe > Zn >Cu > Pb. In sediment, Zn, Pb, and Cu concentrations from one or more of the sampling sites exceeded sediment quality guidelines provided by CCME (1999) and/or ANZECC (2000). Metal concentrations in sediment decreased in the following sequence: Fe > Al > Zn > Pb > Mn > Cu. Concentrations in macroalgae (*Enteromorpha* spp.) were also relatively high and were between “moderately contaminated” to “uncontaminated” for Cu and Pb. Macroalgae as a biomonitor recorded relatively higher concentrations when compared to the other mediums with higher concentrations recorded between spring (SO4) and summer (SO1) and this could be attributed to the metabolic processes including the presence of sufficient algae biomass. Metal concentrations in *Enteromorpha* spp. decreased in the following sequence: Fe > Al > Mn > Zn > Pb > Cu. In light of the above, it is necessary that source identification and continuous monitoring of metal concentrations in the Zandvlei estuary be undertaken.

Keywords: estuary, metals, water, sediments, macroalgae, Zandvlei, *Enteromorpha*, biomonitor.

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DEDICATION

This work is dedicated to:

My creator (GOD) and ancestors (*oMiya, Gcwanini, Sbewu, Salakulandelwa, Rhenqwa, Sjekula, Mancoba, Ngoma, Vezi - Camagu!)*

My grandmother and late grandfather, Nomvula Martha Sidondi and Thembekile Sidondi.

My late uncle and aunt, Patutu “Star” Sidonti and Nothandekile Qwebi

My mother, Nomathamsanqa Sidondi.

My son, Othalive Zele.

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GLOSSARY

Terms/Acronyms/Abbreviations	**Definition/Explanation**
ANZECC	Australian and New Zealand Environment and Conservation Council
ATSDR	Agency for Toxic Substances and Disease Registry
CCME	Canadian Council of Ministers of the Environment
CoCT	City of Cape Town
DWAF	Department of Water Affairs and Forestry
Mg/kg	Microgram per kilogram
Mg/L	Milligram per Litre
SAWS	South African Weather Service
SD	Standard Deviation
WCG	Western Cape Government
WHO	World Health Organisation
Bioaccumulation	It is the process in which contaminants (i.e. metals) enter a food chain and accumulate in biological tissues of aquatic organisms from sources such as water, food and suspended sediments (Wang and Fisher, 1999). In simple terms, it is the increase or build-up of contaminants in organisms, reflecting concentrations that are comparative to those found in the environment.
Biomonitors	Biomonitors are organisms that accumulate contaminants (i.e. metals) in their tissues and may be used to obtain a relative measure of the contaminants in the environment (Phillips and Rainbow, 1994; Hatje, 2016)
Macroalgae	Macroalgae are photosynthetic plant-like organisms (i.e. also known as seaweed) that can be seen with a naked eye and which may be attached to substrata (i.e. rock) or is free-flowing (GWA, 2010).

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

1.1.1. Background

Metals are regarded as one of the main pollutants in coastal environments such as estuaries (Billah et al., 2017). They are introduced into the aquatic environment through the weathering and erosion of rocks and soil and through various human activities involving the processing of metals or the use of materials containing metals (El-Sorogy and Attiah, 2015). Although some metals are naturally present in the environment (Rainbow et al., 1990; Villares et al., 2002), however, it is their presence at higher concentrations that present a potential threat to aquatic systems (Wright and Mason, 1999). They are persistent and toxic pollutants that are typically not removed from aquatic environments by natural processes and they induce a risk for ecosystems and humans (Phillips et al., 2015; Antizar-Ladislao et al., 2015). As a result of the persistent, toxicity and bioaccumulation of metals in the aquatic environment, investigations of the coastal environment have received great attention in many parts of the world (Ryan et al., 2012; Antizar-Ladislao et al., 2015; Nguyen et al., 2020). The investigation into the bioaccumulation and toxicity of metals in aquatic environments is intended to ensure that the appropriate control measures are implemented before irreversible damage occurs (Phillips et al., 2015). While the assessment of metals in aquatic systems typically involves the analysis and monitoring of sediment and the water column, these mediums have been found to have limitations in so far as spatial and temporal patterns of contamination are concerned (Rainbow, 1995; Chakraborty et al., 2014; Phillips et al., 2015). This prompted the need for the use of aquatic organisms such as macroalgae, which are also regarded as bioindicators of metal contamination (Bryan et al., 1985: Rainbow, 1995; Villares et al., 2002). However, apart from the use of macroalgae which provides time-integrated results (Rainbow, 1995; Phillips et al., 2015; Billah et al., 2017; Żbikowski et al., 2007), the analysis of sediment was still regarded as a useful method to study metal pollution (although it does not necessarily predict the toxicity of metals to the biota) (Chakraborty et al., 2014; Antizar-Ladislao et al., 2015). This is because sediments usually act as reservoirs and sinks for metals and have a longer residence time when compared to total dissolved metals in the water column (Mountouris et al., 2002; El-Sorogy and Attiah, 2015). The analysis of sediment is therefore still regarded as a useful method by some researchers, however, a more useful indicator of metal contamination (which was also used in this study) is macroalgae (Żbikowski et al., 2007). Macroalgae (*Enteromorpha* spp.) has a high capacity to bind metals because its cellular wall is rich in hydroxyl, sulphate, and carboxyl groups of polysaccharides which are strong ion-exchangers (Żbikowski et al., 2006). It is also assisted by their local abundance, widespread distribution, and sedentary nature which makes them suitable in situ bioindicators (Phillips et al., 2015). The use of biological indicators such as macroalgae is well documented and has been used extensively in many parts of the world to characterize the status of environmental pollution (Bryan et al., 1985: Rainbow and Phillips, 1993; Rainbow, 1995; Villares et al., 2002). They have also been used to establish spatial and temporal variations in the bioavailable concentrations of metals in coastal and estuarine waters (Misheer et al., 2006). However, in South Africa, there is very limited information available on the use of macroalgae as a biological indicator of metal contamination (Vlachos et al., 1998). Some researchers also found that there is little information available in South Africa on the effects of metal pollutants on estuaries (Nel et al., 2015; Adams et al., 2020).

Estuaries are recognised as one of the most dynamic and productive areas of the coastal zone, where freshwater from land drainage comes into contact and mixes with seawater (Little, 2000; Kennish, 2002). Estuaries are the focal point for various human activities along the coast, as they provide a wide range of opportunities and benefits and contribute significantly to the local and natural economy (Van Niekerk and Turpie, 2012). Since estuaries are close to the ocean, they have for many years been regarded as suitable areas for the disposal of large amounts of both domestic and industrial wastes (Phillips et al., 2015). Furthermore, since these environments are largely subject to intense human occupation, they have also been exposed to run-off from various industrial and urban activities, resulting in the dilution and disposal of waste which introduces contaminants such as metals (De Souza Machado et al., 2016). Like many parts of the world, estuaries in South Africa are also subjected to an influx of various anthropogenic pollutants and pressures that emanate from urban development or catchment land use changes, industrial and agricultural activities, among other activities (Whitfield et al., 2012; Van Niekerk et al., 2013). South Africa has nearly 300 estuaries which occur across three biogeographical regions; warm temperate southern and south-east coast; cool temperate west coast; and subtropical east coast (Emanuel et al., 1992; Whitfield, 1992; Van Niekerk et al., 2020). The South African coastal zone area comprises of a high population density, and this can be attributed to the abovementioned activities, including residential and tourism-related activities. There are approximately 15% of estuaries in South Africa are under severe pollution pressure (Van Niekerk et al., 2013). The Zandvlei estuary in False Bay, Cape Town (i.e. the study area) is a typical example of an estuarine system that has been highly modified by urban, industrial, and agricultural activities (Harding, 1994; C.A.P.E., 2010; WCG, 2018). These anthropogenic activities have exerted a substantial amount of pressure on these productive ecosystems for atleast the past four decades (Cilliers and Adams, 2016). This has necessitated the need to monitor the water quality of these systems in order to protect and manage the water resource in line with section 24 of the Constitution of the Republic of South Africa, 1996 (Act No. 108 of 1996). The National Water Act, 1998 (Act No. 36 of 1998) is administered by the Department of Water and Sanitation (DWS) and mandates it to undertake the monitoring and protection of South African water resources. The Integrated Coastal Management (ICM) Act, 2008 (Act No. 24 of 2008) which is administered by the Department of Forestry, Fisheries and the Environment – Oceans and Coasts (DFFE, formally known as the Department of Environmental Affairs) makes provision in Chapter 4 of the Act for the drafting of estuary specific management plans. Section 33(2) of the ICM Act empowers the Minister responsible for Environmental Affairs with the concurrence of the Minister responsible for Water Affairs to publish a Protocol that will provide guidance for the management of estuaries through the development and implementation of estuarine management plans (EMPs). The National Estuarine Management Protocol (NEMP), as amended (dated June 2021), seeks to specifically address integrated cross-sectoral planning and management of South African estuaries by setting strategic objectives which include the maintenance of the ecological integrity, sustainable use, and protection of estuaries. NEMP also promotes cooperative governance between the different spheres of government as the management of these systems requires an integrated and cooperative management approach. Cilliers and Adams (2016) note that historically, the C.A.P.E. Estuaries Programme in the Western Cape Province has made significant progress in drafting estuarine management plans, which essentially gave effect to the ICM Act.

The water quality monitoring programme for estuaries through EMPs arguably pays less attention to the analysis of metals within these systems. Some EMPs rightfully acknowledge the need to consider metal pollution analysis using conventional analysis of the abiotic matrices (water and sediment), however, this is recommended on an ad-hoc basis and only at problematic sites. The use of living organisms such as algae to monitor metal pollution (biomonitoring) still requires consideration and attention as there is no mention thereof in the existing monitoring plans.

1.1.2. Statement of the research problem

Cape Town comprises of some of the most ecologically diverse and productive coastal environments in South Africa and the world in general and contributes significantly to the City’s economy. Rapid urbanisation and industrialisation coupled with population growth, immigration and tourism poses a significant threat to Cape Town’s estuarine ecosystems. The False Bay coast has been subject to various water quality testing over the past decade and some water quality issues were noted which were mostly linked to stormwater discharge and sewage spills from wastewater treatment facilities (CoCT, 2019). The Zandvlei estuary is a prime example of an estuarine system in the Cape Town Metropole that has been severely affected by urbanisation, industrialisation and population growth. This is evident from the water quality testing results that have been published in various reports by the City of Cape Town over the years and which have led to the closure of the estuary for public access from time to time. The closure of the estuary was, however, mostly due to high levels of faecal coliforms (*Escherichia coli*) (CoCT, 2017; CoCT; 2018). The estuary is used for recreational activities such as sailing, windsurfing, canoeing and fishing (CoCT, 2022). It is important to note that land uses in the Zandvlei catchment ranges from light industry to housing, agriculture and conservation (C.A.P.E., 2010). The estuary comprises of an extensive stormwater drainage system which discharges directly into the estuary (WCG, 2018). A light industrial area is located approximately 2km upstream and is adjacent to the Keysers River which discharges a short distance downstream into Zandvlei estuary. The estuary is also subject to dredging which occurs occasionally in order to remove some large amounts of the sediment deposits to improve water circulation and reduce the risk of flooding. Dredging and the manipulation of the estuary mouth are also likely to affect the circulation of water and mobilisation of contaminants. Seasonal conditions such as elevated rainfall and other changes to physicochemical parameters (pH, salinity, temperature etc.) of the estuary could potentially exacerbate pollution within this particular system. The above human activities are responsible for the extensive modification of the Zandvlei estuary. These activities have a potential of introducing toxic and persistent contaminants such as metals which may significantly alter water quality and potentially threaten both aquatic and human life (Wright and Mason, 1999). Unlike most organic contaminants, metals may not necessarily be eliminated from aquatic systems as they do not undergo environmental degradation or other natural processes which may reduce their toxicity or concentration (Tabudravu et al., 2002; Phillips et al., 2015). Metals enter estuaries from both influent rivers and from direct discharges (i.e. stormwater discharge) and once they are introduced into surface waters, they rapidly become associated with particulates and are found in bottom sediments (Hanson et al., 1993; Wright and Mason, 1999; Phillips et al., 2015; CSIR, 2015). This results in estuaries being the largest receipts of pollutants as sediments are ultimately transferred to the coastal environment (Szefer et al., 1995; Ryan et al., 2012). Metals are of concern particularly in light of global climate change, where predicted changes in pH, temperature and oxygen could potentially alter their behaviour and remobilisation (Schiedek et al., 2007; Adams et al., 2020). In high concentrations, metals may accumulate in different organisms and move through the different trophic levels of a food chain through bioaccumulation and biomagnification which ultimately affects human beings (Chakraborty et al., 2014; Billah et al., 2017). Therefore, the water quality of estuaries in relation to metal contaminants remains of public health concern. Despite potential ecological and human health risks posed by toxic substances such as metals, there is very little research on, and monitoring of, the distribution and accumulation of these compounds in South African estuaries (Van Niekerk et al., 2013).

It is, therefore, arguable that the City of Cape Town may not necessarily have a clear picture of the status of metal pollution, especially in coastal environments such as estuaries as the current water quality tests do not include the monitoring of metals. This is also further confirmed in the Zandvlei estuary management plan (C.A.P.E, 2010) which indicated that there is very limited information on metal contaminants in Zandvlei and the catchment although elevated levels of copper and zinc were once reported in the lower Keysers River. Furthermore, there is currently no literature available on the use of biological indicators such as macroalgae for the analysis of metal concentrations in the Zandvlei estuary. However, it is important to note that the analysis of sediment quality in as far as metal pollution is concerned was undertaken by the Council of Scientific and Industrial Research (CSIR) and a report was published in 2015. Nonetheless, as noted above, sediment analysis does not provide time-integrated bioavailable metal concentrations in the biota (Chakraborty et al., 2014), however, it is a more useful medium when compared to the analysis of water samples (Villares et al., 2001).

In light of the above, biological monitoring and analysis of metal concentrations in this highly urbanised region of the City of Cape Town is therefore necessary to identify the potential vulnerability of this aquatic ecosystem to pollution as well as to prepare baseline information for future monitoring. Therefore, this study will assess spatial and seasonal variations of several metals (Al, Mn, Fe, Zn, Pb, and Cu) in water, sediment and algae samples collected in the Zandvlei estuary.

1.1.3. Research questions

- What is the extent and variations of metal concentrations between the different sites within the study area?
- What is the extent and variations of metal concentrations between the different seasons within the study area?
- Are the recorded concentrations in line with the recommended water and sediment quality guidelines?

1.1.4. Objectives of the study

- To determine spatial variations in metal concentrations found in water, sediment and algae samples in the Zandvlei estuary.
- To determine seasonal variations in metal concentrations found in water, sediment and algae samples in the Zandvlei estuary.
- To determine if measured metal concentrations comply with national and international environmental quality guidelines.

1.1.5. Structure of the thesis

The thesis is comprised of nine main chapters and is organised as follows:

Chapter 1	• Introduce the background of the problem, statement of the research problem, objectives as well as the literature review
Chapter 2	• Presents the materials and methods used for field • sampling and laboratory analysis.
Chapter 3	• Presents the environmental parameteres measured during the different sampling ocassions
Chapter 4	• Comprises of the results and discussion of aluminium concentrations in water, sediment and algae.
Chapter 5	• Comprises of the results and discussion of manganese concentrations in water, sediment and algae.
Chapter 6	• Comprises of the results and discussion of iron concentrations in water, sediment and algae.
Chapter 7	• Comprises of the results and discussion of zinc concentrations in water, sediment and algae.
Chapter 8	• Comprises of the results and discussion of lead concentrations in water, sediment and algae.
Chapter 9	• Comprises of the results and discussion of copper concentrations in water, sediment and algae.
Chapter 10	• Provides the conclusions and recommendations for future research.

1.2. LITERATURE REVIEW

1.2.1. The role and significance of estuaries

The place where land drainage systems meet with the coastal environment is known as an estuary (Allanson and Baird, 1999). The word estuary comes from the Latin '*aestus'* which means tide (Little, 2000). Various definitions of what an estuary is, exist in literature. Whitfield and Elliot (2011) define an estuary as a "semi-enclosed coastal body of water which is connected to the sea either permanently or periodically and has a salinity that is different from that of the adjacent open ocean due to freshwater inputs and includes a characteristic biota". Kennish (2002) states that an estuarine system is comprised of three zones, namely: (i) the nearshore turbid zone – in the open ocean; (ii) the mixing zone, and (iii) a tidal river zone – characterized by a lack of ocean salinity. Freshwater inputs in estuaries are largely driven and dependent on rainfall, while oceanic inputs are primarily driven by tides (Allanson and Baird, 1999). The characteristics of estuaries are usually determined by the physical environment and climate of the region in which they are located (Allanson and Baird, 1999; McQuaid, 2013). Because of the different estuarine characteristics such as sedimentation, geomorphology, and salinity among others, the biotic communities found in these complex systems also differ considerably (Kennish, 2002). Estuaries are highly variable both in space and time and are regarded as important ecological and commercial areas of the coastal zone (Allanson and Baird, 1999; Little, 2000; Kennish, 2002). These areas are some of the most productive habitats on earth, providing significant ecosystem services such as, inter alia, flood and greenhouse gas regulation, and storm protection (Van Niekerk and Turpie, 2012) and act as breeding and nesting grounds for many fish species (McClusky and Elliot, 2004). They also support numerous coastal birds including several endangered species (Kamboo, 2006). Estuaries provide freshwater (both surface and groundwater), nutrients, and sediments to the coastal environment, thereby supporting important ecological processes and the productivity of some fisheries (e.g. prawns and line fishery). In addition, estuaries are regarded as focal points for various activities along the coast, as they provide a wide range of opportunities and benefits (Van Niekerk and Turpie, 2012). Kisand et al. (2002) note that food in estuaries is produced by plants with nutrients along with the energy from the sun together with water and carbon dioxide. Nutrients are transported from surrounding areas by streams and rivers which drain into estuaries. Some of the most important plants that contribute to estuaries are microscopic algae called phytoplankton, and other plants such as seagrasses, mangroves, and macroalgae (Kamboo, 2006).

1.2.2. The impact of anthropogenic activities on estuaries

Over the past decades, it became evident that anthropogenic activities are responsible for the extensive degradation of coastal habitat areas in many parts of the world. Some of these anthropogenic activities introduce a wide range of organic and inorganic contaminants derived from various sources such as agricultural runoff, municipal and industrial waste, boating, and other sources (Machado et al., 2002). Estuaries became (and largely remain) sinks of various pollutants and as a result, they are now considered as some of the most degraded habitats on earth (McClusky and Elliot, 2004). Various other activities such as urban development, agriculture, dredging, oil and gas production, fishing, and shipping also negatively affect estuarine and other coastal habitats. Kennish (2002) argues that pollution remains a major problem worldwide and this is due to a wide range of point and non-point pollution sources which are responsible for increasing nutrients, pathogens, and chemical contamination in estuarine waters (Kennish, 2002). The water quality of estuaries remains a concern due to the increased exposure of estuaries to contaminants and the impact these contaminants would potentially have especially on threatened estuarine ecosystems. Almost all estuaries are affected by human activities in some way, and according to Kennish (2002), this may potentially become more widespread and severe in the future due to population growth in coastal areas, which is projected to reach about six billion people by 2025. Developing countries in South America, Africa, and Asia are likely to be mostly affected as predictions indicate that these impacts are likely to emerge in the coastal zone of developing nations where government regulations and control systems are less stringent or lacking compared to most developed nations (Kennish, 2002). There are several pollutants that are reportedly found in estuaries across the globe. Some of the pollutants that are negatively affecting estuaries include (but are not limited to) petroleum hydrocarbons, pathogens, sewage, heavy metals, halogenated hydrocarbon compounds, acid mine waste, suspended solids, sedimentation/ siltation and polycyclic aromatic hydrocarbons (Kennish, 2002).

Metals enter estuarine environments through several pathways, and these include riverine inflows; non-point run-off from land; direct pipeline discharges; marine transportation; and waste dumping (Kennish, 2002; De Souza Machado et al., 2016). The above anthropogenic sources are responsible for emitting several other pollutants such as pathogens; excessive nutrients; sewage; and petroleum hydrocarbons. Significant amounts of pollutants are introduced by industrial and urban activities in marine and freshwater environments, including estuaries, causing disturbances that may lead to environmental degradation (Akcali and Kucuksezgin, 2011). Metal pollution is of great concern as it may cause irreparable damage to aquatic habitats. Furthermore, metals such as lead, chromium, cadmium, arsenic, and vanadium when found in high concentrations may accumulate in organisms, suppressing growth and damaging their tissue (Chakraborty et al., 2014). They may also accumulate through the different levels of the food chain and ultimately affect human beings (Martins et al., 2004; Alkarkhi et al., 2009; Chakraborty et al., 2014). Some of the most severe pollution problems in estuaries are attributed to toxic chemicals, oil spills, nutrient enrichment as well and sewage (Kennish, 2002). It is worth noting that some contaminants are incapable of dissolving in water (hydrophobic) and as a result, tend to rapidly adsorb onto suspended sediment, which then results in increased concentrations of contaminants in sediment when compared to the water column (CSIR, 2015). Many contaminants that are hydrophobic usually have a high bioaccumulation and biomagnification potential that could be a threat to the health of aquatic organisms and ultimately, to that of humans. It is for this reason that there is a need to protect the ecological functioning of aquatic ecosystems, as well as human health by monitoring contamination levels in the environments (CSIR, 2015). In order for aquatic systems such as estuaries to continue functioning and providing humans with services and goods, there is a need for effective management and monitoring of these systems to ensure that they are adequately protected.

1.2.3. Estuaries in South Africa

Many of the estuaries found in South Africa reportedly originate in previously incised bedrock valleys cut, during a period of lowered sea level periods of the Pliocene and Pleistocene (Allanson and Baird, 1999). South Africa has close to 300 relatively small estuaries with more than 70% of them being less than 50 ha (Van Niekerk et al., 2013). There are about 33 estuaries in the cool temperate region, 124 in the warm temperate, 130 in the subtropical region, and 2 in the tropical biogeographical region (**Table 1.2-2**), South African estuaries vary considerably around the coast in relation to their physicochemical and biotic characteristics (Van Niekerk et al., 2013). The spatial definition of estuaries in South Africa does not only include the open water area but also includes adjacent floodplain and habitat (i.e. 5 m topographical contour), therefore, including all areas that support the biological and physical processes of an estuarine system and this is known as the estuarine functional zone (Skowno et al., 2019). Based on a recently revised estuary classification system, estuaries in South Africa have been grouped into four (4) biogeographical regions and 22 distinctive estuarine ecosystem types (Skowno et al., 2019) (**Table 1.2-2**). Prior to the abovementioned reclassification system, estuaries were classified using Whitfield's (1992) classification system which was later discovered to have omitted a large number of small estuaries and micro-systems from its original characterisation (Van Niekerk et al., 2020). The four biogeographical regions comprise the cool temperate, warm temperate, subtropical, and tropical regions (**Figure 1.2-1**) (Van Niekerk et al., 2020). It is important to note that these biogeographical regions were previously classified into three regions (Emanuel et al., 1992; Turpie et al., 2000, Van Niekerk et al., 2013). However, an additional biogeographical region (i.e. tropical region) was included recently and this seems to be linked to revised estuarine functional zone (Skowno et al., 2019; Van Niekerk et al., 2020). Weather conditions such as rainfall patterns are determined by climatic zones in which estuaries are located. Since South Africa has a highly variable climate, the conditions experienced in each region vary greatly (Van Niekerk and Turpie, 2012).

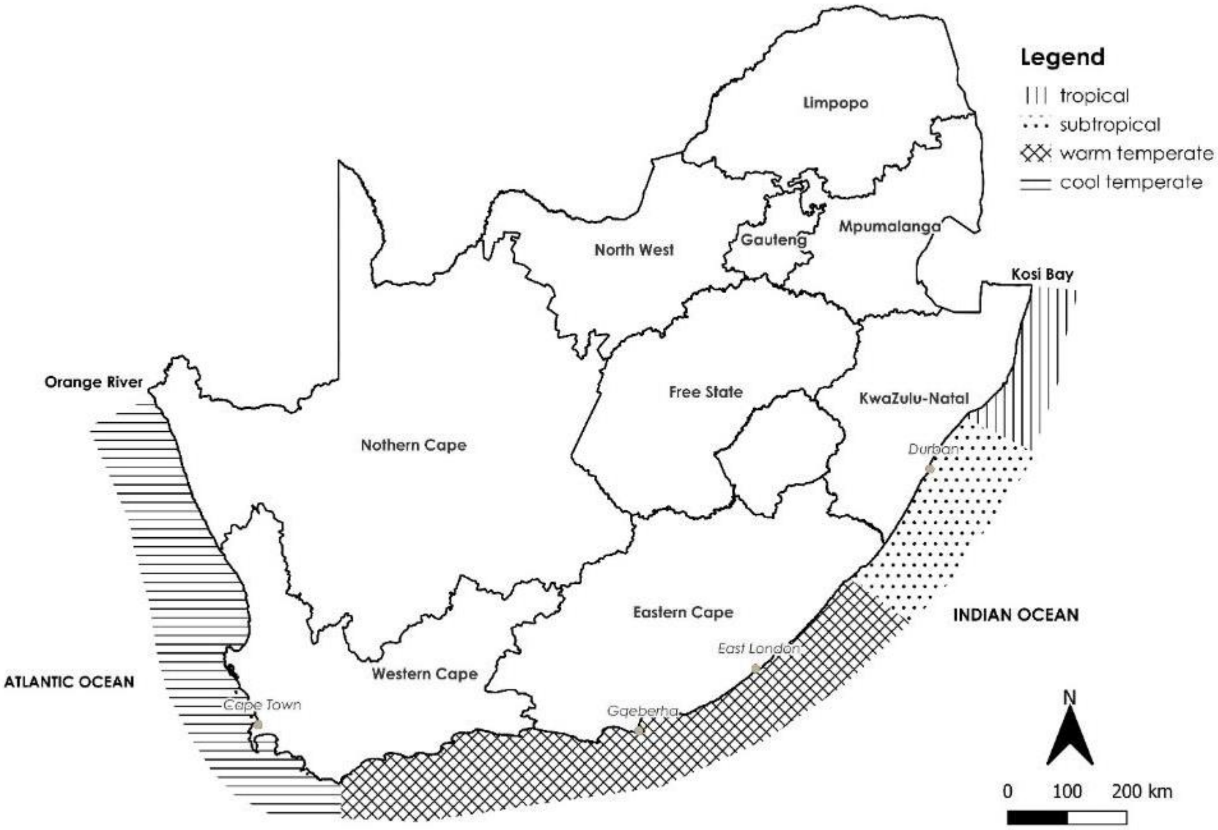

Figure 1.2-1: Map of South Africa showing the four biogeographical regions, namely: cool temperate, warm temperate, subtropical and tropical. (Source: GQIS, adapted from Whitfield and Baliwe, 2013).

The climatic range across the four biogeographic regions has influenced the utilisation of estuaries as well as human settlement patterns within these systems (Van Niekerk et al., 2013). The widely reported pressures that seem to be severely affecting South African estuaries include flow modification, exploitation of living resources, land use and coastal development, pollution, and mouth manipulation (Whitfield et al., 2012; Van Niekerk et al., 2013; Adams et al., 2020; Van Niekerk et al., 2020). Skowno et al. (2019) also noted that estuaries in South Africa are under pressure and that there is a lack of long-term monitoring data to inform conflict resolution and support confident decision-making. About 85% of the estuarine habitat in South Africa is in a poor to fair state and this is seen as a risk since there is a possibility of this percentage increasing further, particularly if the required interventions are not implemented when required or necessary (Van Niekerk et al., 2013). The monitoring of spatial and seasonal variations of contaminants in estuaries therefore becomes important as it provides critical information for assessing environmental health, protecting human health, identifying pollution sources, developing management strategies, and understanding the impacts of climate change. It helps in maintaining the ecological integrity of estuarine ecosystems and ensuring the sustainable use of these valuable resources.

Table 1.2-1: Number of estuaries in each type across four biogeographical regions and classified into 22 estuary ecosystem types (sourced from Van Niekerk et al., 2020)

Estuary type	**Cool temperate**	**Warm temperate**	**Sub-tropical**	**Tropical**	**No. of estuaries in type**	**No. of estuaries in ecosystem types**
Estuarine Lake	4	3	3	2	12	4
Estuarine Bay		1	1		2	2
Estuarine Lagoon	1				1	1
Predominantly Open	3	25	17		45	3
Large Temporarily Closed	9	40	44		93	3
Small Temporarily Closed	8	48	60		116	3
Large Fluvially Dominated	1	1	5		7	3
Small Fluvially Dominated	1	6			7	2
Arid Predominantly Closed	6				6	1
Total	**33**	**124**	**130**	**2**	**289 (290 in total)**	**22**

1.2.4. Legislative context for the management of the coastal environment in South Africa

South Africa is a developing nation that understands the importance of protecting and managing the environment. This is demonstrated by the country's participation and commitments that have been ratified both internationally and locally. There are several legislative frameworks, policies, and agreements that South Africa has agreed to uphold and implement in an effort to ensure the sustainable use, protection, and management of natural resources. The international community (such as the United Nations) which South Africa forms part of, was the first to spearhead initiatives and programmes that aimed at addressing environmental issues. The history of the United Nations and its first environmental conference in Stockholm, Sweden in 1972, is well documented. One of the instrumental international agreements that provided a legal framework for the regulation and management of marine and coastal resources was the 1982 UN Convention on the Law of the Sea, which South Africa ratified together with other member states. There are several other international treaties that South Africa ratified that provided a legal framework for the management of coastal and marine ecosystems and this includes, as an example, the Convention on Wetlands of International Importance Especially as Waterfowl Habitat, 1971 (Ramsar Convention); the Convention on the Conservation of Migratory Species of Wild Animals, 1979 (Bonn Convention); and the Convention on Biological Diversity, 1992. There are several coastal wetlands (including estuaries) in the Western Cape Province of South Africa that have been declared as RAMSAR sites (Cape Nature, 2023). An example of estuaries in the Western Cape that have been declared as RAMSAR sites include the Heuningnes Estuary, Bot-Kleinmond River Estuary as well and the Berg Estuary. South Africa's participation and ratification of international agreements influenced the development of National Legislation which speaks to the protection and management of the environment. The Bill of Rights in Chapter 2 of the Constitution of the Republic of South Africa, 1996 (Act No. 108 of 1996) guarantees everyone within the jurisdiction of South Africa a right to an environment that is not harmful to their health or well-being.

Section 24 of the above Act states the following:

“Everyone has the right:

- *(a) to an environment that is not harmful to their health or well-being; and*
- *(b) to have the environment protected, for the benefit of present and future generations, through reasonable legislative and other measures that—*
    - *i. prevent pollution and ecological degradation;*
    - *ii. promote conservation; and*
    - *iii. secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development.”*

An important national environmental legislation in South Africa that gives effect to Section 24 of the Constitution is the National Environmental Management Act (NEMA), 1998 (Act No. 107 of 1998) which was promulgated after the Environmental Conservation Act, 1989 (Act No. 73 of 1989) (which has mostly been repealed). The National Environmental Management Act is an overarching framework for environmental law in South Africa. There are several Specific Environmental Management Acts (SEMAs), which all fall under the auspices of the overarching NEMA. Some of these SEMA's have been highlighted below and this excludes the National Water Act which is not a SEMA. The National Water Act (NWA), 1998 (Act No. 36 of 1998) was developed in an effort to ensure and enhance the protection of the aquatic ecosystems of South Africa's water resources. The National Environmental Management: Protected Areas Act (NEM:PAA), 2003 (Act No. 57 of 2003) was developed with the objective of ensuring the protection and conservation of ecologically viable areas representative of South Africa's biological diversity and its natural landscapes and seascapes.

The National Environmental Management: Biodiversity Act (NEM:BA), 2004 (Act No.10 of 2004) was developed for "the management and conservation of South Africa's biodiversity within the framework of the NEMA, the protection of species and ecosystems that warrant national protection, and the use of indigenous biological resources in a sustainable manner, amongst other provisions". The Act indicates that the state is the custodian of South Africa's biological diversity and is committed to respecting, protecting, promoting, and fulfilling the constitutional rights of its citizens. A key SEMA for the coastal environment is the National Environmental Management: Integrated Coastal Management Act (NEM: ICMA), 2008 (Act No. 24 of 2008) which according to DEA (2015) was developed to facilitate the sustainable use and management of South Africa's coastal and estuarine resources. The ICMA requires that estuaries within South Africa be managed in an efficient and coordinated manner in line with the National Estuarine Management Protocol (NEMP) and the National Coastal Management Programme (CMP) which lay out specific objectives for the management of the South African coastal environment, including estuaries (DEA, 2015).

The Local Authority, in this case, the City of Cape Town also has several By-Laws and Policies which are in place for the management of important conservation areas such as the Zandvlei estuary which forms part of the Greater Zandvlei Nature Reserve as declared in terms of Provincial Gazette 6389/27, October 2006 (PN 344/2006). The City of Cape Town's recently approved Coastal By-Law, 2020 and Nature Reserve By-Law, 2021 are some of the key legislations that are in place to ensure the protection and management of coastal areas and nature reserves. Some of the key policies include, inter alia, the Management of Urban Stormwater Impacts Policy, 2009 as well as the Floodplain and River Corridor Management Policy, 2009.

1.2.5. The study area: Zandvlei estuary

1.2.5.1. History and development

The Zandvlei estuary appears in charts of the Cape Colony (1679 – 1712) as a small bay connected to False Bay (Morant and Grindley, 1982). According to early sources, the estuary was reputedly named by Jan van Riebeeck of the Dutch East India Company in 1665 (Burman, 1962; Morant and Grindley, 1982; Thornton et al., 1995). The spelling of the estuary's name had changed over the past decades from "Sand Vlei" to "Zand Valei" and the name was officially changed (in spelling) by the City of Cape Town to "Zandvlei" in 1981 (McQuaid, 2013). Before 1866 when water levels in the estuary were significantly reduced by a severe drought, little was known about activities in (and around) the Zandvlei estuary (Morant and Grindley, 1982). However, some of the documented activities which occurred before the drought of 1866 include the establishment of a cattle post along the shores of the estuary in 1673 by the Dutch

East India Company and this was (reputedly) the beginning of modern human activities in the region which continued for over centuries (Morant and Grindley, 1982). In 1774, the establishment of a fortified post on the western side of the estuary was also seen (Morant and Grindley, 1982). According to Brown and Magoba (2009), the estuary at times would dry out completely and this happened under natural conditions. When there was a drought in 1886, there were plans to reclaim the land within the lake by drying it out completely. The first step taken towards this was to close the connection (outlet/mouth) between the lake and the sea. However, no plans were in place to divert rivers feeding into the estuary and as a result, in winter, water entered the estuary through influent rivers (Morant and Grindley, 1982). In 1882 a railway was constructed on the north-western edges of the estuary which connected Cape Town and Muizenburg. After the construction of the railway line, access to the lake increased significantly. The improved access further resulted in the extensive use of the estuary where major recreational activities were held. The first rowing regatta reportedly took place in 1884 June and the second one was in September. In 1907, the Imperial Yacht Club was established due to increased sailing activities within the estuary. However, the club was disbanded in 1946 due to low water levels, weed infestation, and siltation which hampered boating activities. Plans were also put forward to dredge the lake in 1929; however, these plans were subsequently suspended due to World War II (Morant and Grindley, 1982). In 1978, a 22-ha portion along the northern shore of the estuary was proclaimed as the Zandvlei Bird Sanctuary. In 2006, a 204-ha area (inclusive of the above 22ha) was declared as the Greater Zandvlei Estuary Nature Reserve in terms of Provincial Gazette 6389/27, October 2006 (PN 344/2006) (CoCT, 2011).

1.2.5.2. Physical characteristics

Zandvlei is a long, shallow coastal estuarine lake situated on the north-western shore of False Bay (34°05' S; 18°28' E) (**Figure 1.2-2**), east of Muizenburg, about 20 kilometres south of Cape Town (Harding, 1994; McQuaid, 2013). The estuarine lake forms part of the estuaries located in the Cape Floristic Region and it falls within the cool-temperate biogeographic region in South Africa (Quick and Harding, 1994; WCG, 2018). The Zandvlei estuary has a sporadic tidal connection to the sea and is classified as a temporarily open/closed estuary (Whitfield and Baliwe, 2013). It is also the largest of the eight estuaries situated in False Bay, as it covers about 155 hectares which makes up approximately 80% of the estuarine area in False Bay (Brown and Magoba, 2009; McQuaid, 2013). Zandvlei is divided into three components (Morant and Grindley, 1982; Brown and Magoba, 2009), namely: a) the Marina Da Gama (residential area) with a water surface area (WSA) of approximately 32.6 hectares, b) the main vlei (the lake itself) (WSA of approximately 57 hectares), and c) the Westland wetland (WSA of approximately 31 hectares) (**Figure 1.2-3**). The lake has a depth of between 0.7 to 1.3 metres, the maximum depth is approximately 2 metres (Harding, 1994), it is about 2.6 km long and is approximately 0.5 km wide, at its widest point, and this is excluding the Marina Da Gama residential area (Brown and Magoba, 2009). Prior to the modification of the estuary, when it was in its natural state, water levels were reported to fluctuate between 2.5 to 3.0 metres above mean sea level (AMSL) which resulting in flooding in some areas within the estuary and this was before the level of the sand berm was broken (Brown and Magoba, 2009). The mouth of the estuary is artificially breached even though water levels were recently reported to be only approximately +1.3 to +1.4 metres above mean sea level, the breaching is necessary due to low-lying residential areas such as the Marina Da Gama (Brown and Magoba, 2009). According to McQuaid (2013), the formation of a sand bar during the summer period closes the mouth of the estuary and thus prevents seawater from flowing into the estuary. It is normally during the winter period where seawater comes into contact (and mixes) with the water in the estuary and this is due to the absence of a sand bar on the mouth of the estuary. The Zandvlei estuary lies in a Mediterranean climatic region and receives its annual rainfall from May to September, averaging between 400 to 600 mm (Thornton et al., 1995) and also experiences a warm to hot dry summer (Morant and Grindley, 1982). The climate of the False Bay coastline is also reported as being characterized by winds that are predominantly South to South East during the dry season (i.e. spring and summer), and North to North West during winter (mean wind speed for 1992 was 6.0 $\mathrm{m}.s^{-1}$) (Harding, 1994).

The Zandvlei catchment comprising an area of 9,655 ha is drained by several rivers and streams which include the Little Princess Vlei Stream, Westlake Stream, the Sand River, Diep River, Keysers Rivers, and Langvlei Canal (C.A.P.E., 2010). However, it is important to note that the Keysers, Sand, and Westlake Rivers are considered as the main rivers that feed into the estuary (Brown and Magoba, 2009). The Diep River name changes and is called the Sand River downstream of little Princessvlei. The Sand River system drains the southern end of Table Mountain and an area south of Wynberg Hill. According to McQuaid (2013), this river is the most important river that runs into the lake. The Westlake River rises on Steenberg Mountain and flows near a suburb called Lakeside and into Zandlvei (Brown and Magoba 2009; McQuaid, 2013). The Keysers River drains Constantiaberg and flows into the lake in the northwestern corner (Brown and Magoba, 2009; McQuaid, 2013).

Land use in the catchment is reported as varying from industry to housing, agriculture, forestry, and conservation (**Figure 1.2-3**). Recreational use in Zandvlei included a limited amount of fishing, however, this has since changed to ‘catch and release’ unless it is an alien fish species and bait collection is prohibited. Other recreational activities include bird watching, boating activities, picnicking, hiking, and sporting events (C.A.P.E., 2010).

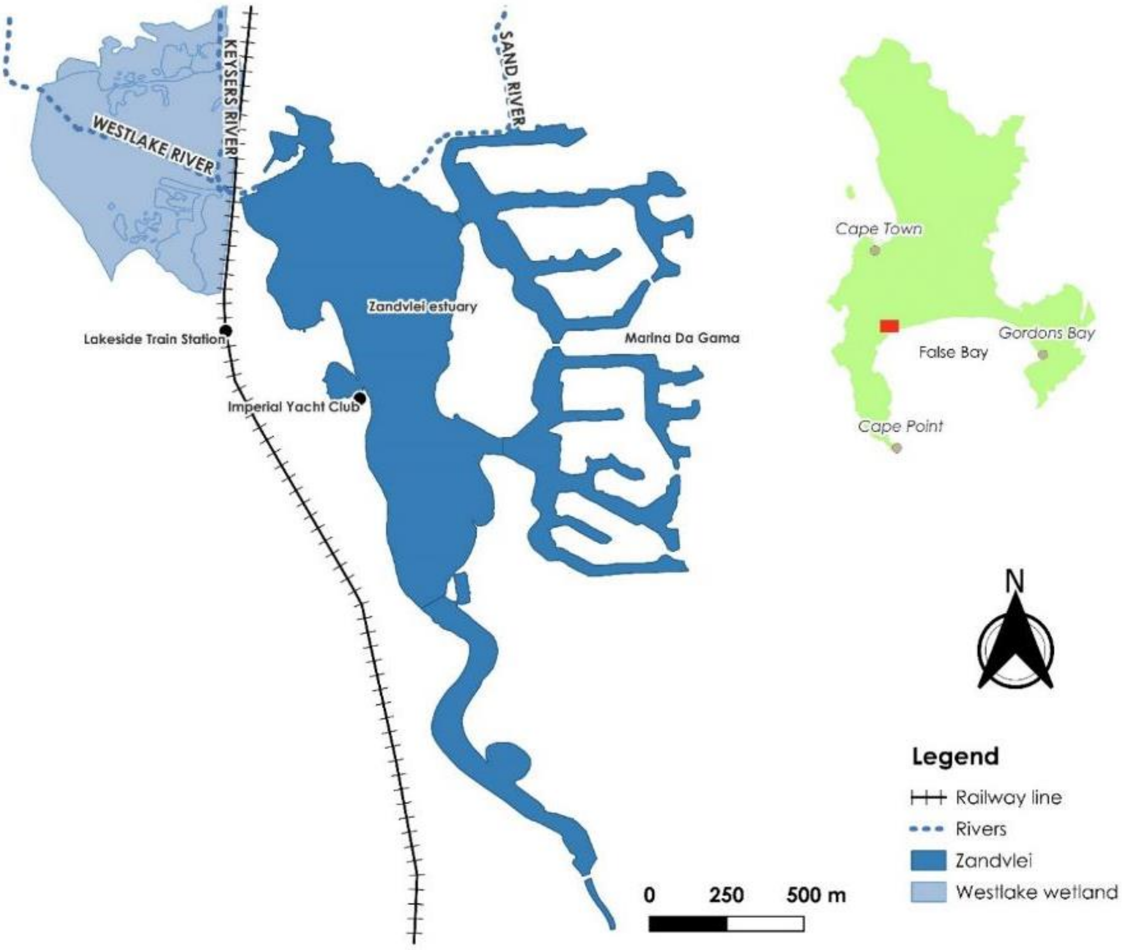

Figure 1.2-2: Map of the Zandvlei estuary and its influent rivers, namely: Westlake, Keysers, and Sand River. The location of the study area is shown with a red polygon on the City of Cape Town map (green map on the right-hand side) (Source: QGIS, Sidondi, L)

Previous studies conducted on the Zandvlei estuary also indicate that the lake including the Marina da Gama has for many years been dominated by pondweed (*Stuckenia pectinata*) (Harding, 1994). This plant plays a critical role in the estuary, more especially in the functioning of the lake ecosystem (Quick and Harding, 1994). It increases water clarity by reducing sediment resuspension, removing nutrients from the water, and providing feeding areas for juvenile fish and other aquatic fauna (Quick and Harding, 1994). The pondweed in the estuary is managed with a floating mechanical harvester which cuts the plant to ensure the continuity of recreational activities such as sailing without impairing the function of the plant (Harding, 1994). As far as algae is concerned, there are several species which are reportedly growing in the Zandvlei estuary; these include *Nittela sp*., *Lamprothanmium sp*. (Morant and Grindley, 1982) and *Enteromorpha spp*. and *Cladophora sp* (Thornton et al., 1995)

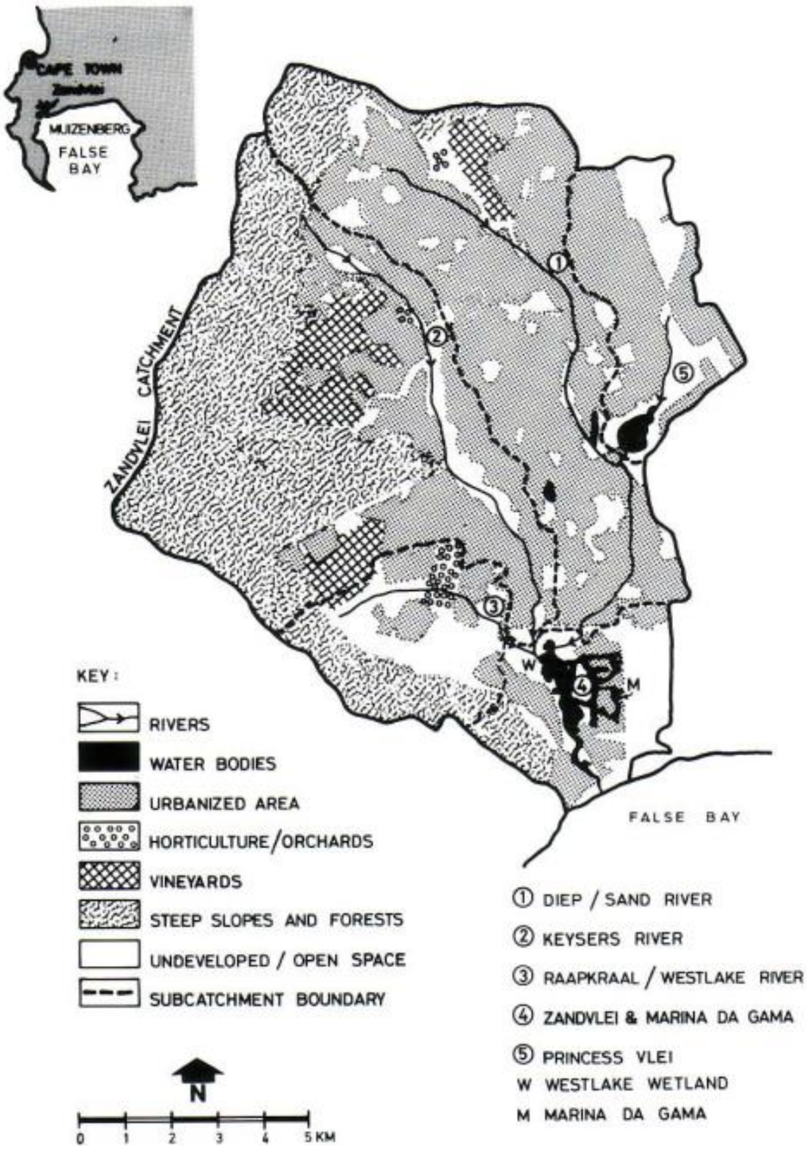

Figure 1.2-3: Map of the Zandvlei estuary and surrounding land uses (Map sourced from Quick and Harding, 1994)

1.2.6. Pollution of Cape Town estuaries

The cool temperate region (where the study area lies) has the highest percentage (63%) of estuaries (about 33%) that are under severe pollution pressure (Skowno et al., 2019). The City of Cape Town has highlighted in its State of the Environment Report (CoCT, 2022), that stormwater runoff, municipal wastewater, agricultural runoff, and industrial wastewater are some of the identified sources of pollution that negatively affects some of its aquatic ecosystems. Rapid urbanisation due to an increase in coastal population growth is recognised as one of the key driving forces which have led to an increase in pollution levels within the coastal environment. Ageing infrastructure coupled with South Africa’s energy crisis is recognised as one of the key problems which contribute to sewer overflows in some wastewater treatment facilities (i.e. pump stations) within the City of Cape Town, and this is in addition to other challenges relating to materials disposed of in the sewer system which causes blockages and mechanical failures (CoCT, 2022). Sewer discharges or spillages into estuaries can have a detrimental impact on the health and functioning of these ecosystems and may also pose a threat to human health. The Zandvlei estuary has also been affected by sewage spillages emanating from malfunctioning pump stations as a result of electricity outages due to a struggling electricity grid in South Africa. According to Van Niekerk et al. (2013), contaminated stormwater runoff from commercial and industrial areas is one of the key pollution issues faced by estuaries in urban areas. Since commercial and industrial areas are production/manufacturing areas, an array of pollutants such as trace metals, hydrocarbons, nutrients, etc. are released into the environment and transported in stormwater runoff which discharges them into rivers and estuaries (Binning and Baird, 2001, Van Niekerk et al., 2013; McDonald et al., 2022). Stormwater runoff from fertilised gardens and agricultural areas (i.e. wine farms etc.) also contributes to the pollution of estuaries. Illegal discharges into the City’s stormwater system in commercial and industrial areas is a challenge that has been noted by the City of Cape Town Municipality (CoCT, 2022). This may adversely affect the water quality and ecological functioning of these aquatic ecosystems. Poor water quality reduces the resilience and nursery function of these ecosystems; kills aquatic life (i.e. invertebrates and fish); threatens habitat diversity; and increases the vulnerability of estuaries to invasive species, parasites, pathogens, and diseases. Poor water quality also impacts estuarine resilience to natural stresses such as climate change over an extended period (Skowno et al., 2019). Urban development is also responsible for the pollution and modifications of these aquatic ecosystems. According to Skowno et al. (2019), approximately 29% of South African estuaries are subject to severe pressure from habitat modification due to urban, industrial, agricultural, and infrastructural development, and most of these are recorded in the Cool Temperate region (63%). Developments such as road bridges, residential units (Marinas), as well as activities such as dredging, are some examples highlighted by Van Niekerk et al. (2013) and Skowno et al. (2019) of activities which have a detrimental impact on estuaries as they lead to various negative outputs (i.e. sedimentation, changes in flow water velocity etc.) that impact on the functioning of an estuary. The study area (i.e. Zandvlei) is a typical example of an estuarine system that is severely affected by several human activities (almost all the key pressures highlighted above). Van Niekerk et al. (2013) have also identified emerging pressures that are in need of further investigation as they are likely to increase toxic substances and metal pollution in sediments. It is important to note that rivers feeding into estuary are in many instances the vehicle which transports contaminants into these systems. For instance, elevated levels of copper and zinc were once reported in one of the rivers feeding into the study area (C.A.P.E., 2010).

1.2.7. Metal contaminants

Metals occur naturally in the environment and are vital for survival. They are needed for plants in various physiological processes as a form of micronutrients (Manganese (Mn), Iron (Fe), Zinc (Zn), and Cobalt (Co)) (Thorat et al., 2023). However, at high concentrations, these essential trace elements become toxic (Chakraborty et al., 2014; Thorat et al., 2023). Notably, metals such as Chromium (Cr), Cadmium (Cd), and Lead (Pb) are considered highly toxic even at low concentrations (Sodhi et al., 2022; Thorat et al., 2023). Metals are introduced to the environment through various natural processes such as weathering of rocks and erosion of soils, volcanic eruptions, and also through various anthropogenic activities (i.e. waste disposal, industrial or domestic effluents, dredging, etc.) (Bryan et al., 1985; El-Sorogy and Attiah, 2015; De Souza Machado et al., 2016). Anthropogenic activities have introduced additional processes that have increased the rate of redistribution of metals into the environment (Garrett, 2000). The toxicity and persistence of metals could be moved through bioaccumulation to a higher trophic level by a process known as bioconcentration and biomagnification and may pose a threat to both human and animals (Phillips et al., 2015; Antizar-Ladislao et al., 2015; Billah et al., 2017). Bioconcentration is known as a process where contaminant materials (i.e. metals) are accumulated and sequestrated by organisms directly from the ambient environment (Connell et al., 1999). Biomagnification is defined as the increase in contaminant concentration in an organism due to bioconcentration and trophic transfer (Hoffman et al., 2002; Connell et al., 1999). The release of metals as a result of anthropogenic activities has been a cause of public health concern across many parts of the world. One of the first widely documented instances of metal contamination in the aquatic environment occurred in 1953 at Minamata Bay in Japan (Phillips and Rainbow, 1994; Harada, 1995; Eto, 2000). The death of living organisms such as fish and birds were noted in Minamata in the early 1950s and this was followed by the mortality of animals such as cats in 1953 (Phillips and Rainbow, 1994). In 1956, humans were also starting to show strange symptoms relating to the central nervous system (Phillips and Rainbow, 1994; Harada, 1995). It was later discovered that a chemical plant was releasing effluent containing mercury and other pollutants such as copper, lead, manganese, arsenic, selenium, and thallium. The pollution of the aquatic environment in Minamata Bay resulted in a neurological syndrome caused by severe mercury poisoning, with symptoms including general muscle weakness, narrowing of the field of vision, and damage to hearing and speech (Crichton, 2012). Mercury bioaccumulated in aquatic food chains reaching its highest concentrations in fish and shellfish in Minamata Bay and the Shiranui Sea, which when eaten by the local population resulted in mercury poisoning (Phillips and Rainbow, 1994; Eto, 2000; Crichton, 2012). As of March 2001, 1784 of the 2265 victims that were identified, died and it was only in 2004 that the corporation responsible for the chemical factory was ordered to clean up its contamination (Crichton, 2012). There are also other parts of the world that have experienced similar events where aquatic life and humans have been negatively affected by the release of toxic chemicals such as metals into the environment. This is the reason why monitoring of these contaminants in the aquatic environment is critically important not only for the ecosystems or ecological functioning of these systems but also for the economy.

This study has obtained results for several chemical elements but only the following metals were discussed: Al, Mn, Fe, Zn, Pb, and Cu. Also, since some of the chemical elements discussed are regarded as “heavy metals” and others as “trace metals”, the author of this study decided to make reference to both elements as “metals” instead of referring to them as heavy or trace metals.

1.2.7.1. Aluminium

Aluminium is the most abundant metallic element and constitutes about 8% of Earth’s crust (WHO, 1997). It is described as a highly reactive element that is never found as a free metal in nature (ATSDR, 2008). Aluminium is found naturally in the environment as silicates, oxides, and hydroxides, combined with other elements, such as sodium and fluoride, and as complexes with organic matter (WHO, 2003a). In nature, aluminium has only one oxidation state (+3) and as a result, its transport and distribution in the environment depend on the physicochemical parameters of the local environmental system as well as its coordination chemistry (WHO, 1997). The aluminium metal is light in weight and silvery-white in appearance (ATSDR, 2008). Aluminium is released to the environment through natural processes and also from anthropogenic sources. According to WHO (1997) natural processes far outweigh direct anthropogenic contributions to the environment. Naturally, aluminium is commonly found in soil, minerals, rocks, and clays (ATSDR, 2008). Aluminium is highly concentrated in dusts from activities such as mining and agriculture, and in particulate matter from coal combustion (WHO, 1997). It is used in the construction, automotive, and aircraft industries; in the production of metal alloys, in the electric industry; in cooking utensils; and in food packaging. Its compounds are also used as antacids, antiperspirants, and food additives (ATSDR, 2008). As mentioned, it is important to note that since aluminium occurs naturally and is ubiquitous within the Earth’s crust, natural releases through weathering far outweigh or exceed anthropogenic releases of aluminium to the air, water and land (ATSDR, 2008). The solubility and bio-availability of aluminium in aquatic systems is strongly dependent on the pH of the receiving waters (DWAF, 1996). In surface waters, aluminium is usually very low (<0.1 mg/L), however, the concentration of soluble aluminium increases due to the increased solubility of aluminium oxide and salts in acidic waters (ATSDR, 2008). Furthermore, the toxicity of aluminium is dependent on the species and life stage of the organism, the concentration of calcium in the water as well as the pH (DWAF, 1996). According to the WHO (2010), there is no indication that aluminium is carcinogenic. This is also confirmed by the ATSDR (2008) where it is was found that the higher than expected levels of cancer mortality rate in workers from an aluminium industry was most likely due to other carcinogens such as polycyclic aromatic hydrocarbons (PAHs) and tobacco smoke which they were also exposed to.

1.2.7.2. Manganese

Manganese is regarded as an essential micronutrient for plants and animals (DWAF, 1996). It is ubiquitous in the environment, usually occurring with iron and is the eighth most abundant metal in nature, making up about 0.1% of the Earth's crust (WHO, 2004; Mitra et al., 2022). In aquatic ecosystems, manganese does not occur naturally as a metal but is found in various salts and minerals, frequently in association with iron compounds (DWAF, 1996; WHO, 2011). Manganese is found in over 10 oxidation states but the most environmentally and biologically important manganese compounds are those that contain the soluble manganous (Mn^{2+}); insoluble manganic (Mn^{4+}) or permanganates (Mn^{7+}) (DWAF, 1996: WHO, 2011). Some of the significant natural sources of manganese include soils, sediments and rocks (DWAF, 1996). It is a widely used element for the manufacture of iron and steel alloys and manganese compounds (WHO, 2011). It is also commonly used in the manufacturing of batteries, glassware, fireworks, fertilizers, fungicides, paints, cosmetics animal supplementation and other various products (ATSDR, 2012; Rodrigues et al., 2019). Anthropogenic sources of manganese in waterbodies include municipal wastewater discharges, sewage sludge, mining and mineral processing, emissions from production of alloy, steel, and iron industries and the combustion of fossil fuels (WHO, 2004). Elevated manganese concentrations in surface water may be associated with industrial pollution and mining effluents (WHO, 1981). While manganese is required and important for a variety of physiological activities, excessive intake may however result in significant toxicity (Mitra et al., 2022). Since manganese is required in small doses to support life and some biological functions, including the fact that it is ubiquitous in the environment, it has been overlooked as a potential contaminant (Queiroz et al., 2021). Furthermore, it was found that in coastal areas such as estuaries, there are a few studies that have reported manganese as a potentially toxic element since concentrations are usually low in these ecosystems (Queiroz et al., 2021). Toxic effects are likely to manifest in living organisms if and when manganese concentrations exceed the recommended reference or guideline concentration values stipulated by various international environmental institutions and government organisations. Rodrigues et al. (2019) listed several studies conducted in Brazil that reported on the effects caused by acute or chronic exposure to manganese in different biological models and one of the most prevalent effects in animal and cellular models is neurotoxicity, followed by liver changes. Humans are also subject to similar effects as those reported in animals and cellular models, the most prominent being neurotoxicity, including cognitive and learning deficits and childhood hyperactivity (Bouchard et al. 2018; Rodrigues et al., 2019).

1.2.7.3. Iron

Iron is the second most abundant metal and fourth most abundant element in the earth's crust, and accounts for about 5% of the Earth's crust (WHO, 2003b). Iron is usually found in nature in the form of its oxides, as it readily combines with oxygen- and sulfur-containing compounds to form oxides, hydroxides, carbonates, and sulphides (WHO, 2003b). Iron naturally occurs in many minerals with the main ones being magnetite, hematite, goethite, pyrrhotite, siderite, ilmenite and pyrite. It usually occurs in soils (especially clays) and is found in waterbodies as a result of runoff, erosion of clay-based soils, and other geologic sources (Environment and Climate Change Canada, 2019). Iron in water occurs in the two most common states which include the reduced ferrous (Fe^{2+}) and the oxidised ferric, (Fe^{3+}) states (DWAF, 1996). This means that Fe^{2+} is the dominant form of iron under reducing conditions, while Fe^{3+} is the dominant form under oxidizing conditions. Iron is an essential micronutrient for all organisms as it plays a key role in the physiological processes of all living organisms (ANZECC and ARMCANZ, 2000; WHO, 2003b; Kamble et al., 2013). It is required in the enzymatic pathways of chlorophyll and protein synthesis, as well as other respiratory related functions (DWAF, 1996). Some iron compounds are used as food colourants and for the treatment of iron deficiency in humans. Other human uses of iron comprise of its inclusion in constructional material (among others) for drinking-water pipes, and iron oxides as pigments in paints and plastics. Various iron salts are also used as coagulants in water treatment (WHO, 2003b). Naturally, iron is released through the weathering of ores and rocks as well as the leaching of sandstones which release iron oxides and iron hydroxides, while anthropogenic sources of iron emanate from the burning of coal, acid mine drainage, mineral processing, sewage, landfill leachates and the corrosion of iron and steel (DWAF, 1996).

1.2.7.4. Zinc

Zinc is an essential trace element for numerous biological functions and is found widely in nature (Eisler, 1993; DWAF, 1996; CCME, 2018). Elemental zinc does not occur in the natural environment (WHO, 2001). It is usually found in two oxidation states (Zn^0 and Zn^{2+}) in the environment whilst the metallic form is found only in highly reducing environments (ATDSR, 2005: CCME, 2018). It is a transition element that can form complexes with a range of different compounds, such as zinc chloride, zinc sulfate and zinc oxide (ATDSR, 2005; WHO, 2001). The metal zinc is insoluble in water, however, several of its salts are freely soluble (CCME, 2018). Zinc is found in varying amounts in most rocks and many minerals (WHO, 2001). It is introduced into the aquatic environment through natural (i.e. weathering and erosion,) and anthropogenic (i.e. industrial) activities (DWAF, 1996). Zinc is found in both suspended and dissolved forms in aquatic systems; however, it is usually partitioned into suspended and bottom sediments (Eisler, 1993). The behaviour (i.e. mobility, fate and bioavailability) of zinc in aquatic systems is controlled by several physical and chemical processes such as salinity, pH, alkalinity, redox potential (Eh), and dissolved organic matter (WHO, 2001; CCME, 2018). Water with low pH and alkalinity as well as high ionic strength tend to result in elevated dissolved zinc concentrations (DWAF, 1996). In the air, natural zinc inputs are mainly emitted by igneous emissions and forest fires while erosion is one of the largest natural emissions of zinc in water (WHO, 2001). The main anthropogenic sources of zinc include iron and steel production, corrosion of galvanized structures, coal and fuel combustion, mining, zinc production facilities, waste disposal and incineration, and the use of zinc-containing fertilizers and pesticides (WHO, 2001). Anthropogenic and natural sources are of a similar magnitude. Zinc compounds are used in many industries such as the rubber industry, dentistry, medicine, paint, cosmetics and household products (ATSDR, 2005). It is also used in automotive equipment, as well as storage and dry cell batteries (WHO, 2001). In some industries (i.e. steel industry), the carbonate, hydroxide and oxide forms of zinc are widely used as a protective coating for iron and steel products such as sheet metal, pipes, and wire and this is due to the relatively resistant characteristic of zinc to corrosion and rust (DWAF, 1996; WHO, 2001; CCME, 2018). The widespread use and occurrence of zinc in the environment may have a negative impact on living organisms, especially if found in relatively high concentrations. For instance, aqueous Zinc (Zn^{2+}) ion is known to be the most toxic to aquatic organisms (i.e. fish) at relatively low concentrations. (DWAF, 1996; ANZECC, 2000). Zinc in high concentrations may result in negative effects on reproduction, biochemical and physiochemical reactions as well as behavioural effects in aquatic organisms (WHO, 2001). Zinc deficiency in algae limits carbon assimilation, which shows that zinc could be a limiting element for algal growth (Malasarn et al., 2013).

1.2.7.5. Lead

Lead is a non-essential element that is toxic to the environment at higher concentrations. It is usually found in lead-containing ore deposits that are widely distributed throughout the world (WHO, 2003c; ATSDR, 2020). It is a non-biodegradable metal that is found in relatively low concentrations in the natural environment. Lead exists in several oxidation states (i.e. 0, I, II and IV), however, it is highly unlikely to be found in its elemental state (Pb^0) in nature (DWAF, 1996; CCME, 1999a). Common oxidation states in which lead occurs in the natural environment include the monovalent (Pb^{1+}), divalent (Pb^{2+}), and tetravalent (Pb^{4+}) states, with Pb^{2+} being the most common (CCME, 1999a). Divalent lead (Pb^{2+}) is known as the stable ionic species present in the environment and is regarded to be the form in which most lead is bioaccumulated by aquatic organisms (DWAF, 1996). The bioavailability of the divalent lead (Pb^{2+}) in aquatic systems is influenced by physicochemical parameters such pH, whereby decreasing pH increases the bioavailability of Pb^{2+} (DWAF, 1996). In aquatic environments, lead minerals are usually found in lower concentrations, and this is possibly due to lead and its minerals being almost insoluble in water (DWAF, 1996). Lead is mainly released (through natural processes) into the aquatic environment through the weathering of sulphide ores (DWAF, 1996). Once lead is introduced into the aquatic system it can either be deposited in suspended sediments which are usually associated with particulate matter, such as iron and manganese oxides, or it can be precipitated out of solution with carbonate or sulphide (CCME, 1999a). Therefore, sediment can act as a sink of most of the metal contaminants (including lead) that are released into the aquatic environment. Most of the lead released into the environment is from an anthropogenic source (DWAF, 1996; ATSDR, 2020). Lead levels are increasing rapidly because of anthropogenic activities such as industrial and municipal wastewater discharge; mining, manufacturing, milling and smelting of lead and metals associated with lead (e.g. zinc, copper, silver, arsenic and antimony); and combustion of fossil fuels. The use of leaded petrol before it was phased out in many parts of the world, including South Africa in 2006, was also one of the main anthropogenic sources of lead in the environment. Although leaded petrol was phased out and is no longer in use, elevated levels of Pb were still found in soil alongside busy roads compared to that alongside less heavily trafficked roads (Mathee, 2014). Lead is still being used in some paints and although there are regulations which were promulgated for the controlled use of lead in paints whereby lead content of 600 ppm or higher is regarded as a hazardous substance, the adherence and enforcement of such regulations remains questionable (Mathee, 2014). When exposed to elevated amounts (i.e. < 0.05 mg/l), lead is toxic to human and children are particularly at higher risk of lead poisoning as the severity of poisoning increases when they come into contact with lead dust (ATSDR, 2020). Lead poisoning can result in several effects, namely: neurological, renal, cardiovascular, haematological, immunological, reproductive, and developmental effects (ATSDR, 2020).

1.2.7.6. Copper

Copper is regarded as an essential micronutrient for living organisms. Copper occurs naturally in rock, soil, sediment, water, and to some extent, in the air (ATSDR, 2022). Copper occurs in four oxidation states (Cu^0, Cu^{1+}, Cu^{2+}, and Cu^{3+}) with Cu^{1+} and Cu^{2+} being most common (DWAF, 1996; CCME, 1999b). It is usually found in low concentrations in aquatic (i.e. estuarine) and marine environments, but can be toxic if concentrations are relatively higher than those required for the optimum growth of algae (ANZECC and ARMCANZ, 2000). The natural source of copper is the Earth's crust, hence copper also occurs naturally in plants and animals (ATSDR, 2022). The natural occurrence of copper in the aquatic environment is as a result of weathering or dissolution of copper minerals (DWAF, 1996). Although metallic copper is insoluble in water, however copper salts such as Cu^{2+} and Cu^{1+} are highly soluble (DWAF, 1996). Copper is widely used in several industries including manufacturing, automotive, construction, electrical, and smelting (CCME, 1999b; ATSDR, 2022). Specific uses where copper is utilised may include, inter alia, marine antifouling paints, plumbing, electrical wiring, electrical devices, cookware, animal feed, fertilizers, wood preservatives, and roofing, (Henckens and Worrell, 2020). In light of the above, and according to DWAF (1996) the main anthropogenic sources of copper in aquatic environments (such as estuaries), include, inter alia, sewage treatment plant effluents, corrosion of brass and copper pipes, runoff from fungicides and pesticides containing copper in the treatment of soils, and liquid effluents and atmospheric fallout from activities such as mining, smelting and refining industries, coal-burning, and iron- and steel-producing industries. As an essential micronutrient, copper in human and animals is required for growth, neuroendocrine function lung elasticity, vascular function, neovascularization and iron metabolism (NRC, 2000). In plants, copper plays a key role in physiological functions of such as the formation of chlorophyll, carbohydrate and protein metabolism, and photosynthesis (Mitra et al., 2020). Although copper plays an important role in living organisms, excess intake may result in toxicity and could also harmfully interact with metals such as zinc (ATSDR, 2022). Furthermore, copper deficiency affects important metabolic processes (Mitra et al., 2020).

1.2.8. Accumulation of metals in water and sediment

The monitoring of metal concentrations in aquatic (i.e. freshwater/marine) environments was for many years conducted through the analysis of water, sediment and biological material or biota (Bryan et al., 1985; Rainbow, 1995). However, several shortcomings were identified in the traditional method which involved the analysis of metal concentrations in water (Rainbow and Phillips, 1993). In surface waters, depending on environmental conditions, metals are found in two phases, namely: the dissolved or particulate colloidal phases (Sany et al., 2013; Lange et al., 2020). Dissolved metal concentrations are often low and at the limits of analytical detection which may require pre-concentration (Rainbow and Phillips, 1993; Rainbow, 1995; Villares et al., 2001). The analysis of most metals in water becomes technically difficult and samples are prone to inadvertent contamination during collection and analysis (Villares et al., 2001). Furthermore, dissolved concentrations vary over time due to changes in environmental conditions such as fluvial inputs, salinity, stormwater run-off, weather seasons, tidal cycle, etc. The direct measurement of metals in sediments and in the water is challenging due to the need of defining spatial and temporal patterns of contamination before the interpretation of the observed metals could be meaningful (Binning and Baird, 2001; Phillips et al., 2015). In order to address the above shortcomings, sampling and analysis of water samples would need to be undertaken for an extended period and this is a time-consuming and expensive exercise. Furthermore, dissolved metal concentrations do not provide an assessment of the bioavailable fraction of metal concentrations which are available for uptake and accumulation by marine organisms but only provides the total dissolved concentration fraction present. It is the bioavailable fraction of metal concentrations that are potentially toxic and of ecotoxicological relevance (Rainbow, 1995). The monitoring of metal concentrations in sediment is regarded as an alternative which provides much better results than those obtained in the water column. This is because most metals that are discharged into aquatic systems rapidly become associated with particulate matter and incorporated in sediments (Hanson et al., 1993; Binning and Baird, 2001; Mountouris et al., 2002; Ryan et al., 2012; Sodhi et al., 2022). Metal concentrations in sediments usually exceed those of the overlying water and this is one of the reasons why sediments are regarded as sinks or reservoirs for contaminants in aquatic environments (Bryan and Langston, 1992; Antizar-Ladislao et al., 2015).

Most metals in aquatic systems have been found to be related to suspended particles and sediments. Consequently, the distribution of metals in sediment adjacent to urban areas, particularly industries, can show the impact of anthropogenic inputs on aquatic system and the importance of assessing the potential risks associated with anthropogenic discharges. Since metals are accumulated in sediment over time, the analysis of metals in sediment provides some degree of time integration, which is regarded as an advantage when compared to the analysis of water (Rainbow, 1995; Binning and Baird, 2001). However, the accumulation of metals in sediments is influenced by, inter alia, sediment characteristics (which vary spatially and, in some instances, seasonally), such as sediment grain size distribution, organic carbon content, physical and chemical properties and the concentration of iron, manganese and aluminium oxides (Rainbow, 1995; Chapman and Wang, 2001; Mountouris et al., 2002). Therefore, the analysis of sediment still presents challenges with respect to spatial and temporal variations in metal concentrations in aquatic systems. Another disadvantage of the analysis of sediments, just like water, provide total metal concentrations and not the bioavailable metal concentrations which are more useful in the assessment of the ecotoxicological effects of metals in the aquatic environment. It is also important to note that metals in sediments can be released back into the water column through resuspension (i.e. as a result of dredging etc.) or desorption and may act as a source of pollution (De Souza Machado et al., 2016).

1.2.9. Macroalgae as biological monitors

Among the primary producers, algae are very important, since they are an important link in the food web. They serve as food organisms to the marine and freshwater environments where they dominate the photosynthetic production processes (Misheer et al., 2006). These algae are pervasive and grow wherever there is water, nutrients and light. Most of the productivity in the near-shore ecosystems is carried out by Chlorophyta, commonly known as green algae (Żbikowski et al., 2007). According to Khaled et al. (2014) the cell wall of algae consists of a range of polysaccharides and proteins, some of them containing anionic carboxyl, sulfate, or phosphate groups that are excellent binding sites for metal retention. Macroalgae are well-known for their multicellular construction, size and attachment to firm substrates and found in fresh or saltwater (Dawes, 1998). McHugh (2003) notes that these plants grow rapidly and can reach sizes of up to 60 m in length. Macroalgae are found to be reliable indicators of metal contamination because of their ability to accumulate metals (Vlachos et al., 1998). They are described as being sessile and can be used to monitor changes in metal levels over time. Their size makes them readily identifiable, and they are easily handled (Vlachos et al., 1998). Other studies indicate that macroalgae are used as bio-indicators because of their distribution, longevity, and presence at polluted sites. A report by Bryan (1969) indicates that the binding of metals by macroalgae are strong, with only a minimal exchange between bound metals and ambient water. Shuuluka (2011) reported that seasonal variations may influence the range of metal uptake by marine organisms. The metabolic activity of an organism diminishes due to a decrease in temperature, and this could result in less metal uptake (Lozano et al., 2003). As a result, concentrations are reported as being generally low in spring/summer due to high growth rates and environmental metals that are diluted, and high in winter due to deceleration of metabolic processes (Villares et al., 2001; Shuuluka, 2011).

Metal concentrations in algae are strongly dependent on the environmental parameters (such as temperature, salinity, pH, oxygen, and nutrient concentrations) of the sampling sites (Żbikowski et al., 2006; Shuuluka, 2011). It is important to also note that the ability of algae to accumulate metals is dependent on a number of factors such as the bioavailability of metals within surrounding areas (Sánchez-Rodríguez et al., 2001). According to Khaled et al. (2014) algae bind only free metal ions and the concentration depend on the nature of the suspended particulate matter. An important assumption highlighted by Misheer et al. (2006), underlying biomonitoring, is that metal concentrations found in macroalgae are directly proportional to the bioavailable metal concentration in the aquatic system. This assumption has, however, been confirmed according to Misheer et al. (2006) by several studies, as they have indicated that metal concentrations in macroalgae are many times higher than those found in their surroundings environments, meaning they respond somewhat authentically to gradients in their environments (Haritonidis and Malea, 1995; Haritonidis and Malea, 1999; Żbikowski et al., 2007).

Green algae species such as the *Enteromorpha* spp. (Chlorophyceae) is commonly found in cold temperate to tropical waters, including estuaries and oceanic waters (Dawes, 1998). Say et al. (1990) also notes that *Enteromorpha* spp. is one of the most commonly found algae in estuaries as it is widely distributed in many coastal regions. In South Africa, there are three *Enteromorpha* spp. species that were recorded in freshwaters, namely: *E. basiramosa*; *E. gracillima and E. intestinalis* (Joska and Bolton, 1993). *Enteromorpha intestinalis* is most commonly found in estuarine and marine environments where large growths is recorded in estuarine systems with high nutrient levels such as the Zandvlei estuary (Joska and Bolton, 1993). Their occurrence in aquatic systems is influenced by physical and chemical properties (i.e. salinity, pH, temperature) of environment as they usually occur in areas of relatively higher salinities; alkaline (pH>7) and slow moving waters (Bryan et al., 1985; Joska and Bolton, 1993). As algae are widespread, they have been used to measure metal contamination in many parts of the world (Melville and Pulkownik, 2006). However, even though *Enteromorpha* spp. have been used in several studies as biological indicators, Rainbow (1995) argued that there is uncertainty surrounding the taxonomy of *Enteromorpha* spp. as they are difficult to identify, especially by non-experts and as a result, their lack in robustness questions the effectiveness of their use as bio-indicators. According to Phillips and Rainbow (1994), suitable aquatic biological monitors should be characterised by the following:

- Biomonitors should be sedentary;
- They should be abundant throughout the study area, and sufficiently long-lived;
- They should be easy to sample, hardy enough to survive under laboratory conditions;
- They should provide sufficient tissue for contaminant analysis; and
- They should tolerate brackish water, permitting studies in estuaries (which are often the most contaminated areas of coastal waters).

An observation by Munda (1984) discovered that the *Enteromorpha* spp. absorbed more Zn, Mn and Co at low salinity compared to high salinity. Furthermore, several studies have shown that the levels of Co, Fe, Cd, Mn, Cu and Zn in *Enteromorpha* spp. increased due to increasing water concentrations (Bryan et al., 1985). One of the advantages of using *Enteromorpha* spp. as reported by Bryan et al. (1985) is their ability of reflecting changes in ambient metal levels more rapidly when compared to species such as *Fucus vesiculosus*.

CHAPTER 2

MATERIALS AND METHODS

2.1. SAMPLING SITES AND SAMPLING PROCEDURES

The study area is located on the north-western shore of False Bay in the City of Cape Town Metropole (34°05'S; 18°28'E) (**Figure 2.1-1**). Please see Chapter 1 (Literature Review) for a detailed description of the study area.

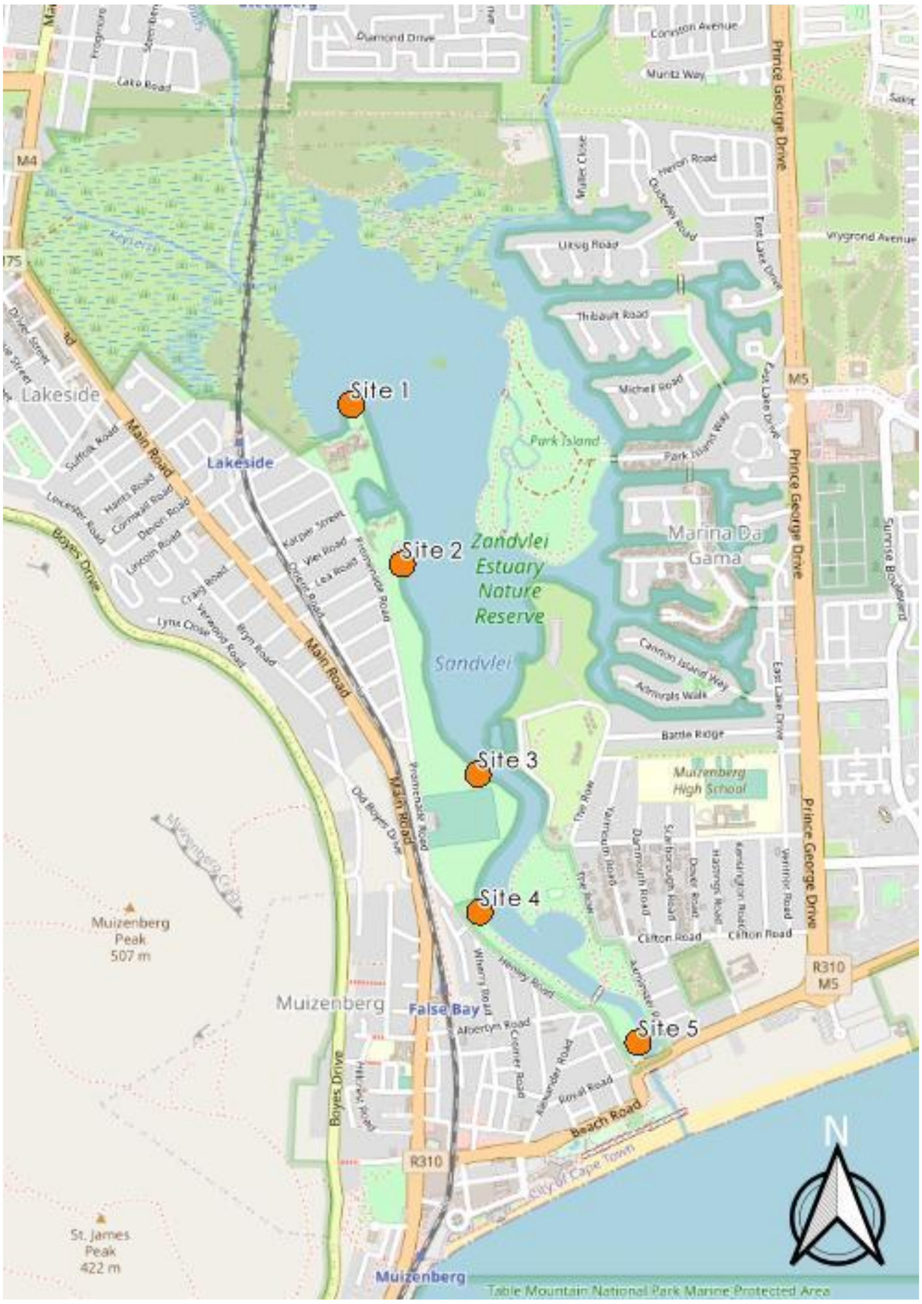

Figure 2.1-1: Study area with sampling sites (Source: QGIS Open Street Map)

2.1.1. Sample collection and preservation: water, sediment and algae

The sampling sites are located along the western boundary of the study area. The sites are located within intervals ranging between 400 m to 600 m and were chosen based on access and for obtaining representative samples (**Figure 2.1-1**). Sites 1 and 2 are located within the upper reaches of the estuary, next to the Sea Scout Base and Imperial Yacht Club, respectively. Sites 3 and 4 are within the middle to lower reaches of the estuary and Site 5 is located near the estuary mouth just before Royal Road, Muizenberg. Sampling commenced on January 2017 and concluded by November 2017. Samples (water, sediment and algae) were collected from the study area using 20 mℓ plastic pill vial containers from each of the five sampling sites indicated above. Five replicates were collected for each sample. Sediment samples were collected at a depth of approximately 10 – 15 cm at each sampling site. Algae samples were collected by hand (using hand gloves) and placed in the plastic pill vial containers. Samples were immediately stored in an icebox for transportation to the laboratory and stored at -18°C for further analysis. Sampling equipment used to collect samples was appropriately labelled and cleaned with distilled water before and after each sampling occasion. Sampling was conducted during both the wet and dry season in four sampling occasions [SO1 (January); SO2, (April); SO3, (August); SO4, (November)] to evaluate variation in metal concentrations of environmental samples. A total of 75 samples were collected during each sampling occasion.

2.1.2. Environmental parameters

The physical and chemical parameters of the study area was measured during the sample collection process. The physicochemical parameters that were measured included temperature (°C), pH, dissolved oxygen (mg/L), electrical conductivity (μS/cm) and salinity (ppt). These were measured during each sampling occasion at each sampling site with a YSI ProDDS multi-parameter instrument. The equipment was checked and calibrated according to the manufacturers' specifications. Rainfall data measured in mm was obtained from the South African Weather Services for the sampling duration. The estuary mouth opening and closing periods was obtained from the City of Cape Town for the abovementioned sampling period.

2.1.3. Algae identification and use

The use of plant (green algae) samples in this study was primarily for the comparison of the uptake of metals in plants versus in water and sediments. A shortcoming of this research was the lack of identification of each algae species collected, as this required specialised expertise. However, it was noted that the collected algae samples were identified as *Enteromorpha* spp.

This determination was reached through the comparison of the external morphology of the collected plants with existing literature.

2.2. METAL ANALYSIS

2.2.1. Acid digestion of environmental samples

The method used to determine the concentrations of the metals was the nitric acid (HNO_3) digestion method. Metal analyses was carried out at the Central Analytical Facilities (CAF) from the University of Stellenbosch. Metal concentrations were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). It is important to note that out of the 12 metals (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Pb) analysed in water, sediment and algae samples, six (Al, Mn, Fe, Cu, Zn and Pb) were further discussed in the thesis. This was based on the recorded concentrations from the samples that were analysed.

2.1.1.1. Water samples

Water samples (10 mℓ) were digested with 5 mℓ of 65% nitric acid at 40°C for 60 minutes and then at 120°C for 180 minutes, using a Grant UBD heating block. An acid blank of 10 mℓ of 65% nitric acid was analysed along with all the collected samples to check for possible contamination. After cooling samples at room temperature, the water samples were filtered with Whatman No. 6 (90 mm) filter paper into 20 mℓ volumetric flasks, diluted up to a volume of 20 mℓ with distilled water and subsequently filtered for a second time using 0.45 µm cellulose nitrate ultrafiltration membrane filters (Millipore) into pill vials using syringes. Thereafter, 2 mℓ subsamples were subsequently placed in plastic centrifuge tubes and diluted to 10 ml with distilled water. All digested samples were then stored in the refrigerator for analysis.

The following formula was applied for the calculation of metal concentrations in water samples (metal concentrations are expressed in mg/L for water samples).

$$= [\mathbf{ICP} - \mathbf{Blank}] \times 10$$

2.1.1.2. Sediment and algae samples

Sediment and algae samples were oven dried at 60°C for 48 to 72 hours. A subsample with an exact mass measured between 0.2 - 0.3 g of the sediment and algae samples was digested in 10 mℓ 65% nitric acid at 40 °C for 60 minutes and then at 120°C for 180 minutes, using a Grant UBD heating block. After cooling samples at room temperature, the water samples were filtered with Whatman No. 6 (90 mm) filter paper into 20 mℓ volumetric flasks, diluted up to a volume of 20 mℓ with distilled water and subsequently filtered for a second time using 0.45 µm cellulose nitrate ultrafiltration membrane filters (Millipore) into pill vials using syringes. Thereafter, 1 mℓ subsamples were subsequently placed in plastic centrifuge tubes and diluted to 10 mℓ with distilled water. All digested samples were then stored in the refrigerator for analysis.

The following formula was applied for the calculation of metal concentrations in sediment and algae samples (metal concentrations are expressed in mg/kg for sediment and algae samples).

$$= \frac{\textbf{ICP} - \textbf{Blank}}{\textbf{sample mass (g)}} \textbf{ x [200]}$$

2.2.2. Bioconcentration factors

The bioconcentration factor (BCF) was calculated for each of the six metals. The BCF was calculated using the following equations:

$$\textbf{BCF} = \frac{\text{Metal concentration in algae } (\frac{\text{mg}}{\text{kg}})}{\text{Metal concentration in water } (\frac{\text{mg}}{\text{L}})} \quad \text{and/or} \quad = \frac{\text{Metal concentration in algae } (\frac{\text{Mg}}{\text{kg}})}{\text{Metal concentration in sediment } (\frac{\text{mg}}{\text{L}})}$$

It is important to note that where the BCF exceeds a value of 1, bioaccumulation of the metal in the tissue of an organism (i.e. algae) is considered to have occurred (McGuirk et al., 2020). The classification of bioconcentration factors was adopted from Dallinger and Rainbow (1993), where: macroconcentration is when BCF is >2; microconcentration is when BCF is 1-2; and deconcentration is when BCF is <1.

2.3. STATISTICAL ANALYSIS

The results presented in this study are averages (±SD) of five replicates for each sampling point from the five different sampling sites within the study area. Statistical analysis was conducted using the SigmaPlot (version 14.0) software by SYSTAT Software Inc. Statistical differences between the different sampling sites and sampling occasions for the subject metals were analysed and evaluated using the Kruskal-Wallis One-Way Analysis of Variance (ANOVA) on Ranks and Student Newman Kuels Method for post hoc tests. In cases where there were missing or unequal datasets, the Dunns Method was utilised for post hoc tests. The condition for the significant differences was set at $p<0.05$ for all statistical analysis undertaken for this study.

CHAPTER 3

ENVIRONMENTAL PARAMETERS RESULTS

3.1. PHYSICOCHEMICAL PARAMETERS

Table 3.1-1: Physicochemical parameters recorded for each sampling site during the first sampling occasion in January.

Parameters	Site 1	Site 2	Site 3	Site 4	Site 5
Dissolved oxygen (mg/L)	5.8	5.48	5.42	4.5	3.71
Temperature (°C)	24.4	23.3	23.4	22.9	23.2
Electrical Conductivity (µS/cm)	24608	24765	25943	30107	34678
Salinity (ppt)	15.15	15.63	16.30	19.75	22.67
pH	8.63	8.43	8.01	7.97	7.28

The physicochemical parameters measured for each sampling site included temperature, pH, dissolved oxygen, electrical conductivity and salinity. The dissolved oxygen recording between sites 1 and 5 during the first sampling occasion ranged from 5.8 mg/L to 3.71 mg/L. The estuary temperature in January was found to be slightly higher when compared to other sampling occasions, ranging from 24.4°C to 22.9°C between the different sampling sites. The pH ranged from 8.63 to 7.28 between sites 1 and 5, whilst the salinity was found ranging from 15.15 ppt to 22.67 ppt between sites 1 and 5 (**Table 3.1-1**).

Table 3.1-2: Physicochemical parameters recorded for each sampling site during the second sampling occasion in April.

Parameters	Site 1	Site 2	Site 3	Site 4	Site 5
Dissolved oxygen (mg/L)	13.84	11.2	6.1	4.97	2.42
Temperature (°C)	23.2	22.7	22.6	21.8	21.1
Electrical Conductivity (µS/cm)	28812	30135	31789	32803	36885
Salinity (ppt)	18.65	19.68	20.85	22.3	25.45
pH	8.81	8.63	8.02	4.97	6.8

The dissolved oxygen recorded between sites 1 and 5 during the second sampling occasion ranged from 13.84 mg/L to 2.42 mg/L. The temperature between sites 1 and 5 ranged from 23.2°C to 21.1°C. Electrical Conductivity (EC) values decreased from site 5 (36885 µS/cm) to site 1 (28812 µS/cm). The pH values ranged between 8.81 and 4.94 between the different sampling sites, with site 4 having the lowest pH (4.94) recorded throughout the entire sampling period. Salinity levels increased from site 1 to site 5 with values ranging from 18.65 ppt to 25.45 ppt (**Table 3.1-2**).

Due to technical reasons, there are no physicochemical data available for sampling occasion 3.

Table 3.1-3: Physicochemical parameters recorded for each sampling site during the fourth sampling occasion in November.

Parameters	Site 1	Site 2	Site 3	Site 4	Site 5
Dissolved oxygen (mg/L)	18.51	14.77	9.91	8.63	6.44
Temperature (°C)	22.0	23.0	23.6	21.6	22.6
Electrical Conductivity (μS/cm)	13546.50	22005	22369	21671	22961
Salinity (ppt)	12.46	13.80	12.01	14.05	14.60
pH	9.42	9.23	8.73	8.54	8.14

The dissolved oxygen recorded during the last sampling occasion between the different sampling sites ranged from 18.51 mg/L to 6.44 mg/L. The recorded temperature ranged from 23.6°C to 21.6°C between the different sampling sites. Salinity levels increased as expected from the upper reaches (site 1) down towards the lower reaches (site 5) with values ranging from 12.46 ppt to 14.60 ppt. EC values recorded between the different sampling sites ranged from 13546.50 μS/cm to 22961 μS/cm. pH values ranged from 9.14 to 8.14 between sites 1 and 5 (**Table 3.1-3**).

3.2. RAINFALL DATA

Table 3.2-1: Monthly average daily rainfall (mm) obtained from Rondevlei Nature Reserve station (Source: South African Weather Service)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2017	5.2	0.3	6.1	24.2	4.0	93.1	46.5	51.0	20.4	33.5	27.8	4.6
*** indicates data is missing or is not yet available in the current month --- indicates that data is unavailable or was not requested --- indicates that data is unavailable or was not requested Monthly Daily Rain (mm) Data for station [0004874 X] - RONDEVLEI Measured at 08:00												

The monthly average (mm) rainfall data for the study area was obtained from the closest station, the Rondevlei Nature Reserve weather station and was supplied by the South African Weather Service (SAWS). The data covers the duration in which samples were collected from the Zandvlei estuary. The highlighted months are for the different sampling occasions (**Table 3.2-1**).

3.3. OPENING AND CLOSING OF ESTUARY MOUTH

Table 3.3-1: Opening and closing dates of the Zandvlei estuary mouth sandbar (Source: City of Cape Town, 2017). Shaded rows represent sample collection periods.

Day	Opening Date	Day	Closing Date	Notes
Mon	9-Jan-17	Mon	16-Jan-17	
Tue	7-Feb-17	Mon	13-Feb-17	
Mon	27-Mar-17	Fri	31-Mar-17	
Mon	24-Apr-17	Tue	28-Apr-17	
Mon	22-May-17	Fri	26-May-17	
Thurs	26-Jun-17			Open for Winter
Thurs	27-Jul-17		Sandbar formed naturally	Open
				Mouth open in August
Tues	12-Sep-17		Sandbar formed naturally	
Fri	22-Sep-17	Mon	26-Sep-17	
Wed	1-Nov-17	Thurs	9-Nov-17	
Thurs	30-Nov-17	Tue	5-Dec-17	Close
Rubble Weir 0.4m M.S.L				

The sandbar located at the mouth of the estuary is occasionally opened and closed during specific periods to manage the system so as to avoid flooding of the Marina da Gama residential area and to ensure that the system is well functioning. The periods at which the mouth of the estuary was opened and closed, including the sandbar that formed naturally at the mouth are included in this report (**Table 3.3-1**). This data is particularly important in determining the behaviour of metals in the system in relation to its spatial and temporal distribution.

CHAPTER 4

ALUMINIUM RESULTS AND DISCUSSION

4.1. RESULTS

It should be noted that statistical comparisons presented in this chapter were made between consecutive sites and consecutive occasions.

Table 4.1-1: Mean concentrations (±SD) of aluminium in water, sediment and algae during different sampling occasions at five sampling sites (n=5).

	SAMPLING OCCASIONS (SO)			
ALUMINIUM	SO1 **(Summer)**	SO2 **(Autumn)**	SO3 **(Winter)**	SO4 **(Spring)**
WATER (mg/ℓ)				
Site 1	^{a}0.0464^{a} (±0.0911)	^{a}0.0228^{a} (±0.0511)	^{a}0.2076^{a} (±0.2681)	ND
Site 2	^{a}0.0325^{a} (±0.0726)	^{a}0.0135^{a} (±0.0195)	^{a}0.2431^{a} (±0.2430)	ND
Site 3	^{a}0.0019^{a} (±0.0042)	^{b}0.3563^{b} (±0.2080)	^{a}0.0788^{c} (±0.1179)	ND
Site 4	<LD	^{b}0.3938^{b} (±0.1779)	^{b}2.0438^{b} (±2.7055)	ND
Site 5	<LD	^{c}0.0309^{a} (±0.0305)	^{b}0.0199^{a} (±0.0232)	ND
SEDIMENT (mg/kg)				
Site 1	^{a}986.86^{a} (±181.56)	^{a}1448.81^{b} (±246.13)	^{a}1128.81^{c} (±190.29)	^{a}794.28^{d} (±121.12)
Site 2	^{a}1229.91^{a} (±247.36)	^{a}1399.36^{a} (±406.64)	^{a}1242.21^{a} (+297.10)	^{a}948.86^{a} (±106.18)
Site 3	^{b}606.17^{a} (±226.72)	^{a}901.26^{a} (±279.44)	^{a}979.04^{a} (±309.31)	^{a}1062.32^{a} (±156.22)
Site 4	^{c}923.95^{a} (±191.91)	^{a}1562.04^{a} (±923.63)	^{a}1357.36^{a} (±723.22)	^{b}189.36^{b} (±26.21)
Site 5	^{d}250.55^{a} (±66.75)	^{b}452.96^{b} (±88.83)	^{b}466.32^{b} (±48.42)	^{c}301.32^{c} (±40.10)
ALGAE (mg/kg)				
Site 1	^{a}1587.81^{a} (±960.79)	^{a}1525.49^{a} (±438.18)	^{a}307.782^{b} (±127.12)	^{a}596.46^{b} (±335.08)
Site 2	^{a}1310.95^{a} (±639.94)	^{a}497.88^{b} (±27.88)	^{a}266.91^{c} (±31.08)	^{a}325.13^{c} (±119.27)
Site 3	^{b}3688.73^{a} (±818.35)	^{a}1302.49^{b} (±539.51)	^{a}999.43^{b} (±454.50)	^{a}713.15^{b} (±209.80)
Site 4	^{c}60.24^{a} (±44.13)	^{b}2662.61^{b} (±859.27)	^{a}2220.69^{b} (±1778.35)	^{a}3548.74^{b} (±922.99)
Site 5	^{d}4517.35^{a} (±9620.77)	^{c}915.03^{a} (±879.69)	^{a}1937.46^{a} (±3401.13)	ND

LD = Limit of Detection
ND = No Data
The statistical differences between sites per sampling occasion are indicated by superscripted letters to the left of mean values.
The statistical differences between sampling occasions per site are indicated by superscripted letters to the right of mean values.
Water, sediment and algae samples were not statistically compared with each other.

Table 4.1-2: Bioconcentration factors of algae for aluminium.

Aluminium		SO1 (Summer)	SO2 (Autumn)	SO3 (Winter)	SO4 (Spring)
Site 1	A/W	34220	66907.46	1482.57	
	A/S	1.6	1.05	0.27	0.75
Site 2	A/W	40336.92	36880	1097.94	
	A/S	1.07	0.36	0.21	0.34
Site 3	A/W	1941436.84	3655.60	12683.12	
	A/S	6.09	1.45	1.02	0.67
Site 4	A/W		6761.33	1086.55	
	A/S	0.07	1.70	1.64	18.74
Site 5	A/W		29612.62	97359.80	
	A/S	18.03	2.02	4.15	

Shaded areas represent sites with either no data or where the recorded concentrations were below the level of detection.

A/W = $\frac{Algae\ metal\ concentration\ (\frac{mg}{kg})}{Water\ metal\ concentration\ (\frac{Mg}{L})}$ A/S = $\frac{Algae\ metal\ concentration\ (\frac{mg}{kg})}{sediment\ metal\ concentration\ (\frac{mg}{kg})}$

4.1.1. Water

4.1.1.1. Comparisons of metal concentrations between sites per occasion

The results showed statistical differences ($p<0.05$) in aluminium (Al) concentrations recorded between sites 2 and 3 as well as sites 4 and 5 in SO2. Statistical differences ($p<0.05$) in Al concentrations ($p<0.05$) were also recorded between sites 3 and 4 in SO3. No statistical differences ($p>0.05$) in Al concentrations were recorded between the consecutive comparisons of the different sampling sites in SO1. No data was recorded for SO4 (**Table 4.1-1**).

4.1.1.2. Comparisons of metal concentrations between sampling occasions per site

The results showed no statistical differences ($p>0.05$) in Al concentrations were recorded between the different sampling occasions at sites 1, 2 and 5. Pairwise multiple comparisons of consecutive sampling occasions revealed that Al mean concentrations recorded at site 3 were statistically different between all sampling occasions ($p<0.05$). Pairwise multiple comparisons of consecutive sampling occasions at site 4 revealed statistical differences ($p<0.05$) in Al concentrations recorded between SO1 and SO2. No data was recorded for SO4 (**Table 4.1-1**).

4.1.2. Sediment

4.1.2.1. Comparisons of metal concentrations between sites per occasion

Pairwise multiple consecutive comparisons of the different sampling sites show statistical differences ($p<0.05$) in mean Al concentrations recorded between sites 2 and 3, sites 3 and 4 as well as sites 4 and 5 in SO1. No statistical differences ($p>0.05$) were recorded in the pairwise consecutive comparisons between sites 1 and 2, sites 2 and 3 as well as sites 3 and 4 in SO2 and SO3. However, significant differences ($p<0.05$) in mean Al concentrations were recorded in the pairwise consecutive comparisons between sites 4 and 5 in SO2 and SO3. In SO4, the results showed no statistical differences ($p>0.05$) in Al concentrations recorded between sites 1 and 2 as well as sites 2 and 3. However, significant differences ($p<0.05$) in Al concentrations were recorded between sites 3 and 4, as well as sites 4 and 5 on SO4 (**Table 4.1-1**).

4.1.2.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparisons of consecutive sampling occasions at site 1 revealed that all consecutive occasions differed significantly ($p<0.05$) from each other. The results showed no statistical differences ($p>0.05$) in Al concentrations recorded between the different sampling occasions at sites 2 and 3. At site 4, there results revealed no statistical differences ($p>0.05$) between SO1, SO2 and SO3. However, there were significant differences ($p<0.05$) in Al concentrations recorded between SO3 and SO4 at site 4. Pairwise multiple comparisons of consecutive sampling occasions have revealed statistical differences ($p<0.05$) between the following sampling occasions at site 5: SO1 and SO2 as well as SO3 and SO4 (**Table 4.1-1**).

4.1.3. Algae

4.1.3.1. Comparisons of metal concentrations between sites per occasion

The results showed no statistical differences ($p>0.05$) in Al concentrations recorded between sites 1 and 2 on SO1. However, the results showed significant differences ($p<0.05$) in Al concentrations recorded between sites 2 and 3, sites 3 and 4 as well as sites 4 and 5 on SO1. The results show no statistical differences ($p>0.05$) in Al concentrations recorded between sites 1 and 2 as well as sites 2 and 3 on SO2. However, statistical differences ($p<0.05$) in Al concentrations were recorded between sites 3 and 4 as well as sites 4 and 5. No statistical differences ($p>0.05$) in mean Al concentrations were recorded between pairwise consecutive sampling sites on SO3 and SO4 (**Table 4.1-1**).

4.1.3.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparisons of consecutive sampling occasions revealed the following differences at site 1: SO1 and SO2 ($p>0.05$); SO2 and SO3 ($p<0.05$); and SO3 and SO4 ($p>0.05$). The results showed statistical differences ($p>0.05$) in Al concentrations recorded between SO1 and SO2 as well as between SO2 and SO3 at site 2. No statistical differences ($p>0.05$) in Al concentrations were recorded between SO3 and SO4 at site 2. Pairwise multiple comparisons of consecutive sampling occasions at site 3 revealed no statistical differences in mean Al concentrations recorded between SO1 and SO2 ($p<0.05$).

The results showed no statistical differences ($p>0.05$) in Al concentrations recorded between SO2 and SO3 as well as between SO3 and SO4 at site 3. At site 4, the results showed statistical differences in Al concentrations recorded between SO1 and SO2 ($p<0.05$). However, no statistical differences ($p>0.05$) in Al concentrations were recorded between SO2 and SO3 as well as between SO3 and SO4 at site 4. The results showed no statistical differences ($p>0.05$) in Al concentrations recorded between the different sampling occasions at site 5 (**Table 4.1-1**).

4.1.3.3. Bioconcentration factors of algae for aluminium

The bioconcentration factors of Al in *Enteromorpha* spp. are presented in **Table 4.1-2**. The results show a significantly high bioconcentration of Al in *Enteromorpha* spp. (>2) relative to water, ranging from 1086.55 to 1941436.84. The bioconcentration factors of Al in *Enteromorpha* spp. relative to sediment were considerably lesser when compared to water, ranging from 0.07 to 18.74. The bioconcentration factor of <1 for Al in *Enteromorpha* spp. relative to sediment was recorded at sites 1 (SO3, SO4); 2 (SO2, SO3, SO4); 3 (SO4); and 4 (SO1).

4.2. DISCUSSION

4.2.1. Water

Mean Aluminium (Al) concentrations (mg/ℓ) found in water at the different sampling sites within the study area ranged from <LD to 2.0438±2.7055 (**Table 4.1-1**). Spatial variations in Al concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 2 (0.0135±0.0195) and 3 (0.3563±0.2080) as well as sites 4 (0.3938±0.1779) and 5 (0.0309±0.0305) on SO2 (autumn). The increase in Al concentrations ($p<0.05$) recorded between sites 2 (0.0135±0.0195) and 3 (0.3563±0.2080) on SO2 (autumn) may be due to land-based discharges or urban run-off, particularly since there is a stormwater drainage outlet located close to site 3 (**Figure 4.2-1**). Other factors that may have also influenced the recorded Al concentrations include the gradient of the estuary, the closing and opening of the estuary mouth, and physicochemical parameters. It is important to note that metals exist in water either as particulate colloidal and/or dissolved phases (Sany et al., 2013). According to the Zandvlei Estuarine Management Plan draft prepared by the Western Cape Government (WCG, 2018), circulation in the estuary is impeded or affected by the dense growth of pondweed (*Stuckenia pectinata*) and deeper channels found in some areas, including the marina. The relatively deeper channels could potentially prevent the movement of metals as water circulation becomes slow or even stagnant, and this could be a reason for elevated concentrations in the central and upper parts of the estuary. The estuary mouth was also closed on SO2 (autumn) (**Table 3.3-1**) and this could have contributed to stagnant waters within the system. No statistical differences ($p>0.05$) were recorded between the different consecutive sampling sites in SO1 (summer) (**Table 4.1-1**). According to Sany et al. (2013) elevated metal concentrations in the water column may be linked to an increase in salinity and decrease in pH and redox potential. Toxic effects of Al are usually found over the pH range of 4.4 to 5.4 with maximum toxicity occurring around pH 5.0 to 5.2 (DWAF, 1996; ANZECC, 2000). In most surface waters, Al concentrations are very low and when found in high concentrations, this usually occurs when the pH is less than 5 (DWAF, 1996). Salinity concentrations recorded for the study area increased from 19.68 ppt (site 2) to 25.45 ppt (site 5), while the pH decreased from 8.63 (site 2) to 6.8 (site 5) on SO2 (autumn) (**Table 3.1-2**). The increase in salinity and Al concentrations appears to be in line with the findings by Sany et al. (2013). However, a decline in Al concentrations ($p<0.05$) was found between sites 4 (0.3938±0.1779) and 5 (0.0309±0.0305) on SO2 (**Table 4.1-1**). This may be ascribed to a potential reduction in anthropogenic inputs and water circulation which seems to be characterised by stagnant waters, which could potentially hinder/limit the movement of metals within the estuary during a period with relatively low rainfall (i.e. 24 mm).

The lower parts of the estuary (i.e. site 4 or 5) are to some extent expected to record lower mean concentrations as compared to the upper parts which are largely influenced or affected by influent rivers (Keysers, Westlake, Sand/Diep River) (**Figure 4.2-1**) and as well the extensive stormwater drainage system, including activities from the marina. However, since there are other factors (i.e. physicochemical parameters, rainfall, etc.) that influence the availability and uptake of metals in the water column, this probability cannot always be met or realised. The World Health Organisation (WHO, 1997) noted that the concentration of Al in natural waters can differ significantly depending on various physicochemical and mineralogical factors. Dissolved Al concentrations in waters with near-neutral pH values usually range from 0.001 to 0.05 mg/ℓ but rise to 0.5 to 1 mg/ℓ in more acidic waters or water rich in organic matter (WHO, 1997). The pH values recorded for this study were mostly alkaline or slightly above neutral (i.e., above 7), except for site 4 (SO2) which recorded a pH of 4.97 and a mean concentration of 0.3938±0.1779 mg/ℓ. There seems to be a link between low pH values and high mean metal concentrations. Aluminium is commonly introduced into the environment through industrial processes or product production (i.e., cosmetics, chemicals, etc.) or domestic run-off (including that of agricultural activity (Erasmus, 2004). The source of metals in urban run-off (stormwater) is mainly from industries, buildings, vehicle parts and components, fuel and oils, roofs with metallic elements, and other metallic structures from roads, etc. (Brown and Peake, 2006; Barbosa et al., 2012; Sakson et al., 2018). The study area is characterized by an extensive stormwater drainage system, consisting of stormwater drains that discharge directly into the estuary, some of which discharge into the three rivers which feed directly into the estuary (C.A.P.E., 2010). There are several stormwater outlets located around the different sampling sites (**Figure 4.2-1**). Furthermore, approximately 14 pump stations are located around the estuary, some of which have reportedly been overflown as a result of malfunction, vandalism, and/or blockages and this has negatively affected the water quality of the estuary (C.A.P.E., 2010). An industrial area is located approximately 2km north of the estuary. This area is regarded as a potential source of illegal discharges and contaminated stormwater run-off (WCG, 2018). The extensive nature of the stormwater system around the estuary creates an opportunity for contaminants, including metals from neighbouring (i.e., Marina Da Gama) and upstream activities to enter the estuary. The rainfall data obtained from the South African Weather Services (SAWS) shows a monthly average rainfall of 24.2 mm for SO2 (autumn) and a much higher monthly average rainfall of 51.0mm for SO3 (winter) (**Table 3.2-1**).

The results show statistical differences in Al concentrations ($p<0.05$) in the pairwise consecutive comparisons between sites 3 (0.0788±0.1179) and 4 (2.0438±2.7055) on SO3 (winter) (**Table 4.1-1**). The highest mean Al concentration was recorded at site 4 on SO3 (winter). However, the recorded Al concentrations at site 4 (SO3) varied significantly in temporal scales as the standard deviation was significantly high. Elevated Al concentrations at site 4 may be attributed to fluvial and stormwater discharges. The high rainfall levels recorded in winter (SO3) may have also contributed to the concentration recorded at site 4 (2.0438±2.7055) on SO3 (winter) (**Table 4.1-1**). Some of the recorded mean Al concentrations within the different sampling sites on each sampling occasion exceeded the recommended water quality guidelines for the protection of aquatic ecosystems as stipulated by the Department of Water Affairs and Forestry (DWAF, 1996), the Canadian Council of Resource and Environment Ministers Guidelines (CCREM, 1987), and the Australian and New Zealand Environment and Conservation Council (ANZECC, 2000) (**Table 10.1-1**). Sites that have exceeded the different water quality guidelines are listed in **Table 10.1-1**. Some of the sites that exceeded guidelines are near stormwater outlets and also not far from the influent rivers, Elevated Al concentrations in surface waters can cause toxic effects to aquatic organisms (ANZECC, 2000).

Figure 4.2-1: Map showing sampling sites and stormwater drainage outlets within the Zandvlei estuary (Source: Google Maps; CoCT open data portal)

The results show no significant differences (p>0.05) in the mean Al concentrations recorded between the different sampling occasions at sites 1 and 2 (**Table 4.1-1**). However, comparisons of temporal variations in Al concentrations show significant differences (p<0.05) in the pairwise consecutive comparisons between the different sampling occasions at site 3. The results reveal that the summer period (SO1) (0.0019±0.0042) recorded a lower Al concentration when compared to autumn (SO2) (0.3563±0.2080) and winter (SO3)

(0.0788±0.1179) at site 3 with the highest mean Al concentration at site 3 recorded in autumn (SO2) (0.3563±0.2080) (**Table 4.1-1**). The above may be attributed to changes in anthropogenic load inputs, rainfall levels and physicochemical parameters such as salinity and pH. According to De Souza Machado et al. (2016) elevated rainfall may intensify the adsorption and precipitation processes due to high river flows which ultimately results in less dissolved metal in the water column. This may be the explanation for the relatively low mean Al concentration recorded in winter (SO3) at site 3. The estuary mouth was closed on SO2 (autumn) and open on SO3 (winter) (**Table 3.3-1**). The closing of the estuary mouth results in water being stagnant in the estuary which may have potentially contributed to metals being dissolved in the water column, hence SO2 recorded statistically higher mean Al concentrations when compared to when the mouth was open in SO3 (winter) at site 3.

Figure 4.2-2: Map showing the proximity of sites 3 and 4 from existing stormwater drainage outlets within the study area (Source: Google Maps; CoCT open data portal)

The results show significant differences ($p<0.05$) in mean Al concentrations between SO1 (0.0000±0.0000) and SO2 (0.3938±0.1779) as well as between SO2 (0.3938±0.1779) and SO3 (2.0438±2.7055) at site 4 (**Table 4.1-1**), while there was a decline ($p<0.05$) in mean Al concentrations recorded between SO2 (0.3563±0.2080) and SO3 (0.0788±0.1179) at site 3. The seasonal variations recorded at site 4 may be due to several factors including rainfall, anthropogenic inputs, estuarine circulation and physicochemical parameters such as pH and salinity. There is a stormwater drainage outlet located near site 4 which may discharge Al containing wastewater from time to time (**Figure 4.2.2**).

4.2.2. Sediment

The mean Al concentrations (mg/kg) recorded in sediment at the different sampling sites within the Zandvlei estuary ranged from 189.36±26.21 to 1562.04±923.63 (**Table 4.1-1**). Spatial variations in Al concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 2 (1229.91±247.36) and 3 (606.17±226.72), sites 3 (606.17±226.72) and 4 (923.95±191.91) as well as sites 4 (923.95±191.91) and 5 (250.55±66.75) on SO1 (summer). This may be due to several environmental factors such as salinity, pH, anthropogenic inputs, redox potential, and sediment grain size distribution (Davies et al., 1991). The highest mean Al concentration on SO1 (summer) was recorded at site 2 and from there mean Al concentrations fluctuated between the different sites, with the lowest mean Al concentrations recorded at site 5 (**Table 4.1-1**). The fluctuating mean Al concentrations between the different sampling sites on each occasion may also be due to different levels of anthropogenic inputs from the various stormwater drainage outlets. Physicochemical factors such as salinity, density, flow velocity, and suspended matter composition also influence the distribution and fate of metals in estuaries (De Souza Machado et al., 2016). Chapman and Wang (2001) noted that the partitioning of contaminants between sediments and overlying water is controlled by salinity. This is because the water chemistry and ionic strength, which both affect the movement of metals in estuaries, is influenced by salinity (Benoit et al., 1994; De Souza Machado et al., 2016). Elevated salinity leads to an increased competition between cations and metal ions and this results in desorption of metals from sediment and suspended matter as a result of increasing salinity (Mwanuzi and De Smedt, 1999). The recorded salinity levels in this study ranged from 15.15ppt to 22.67ppt on SO1 (summer) (**Table 3.1-1**). The lowest salinity concentration was recorded at site 1 (upstream, near influent rivers) and the highest was recorded at site 5 (downstream, near estuary mouth). While the pH decreased from 8.63 to 7.28 between sites 1 and 5. Site 5 on SO1 (summer) recorded the highest salinity (22.67ppt), lowest pH (7.28) and metal concentration (250.55±66.75) and this seems to be in line with the above discussion regarding the effect of physicochemical parameters. The results show statistical differences in Al concentrations ($p<0.05$) recorded between sites 4 (1562.04±923.63) and 5 (452.96±88.83) on SO2 (autumn), including sites 4 (1357.36±723.22) and 5 (466.32±48.42) on SO3 (winter) (**Table 4.1-1**). The relatively high concentrations recorded at site 4 may be attributed to a combination of factors such as physicochemical parameters, rainfall, sediment grain size distribution and a local source of contamination such as the nearby stormwater drainage outlets. Elevated rainfall on both SO2 (autumn) (24.2mm) and SO3 (winter) (51.0mm) may have contributed to the above spatial variations between sites 4 and 5 on SO2 and SO3.

Statistical differences in Al concentrations ($p<0.05$) were recorded in the pairwise consecutive comparisons between sites 3 (1062.32±156.22) and 4 (189.36±26.21) as well as sites 4 and 5 (301.32±40.10) on SO4 (spring) (**Table 4.1-1**). A consistent pattern was noted in which site 5 recorded statistically lower mean Al concentrations when compared with the other sampling sites in each sampling occasion, except for SO4. This may potentially be attributed to the location of the site, as it is close to the estuary mouth, which is further away from the upstream in-flowing rivers and the marina. The distribution of sediment grain size may have also played a significant role in the accumulation of metals in sediment within the study area. A study conducted by the CSIR (2015) noted that the sediments collected in 29 of the 41 (71%) sampling stations in the Zandvlei estuary and the marina were dominated by mud and the remaining stations mainly characterised by fine-grained sand. Sediments were classified based on the Wentworth scale, which includes different grain size classes, as presented in **Table 4.2-1** (CSIR, 2015). The sediment in the main body of the estuary was less muddy with a few exceptions (where the content of mud was notably high) at sampling stations 78 and 75 (CSIR, 2015). These two sampling stations (i.e., 78 and 75) were located close to sampling sites 1 and 2, respectively, of this study. Maurer (2019) found that the coarsest sediment sampled was most prominent at the lower parts and the finest sediment at the upper parts of the Zandvlei estuary. Maurer (2019) collected sediment samples for analysis during the same period in which samples were collected for this study. Both the CSIR (2015) and Maurer (2019) studies noted that the upper parts of the estuary were characterised by muddy/fine sediments and the lower parts by coarse/sandy sediments.

Table 4.2-1: Sediment grain size classes in terms of the Wentworth Scale (CSIR, 2015)

Classes	Size
Mud	(<0.063 mm)
Very fine-grained sand	(0.063 – 0.125 mm)
Fine-grained sand	(0.125 – 0.250 mm)
Medium-grained sand	(0.25 – 0.50 mm)
Coarse-grained sand	(0.5 – 1.0 mm)
Very coarse-grained	(1.0 – 2.0 mm),
Gravel	(>2.0 mm)

According to Nduka and Orisakwe (2011) fine sediments have a significant capacity to retain metals and as a result can adsorb an increased concentration of metals. This is due to the large surface area to volume provided by fine grained particles for adsorption (CSIR, 2015).

The small surface area to volume and the absence of surface electric charges on course/sand grains usually results in contaminant concentrations that are of magnitude lower than in fine grained (mud dominated) sediments (CSIR, 2015). Based on the distribution of the sediment grain sizes of the study area, it is expected that sediments in the upper parts of the estuary may accumulate higher metal concentrations when compared to sediments in the lower parts of the estuary. However, since sediment grain size distribution is not the only factor which influences the accumulation of metals in sediment, the above expectation may not be realised in some instances. For example, mean sediment Al concentrations recorded in this study appear to be widely distributed and vary across the different sampling sites. Site 4 recorded relatively higher mean concentrations (expect for in SO4) when compared to site 5 which is closer to the estuary mouth and comprises of coarser-grained sediments. The higher concentration recorded at site 4 could be attributed to not only sediment grain size but also to the extent of anthropogenic inputs, as well as the estuary gradient which results in the slow movement of water and other materials within the estuary. The relatively higher mean Al concentrations at site 4 show that correlations exist between decreasing grain size and increasing metal concentrations (Chapman and Wang, 2001). However, the relatively low concentrations recorded at site 5 in the different sampling occasions (except for SO4) may be an indication of other factors at play such as strong tidal currents which results in coarser sediment and low organic content (Acevedo-Figueroa et al., 2006). Izegaegbe et al. (2020) also recorded low metal concentrations at a site located near the estuary mouth in the Mhlathuze estuary in KwaZulu Natal which was attributed to strong tidal currents which prevent the deposition of fine sediments, limiting the accumulation of metals in sediment. It is also noted that the gradient of the estuary also appears to play a role in the movement of water and ultimately the distribution of metals within the system. This is because some of the water coming into the estuary from land based (stormwater) run-off and influent rivers, is stagnant in some parts of the estuary, where dense vegetation (pondweed) occurs and deep channels are found (WCG, 2018).

Seasonal (temporal) variations in Al concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between the different sampling occasions at site 1 (**Table 4.1-1**). The results show elevated Al concentrations in both autumn (SO2) and winter (SO3) as compared to other seasons at site 1. This may be ascribed to increased fluvial and other land-based discharges due to elevated rainfall. Site 1 is located close to the influent rivers which are heavily impacted by upstream anthropogenic activities. According to WCG (2018) the nearby industrial area is regarded as a potential source of illegal discharges and contaminated stormwater runoff. The above industrial area is located adjacent to the Keysers River which discharges a short distance downstream into the Zandvlei estuary. Sewer overflows and the dumping of waste and litter in the stormwater drainage system (WCG, 2018) may also result in elevated metal concentrations in the Zandvlei estuary. No significant differences in mean Al concentrations (p>0.05) were recorded in the pairwise consecutive comparison between the different consecutive sampling occasions at sites 2 and 3 (**Table 4.1-1**). The results show a significant decline (p<0.05) in mean Al concentrations between winter (SO3) (1357.36±723.22) and spring (SO4) (189.36±26.21) at site 4. The relatively high concentration recorded in winter could be due to elevated rainfall which transports metals and particulate matter. Seasonal variations in mean Al concentrations also show statistical differences (p<0.05) in the pairwise consecutive comparisons between summer (SO1) and autumn (SO2) as well as between winter (SO3) and spring (SO4) at site 5 (**Table 4.1-1**). The highest mean Al concentration was recorded in autumn (SO2) followed by winter (SO3) when compared to other sampling occasions at site 1 (**Table 4.1-1**). The average monthly rainfall recorded for autumn (SO2) and winter (SO3) was 24mm and 51.0mm, respectively (**Table 3.2-1**). The comparison of seasonal variations in mean Al concentrations show that the dry season (spring [SO4] and summer [SO1]) recorded low mean Al concentrations when compared to the wet season (autumn [SO2] and winter [SO3]). This may be attributed to rainfall, which increases land-based run-off and river discharges, which are polluted by contaminants such as Al, among others. However, some researchers (Lim et al., 2006; Li et al., 2009) argue that rainwater causes increased mobility and dilution, which decreases metal concentrations in sediments. This was not the case for this study as the recorded mean Al concentrations (**Table 4.1-1**) were higher during the wet season.

Factors which may be contributing to increased metal concentrations in wet season include the gradient of the estuary (i.e. deeper channel upstream) and high levels of pondweed which slows down water flow, even in the wet season. This may result in more metals settling into the sediment, causing accumulation of metals in sediments in certain parts of the estuary, especially in the upper and central parts. Moreover, if the estuary mouth is closed, water flow is restricted. Therefore, the influx of metals from land-based sources and the influent rivers end up accumulating in the sediment as the metal-containing particles settle after rain events.

Table 4.2-2: Comparison of mean sediment Al concentrations recorded in Zandvlei and others [data extracted from Izegaegbe et al. (2020)]

Estuaries	Al (mg/kg)
Zandvlei, South Africa[a]	(911.5875 ±243.857)
Mhlathuze, South Africa[b]	(12668 ±6958)

[a]present study; [b]Izegaegbe et al. (2020);

The concentrations recorded in this study were compared to those recorded for the Mhlathuze Estuary, South Africa (Izagaebe et al., 2020) (**Table 4.2-2**), since there are no sediment quality guidelines for Al from South Africa's Department of Water Affairs (DWAF) (1996), Canadian Sediment Quality Guidelines (CCME, 2001) as well as the Australian and New Zealand

Environment and Conservation Council (ANZECC, 2000) (**Table 10.1-2**). Concentrations recorded for Al in the Mhlathuze estuary were significantly higher than those recorded in the current study. This may largely be attributed to the fact that Mhlathuze is in close proximity to a vast industrial and shipping complex as compared to the Zandvlei estuary, which is located within a residential area with some upstream industrial and other activities within its surroundings. It was expected that the Zandvlei estuary is likely to record lower Al concentrations when compared to Mhlathuze. This is because of the nature and extent of anthropogenic activities in and around the Zandvlei estuary, which are not at the same scale as in Mhlathuze. It is however, acknowledged that neighbouring and upstream activities in the study area (i.e., recreational, agricultural, commercial, light, and heavy industrial activities) may likely affect the water quality of the estuary through the discharge and run-off of chemicals and household products which may be the source of Al and other metals in the estuary.

4.2.3. Algae

The mean Al concentrations (mg/kg) recorded in *Enteromorpha* spp. at the different sampling sites within the Zandvlei estuary ranged from 60.24±44.13 to 4517.35±9620.77 (**Table 4.1-1**). Spatial variations in Al concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 2 (1310.95±639.94) and 3 (3688.73±818.35), sites 3 and 4 (60.24±44.13) as well as sites 4 and 5 (4517.35±9620.77) on SO1 (summer). The results show a fluctuation in mean Al concentrations recorded between the different sampling sites on SO1 (summer) and this may be attributed to local and seasonal environmental conditions which influence the growth and morphology, life span of the algae species as well as the interactions among metal ions resulting in competition in binding sites (Villares et al., 2002; Chakraborty and Owens, 2014). The different levels of anthropogenic inputs from the various stormwater drainage outlets located along the estuary as well as street run-off from adjacent properties may have also contributed in the spatial variations in Al concentrations recorded between the different sampling sites. For instance, if one of the stormwater outlets discharges more Al containing stormwater at a particular point, and with the estuary being stagnant in most parts, that particular area may have higher concentrations of Al in algae, if conditions are favourable for the accumulation of that metal in algae. According to Żbikowski at el. (2006) metal concentrations in algae may be affected by various environmental conditions including the concentrations of metals in water, light intensity, pH and salinity among other factors. Physicochemical parameters such as salinity and pH seem to have had little effect on the spatial variations recorded on SO1 (summer). This is evident from site 4 which recorded a mean Al concentration value of 60.24±44.13 (**Table 4.1-1**) and a salinity concentration of 19.75ppt (**Table 3.1-1**), whereas site 3 recorded a concentration of 3688.73±818.35 and a salinity concentration of 16.30ppt Site 5 (SO1) recorded the highest Al concentration

(4517.35±9620.77). However, the recorded standard deviation was relatively high and demonstrates that the Al concentrations recorded at site 5 varied significantly. The results show significant differences in mean Al concentrations ($p<0.05$) recorded between sites 3 (1302.49±539.51) and 4 (2662.61±859.27) as well as sites 4 and 5 (915.03±879.69) on SO2 (autumn) (**Table 4.1-1**). Similar to SO1 (summer), the spatial variations found in mean Al concentrations recorded between the above sites on SO2 show a fluctuation in the recorded concentrations. As discussed above, this fluctuation in mean Al concentrations recorded in *Enteromorpha* spp. may be due to several reasons, which include the location of sampling sites, metal concentrations in solution, the interaction between metals and other elements, dilution of metal concentrations due to growth (metabolic factors), and physicochemical parameters such as pH and salinity (Villares et al., 2002). The results further clearly demonstrate *Enteromorpha* spp. as a macroconcentrator of Al as the bioconcentration was >2 in relation to concentrations recorded in water (Dallinger and Rainbow, 1993) (**Table 4.1-2**). This means that Al was bioconcentrated in *Enteromorpha* spp. at extremely high levels relative to Al concentrations recorded in water. However, the bioconcentration factors of Al in *Enteromorpha* spp. relative to sediment concentrations were much lesser to a point where deconcentration (<1) was noted at sites 1 (SO3, SO4); 2 (SO2, SO3, SO4); 3 (SO4); and 4 (SO1) (**Table 4.1-2**).

The bioavailability of metals is enhanced by physicochemical parameters such as a pH, which can reduce the hydrophilic complexations of a dissolved metal by increasing the availability of the free metal ion (Phillips and Rainbow, 1994). The free metal ion binds on the membrane transport protein and/or replaces a major ion as it enters an organism, and this usually occurs at low pH acidic streams, which often promote the percentage contribution free metal ion, which affects uptake and toxicity of metals (Phillips and Rainbow, 1994). The low pH of 4.97 recorded for site 4 in SO2 seems to have contributed to the statistically higher mean Al concentration (2662.61±859.27) recorded at site 4. It is, however, important to note that statistically significantly higher concentrations were recorded at sites with alkaline pH values (above 7) as well, and this may well be an indication of other factors, which may also influence the bioaccumulation of metals by macroalgae. Żbikowski et al. (2006) noted that it is difficult to clearly pinpoint the main factors that could influence the accumulation of metals in algae because of the different factors (i.e. pH, salinity, temperature, etc.) interacting with each other. Temporal (seasonal) variation in mean Al concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO2 (autumn) and SO3 (winter) at site 1 (**Table 4.1-1**). The recorded mean Al concentrations in SO3 (winter) at site 1 were statistically lower than the concentrations recorded in SO1 (summer) and SO2 (autumn). The reduction of concentrations during the winter period may also be likely influenced by less anthropogenic activities as result of heavy rainfall. Sany et al. (2013) indicated that the reduction of pollution levels coincided with the decrease of anthropogenic activities during the rainy season.

Statistical differences ($p<0.05$) in mean Al concentrations were also recorded in the pairwise consecutive comparisons between SO1 (summer), SO2 (autumn) and SO3 (winter) at site 2 (**Table 4.1-1**). Sites 3 and 4 show statistical differences ($p<0.05$) in Al concentrations in the pairwise comparisons between SO1 (summer) and SO2 (autumn). There were no statistical differences ($p>0.05$) in the pairwise consecutive comparisons between the different sampling occasions at site 5. Seasonal variations in Al concentrations recorded in this study show significantly higher mean concentrations in the dry seasons as compared to the wet season except for site 4. Some researchers have found concentrations of chemical elements in algae generally lower in the warmer months. This was attributed to the metabolic activity of algae, which is usually high in summer and results in the dilution of the accumulated metals whereas in winter, after slowing down of metabolic processes, the element content is usually higher (Hou and Yan, 1998; Villares et al., 2002). The abovementioned impact of the metabolic activity of algae on metal concentrations did not correlate with the findings of this study, as there were concentrations that were relatively high in both summer and winter months (**Table 4.1-1**). Villares et al. (2002) also notes that the metabolic activity of algal species could not explain all the variation in their study. A study conducted by Wright and Mason (1999) recorded higher concentrations of metals in winter for *Enteromorpha* spp. and noted a similar pattern in metal levels in sediments and in various invertebrates. The above findings clearly indicate that seasonal variation appears to be largely influenced by environmental factors, such as physicochemical parameters and discharges into the estuary, to name a few. These factors place a greater role than the metabolic and reproductive nature of macroalgae.

CHAPTER 5

MANGANESE RESULTS AND DISCUSSION

5.1. RESULTS

It should be noted that statistical comparisons presented in this chapter were made between consecutive sites and consecutive occasions.

Table 5.1-1: Mean concentrations (±SD) of manganese in water, sediment and algae during different sampling occasions at five sampling sites (n=5).

	SAMPLING OCCASIONS (SO)			
MANGANESE	SO1 (**Summer**)	SO2 (**Autumn**)	SO3 (**Winter**)	SO4 (**Spring**)
		WATER (mg/ ℓ)		
Site 1	$^{a}0.0227^{a}$ (±0.0076)	$^{a}0.0339^{a}$ (±0.0077)	$^{a}0.0339^{a}$ (±0.0250)	ND
Site 2	$^{a}0.0140^{a}$ (±0.0076)	$^{b}0.0260^{a}$ (±0.0083)	$^{a}0.0240^{a}$ (±0.0094)	ND
Site 3	$^{a}0.0176^{a}$ (±0.0029)	$^{b}0.0207^{a}$ (±0.0028)	$^{a}0.0204^{a}$ (±0.0068)	ND
Site 4	$^{a}0.0181^{a}$ (±0.0040)	$^{b}0.0233^{a}$ (±0.0041)	$^{a}0.0397^{b}$ (±0.0196)	ND
Site 5	$^{a}0.0239^{a}$ (±0.0036)	$^{c}0.0350^{b}$ (±0.0038)	$^{a}0.0203^{c}$ (±0.0034)	ND
		SEDIMENT (mg/kg)		
Site 1	$^{a}7.62^{a}$ (±1.54)	$^{a}26.59^{b}$ (±14.84)	$^{a}14.94^{b}$ (±4.42)	$^{a}17.15^{b}$ (±7.64)
Site 2	$^{b}27.29^{a}$ (±9.21)	$^{a}32.04^{a}$ (±18.12)	$^{b}8.38^{b}$ (±4.68)	$^{a}13.11^{c}$ (±3.35)
Site 3	$^{c}3.15^{a}$ (±1.13	$^{b}2.33^{a}$ (±0.59)	$^{c}3.26^{a}$ (±1.43)	$^{b}1.82^{a}$ (±0.36)
Site 4	$^{c}3.70^{a}$ (±0.81)	$^{b}2.87^{a}$ (±0.70)	$^{d}4.84^{a}$ (±2.24)	$^{c}1.22^{b}$ (±0.33)
Site 5	$^{d}5.11^{a}$ (±0.83)	$^{c}3.53^{b}$ (±0.47)	$^{e}5.88^{c}$ (±0.59)	$^{d}4.47^{d}$ (±1.74)
		ALGAE (mg/kg)		
Site 1	$^{a}531.91^{a}$ (±464.65)	$^{a}814.04^{a}$ (±291.69)	$^{a}250.94^{a}$ (±123.87)	$^{a}1008.93^{b}$ (±471.34)
Site 2	$^{b}627.99^{a}$ (±223.00)	$^{b}405.02^{a}$ (±203.05)	$^{a}134.88^{b}$ (±136.02)	$^{a}152.80^{b}$ (±88.70)
Site 3	$^{c}243.10^{a}$ (±92.93)	$^{c}28.13^{b}$ (±16.15)	$^{a}19.81^{b}$ (±7.71)	$^{a}502.44^{c}$ (±653.11)
Site 4	$^{d}24.62^{a}$ (±6.82)	$^{d}258.05^{b}$ (±248.49)	$^{b}585.14^{b}$ (±270.20)	$^{a}241.40^{b}$ (±176.38)
Site 5	$^{d}90.54^{a}$ (±91.17)	$^{d}79.31^{a}$ (±34.21)	$^{c}20.30^{b}$ (±2.75)	ND

ND = No Data
The statistical differences between sites per sampling occasion are indicated by superscripted letters to the left of mean values.
The statistical differences between sampling occasions per site are indicated by superscripted letters to the right of mean values.
Water, sediment and algae samples were not statistically compared with each other.

Table 5.1-2: Bioconcentration factors of algae for manganese.

Manganese		SO1 (Summer)	SO2 (Autumn)	SO3 (Winter)	SO4 (Spring)
Site 1	A/W	23432.16	24012.98	7402.36	
	A/S	69.80	30.61	16.80	58.83
Site 2	A/W	44856.43	15577.69	5620	
	A/S	23.01	12.64	16.10	11.66
Site 3	A/W	13812.5	1358.93	971.08	
	A/S	77.17	12.07	6.08	276.07
Site 4	A/W	1360.22	11075.10	14739.04	
	A/S	6.65	89.91	120.90	197.87
Site 5	A/W	3788.28	2266	1000	
	A/S	17.72	22.47	3.45	

Shaded areas represent sites with either no data or where the recorded concentrations were below the level of detection.

A/W = $\frac{Algae\ metal\ concentration\ (\frac{mg}{kg})}{Water\ metal\ concentration\ (\frac{Mg}{L})}$ A/S = $\frac{Algae\ metal\ concentration\ (\frac{mg}{kg})}{sediment\ metal\ concentration\ (\frac{mg}{kg})}$

5.1.1. Water

5.1.1.1. Comparisons of metal concentrations between sites per occasion

The results showed no statistical differences in manganese (Mn) concentrations recorded between the different sampling sites on SO1 and SO3 ($p>0.05$). Pairwise multiple comparisons of consecutive sampling sites have revealed the following differences on SO2: sites 1 and 2 ($p<0.05$); sites 2 and 3 ($p>0.05$); sites 3 and 4 ($p>0.05$); and sites 4 and 5 ($p<0.05$). No data was recorded for SO4 (**Table 5.1-1**).

5.1.1.2. Comparisons of metal concentrations between sampling occasions per site

The results showed no statistical differences in Mn concentrations recorded between the different sampling occasions at sites 1, 2 and 3 ($p>0.05$). At site 4, the results revealed no significant differences ($p>0.05$) in Mn concentrations recorded between SO1 and SO2, whilst there were statistical differences ($p<0.05$) in Mn concentrations recorded between SO2 and SO3. Pairwise multiple comparisons of consecutive sampling occasions at site 5 revealed statistical differences ($p<0.05$) between the different sampling occasions (**Table 5.1-1**). No data was recorded for SO4 (**Table 5.1-1**).

5.1.2. Sediment

5.1.2.1. Comparisons of metal concentrations between sites per occasion

Pairwise multiple comparison of consecutive sampling sites on SO1 revealed statistical differences (p<0.05) in Mn concentrations recorded between sites 1 and 2, sites 2 and 3, as well as sites 4 and 5 (p<0.05). However, there were no statistical differences recorded between sites 3 and 4 (p>0.05) on SO1. Pairwise multiple comparison of consecutive sampling sites on SO2 showed statistical differences in Mn concentrations recorded between sites 2 and 3 as well as sites 4 and 5 (p<0.05). On SO3, the results showed statistical differences in Mn concentrations recorded between the different sampling sites (p<0.05). Pairwise multiple comparison of consecutive sampling sites on SO4 revealed statistical differences between sites 2 and 3, sites 3 and 4 as well as sites 4 and 5 (p<0.05) (**Table 5.1-1**).

5.1.2.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparisons of consecutive sampling occasions at site 1 revealed statistical differences in Mn concentrations recorded between SO1 and SO2 (p<0.05). However, the results showed no statistical differences in Mn concentrations recorded between SO2 and SO3 as well as between SO3 and SO4 (p>0.05). At site 2, the results showed statistical differences in Mn concentrations recorded between SO2 and SO3 and also between SO3 and SO4 (p<0.05). No statistical differences (p>0.05) in Mn concentrations were recorded between the different sampling occasions at site 3. Pairwise multiple comparisons of consecutive sampling occasions at site 4 have revealed no statistical differences in Mn concentration between SO1 and SO2 as well as between SO2 and SO3 (p>0.05). However, the results showed no significant differences (p>0.05) in Al concentrations between SO3 and SO4 (p<0.05). At site 5, the results show statistical differences (p<0.05) in Mn concentrations between the different sampling occasions (**Table 5.1-1**).

5.1.3. Algae

5.1.3.1. Comparisons of metal concentrations between sites per occasion

Pairwise multiple comparisons of consecutive sampling sites on sampling occasions 1 and 2 have shown statistical differences in Mn concentrations between sampling sites 1, 2, 3 and 4 (p<0.05). On SO3, the results showed statistical differences in Mn concentrations recorded between sampling sites 3 and 4 as well as sites 4 and 5 (p<0.05). However, no significant differences (p>0.05) in Mn concentrations were recorded between sites 1 and 2 as well as sites 2 and 3. The results revealed no statistical differences in Mn concentrations between the different sampling sites on SO4 ($p>0.05$) (**Table 5.1-1**).

5.1.3.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparisons of consecutive sampling occasions at site 1 show no statistical differences in Mn concentrations between SO1 and SO2 as well as between SO2 and SO3 ($p>0.05$). However, statistical differences in Mn concentrations were recorded between SO3 and SO4 at site 1 ($p<0.05$). The results showed significant differences ($p<0.05$) in Mn concentrations recorded between SO2 and SO3 at site 2. Pairwise multiple comparisons of consecutive sampling occasions at site 3 revealed statistical differences ($p<0.05$) in Mn concentrations recorded between SO1 and SO2 as well as SO3 and SO4 ($p<0.05$), while Mn concentrations recorded between SO2 and SO3 recorded no statistical differences at site 3 ($p>0.05$). At site 4, the results showed statistical differences in Mn concentrations between SO1 and SO2 ($p<0.05$), while there were no significant differences ($p>0.05$) recorded between SO2 and SO3 as well as SO3 and SO4. Pairwise multiple comparison of consecutive sampling occasions at site 5 have shown statistical differences in Mn concentrations recorded between SO2 and SO3 ($p<0.05$). However, the results showed no statistical differences ($p>0.05$) in Mn concentrations recorded between SO1 and SO2 at site 5 (**Table 5.1-1**).

5.1.3.3. Bioconcentration factors of algae for manganese

The bioconcentration factors of Mn in *Enteromorpha* spp. are presented in **Table 5.1-2.** The results show a high amount of Mn that is bioconcentrated in *Enteromorpha* spp. (>2) as compared to Mn concentrations recorded in water. Furthermore, as expected, the bioconcentration factor relative to sediment was much lesser in comparison to those relative to water. Site 4 (SO3) recorded the highest bioconcentration factor relative to water, while site 3 (SO4) recorded the highest bioconcentration factor relative to sediment.

5.2. DISCUSSION

5.2.1. Water

Mean Mn concentrations (mg/ℓ) recorded in water at the different sampling sites within the study area ranged from 0.0140±0.0076 to 0.0397±0.0196 (**Table 5.1-1**). Spatial variations in Mn concentrations show significant differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 (0.0339±0.0077) and 2 (0.0260±0.0083) as well as sites 4 (0.0233±0.0041) and 5 (0.0350±0.0038) on SO2 (autumn). The relatively higher mean Mn concentration recorded at site 1 (SO2) may be linked to the proximity of the site from influent rivers which discharge directly into the estuary as well as the gradient of the estuary (**Figure 4.2-1**). The results show an increase in Mn concentrations at site 5 (SO2) and this may be linked to anthropogenic discharges (i.e. stormwater drainage, street run-off etc.), estuarine circulation as a result of rainfall (24.2 mm), as well as the closure of the estuary mouth (**Table 3.3-1**). An increase in metal concentrations in the water column may also be linked to an increase in salinity and a decrease in pH and redox potential (Sany et al., 2013). This is because elevated salinity leads to an increased competition between cations and metals for binding sites which ultimately pushes the metals into the overlying water (Nduka and Orisakwe, 2011). The recorded salinity concentrations increased from 18.65ppt (site 1) to 25.45ppt (site 5) and pH decreased from 8.81 (site 1) to 4.97 (site 4) on SO1 (summer) (**Table 3.1-1**). The acidity level (pH) recorded for site 4 (SO2) does not appear to have had an impact in the recorded mean concentration at site 4 (0.0233±0.0041) as it was significantly lower ($p<0.05$) than the concentration recorded at site 5 (0.0350±0.0038) which had a pH level of 6.8. Salinity and some of the factors discussed above may have had influenced the spatial variations recorded on SO2 (autumn). Anthropogenic sources of manganese in waterbodies include municipal wastewater discharges, sewage sludge, mining and mineral processing, emissions from production of alloy, steel, and iron industries and the combustion of fossil fuels (WHO, 2004). As indicated in the previous chapter, a nearby industrial regarded as a potential source of illegal discharges and contaminated stormwater run-off (WCG, 2018).

Accidental wastewater discharges have been reported in some of the 14 pump stations located around the estuary (C.A.P.E., 2010). Manganese appears to emanate from the water entering the estuary from inflowing rivers, stormwater run-off and accidental wastewater discharges that are reported from time to time. It is also important to note that the estuary mouth was closed during sample collection on SO2 (autumn). The gradient and vegetation (i.e., pondweed) of the estuary also plays an important role in the movement of water (and metals) in the estuary. Pondweed (*Stuckenia pectinata*) occurs naturally in the Zandvlei estuary and has a tendency of forming (during summer period) dense clumps which are usually concentrated in upper sections of the estuary (McQuaid, 2013). These dense clumps or mats of *Stuckenia pectinata* often reduce light penetration, intensify flooding, impede recreational activities (i.e., fishing and boating) and decrease current flow, which leads to stagnation (Quick and Harding, 1994; C.A.P.E., 2011). The stagnant waters influence the movement of metals in the water column and may therefore result in some metals settling in the sediment, especially if the environmental conditions of the estuary allow for such to occur. The results show no significant differences ($p>0.05$) in Mn concentrations recorded between the different consecutive sampling sites on SO1 (summer) and SO3 (winter) (**Table 5.1-1**). This may be due to local and seasonal environmental conditions (i.e. water properties, estuarine circulation, river and groundwater discharge, water flow velocities, sediment input and resuspension, etc.) which influence the movement and fate of metals (De Souza Machado et al., 2016). The recorded mean Mn concentrations were well below the recommended Target Water Quality Range (TWQR) concentration by DWAF (1996) as well as the guideline concentration values recommended by AZNECC (2000) and CCME (2019) (**Table 10.1-1**).

Seasonal (temporal) variations in mean Mn concentrations show no significant differences ($p>0.05$) in the pairwise consecutive comparisons between the different sampling occasions at sites 1, 2 and 3 (**Table 5.1-1**). This could mean that the anthropogenic inputs of Mn were consistent to throughout the different seasons at sites 1, 2 and 3. The results show significant differences in mean Mn concentrations recorded between autumn (SO2) (0.0233a±0.0041) and winter (SO3) (0.0397±0.0196) at site 4 ($p<0.05$) (**Table 5.1-1**) and this may be attributed to elevated rainfall which washes away contaminants that may have been deposited in various places and these end up in waterbodies. A monthly average rainfall of 24.2 mm and 51 mm was recorded for autumn (SO2) and winter (SO3), respectively (**Table 3.2-1**). Sany et al. (2013) highlighted that elevated rainfall promotes increased land-based run-off and fluvial discharges which may contain pollutants. The results show significant differences in mean Mn concentrations recorded between the different sampling occasions at site 5 ($p<0.05$) (**Table 5.1-1**). Site 5 recorded low pH values and high salinity levels when compared to other sites in each sampling occasion (**Tables 3.1-1, 3.1-2,** and **3.1-3**). According to Sany et al. (2013) elevated metal concentrations may be affected by low pH and increased salinity levels. However, mean concentrations recorded in this study at sites with low pH and high salinity levels were relatively low and were within the same range as those recorded in sites with high pH and low salinity levels. This could be an indication that Mn was either in low concentrations in the water column and adsorbed in sediments/organisms and/or the anthropogenic input of Mn was also low. Mn appears to be widely distributed in the water column as some sites in the upper parts of the estuary recorded similar concentrations as those in the lower parts of the estuary.

5.2.2. Sediment

The mean Mn concentrations (mg/kg) recorded in sediment at the different sampling sites within the study area ranged from 1.22±0.33 to 27.29±9.21 (**Table 5.1-1**). Spatial variations in Mn concentrations (±SD) show significant differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 (7.62±1.54) and 2 (27.29±9.21), sites 2 and 3 (3.15±1.13) as well as sites 4 (3.70±0.81) and 5 (5.11±0.83) on SO1 (summer). The results show a relatively higher mean concentration at site 2 (SO1) which may be attributed to the proximity of the site to pollution sources as the site is located close to a stormwater drainage outlet, a small yacht club as well as a parking area. Manganese is often used in the leather, textile and other production industries, in the manufacturing industry (i.e. chemical, glass), and can be used as a fertilizer (WHO, 2001). Urban run-off is responsible for the disposal or transportation of several contaminants such as oils, grease, toxic chemicals from motor vehicles, pesticides and nutrients from lawns and gardens, and heavy metals from roofs, motor vehicles, and other sources (EPA, 2003). The industrial area located north of the study area has over 200 businesses which include various manufacturing activities (COCT, 2017). Western Cape Government (2018) regards this area as a potential source of illegal discharges. Other factors which may have contributed to the elevated concentrations recorded at site 2, include river discharge or inputs, estuary gradient, opening and closing of the estuary mouth, as well as sediment grain size distribution. The results also show similar spatial variations in Mn concentrations recorded between the different sampling sites on SO2 (autumn), where site 2 also recorded a relatively higher mean concentration when compared to the other sampling sites, particularly sites 3 to 5 (**Table 5.1-1**). However, on SO3 (winter), the results show a significantly higher Mn concentration recorded at site 1 and thereafter mean Mn concentrations decreased in a fluctuating pattern. The elevated concentration recorded at site 1 may be ascribed to river inputs from upstream industrial and other activities which ultimately ends up draining into the estuary due to elevated rainfall recorded in winter (SO3). The recorded monthly average rainfall by SAWS was 5.2 mm (SO1 [summer]), 24.2 mm (SO2 [autumn]), 51.0 mm (SO3 [winter]), and 27.8 mm (SO4 [spring]) (**Table 3.2-1**). High rainfall levels result in increased land-based inputs (i.e. stormwater and industrial effluents) and river discharges which may significantly affect the distribution of metals in the estuary (Sany et al., 2013).

The gradient of the estuary may have also played a role in the settling of Mn in sediments found in the upper/northern parts (i.e. site 1 or 2). The main body of the estuary consists of deeper channels and this is mainly due to the removal of pondweed for recreational activities (WCG, 2018). Recreational areas are kept clear of pondweed to a depth of 0.5 m for all canals in the marina, and to as deep as possible in the main body of the estuary (WCG, 2018). This directly affects the transportation of metals in the estuary and may therefore be responsible for the concentration recorded at the northern/upper parts of the study area. Nduka and Orisakwe

(2011) found that the rate of adsorption and desorption of metals to and from sediments was controlled by the water chemistry of the receiving environment. De Souza Machado et al. (2016) underlined that the distribution and fate of metals within an estuary is influenced by factors such as estuarine circulation, river and groundwater discharge, tidal flooding, sediment input and re-suspension, exchange with neighbouring environments, water properties (e.g. salinity, redox and pH) and the presence of organisms. Sediment grain size plays a significant role in the distribution of metals in sediments (Chapman and Wang, 2001; Sany et al., 2013; CSIR, 2015). Fine-grained sediments have a significant capacity to retain metals because of their greater surface area and as such, correlations usually exist between decreasing grain sizes and increasing metal concentrations (Chapman and Wang, 2001; CSIR, 2015). As discussed in chapter 4, an assessment of sediment grain size at the Zandvlei estuary by the CSIR (2015) and Maurer (2019) found that coarsely grained sediments (i.e. sand) were mostly found in the southern/lower parts of the estuary and fine-grained sediment (i.e. mud) were found in the northern/upper parts of the Zandvlei estuary. In light of this, it was expected that sediments in the northern/upper parts of the estuary will record higher metal concentrations when compared to sediments in the southern/lower parts of the estuary. The results show the mean Mn concentrations in sediment are in line with the above expectation as the concentrations recorded at sites 1 and 2 were higher than mean Mn concentrations recorded at sites 3, 4 and 5 in all sampling occasions (**Table 5.1-1**). Salinity ranged from 18.65ppt (site 1) to 25.45ppt (site 5) on SO2, while the pH ranged from 8.81 (site 1) to 6.8 (site 5) (**Table 3.1-2**). Salinity is responsible for changes in water chemistry and ionic strength which affects metal mobilisation (Benoit et al., 1994). Salinity also significantly increases the concentration of carbonate and bicarbonate ions which enhances the water pH buffering capacity. As a result of this, pH is expected to have an impact on metal behaviour at low salinities (De Souza Machado et al., 2016). Low salinities are recorded in the northern/upper parts of the estuary where high Mn concentrations were recorded (**Table 5.1-1**). Site 5 recorded a statistically higher mean concentration ($p<0.05$) as compared to site 4 on SO1 (summer) and SO2 (autumn), which may be due to sediment grain size as well as the closure of the estuary mouth which prevents flushing of contaminants out of the system. Statistical differences ($p<0.05$) in mean Mn concentrations were also recorded in the pairwise comparisons between sites 2 and 3, sites 3 and 4, as well as sites 4 and 5 on SO4 (spring) (**Table 5.1-1**). The mean concentration (±SD) recorded at site 2 (SO4) (13.11±3.35) was statistically significantly higher than the mean concentrations recorded at site 3 (1.82±0.36), site 4 (1.22 ±0.33) and 5 (4.47±1.74) on SO4 (spring) (**Table 5.1-1**). This may be linked to several factors which include stormwater drainage, physicochemical parameters (i.e. salinity, pH etc.), and gradient of the estuary as well as sediment grain size. Site 2 is located near a stormwater drainage outlet (**Figure 5.2-1**). It is also located in an area with deeper channels as a result of the removal of pondweed (WCG, 2018).

Figure 5.2-1: Map showing the proximity of site 2 from stormwater drainage outlets within the study area (Source: Google Maps; CoCT open data portal)

Temporal (seasonal) variations in mean Mn concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO1 (summer) (7.62±1.54) and SO2 (autumn) (26.59 ±14.84) at site 1 (**Table 5.1-1**). The relatively high Mn concentrations recorded in autumn (SO2) at site 1 may be attributed to elevated river inputs and stormwater run-off due to an increase in rainfall. During the wet season (i.e. autumn and winter), increased freshwater runoff can carry higher loads of metals from land-based sources, resulting in elevated metal concentrations. Urban runoff is regarded as a significant pathway of pollutants such as metals, as it carries untreated contaminants to nearby aquatic systems (Fletcher et al., 2013). According to McDonald et al. (2021) elevated metal concentrations in stormwater is mainly attributed to anthropogenic sources, such as metal surfaces (roofing and construction sites), vehicles (brake and tyre wear, exhaust) and runoff from commercial and industrial activities. The movement of metals is strongly associated with rainfall and melting (Taka et al., 2022). The results show significant differences ($p<0.05$) in mean Mn concentrations recorded between SO2 (autumn) (32.04±18.12) and SO3 (winter) (8.38b±4.68) as well as SO3 (winter) and (SO4) (spring) (13.11±3.35) at site 2 (**Table 5.1-1**). The above recorded statistical differences at site 1 may be attributed to the increased rainfall levels recorded in autumn (SO2) which may have potentially increased pollutants entering the site. Elevated metal concentrations in sediments are also caused by either low remobilisation rates into water or through a process known as precipitation (Rainbow, 1995; Chakraborty and Owens, 2014). Seasonal fluctuations (i.e. tidal and seasonal currents as well as changes to pollution load) may also be responsible for the temporal variations recorded in mean Mn concentrations (Sany et al., 2013).

Site 1 is located near the upstream influent rivers and therefore receives river discharge and land-based inputs. However, as much as rainfall may increase pollution load into the estuary, rainwater is also responsible for increased mobility and dilution which decreases metal concentrations in sediment (Li et al., 2009; Sany et al., 2013). This may have been a factor in the temporal variations recorded in mean Mn concentrations ($p<0.05$) between SO2 (autumn) and SO3 (winter) at site 1 (**Table 5.1-1**). No statistical differences ($p>0.05$) in mean Mn concentrations were recorded between the different sampling occasions at site 3. The results show significant differences in mean Mn concentrations recorded between SO3 (winter) and SO4 (spring) at site 4. A statistically significantly lower mean Mn concentrations was recorded in spring (SO4) when compared to winter (SO3) at site 4 and this may be due to factors such as decreased land-based run-off and river discharges as a result of lower rainfall levels in spring. The recorded monthly average rainfall for SO4 (spring) was 27.8 mm compared to the 51.0 mm recorded for SO3 (winter) (**Table 3.2-1**). As discussed above, sediment grain size played a key role in controlling sediment metal concentrations (Chapman and Wang, 2001) as there was link between sites consisting of coarse-grained sediments and the recorded low metal concentrations for those particular sites. Statistical differences ($p<0.05$) in mean Mn concentrations were also recorded in the pairwise consecutive comparisons between all the different sampling occasions at site 5 (**Table 5.1-1**). A fluctuation in mean concentrations was noted between the different occasions at site 5 and this may be attributed to seasonal fluctuations, including opening and closing of the estuary mouth.

Table 5.2-1: Comparison of mean sediment Mn concentrations recorded in Zandvlei and others [data extracted from Izegaegbe et al. (2020)]

Estuaries	**Mn (mg/kg)**
Zandvlei, South Africa[a]	9.47±3.75
Mhlathuze, South Africa[b]	356±230
Swartkops. South Africa[c]	114±94
St Louis Estuary, Senegal[d]	55.7±230
Brisbrane Estuary, Australia[e]	386±428

[a]present study; [b]Izegaegbe et al. (2020); [c]Diop et al. (2015); [d]Duodu et al. (2017); [e]Hamzeh et al. (2014)

There are no recommended sediment quality guidelines available for Mn from DWAF (1996), ANZECC (2000) and CCME (2001) (**Table 10.1-2**). The sediment concentrations recorded in this study was compared to other studies done on estuaries in Southern and Western Africa. The Zandvlei estuary recorded the lowest mean concentration for Mn in sediment when compared to other estuaries listed in **Table 5.2-1**. The recorded concentrations for the Mhlathuze, Swartkops, and St Louis estuaries are much higher than the mean concentration recorded for Mn in the Zandvlei estuary. This shows that the sediment in Zandvlei estuary is, in relative terms, less contaminated with Mn.

5.2.3. Algae

The mean Mn concentrations (mg/kg) in algae (*Enteromorpha* spp.) ranged from 19.81±7.71 to 1008.93±471.34 (**Table 5.1-1**). The comparison of the spatial variations in Mn concentrations show statistical differences ($p<0.05$) in pairwise consecutive comparisons between sites 1 and 2, sites 2 and 3, as well as sites 3 and 4 on SO1 (summer) and SO2 (autumn). A statistically significant ($p<0.05$) increase in Mn concentrations was noted between site 1 (531.91±464.65) and site 2 (627.99±223.00) on SO1 (summer). Site 2 is located near a stormwater drainage outlet which may be responsible for the discharge of metals such as Mn, resulting in elevated levels of Mn at site 2 on SO1 (summer). The results show a significant decline in Mn concentrations ($p<0.05$) recorded in the pairwise consecutive comparisons between sites 2 (627.99±223.00) and 3 (243.10±92.93) as well as sites 3 (243.10±92.93) and 4 (24.62 ±6.82) on SO1 (summer) (**Table 5.1-1**). This could be attributed to the gradient of the estuary which comprises of a deeper channel upstream and therefore standing waters within the northern/upper and central parts of the estuary. The elevated concentrations upstream could be due to river inputs, and stormwater discharges from the industrial area and other activities within the catchment. The recorded monthly average rainfall for SO1 (summer) was 5.2 mm (**Table 3.2-1**). The estuary mouth was also closed during sample collection on SO1 (summer). The above seems to suggest that there was limited water flow and distribution of metals in the estuary as most of the water was probably stagnant in areas with deeper channels (i.e. upstream). Furthermore, Żbikowski et al. (2006) noted that there are several other environmental factors which play a key role in the accumulation of metals in algae, and these include: the concentration of metals in water, salinity, pH, interactions between different metals, light intensity, and metabolic factors such as dilution of metal contents due to algal growth. One or a combination of the above factors may have potentially resulted in the recorded Mn concentrations in this study. The bioconcentration factors of Mn in *Enteromorpha* spp. relative to Mn concentrations in water have shown the macroconcentration (BCF >2) of Mn in *Enteromorpha* spp. and this is also evident with sediments, but to a much lesser degree (**Table 5.1-2**). The bioconcentration rates in *Enteromorpha* spp. appears to suggest that most of the metals are removed from the aquatic environment and are stored in plants such as *Enteromorpha* spp.

On SO2 (autumn), Mn concentrations decreased significantly ($p<0.05$) between sites 1 and 2 as well as sites 2 and 3 (**Table 5.1-1**). Site 1 recorded the highest mean concentration on SO2 (autumn). This may be due to the proximity of the site to the upstream influent rivers as well as physical and chemical properties (among other factors). Struck et al. (1997) noted that the uptake of metals such as Mn was found to increase with decreasing salinity. Sites 1 and 2 in all sampling occasions recorded lower salinity levels (12.46 to 19.68 ppt) (**Tables 3.1-1**, **3.1-2**, and **3.1-3**) and higher mean Mn concentrations when compared to sites 4 and 5, which recorded higher salinity levels (i.e. 16.30 to 25.45 ppt) and lower mean Mn concentrations, especially site 5 near the estuary mouth. No statistical differences ($p>0.05$) were found in concentrations recorded at sites 1, 2 and 3 on SO3. Spatial variations in Mn concentrations show statistical differences in the pairwise consecutive comparisons between sites 3 and 4, as well as sites 4 and 5 ($p<0.05$) on SO3 (winter). No statistical differences ($p>0.05$) were recorded in concentrations recorded between the different sampling sites on SO4 (**Table 5.1-1**). Site 4 (SO3) recorded the highest mean Mn concentration (585.14±270.20) when compared to other sampling sites within SO3 (winter). This may be attributed to the slowing down of metabolic processes, which results in higher metal content in algae, among other possible factors. (Villares et al., 2002; Żbikowski et al., 2006). The hardening of the many surfaces in the Zandvlei catchment as a result of urban development may result in water flowing directly into the estuary instead of infiltrating into the soil and groundwater. SO3 was in the winter season and therefore recorded a significant amount of rainfall (51.0 mm) which may have contributed to the transportation of metal contaminants from neighbouring and upstream activities (i.e. industrial and agriculture) into the estuary. However, it is important to note that the movement of metals in an estuary is not only affected by rainfall, river input or tidal flows but may also be affected by several physical and chemical parameters, such as salinity which changes water chemistry and ionic strength, ultimately affecting metal mobilisation (Benoit et al., 1994; De Souza Machado et al., 2016).

The comparison of the temporal (seasonal) variations in Mn concentrations show statistical differences ($p<0.05$) in pairwise consecutive comparisons between SO3 (winter) (250.94±123.87) and SO4 (spring) (1008.93±471.34) at site 1 (**Table 5.1-1**). The spring season (SO4) at site 1 recorded the highest mean concentration when compared to all other sites from the different sampling occasions. High levels of Mn in spring (SO4) at site 1 may be due to land-based inputs from upstream activities, including a combination of other factors (i.e. salinity, pH, metabolic factors, etc.). It has been found to be difficult to determine explicitly the main factors affecting seasonal changes in metal accumulation in algae as this may be caused by the interactions between various factors (Vasconcelos and Leal, 2001; Szefer, 2002; Żbikowski et al., 2007). Statistical differences ($p<0.05$) in Mn concentrations were also recorded in the pairwise consecutive comparisons between SO2 (autumn) (405.02±203.05) and SO3 (winter) (134.88±136.02) at site 2 (**Table 5.1-1**). Winter recorded low concentrations when compared to autumn at site 2 and this may be caused by seasonal fluctuations and the interaction of Mn with other metals on site, including the availability or dominance of algae during the winter season (Żbikowski et al., 2006). During the winter period (high rainfall), *Enteromorpha* in the Zandvlei estuary is likely to be less dominant due to low salinity conditions (Muhl, 2003). This may affect the accumulation of metals in the available macroalgae. Temporal variations in Mn concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO1 (summer) (243.10±92.93) and SO2 (autumn) (28.13±16.15) as well as SO3 (winter) (19.81±7.71) and SO4 (spring) (502.44±653.11) at site 3 (**Table 5.1-1**). Statistical differences ($p<0.05$) in Mn concentrations were recorded in the pairwise consecutive comparisons between SO1 (summer) (24.62±6.82) and SO2 (autumn) (258.05±248.49) at site 4. This may have been due to algae metabolic processes. Metal concentrations in algae are usually low in the summer months due to growth rates resulting in dilution of the accumulated metals. However, in winter, metal concentrations in algae are usually higher due to the slowing down of metabolic processes (Lyngby and Brix, 1984; Hou and Yan, 1998; Villares et al., 2002; Żbikowski et al., 2007). The growth of macroalgae is however, also affected by high or low salinity (over an extended period) as this reduces growth because of the enzymes and reduced turgor pressure slowing cell division (Lobban and Harrison, 1994; Muhl, 2003).

The results further show significant differences ($p<0.05$) in mean Mn concentrations recorded between SO2 (autumn) (79.31±34.21) and SO3 (winter) (20.30±2.75) at site 5 (**Table 5.1-1**). Although winter is expected to record higher metal concentrations as a result of the slowing of metabolic processes, this study found the opposite, where winter recorded the lowest Mn concentrations from the different sampling sites (except for site 3) and this may be due to an interaction of different factors, as noted above. The bioaccumulated Mn concentrations seem to suggest some level of contamination of the study area. Although Mn occurs naturally in the environment, the bioaccumulated concentrations appear to be influenced by anthropogenic sources. Occasional sewer overflows were reported in the Zandvlei catchment area (C.A.P.E., 2010; WCG, 2018), and there is an industrial area located 2km north of the site and which appears to be discharging some of its stormwater directly in one of the influent rivers which feeds into the estuary. The above anthropogenic sources may have contributed to the elevated levels of Mn recorded in the sampled macroalgae.

CHAPTER 6

IRON RESULTS AND DISCUSSION

6.1. RESULTS

It should be noted that statistical comparisons presented in this chapter were made between consecutive sites and consecutive occasions.

Table 6.1-1: Mean concentrations (±SD) of iron in water, sediment and algae during different sampling occasions at five sampling sites (n=5).

	SAMPLING OCCASIONS (SO)			
IRON	SO1 (**Summer**)	SO2 (**Autumn**)	SO3 (**Winter**)	SO4 (**Spring**)
WATER (mg/ℓ)				
Site 1	^{a}0.0583^{a} (±0.0754)	^{a}0.0103^{a} (±0.0230)	^{a}0.2835^{b} (±0.3385)	ND
Site 2	^{a}0.0420^{a} (±0.0940)	^{a}0.0176^{a} (±0.0393)	^{a}0.4016^{b} (±0.3890)	ND
Site 3	<LD	^{a}0.1110^{b} (±0.1378)	^{a}0.1875^{b} (±0.1361)	ND
Site 4	<LD	^{b}0.2367^{b} (±0.1631)	^{a}1.8990^{c} (±2.3371)	ND
Site 5	<LD	^{c}0.0483^{b} (±0.0954)	^{a}0.1461^{c} (±0.1010)	ND
SEDIMENT (mg/kg)				
Site 1	^{a}1040.01^{a} (±275.52)	^{a}1839.65^{b} (±491.10)	^{a}1284.14^{c} (±540.07)	^{a}1009.69^{d} (±187.30)
Site 2	^{b}5834.36^{a} (±3788.34)	^{b}6744.90^{a} (±5130.36)	^{a}1448.44^{b} (±576.33)	^{b}1828.28^{b} (±509.02)
Site 3	^{c}513.45^{a} (±268.67)	^{c}726.48^{a} (±262.87)	^{a}904.45^{a} (±357.81)	^{c}742.11^{a} (±88.29)
Site 4	^{d}1000.95^{a} (±422.36)	^{d}2160.94^{b} (±2176.14)	^{a}3429.42^{b} (±5514.62)	^{d}197.81^{c} (±28.64)
Site 5	^{e}400.39^{a} (±106.80)	^{e}580.85^{a} (±130.75)	^{b}600.01^{a} (±66.09)	^{e}532.09^{a} (±119.67)
ALGAE (mg/kg)				
Site 1	^{a}3018.42^{a} (±951.75)	^{a}1934.56^{a} (±736.91)	^{a}455.09^{a} (±172.32)	^{a}951.51^{a} (±651.55)
Site 2	^{b}1816.89^{a} (±641.34)	^{b}821.27^{b} (±45.66)	^{a}354.25^{c} (±83.86)	^{a}519.94^{c} (±172.82)
Site 3	^{c}4764.45^{a} (±606.10)	^{c}1347.84^{b} (±519.47)	^{a}781.94^{b} (±210.50)	^{a}1057.09^{b} (±395.58)
Site 4	^{d}539.73^{a} (±164.05)	^{d}2913.35^{b} (±569.38)	^{a}1965.49^{b} (±1347.57)	^{a}3253.38^{b} (±760.65)
Site 5	^{e}1573.43^{a} (±870.59)	^{d}2444.57^{a} (±1240.90)	^{a}1850.15^{a} (±836.29)	ND

LD = Limit of Detection
ND = No Data.
The statistical differences between sites per sampling occasion are indicated by superscripted letters to the left of mean values.
The statistical differences between sampling occasions per site are indicated by superscripted letters to the right of mean values.
Water, sediment and algae samples were not statistically compared with each other.

Table 6.1-2: Bioconcentration factors of algae for iron.

Iron		**SO1 (Summer)**	**SO2 (Autumn)**	**SO3 (Winter)**	**SO4 (Spring)**
Site 1	A/W	51773.93	187821.36	1605.26	
	A/S	2.90	1.05	0.35	0.94
Site 2	A/W	43259.29	46663.07	882.10	
	A/S	0.31	0.12	0.24	0.28
Site 3	A/W		12142.70	4170.35	
	A/S	9.28	1.86	0.86	1.42
Site 4	A/W		12308.20	1035.013	
	A/S	0.54	1.35	0.57	16.45
Site 5	A/W		50612.22	12663.59	
	A/S	3.93	4.21	3.08	

Shaded areas represent sites with either no data or where the recorded concentrations were below the level of detection.

A/W = $\frac{Algae\ metal\ concentration\ (\frac{mg}{kg})}{Water\ metal\ concentration\ (\frac{Mg}{L})}$ A/S = $\frac{Algae\ metal\ concentration\ (\frac{mg}{kg})}{sediment\ metal\ concentration\ (\frac{mg}{kg})}$

6.1.1. Water

6.1.1.1. Comparison of metal concentrations between sampling sites per occasion

The results showed no statistical differences ($p>0.05$) in iron (Fe) concentrations recorded between the different sampling sites on SO1 and SO3. Pairwise multiple comparison of consecutive sampling sites on SO2 revealed statistical differences ($p<0.05$) in Fe concentrations recorded between sites 3 and 4 as well as sites 4 and 5. No data was recorded for SO4 (**Table 6.1-1**).

6.1.1.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions at sites 1 and 2 revealed statistical differences ($p<0.05$) in Fe concentrations recorded between SO2 and SO3. At site 3, the results showed statistical differences in Fe concentrations recorded between SO1 and SO2 ($p<0.05$). However, no significant differences ($p>0.05$) in Fe concentrations were recorded between SO2 and SO3 at site 3. Pairwise multiple comparison of consecutive sampling occasions at sites 4 and 5 showed statistical differences ($p<0.05$) in Fe concentrations between the different sampling occasions. No data was recorded for SO4 (**Table 6.1-1**).

6.1.2. Sediment

6.1.2.1. Comparisons of metal concentrations between sites per occasion

Pairwise multiple comparison of consecutive sampling sites on SO1, SO2 and SO4 revealed statistical differences ($p<0.05$) between the different sampling sites. On SO3, the results showed no significant differences ($p>0.05$) in Fe concentrations recorded between sites 1 and 2, sites 2 and 3 as well as sites 3 and 4. However, significant differences ($p<0.05$) in Fe concentrations were recorded between sites 4 and 5 (**Table 6.1-1**).

6.1.2.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions revealed significant differences in Fe concentrations recorded between the different sampling occasions at site 1 ($p<0.05$). The results showed no statistical differences ($p>0.05$) in Fe concentrations between SO1 and SO2 at site 2. However, significant differences ($p<0.05$) in Fe concentrations were recorded between SO2 and SO3 at site 2. The results show no statistical differences ($p>0.05$) in Fe concentrations recorded between the different sampling occasion at sites 3 and 5. Pairwise multiple comparison of consecutive sampling occasions at site 4 revealed statistical differences between SO1 and SO2 ($p<0.05$) and also between SO3 and SO4 ($p<0.05$). However, no statistical differences ($p>0.05$) in Fe concentrations were recorded between SO2 and SO3 (**Table 6.1-1**).

6.1.3. Algae

6.1.3.1. Comparisons of metal concentrations between sites per occasion

Pairwise multiple comparison of consecutive sampling sites on SO1 revealed statistical differences ($p<0.05$) in Fe concentrations recorded between the different sampling sites. On SO2, the results showed statistical differences ($p<0.05$) in Fe concentrations recorded between sites 1 and 2, sites 2 and 3 as well as sites 3 and 4. No statistical differences ($p>0.05$) in Fe concentrations were recorded between the different sampling sites on SO3 and SO4 (**Table 6.1-1**).

6.1.3.2. Comparisons of metal concentrations between sampling occasions per site

There results showed no statistical differences in Fe concentrations recorded between the different sampling occasion at sites 1 and 5 ($p>0.05$). Pairwise multiple comparison of consecutive sampling occasions at site 2 revealed statistical differences ($p<0.05$) in Fe concentration between SO1 and SO2 as well as between SO2 and SO3. At sites 3 and 4, the results showed statistical differences in Fe concentrations recorded between SO1 and SO2 ($p<0.05$), while no statistical differences in Fe concentrations were recorded between SO2 and SO3 as well as between SO3 and SO4 ($p>0.05$) (**Table 6.1-1**).

6.1.3.3. Bioconcentration factors of algae for iron

The bioconcentration factors of Fe in *Enteromorpha* spp. are presented in **Table 6.1-2**. The highest bioconcentration of Fe in *Enteromorpha* spp. relative to water concentrations was recorded at site 1 (SO2). The autumn season (SO2) show a consistently higher bioconcentration of Fe in *Enteromorpha* spp. relative to water. The bioconcentration factor of Fe in *Enteromorpha* spp. relative to sediment was <1 at site 1 (SO3), site 2 (SO1, SO2, SO3, SO4), and site 4 (SO1, SO3). The highest bioconcentration of Fe in *Enteromorpha* spp. relative to sediment (16.45) was recorded at site 4 (SO4).

6.2. DISCUSSION

6.2.1. Water

Mean Fe concentrations (mg/ℓ) found in water at the different sampling sites within the Zandvlei estuary ranged from <LD to 1.90±2.34 (**Table 6.1-1**). Spatial variations in Fe concentrations showed statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 3 (0.1110±0.1378) and 4 (0.2367±0.1631) as well as sites 4 (0.2367±0.1631) and 5 (0.0483±0.0954) on SO2 (autumn). The mean Fe concentrations recorded at the above sites on SO2 were well below the 0.3 mg/ℓ water guideline concentration value recommended by CCREM (1987) and ANZECC (2000). However, sites 2 and 4 on SO3 (winter) exceeded the above-mentioned water quality guidelines by CCREM (1987) and ANZECC (2000) (**Table 10.1-1**). It is important to note that the ANZECC guideline value is used as an interim indicative working value and is therefore not an established or approved figure. No guideline value was available for Fe from DWAF (1996) (**Table 10.1-1**). It is important to note that Fe is often found in water in two oxidation states, namely, ferrous (Fe^{2+}) and ferric (Fe^{3+}) or as organic ferrous and ferric complexes (DWAF, 1996; ANZECC, 2000; Thorat et al., 2023). Iron is generally present in the ferric state in the water column and the concentrations are usually low (DWAF, 1996; ANZECC, 2000). Anthropogenic activities that are likely to introduce Fe into the environment include the corrosion of iron and steel, burning of coal, acid mine drainage, mineral processing, sewage and landfill leachates (DWAF, 1996). The results show no statistical differences ($p>0.05$) in Fe concentrations recorded between the different sampling sites on SO1 (summer) and SO3 (winter). Site 4 on SO3 (winter) recorded the highest Fe concentrations, however, the recorded standard deviation is very high, which is an indication that the recorded concentrations varied significantly. Furthermore, the relatively high mean Fe concentration recorded at site 4 (SO3) was not statistically different ($p>0.05$) from the concentrations recorded from the other sites on SO3 (**Table 6.1-1**). The concentrations recorded at site 4 on both SO2 (autumn) and SO3 (winter) seems to suggest that there may be a local source of Fe at that site. Sites 2 and 4 (SO3) which both exceeded the guidelines are located close to a stormwater drainage outlet **(Figure 4.2-2**) which may be responsible for discharging stormwater containing Fe into the estuary, particularly in the winter period (SO3).

Temporal (seasonal) variations in Fe concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO2 (0.0103±0.0230) and SO3 (0.2835±0.3385) at both sites 1 and 2 (**Table 6.1-1**). The increase in mean Fe concentrations recorded between SO2 (autumn) and SO3 (winter) at both sites 1 and 2 may be attributed to elevated rainfall levels in the winter period. The recorded monthly average rainfall level for winter (SO3) was 51.0 mm as compared to 24.2 mm recorded for SO2 (autumn) (**Table 3.2-1**). Corrosion of iron and steel from neighbouring facilities may also contribute to the elevated Fe concentrations. In addition, the estuary is also affected by sewer overflows from malfunctioning sewer pump stations (C.A.P.E., 2010; WCG, 2018). Sites 1 and 2 are located upstream and are likely affected by river discharges from the influent rivers, one of which appears to be affected by potential illegal discharges from an industrial area (WCG, 2018). Seasonal variations in Fe concentrations also show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO1 (summer) and SO2 (autumn) at site 3 (**Table 6.1-1**). This may also be attributed to elevated rainfall resulting in the increase in fluvial and stormwater discharges including run-off from nearby land uses (i.e. road surfaces, etc.). The monthly average rainfall recorded for SO2 (autumn) was 24.2mm, which was higher than the 5.2mm recorded for SO1 (summer) (**Table 3.2-1**). SO1 (summer) recorded low concentrations across the different sites and the recorded rainfall levels were relatively low (as indicated above). Therefore, the increase in Fe concentrations during the autumn and winter period seems to be linked to the increased rainfall. Statistical differences ($p<0.05$) in Fe concentrations were recorded between the different sampling occasions at sites 4 and 5 (**Table 6.1-1**). The seasonal variations recorded at sites 4 and 5 were similar to those recorded at other sampling sites as they all show increased Fe concentrations in the winter season as compared to the summer period, which recorded relatively low mean Fe concentrations. The relatively low concentrations recorded in the water column may be due to the sorption of metals from the overlying waters by sediments (De Souza Machado et al., 2016).

6.2.2. Sediment

The mean Fe concentrations (mg/kg) recorded in sediment at the different sampling sites ranged from 197.81±28.64 to 6744.90±5130.36 (**Table 6.1-1**). The comparison of spatial variations in Fe concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between the different sampling sites on SO1 (summer), SO2 (autumn) and SO4 (spring). The results show similar spatial variations or fluctuations in mean Fe concentrations recorded (i.e. site 1 < site 2 > site 3 < site 4 > site 5) between the different sampling sites on each of the above-mentioned sampling occasions, except for site 4 on SO4 (**Table 6.1-1**). The results also show a statistically significant increase in mean Fe concentrations was recorded between sites 1 and 2 on each of the above-mentioned sampling occasions (i.e. SO1, SO2 and SO4). The relatively high mean Fe concentrations recorded at site 2 (5834.36±3788.34 [SO1]; 6744.90±5130.36 [SO2]; 1828.28±509.02 [SO4]) when compared to other sampling sites on SO1, SO2 and SO4 (**Table 6.1-1**) may be attributed to factors such as anthropogenic inputs, sediment grain size, estuary gradient, stormwater drainage, river discharges as well as physicochemical parameters. Anthropogenic inputs include land-based discharges from the stormwater drainage system and run-off from parking areas and other facilities within or adjacent to the estuary. Elevated metal concentrations have been found to be associated with fine-grained sediments (i.e. decreasing grain size) due to their high surface to volume ratio and absorption capabilities (Phillips and Rainbow, 1994; Binning and Baird, 2001; Chapman and Wang, 2001; Chakraborty and Owens, 2014). As discussed in the previous chapters, fine-grained sediments were predominantly found in the northern/upper parts of the estuary, while coarse-grained sediments were predominantly found in the southern/lower parts of the estuary (CSIR, 2015; Maurer, 2019). This means that higher Fe concentrations were expected in the upper (northern) parts of the estuary, and lower Fe concentrations were expected in the lower (southern) parts. This expectation was realised by the results of this study. The estuary comprises of standing waters (Day et al., 2020) and deeper channels upstream where dense growth of pondweed is found (WCG, 2018). This could potentially result in some metals being trapped or confined in some of these areas and, therefore, possibly aiding the adsorption process which removes metals from the water column and stores it in the sediment (Sany et al., 2013).

The results show a statistically significant ($p<0.05$) decline in Fe concentrations between sites 2 and 3 on each occasion (except for SO3) (**Table 6.1-1**). This may be due to fluctuating river flow inputs and land-based discharges (i.e. stormwater run-off), sediment grain size and the estuary gradient. A relatively shallow gradient of the estuary is evident from site 3 (towards the estuary mouth) and this may potentially contribute to the relatively low Fe concentrations recorded at this particular site, considering that most of the standing waters and deeper channels are predominantly upstream where large amounts of pondweed are usually found (WCG, 2018). **Table 6.1-1** shows a statistical ($p<0.05$) increase in Fe concentrations between site 3 and 4 on SO1 (summer) and SO2 (autumn), while a decline, however not statistically significant ($p>0.05$), in Fe concentrations was recorded between sites 3 and 4 on SO4. The increase in Fe concentrations at site 4 may be linked to fluctuating land-based discharges. Physicochemical parameters such as salinity and pH seem to have had a limited effect on the recorded Fe concentrations and this is evident from the similar spatial variations recorded between the different sampling occasions. A statistical decline in Fe concentrations ($p<0.05$) was recorded between sites 4 and 5 on SO1 (summer), SO2 (autumn) and SO3 (winter). This may be attributed to the location of site 5 as it is close to the estuary mouth and further away from the influent rivers (**Figures 4.2-1** and **8.2-1**). It may also be linked to sediment grain size as the site comprises of course grained sediment (Maurer, 2019). Izegaegbe et al. (2020) also recorded low concentrations in sediment at a site located within the estuary mouth and have attributed this to coarser sediment and low total organic content. The sandy / coarse-grained sediment found within or close to the estuary mouth (i.e. site 5) may be linked to strong tidal currents which may prevent the deposition of fine sediments and therefore resulting in low metal accumulation in sediment (Acevedo-Figueroa et al., 2006). No statistical differences were recorded between sites 1 and 2 as well as sites 2 and 3 on SO3 (winter). The high standard deviation recorded at site 3 on SO3 (winter) (**Table 6.1-1**) reflected a wide variation of Fe concentrations. The mean Fe concentrations recorded in sediment at the Zandvlei estuary were compared with other estuaries in South Africa and abroad. There are no sediment quality guidelines available for Fe in ANZECC (2000) and CCME (2001) (**Figure 10.1-2**). There are also currently no sediment quality guidelines for the estuarine environment in South Africa. The comparison of Fe concentrations recorded in this study and other estuaries (**Table 6.2-1**) show that this study recorded low mean Fe concentrations when compared to the other estuaries. The highest Fe concentration was recorded in the Mhlathuze estuary followed by the Brisbrane Estuary in Australia.

Table 6.2-1: Comparison of mean sediment Fe concentrations recorded in Zandvlei and other estuaries [data extracted from Izegaegbe et al. (2020)]

Estuaries	Fe (mg/kg)
Zandvlei, South Africa[a]	1641±1052
Mhlathuze, South Africa[b]	17094±7135
St Louis Estuary, Senegal[c]	1819±1783
Brisbrane Estuary, Australia[d]	15784±2518

[a]present study [b]Izegaegbe et al. (2020) [c]Duodu et al. (2017) [d]Hamzeh et al. (2014)

Temporal (seasonal) variation in mean Fe concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between different sampling occasions at site 1 (**Table 6.1-1**). The results show a statistical increase in Fe concentrations between SO1 (summer) and SO2 (autumn) at sites 1 and 4 and this may be attributed to changes to anthropogenic inputs and land-based discharges. Increased river discharges may have led to high suspended material input within the system, which could intensify the adsorption and precipitation process (De Souza Machado et al., 2016). The removal of metals in the water column through precipitation or the low remobilisation rates into water can result in elevated concentrations in sediment (Rainbow, 1995). The above processes could have been responsible for the increased Fe concentrations recorded on SO2 (autumn) at sites 1 and 4 (**Table 6.1-1**). The bioavailability of contaminants in sediment can be significantly influenced by factors such as river flow and tidal flushing and these factors together with coastal and other processes can influence the distribution of salinity within an estuary (Chapman and Wang, 2001; Valle-Levinson, 2010). The water chemistry of estuaries has been found to be affected by salinity, among other factors (Benoit et al., 1994). Salinity is also one of the controlling factors for the partitioning of metals between water and sediment, therefore affecting the rate of adsorption and desorption of metals to and from sediments (Chapman and Wang, 2001; Sany et al., 2013). Elevated salinity increases competition between cations and metals for binding sites (Nduka and Orisakwe, 2011). Therefore, increased salinities and a decrease in pH may contribute to elevated metal concentrations in the system, which directly affects metal concentrations in sediment. The recorded salinity and pH were 15.15ppt and 8.63, respectively, for SO1 (summer) at site 1 (1040.01±275.52) and 18.65ppt and 8.81, respectively, for SO2 (autumn) at site 1 (1839.65±491.10) (**Table 3.1-2**). The monthly average rainfall for SO1 and SO2 was 5.2 mm and 24.2 mm, respectively (**Table 3.2-1**). As indicated above, autumn (SO2) recorded higher salinity and Fe concentrations when compared to summer (SO1) which recorded lower salinity and Fe concentrations. The acidity level (pH) does not appear to have had an impact on the recorded Fe concentrations in sediment. The results also show a statistical decline in Fe concentrations recorded between SO2 (autumn) and SO3 (winter) at sites 2 and 3 and this could be attributed to the elevated rainfall, which results in increased mobility and dilution, therefore, decreases metal concentrations in sediment (Sany et al., 2013). A statistical decline ($p<0.05$) in Fe concentrations was found between SO3 (winter) and SO4 (spring) at sites 1 and 4 (**Table 6.1-1**) and this may be attributed to changes in anthropogenic activities over time, such as the establishment or closure of industrial or construction activities which can lead to temporal variations in metal concentrations.

6.2.3. Algae

The mean Fe concentrations (mg/kg) recorded in algae (*Enteromorpha* spp.) at the different sampling sites ranged from 354.25±83.86 to 4764.45±606.10 (**Table 6.1-1**). The comparison of spatial variations in Fe concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between the different sampling sites on SO1 (summer). The results show fluctuating Fe concentrations ($p<0.05$) between the different sampling sites on SO1 (summer) with the highest Fe concentration recorded at site 3 (4764.45±606.10) (**Table 6.1-1**). A similar fluctuating pattern was found in mean Fe concentrations recorded in sediment. The abovementioned fluctuation in mean Fe concentrations recorded in algae at the different sampling sites on SO1 (summer) may be attributed to Fe being predominantly attached to extracellular sites which are largely affected by physicochemical parameters, which ultimately affect the biosorption of metals on algae surfaces (Mohamed and Khaled, 2005). Site 1 recorded statistically higher Fe concentrations on SO1 (summer) and SO2 (autumn) (**Table 6.1-1**). The relatively high Fe concentrations recorded in *Enteromorpha* may be an indication of the role of Fe as an essential element for biological activity (Mohamed and Khaled, 2005). Furthermore, the relatively high Fe mean concentrations in sediment could also potentially explain the high Fe concentrations recorded in algae (Billah et al., 2017). The spatial distribution and relatively high concentrations of Fe recorded at some of the sites within the different sampling occasions could also be attributed to factors such as the ability of algae to bioaccumulate Fe from surrounding environments (Chakraborty and Owens, 2014), and contamination from industrial and other activities (Eisler, 1981). Spatial variations in Fe concentrations also show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 (1934.56±736.91) and 2, sites 2 (821.27±45.66) and 3, as well as sites 3 (1347.84±519.47) and 4 (2913.35±569.38) (**Table 6.1-1**). The results show similar spatial variations in Fe concentrations between sites 1 to 3 on SO1 (summer) and SO2 (autumn). The relatively high mean Fe concentrations recorded at site 1 on both SO1 (summer) and SO2 (autumn) (**Table 6.1-1**) could be attributed to anthropogenic inputs and other land-based or stormwater discharges. Iron recorded some of the highest bioconcentration factors in *Enteromorpha* spp. relative to water (**Table 6.1-2**). This may be due to the fact that Fe is one of the basic elements for most living organisms, and it is usually more abundant in the aquatic environment (Hassan et al., 2008). However, the bioconcentration factors of Fe in *Enteromorpha* spp. relative to sediments show instances of deconcentration (<1), particularly at site 1 (SO3), site 2 (SO1, SO2, SO3, SO4), and site 4 (SO1, SO3) (**Table 6.1-2**).

As discussed in previous chapters, some of the influent rivers are likely to be affected by potential discharges from the nearby industrial area as well as sewer overflows from the various pump stations (WCG, 2018) and all of these and other activities may result in elevated levels of Fe within the system. The spatial distribution of Fe within the system is also likely affected by the extensive stormwater drainage system which discharges directly into the estuary from the neighbouring communities (C.A.P.E., 2010). Corrosion of iron and steel from the neighbouring communities may also be one of the contributing factors to some of the Fe concentrations recorded at some sampling sites on the different sampling occasions. No statistical differences ($p>0.05$) were recorded between the different sampling sites on SO3 (winter) and SO4 (spring) (**Table 6.1-1**). This may be attributed to algal metabolic processes during the winter season as well as physical and chemical parameters of the estuary during both winter and spring. Elevated rainfall in the winter period may have distributed metals across the different sites instead of metals being accumulated in areas with standing waters.

Temporal (seasonal) variations in Fe concentrations (±SD) show statistical differences ($p<0.05$) in pairwise consecutive comparisons between SO1 (summer) (1816.89 ±641.34) and SO2 (autumn) (821.27±45.66), as well as SO2 and SO3 (winter) (354.25±83.86) at site 2 (**Table 6.1-1**). The above statistical differences at site 2 shows a decline in Fe concentrations between the three consecutive seasons (i.e. SO1>SO2>SO3). Seasonal variations in metal accumulation in algae may be influenced by several factors such as pH, salinity, light intensity, metabolic activity, metal concentrations in water and the interactions between chemical elements and as a result of this, Żbikowski et al. (2006) highlights that it is difficult to explicitly identify the main factor that may cause a particular seasonal change as the above factors also interact with each other (Szefer, 2002). Several studies have found that metal concentrations decrease in algae during periods of growth (i.e. summer) and higher concentrations recorded during periods of slower metabolic activity (i.e. winter) (Hou and Yan, 1998; Villares et al., 2002; Żbikowski et al., 2006; Żbikowski et al., 2007; Ryan et al., 2012; Chakraborty and Owens, 2014). However, the seasonal variations found in Fe concentrations in this study show the opposite, which means that higher metal concentrations were recorded during the summer period (i.e. growth period) and lower concentrations during the winter season when metabolic processes have slowed down. Drude de Lacerda et al. (1985) also found similar higher concentrations in summer and ascribed this to elevated metal concentrations in water due to a potential increase in land-based discharges. Another possible reason for high metal concentrations recorded in summer may be the existence of higher rates of photosynthesis and respiration during this season, which would support the bioaccumulation of metals. Also, the existence of large amounts of algae could contribute to the aforementioned accumulation (Villares et al., 2002). The results also show similar seasonal variations in Fe concentrations recorded between SO1 (summer) (4764.45±606.10) and SO2 (autumn) (1347.84±519.47 mg/kg) at site 3 (**Table 6.1-1**). The lower concentrations recorded in autumn may potentially be attributed to increased rainfall and low salinity. During the winter period (high rainfall), *Enteromorpha* spp. in the Zandvlei estuary is likely to be less dominant due to low salinity conditions (Muhl, 2003). This may affect the assimilation of metals as there would be less algae biomass available for metal uptake. Seasonal variations in Fe concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO1 (summer) (539.73±164.05) and SO2 (autumn) (2913.35±569.38) at site 4 (**Table 6.1-1**). SO2 recorded statistically higher Fe concentrations at site 4 and this could be ascribed to the growth effect of algae, as the recorded concentrations increased when growth was slowing down (Villares et al., 2002; Żbikowski et al., 2007).

CHAPTER 7

ZINC RESULTS AND DISCUSSION

7.1. RESULTS

It should be noted that statistical comparisons presented in this chapter were made between consecutive sites and consecutive occasions.

Table 7.1-1: Mean concentrations (±SD) of zinc in water, sediment and algae during different sampling occasions at five sampling sites (n=5).

	SAMPLING OCCASIONS (SO)			
ZINC	SO1 (**Summer**)	SO2 (**Autumn**)	SO3 (**Winter**)	SO4 (**Spring**)
WATER (mg/ℓ)				
Site 1	$^{a}0.1818^{a}$ (±0.1511)	$^{a}1.1085^{b}$ (±0.4763)	$^{a}0.1066^{c}$ (±0.1341)	ND
Site 2	$^{a}0.0801^{a}$ (±0.0333)	$^{b}0.2112^{a}$ (±0.1610)	$^{a}0.0239^{b}$ (±0.0418)	ND
Site 3	$^{a}0.1560^{a}$ (±0.0676)	$^{b}0.1257^{a}$ (±0.1171)	$^{a}0.0388^{a}$ (±0.0856)	ND
Site 4	$^{a}0.1306^{a}$ (±0.0462)	$^{b}0.1015^{a}$ (±0.0600)	$^{a}0.1478^{a}$ (±0.2620)	ND
Site 5	$^{a}0.2026^{a}$ (±0.0444)	$^{b}0.2114^{a}$ (±0.1391)	$^{b}0.4677^{a}$ (±0.2867)	ND
SEDIMENT (mg/kg)				
Site 1	$^{a}1040.01^{a}$ (±275.52)	$^{a}22.70^{b}$ (±4.01)	$^{a}75.74^{b}$ (±54.61)	$^{a}25.77^{b}$ (±21.19)
Site 2	$^{b}5834.36^{a}$ (±3788.34)	$^{a}26.97^{b}$ (±8.51)	$^{a}40.05^{b}$ (±13.64)	$^{a}5.94^{c}$ (±2.89)
Site 3	$^{b}513.45^{a}$ (±268.67)	$^{b}7.03^{a}$ (±4.17)	$^{b}15.55^{a}$ (±11.80)	$^{a}5.90^{a}$ (±5.59)
Site 4	$^{b}1000.95^{a}$ (±422.36)	$^{b}8.40^{b}$ (±4.89)	$^{b}12.50^{b}$ (±3.75)	$^{a}5.87^{b}$ (±9.16)
Site 5	$^{b}400.39^{a}$ (±106.80)	$^{b}7.07^{a}$ (±9.87)	$^{c}14.34^{a}$ (±6.37)	$^{a}37.37^{a}$ (±34.62)
ALGAE (mg/kg)				
Site 1	$^{a}31.65^{a}$ (±18.24)	$^{a}33.48^{a}$ (±7.76)	$^{a}28.14^{a}$ (±6.36)	$^{a}31.35^{a}$ (±13.26)
Site 2	$^{a}20.08^{a}$ (±5.61)	$^{b}34.33^{a}$ (±54.20)	$^{a}27.10^{a}$ (±11.47)	$^{a}48.31^{a}$ (±19.89)
Site 3	$^{b}52.16^{a}$ (±12.87)	$^{c}6.93^{b}$ (±3.80)	$^{b}15.79^{c}$ (±6.50)	$^{a}26.09^{d}$ (±9.13)
Site 4	$^{c}0.68^{a}$ (±0.95)	$^{c}31.19^{b}$ (±12.49)	$^{b}60.89^{c}$ (±6.22)	$^{a}38.22^{d}$ (±16.01)
Site 5	$^{d}86.95^{a}$ (±186.73)	$^{d}11.32^{a}$ (±7.90)	$^{b}47.11^{a}$ (±52.71)	ND

ND = No Data
The statistical differences between sites per sampling occasion are indicated by superscripted letters to the left of mean values. The statistical differences between sampling occasions per site are indicated by superscripted letters to the right of mean values. Water, sediment and algae samples were not statistically compared with each other.

Table 7.1-2: Bioconcentration factors of algae for zinc.

Zinc		SO1 (Summer)	SO2 (Autumn)	SO3 (Winter)	SO4 (Spring)
Site 1	A/W	174.09	30.20	263.98	
	A/S	0.03	1.47	0.37	1.22
Site 2	A/W	250.69	162.55	1133.89	
	A/S	0.00	1.27	0.68	8.13
Site 3	A/W	334.36	55.13	406.96	
	A/S	0.10	0.99	1.02	4.42
Site 4	A/W	5.21	307.29	411.98	
	A/S	0.00	3.71	4.87	6.51
Site 5	A/W	429.17	53.55	100.73	
	A/S	0.22	1.60	3.29	

Shaded areas represent sites with either no data or where the recorded concentrations were below level of detection.

$$\text{A/W} = \frac{\text{Algae metal concentration } (\frac{\text{mg}}{\text{kg}})}{\text{Water metal concentration } (\frac{\text{Mg}}{\text{L}})} \qquad \text{A/S} = \frac{\text{Algae metal concentration } (\frac{\text{mg}}{\text{kg}})}{\text{Sediment metal concentration } (\frac{\text{mg}}{\text{kg}})}$$

7.1.1. Water

7.1.1.1. Comparisons of metal concentrations between sites per occasion

The results showed no statistical differences ($p>0.05$) in zinc (Zn) concentrations recorded between the different sampling sites on SO1. On SO2, the results showed statistical differences ($p<0.05$) in Zn concentrations recorded between sites 1 and 2. However, no statistical differences in Zn concentrations were recorded between sites 2 and 3, sites 3 and 4 as well as sites 4 and 5 on SO2 ($p>0.05$). The results revealed no significant differences ($p>0.05$) in Zn concentrations between sampling sites 1 and 2, sites 2 and 3 as well as sites 3 and 4. However, statistical differences ($p<0.05$) in Zn concentrations were recorded between sites 4 and 5 on SO3. No data was recorded for SO4 (**Table 7.1-1**).

7.1.1.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions revealed statistical differences ($p<0.05$) in Zn concentrations recorded between the different sampling occasions at site 1. At site 2, the result showed statistical differences ($p<0.05$) in Zn concentrations recorded between SO2 and SO3. Pairwise multiple comparison of consecutive sampling occasions revealed no statistical differences ($p>0.05$) in Zn concentrations recorded between the different sampling occasion at sites 3, 4 and 5. No data was recorded for SO4 (**Table 7.1-1**).

7.1.2. Sediment

7.1.2.1. Comparisons of metal concentrations between sites per occasion

Pairwise multiple comparison of consecutive sampling sites on SO1 showed statistical differences ($p<0.05$) in Zn concentrations recorded between sites 1 and 2. However, the results showed no statistical differences ($p>0.05$) in Zn concentrations recorded between sites 2 and 3, sites 3 and 4 as well as sites 4 and 5. On SO2, the results revealed statistical differences in Zn concentrations recorded between sites 2 and 3 ($p<0.05$), whilst no statistical differences recorded between sites 3, 4 and 5 ($p>0.05$). Pairwise multiple comparison of consecutive sampling sites on SO3 revealed the following: sites 1 and 2 ($p>0.05$); sites 2 and 3 ($p<0.05$); sites 3 and 4 ($p>0.05$); and sites 4 and 5 ($p<0.05$). The results showed no statistical differences in Zn concentrations between the different sampling sites on SO4 ($p>0.05$) (**Table 7.1-1**).

7.1.2.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions at sites 1 and 4 revealed significant differences ($p<0.05$) in Zn concentrations recorded between SO1 and SO2. However, no significant differences ($p>0.05$) in Zn concentrations were recorded between SO2 and SO3 as well as between SO3 and SO4 at sites 1 and 4. Pairwise multiple comparison of consecutive sampling occasions at site 2 showed statistical differences between SO1 and SO2 as well as SO3 and SO4 ($p<0.05$). The results showed no statistical differences ($p>0.05$) between the different sampling occasions at sites 3 and 5 (**Table 7.1-1**).

7.1.3. Algae

7.1.3.1. Comparisons of metal concentrations between sites per occasion

Pairwise multiple comparison of consecutive sampling sites on SO1 revealed statistical differences ($p<0.05$) in Zn concentrations between sites 2 and 3, sites 3 and 4 as well as sites 4 and 5. However, no statistical differences ($p>0.05$) in Zn concentrations were recorded between sites 1 and 2 on SO1. The results showed statistical differences ($p<0.05$) in Zn concentrations recorded between sites 1 and 2, sites 2 and 3 as well as sites 4 and 5 on SO2. However, no significant differences ($p>0.05$) in Zn concentrations were recorded between sites 3 and 4 on SO2. Pairwise multiple comparison of consecutive sampling sites on SO3 showed the following: sites 1 and 2 ($p>0.05$); sites 2 and 3 ($p<0.05$); sites 3 and 4 ($p>0.05$); and sites 4 and 5 ($p>0.05$). The results showed no statistical differences ($p>0.05$) in Zn concentrations between the different sampling sites on SO4 (**Table 7.1-1**).

7.1.3.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions at sites 1, 2 and 5 revealed no statistical differences in Zn concentrations recorded between the different sampling occasions ($p>0.05$). The results showed statistical differences ($p<0.05$) in Zn concentrations recorded between the different sampling occasions at sites 3 and 4 (**Table 7.1-1**).

7.1.3.3. Bioconcentration factors of algae for zinc

The bioconcentration factors of Zn in *Enteromorpha* spp. are presented in **Table 7.1-2**. The results show a significant variation in the bioconcentration of Zn in *Enteromorpha* spp. in relation to water concentrations between the different sites and occasions, with bioconcentration factors ranging from 5.21 to 1133.89. The results also show a bioconcentration factor of <1 for Zn in *Enteromorpha* spp. relative to sediment concentrations at sites 1 and 2 (SO1, SO2); and sites 3, 4 and 5 (SO1) while the sites occasions in each site recorded a bioconcentration of factor of >1-2 and/or >2. The highest bioconcentration of Zn in *Enteromorpha* spp. relative to sediment (8.13) is recorded at site 2 (SO4).

7.2. DISCUSSION

7.2.1. Water

The mean Zn concentrations (mg/ℓ) recorded in water at sampling sites within the Zandvlei estuary ranged from 0.0239±0.0418 to 1.1085±0.4763 (**Table 7.1-1**). Spatial variations in Zn concentrations (±SD) recorded in water (mg/ℓ) show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 (1.1085±0.4763) and 2 (0.2112±0.1610) on SO2 (autumn). This may be attributed to the proximity of site 1 to the upstream influent rivers which are likely to carry pollutants from upstream activities. Zn can occur in both suspended and dissolved forms in natural aquatic environments (CCME, 2018). The aqueous zinc ion (Zn^{2+}) is known to be the most toxic (ANZECC, 2000). Anthropogenic sources of Zn emanate from various industries such as the manufacturing, iron, steel and pharmaceutical industries. It is used in automotive equipment, storage and dry cell batteries, dental, medical and household products (WHO, 2001). According to the WCG (2018), a nearby industrial area is regarded as potential source of illegal discharges into the Zandvlei estuary through the Keyser River (WCG, 2018). The results show statistical differences in mean Zn concentrations in the pairwise consecutive comparisons between sites 4 (0.1478a±0.2620) and 5 (0.4677±0.2867) on SO3 (winter) (**Table 7.1-1**). This may have been due to elevated rainfall levels (i.e. 51 mm) recorded in winter (SO3) (**Table 3.2-1**) which may have increased urban run-off and transportation of contaminants. The spatial distribution of metals is also largely affected by factors such as river and groundwater discharge, estuarine circulation, tidal flooding, water properties (i.e. salinity, pH and redox), sediment input and re-suspension, exchange with neighbouring environments as well as the presence of organisms (De Souza Machado et al., 2016). According to DWAF (1996) high Zn concentrations can occur in water with low pH. No statistical differences ($p>0.05$) in Zn concentrations were recorded between the different sampling sites on SO1 (summer) (**Table 7.1-1**). The recorded Zn concentrations ranged from 0.02±0.04 to 1.11±0.48. Zinc (Zn) concentrations recorded between the different sampling sites in each sampling occasion (except for site 2, SO3) exceeded the DWAF (1996) target water quality range (TWQR) value (i.e. chronic and acute effect concentration) of 0.036 mg/ℓ for the protection of aquatic ecosystems. The CCME (2018) water quality guidelines for the protection of aquatic life for dissolved Zn was 0.037 mg/ℓ for short-term exposure and 0.007 mg/ℓ for long-term exposure. The short-term exposure guideline was exceeded by most of the sampling sites (except for site 2 on SO3), while the long-term exposure guideline value was exceeded by all the sampling sites on each occasion (**Table 10.1-1**). The recorded Zn concentrations also exceeded the recommended safe concentration value of 0.0024 mg/ℓ (ANZECC, 2000) for the protection of aquatic ecosystems (i.e. for 99% Level of species protection). The ANZECC (2000) default guideline values are based on the targeted level of species protection (%) and also takes into account that some aquatic ecosystems may already be disturbed and therefore the species protection level required may be less. For the different levels of species protection, different guideline values are proposed for Zn. Based on the guideline values provided in **Table 10.1-1** and the ecological condition (i.e. highly disturbed) of the Zandvlei estuary (Day et al., 2020), it may be appropriate to compare the recorded Zn concentrations in **Table 7.1-1** to the guideline value of 0.031 mg/ℓ which provides 80% level of aquatic species protection and takes into account the current state of the estuary, as it is highly disturbed. It was found that almost all the recorded Zn concentrations (except for site 2, SO3) exceeded the above guideline value of 0.031 mg/ℓ (**Table 10.1-1**) and this may be an indication of Zn contamination.

Temporal (seasonal) variations in mean Zn concentrations show significant differences ($p<0.05$) in the pairwise consecutive comparisons between SO1 (summer) (0.1818±0.1511), SO2 (autumn) (1.1085±0.4763) and SO3 (winter) (0.1066±0.1341) at site 1 (**Table 7.1-1**). The results show a fluctuation in Zn concentrations between the different seasons at site 1 and this could be attributed to changes in rainfall patterns and river flows which influence the input of metals from catchment areas. Changes in anthropogenic activities over time, such as the establishment or closure of industrial or construction plants, can lead to temporal variations in metal concentrations. SO2 (autumn) (1.1085±0.4763) at site 2 recorded statistically higher Zn concentrations when compared to other sampling occasions. This may be due to changes in anthropogenic inputs (i.e. river discharge and urban runoff), physicochemical parameters as well as the gradient of the estuary (among other factors). The Zandvlei estuary has standing water (Day et al., 2022), which may restrict the movement or distribution of metals, making them readily available for uptake in the water column or by sediments. Seasonal variations in Zn concentrations show SO3 (winter) recorded a statistically lower concentration than SO2 (autumn) at both sites 1 and 2 (**Table 7.1-1**). Changes in anthropogenic inputs (i.e. pollution load), river discharge and estuarine circulation (among other factors), and water quality properties (i.e. salinity, pH, etc.) may be responsible for the statistically lower concentrations recorded on SO3 (winter) at both sites 1 and 2 (Sany et al., 2013; De Souza Machado et al., 2016). No statistical ($p>0.05$) differences were recorded in the pairwise consecutive comparisons between the different sampling occasions at sites 3, 4 and 5 (**Table 7.1-1**). No data is available for SO4 (spring). Seasonal variations in the Zn concentrations were found only for sites 1 and 2. Both sites are characterised by standing waters (Day et al., 2022) stormwater drainage outlets, deeper channels or gradient as a result of pondweed removal and are affected by upstream influent rivers (WCG, 2018).

7.2.2. Sediment

The mean Zn concentrations (mg/kg) in sediment collected at the sampling sites within the study area ranged from 5.87±9.16 to 5834.36±3788.34 (**Table 7.1-1**). Spatial variations in Zn concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 (1040.01±275.52) and 2 (5834.36±3788.34) on SO1 (summer). The elevated Zn concentration recorded at site 2 (SO1) may be attributed to urban run-off from the nearby stormwater drainage outlet, the parking area and yacht club (**Figure 5.2-1**). Sediment grain size and physicochemical parameters may have also influenced the recorded concentrations between the above sites. The gradient of the estuary where dense growth of *Stuckenia pectinata* (pondweed) is found (i.e. site 2), comprises of deeper channels with standing or stagnant water (WCG, 2018; Day et al., 2020). High nutrient conditions on site have resulted in dense pondweed mats which restrict recreational activities, exacerbate flooding, limit light penetration, restrict current flow and thus increase stagnation (WCG, 2018). The stagnant waters may result in metals being mostly restricted to certain parts of the estuary and when conditions are favourable, be absorbed or deposited in sediments. The SAWS recorded monthly average rainfall in the period covering SO1 was 5.2 mm. Salinity and pH levels recorded for site 2 (SO1) were 15.63 ppt and 8.43, respectively, as compared to site 1 (SO1), which recorded salinity and pH levels of 15.15 ppt and 8.63, respectively (**Table 3.1-1**). According to Sany et al. (2013) increased metal concentrations may be affected by increase in salinity and decrease in redox potential and pH. The above findings by Sany et al. (2013) appears to be in line the findings of this study in so far as sites 1 and 2 (SO1) is concerned. However, since there are other factors which influence metal uptake and distribution (such as sediment grain size, among others), the above may not be always applicable in some instances as the concentrations may be affected by a combination of the different factors (De Souza Machado et al., 2016). The results show significant differences ($p<0.05$) in Zn concentrations recorded between sites 2 (26.97±8.51) and 3 (7.03±4.17) on SO2 (autumn) (**Table 7.1-1**). This may be due to the proximity of the sites to pollution sources, sediment characteristics as well as the gradient of the estuary. Site 3 is located further away from influent rivers and the sediments found in this area is a mixture of both coarse- and fine-grained sediments (Maurer, 2019). De Souza Machado et al. (2016) noted that salinity reduces the availability of metals and thus diminishes their toxicity. However, there are also other factors which play an important role in the distribution and uptake of metals by organisms. The high concentration recorded at site 2 may also be linked to the above discussion regarding the proximity of the site to stormwater drainage outlets, the gradient of the estuary which creates stagnant waters as well as sediment grain size.

Sediment grain size distribution is regarded as one of the most important factors controlling metal concentrations in sediment; and there are usually correlations between decreasing grain size and increasing metal concentrations (Chapman and Wang, 2001). This is due to high surface to volume ratios and absorption abilities (Binning and Baird, 2001). Sediment grain size distribution in the Zandvlei estuary shows coarsely grained sediment in the lower/southern parts towards the estuary mouth and fine-grained sediments were found in the upper/northern parts of the estuary towards the influent rivers (CSIR, 2015; Maurer, 2019). The Zn concentrations recorded in this study were in line with the above expectation relating to decreasing grain size and increasing metal concentrations as there were higher Zn concentrations recorded in the upper parts of the estuary and lower Zn concentrations recorded in the lower parts of the estuary (**Table 7.1-1**). The results show statistical differences ($p<0.05$) in Zn concentrations recorded between sites 2 (40.05±13.64) and 3 (15.55±11.80) as well sites 4 (12.50±3.75) and 5 (14.34±6.37) on SO3 (winter). Zinc concentrations decreased significantly from site 2 to 3 on SO3 (winter) and this may be linked to sediment grain size distribution, as described above. It may also be due to rainfall as rainwater was found to increase mobility and dilution which decreases metal concentrations in sediment (Lim et al., 2006; Li et al., 2009). No statistical differences ($p>0.05$) were recorded between the different sampling sites on SO4 (spring) (**Table 7.1-1**) although the recorded mean Zn concentrations also followed similar fluctuations between the different sites.

Seasonal (temporal) variations in Zn concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO1 (summer) (1040.01±275.52) and SO2 (autumn) (22.70±4.01) at site 1 (**Table 7.1-1**). The elevated concentrations recorded on SO1 could be linked to changes in anthropogenic activities over time, such as the establishment or closure of industrial and construction activities, which can lead to temporal variations in metal concentrations. SO1 received the least amount of rainfall (5.2 mm) of all the sampling occasions (**Table 3.2-1**). Since the estuary is classified as a standing water system (Day et al., 2020), less rainfall further reduces estuarine circulation or water flow within the system, resulting in stagnant water areas which may contain metals that can be deposited on sediment when conditions are favourable. The recorded concentrations at SO1 (site 1) may also be an indication of elevated Zn concentrations discharged into the environment from upstream influent rivers. SO2 (autumn) recorded a monthly average rainfall of 24.2m. This amount of rainfall coupled with physicochemical parameters such as salinity may have improved circulation and the spatial distribution of metals in the estuary (De Souza Machado et al., 2016), potentially resulting in a somewhat even distribution of metals across the sites, instead of metals being concentrated or trapped in areas with standing waters. At site 2, statistical differences ($p<0.05$) in Zn concentrations were recorded in the pairwise consecutive comparisons between SO1 (summer) and SO2 (autumn) as well SO3 (winter) and SO4 (spring) (**Table 7.1-1**). SO1 (summer) recorded a statistically ($p<0.05$) higher concentration than SO2 (autumn) at both sites 2 and 4 and as discussed above, this may be attributed to factors such as rainfall, estuary gradient, anthropogenic inputs (i.e. stormwater, urban run-off, etc.) and physicochemical properties (i.e. salinity, organic content, pH, etc.). SO4 (spring) recorded a statistically lower ($p<0.05$) concentration when compared to SO3 (winter) at site 2. SO4 (spring) recorded a monthly average rainfall of 27.8m when compared to the monthly average rainfall of 51.0 mm recorded for SO3 (winter) (**Table 3.2-1**).

According to WCG (2018) reduced flushing, therefore increased residence time of water in the Zandvlei estuary increases the potential for accumulation of pollutants and increased anoxia particularly of the bottom sediments. The high rainfall levels recorded on SO3 (winter) may have led to increased river discharge which may have assisted in flushing some of the contaminants out of the estuary, hence the lower concentrations recorded on SO4 (spring). Furthermore, the estuary mouth was open during SO3 (winter) (**Table 3.3-1**) to allow the increased water levels to flow into the sea. On SO4 (spring), the estuary mouth was closed. No statistical differences ($p>0.05$) were between the different sampling occasions at sites 3 and 5 (**Table 7.1-1**). Summer (SO1) recorded high concentrations when compared to all of the other sampling occasions and this could potentially be attributed to several factors including seasonal fluctuations (weather, anthropogenic sources, physicochemical parameters, etc.). Zinc concentrations recorded between the different sampling sites on SO1 exceeded the CCME (1999) recommended safe sediment quality guideline concentration (124 mg/kg), while Zn concentrations recorded at all sites during SO2 (autumn), SO3 (winter) and SO4 (spring) were below the recommended sediment quality guideline by CCME (1999). The interim sediment quality guideline (ISQG) values of 200 mg/kg (low value) and 410 mg/kg (high value) are recommended by ANZECC (2000). The high value provides an indication of concentrations at which toxicity-related adverse effects may be evident or observed (ANZECC, 2000). Zinc concentrations recorded at the different sampling sites (except site 5) on SO1 exceeded the high guideline value recommended by ANZECC (2000) and the low guideline value by ANZECC (2000) was exceeded by all the different sites on SO1 (summer) (**Table 10.1-2**). However, it is important to note that all the other sampling sites on the other sampling occasions (i.e. SO2, SO3 and SO4) were below the guideline values by ANZECC (2000) and CCME (1999) (**Table 10.1-2**). Based on the above, it appears that the estuary was contaminated with Zn on the first sampling occasion (summer). CCME (2001) noted that mean Zn concentrations as high as 7366 mg/kg have been measured in sediments from freshwater lakes near mining and smelting operations. For this study, the highest concentration was 5834.36±3788.34 and this may be an indication of the effect of the activities at the nearby industrial area and/or urban activities.

7.2.3. Algae

The mean Zn concentrations (mg/kg) (±SD) in algae (*Enteromorpha* spp.) collected from sampling sites within the estuary ranged from 0.68±0.95 to 86.95±186.73 (**Table 7.1-1**). The highest concentration was recorded at site 5 on SO1 (summer), while the lowest concentration was recorded at site 4 on SO1 (summer). Spatial variations in Zn concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites (220.08±5.61) and 3 (52.16±12.87), sites 3 and 4 (0.68±0.95) as well as sites 4 and 5 (86.95±186.73) on SO1 (summer) (**Table 7.1-1**). The high standard deviation recorded at site 5 (SO1) reflected a wide variation of bioaccumulated Zn concentrations in macroalgae. Similar to other metals, the bioconcentration factors of Zn in *Enteromorpha* spp. relative to water showed high macroconcentration (>2) rates in *Enteromorpha* spp. (**Table 7.1-2**). However, the bioconcentration factors of Zn in *Enteromorpha* spp. relative to sediment were much lower as can be seen in instances of both deconcentration (<1) and microconcentration (1-2) of Zn in *Enteromorpha* spp. (Dallinger and Rainbow, 1993). All five sampling sites on SO1 indicated deconcentration (<1) of Zn in *Enteromorpha* spp. relative to sediment. The deconcentration may have been due to several factors such as the metabolic activity of algae, which is usually high in summer (SO1) and results in the dilution (reduction) of the accumulated metals (Villares et al., 2002). The recorded temperatures ranged from 24.4°C (site 1, SO1) to 23.2°C (site 5, SO1). Salinity ranged from 15.15 ppt (site 1, SO1) to 22.67 ppt (site 5, SO1). The recorded pH values ranged from 8.63 (site 1, SO1) to 7.28 (site 5, SO1). The results show statistical ($p<0.05$) fluctuations in Zn concentrations from the pairwise consecutive comparisons of the above sites on SO1 (summer) (**Table 7.1-1**). This may be due to changes in growth and metabolic rates of macroalgae (i.e. dilution of metal due to algal growth), which are influenced by local and seasonal environmental conditions (i.e. physicochemical parameters), interactions among metal ions resulting in competition for binding sites in macroalgae, and species lifespan and morphology (Villares et al., 2002; Ryan et al., 2012; Chakraborty and Owens, 2014). Zinc at low concentrations is regarded as an essential micronutrient to support algae growth.

However, at elevated concentration, Zn may adversely influence the physiological and biochemical processes of algae growth (Trzcińska and Pawlik-Skowrońska, 2013). According to Brown et al. (1999), Zn concentrations in *Enteromorpha* spp. in uncontaminated and contaminated sites were expected to be in the range of 10-50 mg/kg and 95-130 mg/kg dry weight, respectively. Three concentration ranges of Zn in *Enteromorpha* spp. were proposed by Say et al. (1990) and these are: for uncontaminated sites = <50 mg/kg; for 'moderately' contaminated sites = 50 – 150 mg/kg; and for 'highly' contaminated sites = >150 mg/kg. Comparisons between the above Zn concentrations and the recorded mean Zn concentrations in this study indicate that Zn concentrations recorded at sites 1, 2 and 4 on SO1 (summer) fall under the category of "uncontaminated sites", while sites 3 and 5 on SO1 (summer) fall under the category of "moderately" contaminated sites. The above Zn concentrations by Brown et al. (1999) and Say et al. (1990) are only used as a guide. The source of Zn contamination at the above and other sites may be from iron and steel production from the nearby industrial area, corrosion of galvanized structures, waste disposal, and the use of zinc-containing fertilizers and pesticides from upstream and neighbouring communities. Land use in the Zandvlei catchment ranges from light industry to housing, agriculture, forestry and conservation (WCG, 2018). Urban run-off and stormwater discharges may be responsible for the disposal and transportation of several contaminants from the above land uses, including toxic chemicals from motor vehicles, pesticides and nutrients from lawns and gardens, as well as metals from roofs, motor vehicles, and other sources (EPA, 2003).

Spatial variations in Zn concentrations show statistical differences ($p<0.05$) in Zn concentrations were recorded in the pairwise consecutive comparisons between sites 1 (33.48±7.76) and 2 (34.33±54.20), sites 2 and 3 (6.93±3.80), as well as sites 4 (31.19±12.49) and 5 (11.32±7.90) on SO2 (autumn) (**Table 7.1-1**). Similar to Zn concentrations recorded on SO1 (summer), statistical ($p<0.05$) fluctuations in Zn concentrations were also recorded at the above sampling sites on SO2. Sites 1 and 2 on SO2 (autumn) recorded statistically ($p<0.05$) higher concentrations when compared to sites 3 and 5 within the same sampling occasion and this may be attributed to elevated anthropogenic inputs from influent rivers and stormwater discharges or run-off. Furthermore, increasing salinity may result in the increase of metal concentrations in green algae (Żbikowski et al., 2007). However, some researchers argue that in the event that anthropogenic inputs (i.e. pollutants) are greater and there are low salinity levels, this could also result in elevated metal concentrations in macroalgae (Villares et al., 2001). The above clearly shows that the bioaccumulation of metals in algae is controlled by various factors, including the interaction between the different factors (Żbikowski et al., 2006). On SO3 (winter), statistical differences ($p<0.05$) in Zn concentrations were recorded in the pairwise consecutive comparisons between sites 2 and 3 (**Table 7.1-1**). A statistical decline in Zn concentrations was recorded between sites 2 (27.10±11.47) and 3 (15.79±6.50) on SO3 (winter). This may be attributed to rainfall, the gradient of the estuary and physicochemical parameters. According to Chakraborty and Owens (2014), Zn is usually accumulated by algae from water and the suggested acceptable background concentration levels are below 100 mg/kg for non-polluted areas. The highest mean Zn concentration recorded in this study was 86.95±186.73 mg/kg which may suggest that some of the sites are not contaminated with Zn.

Temporal (seasonal) variations in mean Zn concentrations show statistical differences ($p<0.05$) in terms of the pairwise consecutive comparisons between the different sampling occasions at sites 3 and 4 (**Table 7.1-1**). The concentrations recorded in summer (SO1) was the highest when compared to those recorded in autumn, winter and spring at site 3. It is important to note that there may be different reasons for the recorded seasonal differences, including but not necessarily limited to physicochemical factors such as salinity and pH, metabolic factors, interactions between metals and other elements, and differences in metal concentrations in solution (Villares et al., 2001). While some researchers indicate that seasonal variations are due to an increase in terrestrial inputs, others found that there are several other underlying factors (i.e. metabolic processes) which play an important role in the seasonal variations found in macroalgae. SO3 (winter) at both sites 3 and 4 recorded statistically higher concentrations and this may be attributed to potentially higher concentrations of metal in water because of increased terrestrial inputs. The decrease in salinity as a result of fluvial inputs may also lead to an increase in metal concentrations (Struck et al., 1997). Zinc is also usually associated with urban effluent. Metal concentrations in algae are generally lower in warmer months due to high metabolic activity which results in the dilution of metals, whereas higher concentrations are usually recorded in winter when algae metabolic activity is slowing down (Villares et al., 2002; Żbikowski et al., 2007). The abovementioned algal growth appears to have had a limited impact on the recorded concentrations in this study as there were relatively high concentrations in summer as well as in winter months. Concentrations recorded in this study fluctuated between the different seasons with no particular pattern that could be attributed to a single factor like algae growth. This may be an indication of the interaction of the different environmental factors such as physical and chemical parameters, variations in metal concentrations in solution, as well as interaction between metals and other elements (Żbikowski et al., 2007).

CHAPTER 8

LEAD RESULTS AND DISCUSSION

8.1. RESULTS

It should be noted that statistical comparisons presented in this chapter were made between consecutive sites and consecutive occasions.

Table 8.1-1: Mean concentrations (±SD) of lead in water, sediment and algae during different sampling occasions at five sampling sites (n=5).

	SAMPLING OCCASIONS (SO)			
LEAD	SO1 (**Summer**)	SO2 (**Autumn**)	SO3 (**Winter**)	SO4 (**Spring**)
WATER (mg/ℓ)				
Site 1	$^{a}0.0261^{a}$ (±0.0346)	$^{a}0.0229^{a}$ (±0.0258)	$^{a}0.0015^{b}$ (±0.0015)	ND
Site 2	$^{a}0.0092^{a}$ (±0.0044)	$^{a}0.0043^{a}$ (±0.0031)	$^{a}0.0031^{a}$ (±0.0032)	ND
Site 3	$^{a}0.0139^{a}$ (±0.0089)	$^{a}0.0033^{b}$ (±0.0025)	<LD	ND
Site 4	$^{a}0.0100^{a}$ (±0.0050)	$^{a}0.0034^{a}$ (±0.0040)	$^{b}0.0120^{a}$ (±0.0158)	ND
Site 5	$^{a}0.0257^{a}$ (±0.0165)	$^{a}0.0047^{b}$ (±0.0068)	$^{b}0.0013^{b}$ (±0.0009)	ND
SEDIMENT (mg/kg)				
Site 1	$^{a}5.46^{a}$ (±1.85)	$^{a}5.42^{a}$ (±3.08)	$^{a}12.92^{a}$ (±18.11)	$^{a}25.77^{b}$ (±21.19)
Site 2	$^{b}29.89^{a}$ (±14.99)	$^{b}32.03^{b}$ (±32.65)	$^{a}9.92^{c}$ (±3.27)	$^{b}5.94^{d}$ (±2.89)
Site 3	$^{c}2.90^{a}$ (±0.78)	$^{b}2.40^{a}$ (±1.07)	$^{b}3.84^{a}$ (±3.08)	$^{b}5.90^{a}$ (±5.59)
Site 4	$^{c}2.69^{a}$ (±0.42)	$^{c}3.74^{b}$ (±0.89)	$^{c}5.81^{c}$ (±2.77)	$^{c}5.87^{d}$ (±9.16)
Site 5	$^{c}3.19^{a}$ (±0.94)	$^{d}1.24^{a}$ (±0.19)	$^{d}2.35^{b}$ (±0.23)	$^{d}37.37^{c}$ (±34.62)
ALGAE (mg/kg)				
Site 1	$^{a}8.42^{a}$ (±2.95)	$^{a}8.75^{b}$ (±3.08)	$^{a}1.74^{b}$ (±0.79)	$^{a}31.35^{b}$ (±13.26)
Site 2	$^{a}8.94^{a}$ (±2.54)	$^{a}3.95^{b}$ (±0.53)	$^{a}2.22^{c}$ (±0.50)	$^{a}48.31^{c}$ (±19.89)
Site 3	$^{b}14.89^{a}$ (±2.34)	$^{a}5.59^{b}$ (±2.89)	$^{a}3.03^{c}$ (±1.13)	$^{a}26.09^{d}$ (±9.13)
Site 4	$^{c}2.05^{a}$ (±0.40)	$^{a}12.32^{b}$ (±1.78)	$^{a}8.15^{c}$ (±4.36)	$^{a}38.22^{c}$ (±16.01)
Site 5	$^{d}6.35^{a}$ (±4.43)	$^{a}4.19^{b}$ (±1.27)	$^{a}4.36^{b}$ (±0.47)	ND

LD = Limit of Detection
ND = No Data
The statistical differences between sites per sampling occasion are indicated by superscripted letters to the left of mean values. The statistical differences between sampling occasions per site are indicated by superscripted letters to the right of mean values. Water, sediment and algae samples were not statistically compared with each other.

Table 8.1-2: Bioconcentration factors of algae for lead.

Lead		SO1 (Summer)	SO2 (Autumn)	SO3 (Winter)	SO4 (Spring)
Site 1	A/W	322.61	382.10	1160	
	A/S	1.54	1.61	0.13	1.22
Site 2	A/W	971.74	918.60	716.13	
	A/S	0.30	0.12	0.22	8.13
Site 3	A/W	1071.22	1693.94		
	A/S	5.13	2.33	0.79	4.42
Site 4	A/W	205	3623.53	679.17	
	A/S	0.76	3.29	0.71	6.51
Site 5	A/W	247.08	891.49	3353.85	
	A/S	1.99	3.38	1.86	

Shaded areas represent sites with either no data or where the recorded concentrations were below the level of detection.

$A/W = \frac{Algae\ metal\ concentration\ (\frac{mg}{kg})}{Water\ metal\ concentration\ (\frac{Mg}{L})}$ $A/S = \frac{Algae\ metal\ concentration\ (\frac{mg}{kg})}{Sediment\ metal\ concentration\ (\frac{mg}{kg})}$

8.1.1. Water

8.1.1.1. Comparisons of metal concentrations between sites per occasion

The results showed no statistical differences in lead (Pb) concentrations recorded between the different sampling sites on SO1 and SO2 ($p>0.05$). On SO3, there results showed no statistical differences in Pb concentrations recorded between sites 1 and 2 as well as sites 2 and 3 ($p>0.05$). However, statistical differences in Pb concentrations were recorded between sites 3 and 4 ($p<0.05$) on SO3 (**Table 8.1-1**). No data is available for sampling occasion 4.

8.1.1.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions revealed the following differences at site 1: SO1 and SO2 ($p>0.05$), as well as SO2 and SO3 ($p<0.05$). The results showed no statistical differences in Pb concentrations recorded between the different sampling occasions at sites 2 and 4. Pairwise multiple comparison of consecutive sampling occasions revealed statistical differences ($p<0.05$) in Pb concentrations recorded between the different sampling occasions at site 3. Pairwise multiple comparison of consecutive sampling occasions showed statistical differences ($p<0.05$) between SO1 and SO2 at site 5. However, no statistical differences ($p>0.05$) in Pb concentrations were recorded between SO2 and SO3 at site 5 (**Table 8.1-1**).

8.1.2. Sediment

8.1.2.1. Comparisons of metal concentrations between sites per occasion

Pairwise multiple comparison of consecutive sampling sites on SO1 revealed statistical differences ($p<0.05$) in Pb concentrations between sites 1 and 2 as well as sites 2 and 3. However, the results showed no significant differences ($p>0.05$) in Pb concentrations recorded between sites 3 and 4 as well as sites 4 and 5. Pairwise multiple comparison of consecutive sampling sites on SO2 and SO4 revealed the following differences: sites 1 and 2 ($p<0.05$), sites 2 and 3 ($p>0.05$), sites 3 and 4 ($p<0.05$), sites 4 and 5 ($p<0.05$). Pairwise multiple comparison of consecutive sampling sites on SO3 showed the following differences: sites 1 and 2 ($p>0.05$), sites 2 and 3 ($p<0.05$), sites 3 and 4 ($p<0.05$), sites 4 and 5 ($p<0.05$) (**Table 8.1-1**).

8.1.2.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions at site 1 revealed no statistical differences ($p>0.05$) in Pb concentrations recorded between SO1 and SO2 as well as between SO2 and SO3. However, the results showed statistical differences ($p<0.05$) in Pb concentrations recorded between SO3 and SO4 at site 1. Pairwise multiple comparison of consecutive sampling occasions revealed significant differences ($p<0.05$) in Pb concentrations between the different sampling occasions at sites 2 and 4. The results showed no statistical differences in Pb concentrations recorded between the different sampling occasions at site 3 ($p>0.05$). Pairwise multiple comparison of consecutive sampling occasions showed statistical differences ($p<0.05$) in Pb concentrations between SO2 and SO3 as well as between SO3 and SO4 at site 5 (**Table 8.1-1**).

8.1.3. Algae

8.1.3.1. Comparisons of metal concentrations between sites per occasion

Pairwise multiple comparison of consecutive sampling sites showed statistical differences ($p<0.05$) between sites 2 and 3 as well as sites 4 and 5 on SO1. The results showed no statistical differences ($p>0.05$) in Pb concentrations were recorded between the different sampling sites on SO2 and SO3 as well as between SO3 and SO4 (**Table 8.1-1**).

8.1.3.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions showed statistical differences ($p<0.05$) in Pb concentrations recorded between SO1 and SO2 at site 1. However, the results showed no statistical differences in Pb concentrations recorded between SO2 and SO3 as well as between SO3 and SO4 ($p>0.05$). Pairwise multiple comparison of consecutive sampling occasions has shown the following differences at sites 2 and 4: SO1 and SO2 ($p<0.05$), SO2 and SO3 ($p<0.05$), as well as SO3 and SO4 ($p>0.05$). The results showed statistical differences in Pb concentrations recorded between the different sampling occasions at site 3 ($p<0.05$). Pairwise multiple comparison of consecutive sampling occasions revealed statistical differences ($p<0.05$) in Pb concentrations recorded between SO1 and SO2 at site 5 (**Table 8.1-1**).

8.1.3.3. Bioconcentration factors of algae for lead

The bioconcentration factors of Pb in *Enteromorpha* spp. are presented in **Table 8.1-2**. Lead bioconcentrated in *Enteromorpha* spp. at high levels in relation to water, with bioconcentrations values ranging from 205 to 3623.53. The results show the bioconcentration factors of Pb in *Enteromorpha* spp. relative to sediment ranging from 0.12 to 8.13. Bioconcentration factors of <1 for Pb in *Enteromorpha* spp. relative to sediment concentrations were found at sites 2 (SO1, SO2 and SO3), 3 (SO3), and 4 (SO1, SO3) while bioconcentration factors of 1-2 and >2 were recorded at the remaining sites and sampling occasions. The results show an elevated bioconcentration of Pb in *Enteromorpha* spp. relative to water as compared to sediments.

8.2. DISCUSSION

8.2.1. Water

The mean Pb concentrations (mg/ℓ) recorded in water at sampling sites within the Zandvlei estuary ranged from <LD to 0.0261±0.0346 (**Table 8.1-1**). The results show that the highest concentrations were recorded at site 1 on SO1 (summer). Although the highest concentration was recorded at site 1 (SO1), comparisons of spatial variations in Pb concentrations show no statistical differences ($p>0.05$) in the pairwise consecutive comparisons between the different sampling sites on SO1 (summer) and SO2 (autumn). Statistical differences in Pb concentrations ($p<0.05$) were only recorded in the pairwise consecutive comparisons between sites 3 (0.0000±0.0000) and 4 (0.0120±0.0158) on SO3 (winter). This may be ascribed to estuarine circulation as a result of increased rainfall levels recorded (51.0 mm) in the winter (SO3) months. Local terrestrial inputs or stormwater discharges may also have contributed to the elevated Pb concentration levels recorded at site 4 (SO3) which were statistically higher than the concentrations recorded at site 3 on SO3 (**Table 8.1-1**). According to DWAF (1996) some of the sources of Pb include street run-off, as well as industrial and municipal wastewater discharges. Although South Africa phased out leaded petrol in 2006 and promulgated legislation to control the use of Pb in paint, elevated levels of Pb were still found in soil alongside busy roads compared to that alongside less heavily trafficked roads (Mathee, 2014). The Zandvlei estuary is characterised by an extensive system of stormwater drainage outlets and there are about 14 sewer pump stations within the catchment, some of which have experienced overflows from time to time (C.A.P.E., 2010). An industrial area is located approximately 2km north of the study area, adjacent to the Keysers River and is regarded as a potential source of illegal discharges and stormwater runoff (C.A.P.E., 2010; WCG, 2018). According to the COCT (2017) this industrial area is home to over 250 businesses which include the manufacturing of chemicals and chemical products, computer and electronic products, leather and related products, as well as rubber and plastic products (amongst various other products). Furthermore, there are approximately 12 businesses located there that are unknown (or could not be identified). This may be of concern as it is unknown what type of operations are undertaken from the above businesses and where their waste is disposed of. The above land uses and activities are likely to negatively affect the Zandvlei estuary by introducing and resulting in elevated levels of contaminants within the system. Some of the mean Pb concentrations recorded at the different sampling sites on each occasion (with a few exceptions) exceeded the recommended water quality guidelines values by DWAF (1996); (CCME, 2001); and (ANZECC, 2000) (**Table 10.1-1**). This may therefore be an indication of Pb pollution in the study area and it is likely as a result of the abovementioned land uses and activities around the estuary. As noted in the literature review, elevated Pb concentrations could result in neurological, renal, cardiovascular, haematological, immunological, reproductive, and developmental effects (ATSDR, 2020).

Temporal (seasonal) variations in Pb concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO2 (autumn) (0.0229 ± 0.0258) and SO3 (winter) (0.0015 ± 0.0015) at site 1 (**Table 8.1-1**). The above statistical differences in concentrations recorded between autumn and winter at site 1 shows a decline in Pb concentrations and this may be attributed to seasonal fluctuations in weather condition (i.e. rainfall) and other factors such as physicochemical parameters and anthropogenic inputs. Concentrations of contaminants in aquatic systems have been found to vary widely in temporal scales and this is due to various interacting factors such as flow intensity; tidal and current related effects; and the reoccurrence of contaminating discharges (Phillips and Rainbow, 1994). The results show no significant variation ($p>0.05$) in the recorded Pb concentrations between the different sampling occasions at site 2 and 4. Comparisons of temporal variations in Pb concentrations further show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between the different sampling occasions at site 3. Temporal variations recorded in this study show that warmer months recorded higher concentrations when compared to concentrations in winter. At site 3, the results show SO1 (summer) (0.0139±0.0089) recorded a statistically higher Pb concentration when compared to SO2 (autumn) (0.0033±0.0025) and SO3 (winter) (0.0000±0.0000) (**Table 8.1-1**). This may be ascribed to river flow inputs and land-based discharges which affects estuarine circulation and physicochemical parameters which may ultimately affect the rate of absorption and desorption of metals to and from the water column (Sany et al., 2013). The recorded salinity levels for site 3 on the SO1 (summer) and SO2 (autumn) was 16.30 ppt and 20.85ppt, respectively (**Tables 3.1-1** and **3.1-2**). No physicochemical records are available for SO3. However, it is assumed that salinity levels were probably elevated due to the opening of the estuary mouth and mixing with seawater. The South African Weather Services recorded a monthly average rainfall of 5.2 mm (SO1 – summer), 24.2 mm (SO2 – autumn) and 51.0 mm (SO3 – winter) (**Table 3.2-1**). SO3 (winter) which had the highest amount of rainfall recorded the lowest Pb concentrations and this may be linked to a reduction of anthropogenic activities during the winter period which may have led to the reduction in the level of anthropogenic inputs. Furthermore, increased riverine discharge as a result of elevated rainfall may result in the reduction in water and particle residence times including flushing of contaminants out of the estuary (De Souza Machado et al., 2016). Physicochemical parameters such as salinity and pH may have had an influence on metal concentrations in the water column. According to Sany et al. (2013) elevated metal concentrations may potentially be affected by an increase in salinity and a decrease in pH. The results in this study seem to be less affected by salinity as the elevated salinity levels recorded on SO2 (16.30 ppt) (autumn), at site 3 (**Table 3.1-2**), recorded statistically lower Pb concentrations (0.0033±0.0025) when compared to SO1 (summer) at site 3 (**Table 3.1-1**), which recorded a concentration of 0.0139±0.0089 and a salinity concentration of 20.85 ppt. The recorded pH values for SO1 and SO2 at site 3 was 8.01 and 8.02, respectively (**Tables 3.1-1** and **3.1-2**). The acidity (pH) levels recorded in this study seemed to have no effect in the temporal variations recorded between the different occasions at site 3. The results show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO1 (summer) (0.0257±0.0165) and SO2 (autumn) (0.0047±0.0068) at site 5. The above seasonal variations are similar to those recorded at sites 1 and 3 where Pb concentrations were found to be decreasing from summer to winter. The factors discussed above (i.e. flow intensity; tidal and current related effects etc.) are likely to have also influenced the recorded differences between SO1 (summer) and SO2 (winter) at site 5 (**Table 8.1-1**).

8.2.2. Sediment

The mean Pb concentrations (mg/kg) recorded from sediment collected at the five sampling sites within the Zandvlei estuary ranged from 1.24±0.19 to 37.37±34.62 (**Table 8.1-1**). Comparisons of spatial variations in Pb concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 (5.46±1.85) and 2 (29.89±14.99) as well as sites 2 (29.89±14.99) and 3 (2.90±0.78) on SO1 (summer) (**Table 8.1-1**). A statistically significant increase in Pb concentrations was recorded between sites 1 (5.46±1.85) and 2 (29.89±14.99) on SO1 (summer). This may be attributed to several factors such as stormwater run-off, land-based discharges, sediment grain size, estuary gradient, month manipulation, as well as physicochemical parameters. Site 2 is located opposite a yacht club, a parking area as well as a stormwater drainage outlet. Discharges from the stormwater outlet and street run-off from nearby parking area may have resulted in the elevated levels of Pb recorded at site 2 on SO1 (summer). Lead enters the aquatic environment in various ways, such as precipitation, lead dust fall-off, street run-off as well as industrial and municipal wastewater discharges (DWAF; 1996). The influent rivers (i.e. Keysers River) which discharge at the Zandvlei estuary may have also been contaminated with Pb from the industrial area located along its banks. The gradient of the estuary comprises of a relatively deeper channels with standing water (WCG, 2018; Day et al., 2020). The deeper channels are caused by the continuous removal of pondweed which have been found to exacerbate flooding, limit light penetration, restrict current flow and thus increase stagnation (WCG, 2018). The stagnant waters are likely to result in metals being limited to certain parts (i.e. sites 1 and 2) of the estuary and when conditions are favourable, be absorbed or deposited in sediments. Lead is usually deposited in sediments together with particulate matter such as iron and manganese oxides or could also be precipitated out of solution with carbonate or sulphide (Eisler, 1988; CCME, 1999a). It is worth noting that on SO1 (summer), the estuary mouth was closed as there was limited rainfall and water circulation within the system, and ultimately less movement of metals within the system.

The results show Pb concentrations (±SD) recorded at sites 3 (2.90±0.78), 4 (2.69±0.42) and 5 (3.19±0.94) on SO1 (summer) were statistically lower than the concentrations recorded at sites 1 (5.46±1.85) and 2 (29.89±14.99) (**Table 8.1-1**). A similar pattern was observed between the different sampling sites on other sampling occasions. This spatial variation in Pb concentrations may be ascribed to sediment grain size (among other factors). According to Nduka and Orisakwe (2011) fine sediments have a significant capacity to retain metals and as a result can adsorb an increased concentration of metals. This is due to the large surface area to volume provided by fine grained particles for adsorption (CSIR, 2015). The small surface area to volume and the absence of surface electric charges on course sand grains usually results in contaminant concentrations that are of magnitude lower than in fine grained (mud dominated) sediments (CSIR, 2015). This study relied on sediment grain size analysis by CSIR

(2015) and Maurer (2019). Sediments collected at 29 of the 41 (71%) sampling stations in the Zandvlei estuary and the marina were dominated by mud and the remaining stations mainly characterised by fine-grained sand (CSIR, 2015). The sediment in the main body of the estuary was less muddy with a few exceptions (where the content of mud was notably high) at sampling stations 78 and 75 (CSIR, 2015). These two sampling stations (i.e., 78 and 75) were located close to sampling sites 1 and 2 (of this study), respectively. Maurer (2019) recorded that sediment retained for the 1700 µm and 1180 µm size classes was highest at the lower/southern parts of the estuary and lowest at the middle and upper/northern parts of the estuary. The above size classes relate to very coarse-grained sand in terms of Wentworth Scale (Error! Reference source not found.). Furthermore, according to Maurer (2019) sediment retained for 63 µm and <63 µm size classes was highest at the upper parts and lowest at the middle and lower parts of the estuary. Based on the Wentworth scale, the 63 µm size class relates to very fine-grained sand and the <63 µm size class relates to mud. Considering the above, the coarsest sediment sampled was most prominent at the lower parts and the finest sediment at the upper parts of the Zandvlei estuary (Maurer, 2019). Both the CSIR (2015) and Maurer (2019) studies noted that upper parts of the estuary were largely characterised by muddy/fine-grained sediments and the lower parts by coarse-grained sediments. The abovementioned sediment grain size analysis and findings correlates with the recorded sediment concentrations in this study (**Table 8.1-1**) as higher Pb concentrations were found in fine sediments in the upper parts of the estuary (except for site 5, SO4) and lower concentrations were recorded in course sediment in the lower parts of the estuary.

Spatial variations in Pb concentrations further show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 (5.42±3.08) and 2 (32.03±32.65), sites 3 (2.40±1.07) and 4 (3.74±0.89) as well as sites 4 and 5 (1.24±0.19) on SO2 (autumn) (**Table 8.1-1**). SO2 (autumn) also recorded a similar spatial variation in Pb concentrations as SO1 (summer) where, on both occasions, site 2 recorded statistically significantly higher ($p<0.05$) Pb concentrations than the other sites. This may be ascribed to possible local point and non-point sources of pollution such as street run-off, stormwater discharges and upstream industrial effluents. Site 2 seems to be susceptible to contaminated urban discharges and run-off because of its proximity to the stormwater drainage outlet, parking area and yacht club (**Figure 5.2-1**). According to Davies et al. (1991) the accumulation of metals in sediment is dependent on several external factors such as anthropogenic input, pH, Eh, ionic strength and grain size distribution. The results show a consistent pattern of high mean Pb concentrations recorded at sampling sites in the upper parts of the estuary (i.e. sites 1 and 2), while lower mean Pb concentrations were recorded in the central (i.e. site 3) and lower parts (i.e. site 4 and 5) (**Table 8.1-1**). This may be linked to the gradient of the estuary (i.e. deeper channels upstream) and sediment grain size (among other factors) which appear to have played a significant role in the movement and accumulation of metals in sediment. Statistical differences ($p<0.05$) in Pb concentrations were also recorded in the pairwise consecutive comparisons between sites 2 (9.92±3.27) and 3 (3.84±3.08), sites 3 and 4 (5.81±2.77) as well as sites 4 and 5 (2.35±0.23) on SO3 (winter) (**Table 8.1-1**). Similar to SO1 (summer) and 2 (autumn), statistically higher ($p<0.05$) Pb concentrations were recorded at site 2 on SO3 (winter). This shows that site 2 was probably located close to a local point or non-point source of contamination, hence the high mean Pb concentrations recorded on this particular site. Although SO3 (winter) recorded a high monthly average rainfall level of 51.0 mm (**Table 3.2-1**), the distribution of Pb concentrations in sediment were similar to those recorded in other occasions and this seems to suggest that rainfall did not have much of an effect on spatial variations in metal concentrations in sediment. Site 5 recorded the lowest mean concentration on SO3 (winter), which could be attributed to the location of the site which is closer to the mouth of the estuary and further away from influent rivers and some stormwater drainage outlets. It is important to note that majority of the recorded mean Pb concentrations were well below the interim sediment quality guidelines (ISQGs) for marine/estuarine ecosystems (30.2 mg/kg) (CCME, 1999) (**Table 10.1-2**) except for Pb concentrations recorded at site 2 (SO2) (32.03±32.65) and site 5 (SO4) (37.37±34.62) (**Table 10.1-2**). The standard deviations recorded in both sites 2 (SO2) and 4 (SO4) were high and this seems to be an indication of concentrations that varied widely across the different sites. The above which exceeded the CCME guidelines may also be an indication of some lead pollution in autumn (SO2) and spring (SO4) in the Zandvlei estuary. However, it is important to note that the recorded mean Pb concentrations in this study were well below the recommended sediment quality guidelines by ANZECC (2000).

Comparisons of spatial variations in Pb concentrations further show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 (25.77±21.19) and 2 (5.94±2.89), sites 3 (5.90±5.59) and 4 (5.87±9.16) as well as sites 4 and 5 (37.37±34.62) on SO4 (spring) (**Table 8.1-1**). The above results show that site 1 recorded statistically higher concentrations when compared to site 2 and this may be attributed to the proximity of site 1 to influent rivers which receive stormwater / urban run-off as well as potential illegal discharges from the local industrial area (WCG, 2018). Mean Pb concentrations recorded at sites 2, 3 and 4 on SO4 (spring) ranged from 5.87±9.16 (site 4) to 5.94±2.89 (site 2) (**Table 8.1-1**). The above shows a decrease in concentrations from central sampling sites to lower sampling sites and this may be attributed to sediment grain size, the distance of each site from influent rivers and potential anthropogenic inputs. Interestingly, site 5 on SO4 (spring) recorded the highest Pb concentration (37.37±34.62) when compared to all other sampling sites in each sampling occasion (**Table 8.1-1**). This may be attributed to stormwater run-off as site 5 is also located a few metres away from a stormwater drainage outlet which discharges directly into the estuary (**Figure 8.2-1**). The relatively high concentrations recorded at site 5 may also be attributed to atmospheric deposits of lead and elevated levels in soils alongside busy roads (Mathee, 2014). Furthermore, as previously indicated, the Zandvlei estuary is characterised by an extensive stormwater drainage system which discharges directly into the estuary (C.A.P.E., 2010). The recorded average monthly rainfall for SO4 was 27.8 mm (**Table 3.2-1**) and the estuary mouth was also closed during this period (**Table 3.3-1**). This means that stormwater run-off and other land-based discharges were possibly trapped within the system as there was limited estuarine water circulation.

Figure 8.2-1: Map showing the proximity of site 5 from stormwater drainage outlets within the study area (Source: Google Maps; CoCT open data portal)

Temporal (seasonal) variations in Pb concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO3 (winter) (12.92±18.11) and SO4 (spring) (25.77±21.19) at site 1 (**Table 8.1-1**). This may be attributed to changes to pollution load inputs from the influent rivers and rainfall. SO3 (winter) recorded a monthly average rainfall of 51.0 mm (**Table 3.2-1**) as compared to the monthly average rainfall of 27.8 mm recorded for SO4 (spring). According to Lim et al. (2006) and Li et al. (2009) precipitation increases metal mobility and dilution which decreases metal concentrations in sediments. This may be one of the reasons why SO3 (winter) recorded statistically lower Pb concentrations than SO4 (spring) at site 1. Statistical differences ($p<0.05$) in Pb concentrations were recorded in the pairwise comparisons between the different sampling occasions at both sites 2 and 4 (**Table 8.1-1**). The results show both SO1 (summer) and SO2 (autumn) recording statistically higher Pb concentrations when compared to SO3 (winter) and spring (SO4) at site 2. This may be attributed to seasonal fluctuations in weather conditions as well as physical and chemical properties of the estuary. The estuary mouth was closed on SO1 (summer) and SO2 (autumn) and this could have contributed to the higher concentrations recorded on both occasions, especially since the estuary is characterised by deeper channels (WCG, 2018) and standing water (Day et al., 2020) in the central and northern parts. Another possible cause for elevated Pb concentrations on SO1 (summer) and SO2 (autumn) is an increase in anthropogenic activities due to favourable weather conditions (i.e. less rainfall) and a more stable sediment (Sany et al., 2013). The lower concentrations recorded on SO3 (winter) and SO4 (spring) at site 2 may be attributed to elevated rainfall which may have flushed some of the contaminants out of the system as the estuary mouth was open on SO3 (winter) but was closed on SO4 (spring) (**Table 3.3-1**). The discussion relating to rainwater possibly increasing metal mobility and dilution (Lim et al., 2006; Li et al., 2009) may also be applicable in the temporal variations recorded between SO2 (autumn) and SO3 (winter) as well as between SO3 (winter) and SO4 (spring) at site 2 (**Table 8.1-1**). No statistical differences in Pb concentrations ($p>0.05$) were recorded between the different sampling occasions at site 3 (**Table 8.1-1**). At site 4, SO2 (autumn) recorded statistically higher concentrations when compared to SO1 (summer) and this could be linked to an increase in rainfall which increases land-based discharges and stormwater run-off. However, according to Sany et al. (2013) increased metal concentrations may be linked to an increase in salinity, decrease in pH and redox potential. The recorded salinity concentration for SO1 (summer) and SO2 (autumn) at site 4 was 19.75ppt and 22.3ppt, respectively, whilst the pH was 7.97 for SO1 (summer) and 4.97 for SO2 (autumn) (**Tables 3.1-1** and **3.1-2**). The high salinity and low pH values recorded on SO2 (autumn) may have therefore contributed to the temporal variations recorded between SO1 and SO2 at site 4 (**Table 8.1-1**). The results also show a statistical increase in Pb concentrations between SO2 (autumn) and SO3 (winter) and this may be due to elevated stormwater run-off and land-based discharges as a result of increased rainfall. The statistical increase in Pb concentrations recorded on SO4 (spring) at site 4 may be linked to the closing of the estuary mouth and stormwater run-off. Site 4 is located in close proximity to a stormwater drainage outlet and may therefore be affected by stormwater discharges into the estuary.

Temporal variations in Pb concentrations further show statistical differences ($p<0.05$) in the pairwise comparisons between SO2 (autumn) (1.24±0.19) and SO3 (winter) (2.35±0.23) as well as between SO3 (winter) and SO4 (37.37±34.62) at site 5 (**Table 8.1-1**). The results show significant variations in Pb concentrations recorded at site 5 between SO3 (winter) (2.35±0.23) and SO4 (spring) (37.37±34.62). This may be ascribed to seasonal fluctuations in weather conditions and changes to pollution load from anthropogenic sources. River discharge coupled with seasonal and tidal currents as well as other sediment resuspension events also play a significant role in relation to the partitioning and bioavailability of metal contaminants in estuarine sediments (Chapman and Wang, 2001; Sany et al., 2013). Although mean Pb concentrations were expected to be low between the different sampling occasions at site 5 due to sediment grain size (i.e. course grained sediments), among other factors. However, SO4 (spring) at site 5 recorded a relatively high mean Pb concentration (37.37±34.62) but then again, the standard deviation was also high which shows that the concentrations varied substantially.

Table 8.2-1 Comparison of mean sediment Pb concentrations recorded in Zandvlei with other estuaries (data extracted from Izegaegbe et al., 2020)

Estuaries	Pb (mg/kg)
Zandvlei, South Africa[a]	10.2±7.9
Mhlathuze, South Africa[b]	13.6±5.1
Swartkops. South Africa[c]	32.9±27.6
St Louis Estuary, Senegal[d]	233±35.5
Brisbane Estuary, Australia[e]	25.6±9.2

[a]present study [b]Izegaegbe et al. (2020) [c]Binning and Baird (2001) [d]Diop et al. (2015) [e]Duodu et al. (2017)

The comparison of Pb concentrations recorded in sediment from the Zandvlei Estuary and other estuaries is presented in **Table 8.2-1**. The mean Pb concentration recorded in this study (10.2±7.9) was relatively lower than the Pb concentration recorded in the Mhlathuze estuary (13.6±5.1) which is also located in South Africa. The Brisbane Estuary in Australia followed with a relatively higher Pb concentration (25.6±9.2) followed by the Swartkops Estuary in South Africa (32.9±27.6). The highest mean Pb concentration was recorded in St. Louis Estuary in Senegal (233±35.5) (**Table 8.2-1**). According to Diop et al. (2015), the St. Louis estuary was highly contaminated with Pb which was linked to domestic, mineral and industrial wastewaters.

8.2.3. Algae

The mean Pb concentrations (mg/kg) recorded in macroalgae (*Enteromorpha* spp.) collected at the five sampling sites within the Zandvlei estuary ranged from 1.74±0.79 to 48.31±19.89 (**Table 8.1-1**). Comparisons of spatial variations in Pb concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons recorded between sites 2 (8.94±2.54) and 3 (14.89±2.34), sites 3 and 4 (2.05±0.40) as well as sites 4 and 5 (6.35±4.43). Site 3 recorded the highest mean Pb concentration when compared to other sites on SO1 (summer), and this may have been due to anthropogenic, stormwater run-off, riverine, and atmospheric inputs. Site 3 is located close to a stormwater drainage outlet (**Figure 4.2-2**) which could potentially be a source of Pb. The bioconcentration factors of Pb in *Enteromorpha* spp. relative to water were extremely high, and above the concentration factor of >2, which represents macroconcentation of metals in algae. This shows the ability of *Enteromorpha* spp. to absorb metals within the aquatic environment as compared to concentrations recorded in water samples (Memon et al., 2001). However, similar to other metals, the bioconcentration factors of Pb in *Enteromorpha* spp. relative to sediment was at a much lesser degree (**Table 8.2-2**) and this could be an indication of the accumulation abilities of both mediums (i.e. algae and sediment), among other factors. Lead deconcentrated (<1) in *Enteromorpha* spp. in relation to sediment at site 2 (SO1, SO2 and SO3), site 3 (SO3), and site 4 (SO1, SO3). This may have been due to several internal (i.e. algae metabolic rates) and external factors (physicochemical parameters etc.) (Hanaf, 2022). The proposed concentration ranges of Pb in *Enteromorpha* spp. by Say et al. (1990) provided some form of a guideline concentration value for the extent or degree of contamination within estuaries. The above-mentioned concentration ranges are: <20 mg/kg for relatively "clean or unpolluted" sites, 20 – 60 mg/kg for "moderate" contamination and >60 mg/kg for "high" contamination (Say et al., 1990). Applying the above concentrations as a "guideline" to the current study, it was found that all sampling sites on SO1 (summer) recorded concentrations that were below the "moderate contamination" range. The same was found in the comparison of the above guideline values to the mean Pb concentrations recorded in the different sampling sites on SO2 (autumn) and SO3 (winter). No statistical differences ($p>0.05$) in Pb concentrations were recorded in the pairwise consecutive comparisons between the different sampling sites on SO2 (autumn), SO3 (winter) and SO4 (spring) (**Table 8.1-1**). Although no statistical differences ($p>0.05$) were recorded in the pairwise consecutive comparisons between the different sampling sites on SO4 (spring), mean Pb concentrations recorded in the different sampling sites on SO4 (spring) were within the "moderate" contamination range. The concentrations recorded on SO4 are significantly higher and this may be attributed to a local anthropogenic source of contamination such as the nearby industrial area, including disposal of household products, stormwater run-off and other land-based discharges (C.A.P.E., 2010).

Temporal (seasonal) variations in Pb concentrations showed statistical differences ($p<0.05$) in the pairwise comparisons between SO1 (summer) (8.42±2.95) and SO2 (autumn) (8.75±3.08) at site 1 (**Table 8.1-1**). The above concentrations are similar to those found in sediment between the two sampling occasions, attributed to an indication of seasonal variations that are influenced by environmental factors such as discharges to the estuary and physicochemical parameters (pH, salinity etc.) instead of factors inherent to the organisms themselves such as metabolism or reproduction (Wright and Mason, 1999). Statistical differences ($p<0.05$) in Pb concentrations were also recorded between SO1 (summer) (8.94±2.54) and SO2 (autumn) (3.95±0.53) as well as between SO2 (autumn) and SO3 (winter) (2.22±0.50) at site 2 (**Table 8.1-1**). Many studies have reported higher metal concentrations during the winter period when the metabolic processes of algae have slowed down, resulting in an increase of metal content, while lower concentrations were usually recorded in warmer months when there is high metabolic activity resulting in dilution of metals (Hou and Yan, 1998; Brown et al., 1999; Ryan et al., 2012; Villares et al., 2002; Żbikowski et al., 2007). The current study found higher metal concentrations during growth periods (i.e. warmer months) as recorded between SO1 (summer), SO2 (autumn) and SO3 (winter) at sites 2, 3 and 5. This may potentially be attributed to the existence of higher rates of photosynthesis and respiration during the summer period, which may support the accumulation of metals (Żbikowski et al., 2007). Comparisons of temporal variations in Pb concentrations further show statistical differences ($p<0.05$) between the different sampling occasions at site 3 (**Table 8.1-1**). The results show higher mean Pb concentrations in the summer (SO1) (14.89±2.34) and spring season (SO4) (26.09±9.13) as compared to autumn (SO2) (5.59±2.89) and winter season (SO3) (3.03±1.13) at site 3 and this could be attributed to the level of contaminants in water because of land-based discharges during the summer period (Villares et al., 2002). There is limited movement of water within the estuary during the summer period due to low rainfall levels and an abundance of macroalgae due to high salinities (Muhl, 2003). The seasonal variations where lower Pb concentrations were recorded in winter may also be attributed to physicochemical parameters such as salinity. According to Karsten and Kirst (1989) the photosynthetic and growth rate of algae is reduced by low salinities. High rainfall and low temperatures in winter usually results in low salinities while low rainfall and high temperatures usually result in high salinities in estuaries. Villares et al. (2002) argues that the reduction in solar radiation and temperature is responsible for slowing down metabolic activities in algae.

The low mean Pb concentrations largely recorded in the winter season may potentially be attributed to the fact that *Entermorpha* spp. is not abundant in the Zandvlei estuary winter (Muhl, 2003) and therefore the collected biomass may have had limited capacity to accumulate metals as the collected samples were in relatively small amounts, especially towards the influent rivers. Villares et al. (2002) noted that larger amounts of macroalgae could lead to greater accumulation of metals in aquatic systems. The seasonal variations recorded at site 3 are in contrast to the usual seasonal growth effect which results in minimum concentrations being recorded in summer and maximum concentrations being found in winter. Significant variations ($p<0.05$) in temporal scales were also recorded in the pairwise consecutive comparisons between SO1 (summer) (2.05±0.40), SO2 (autumn) (12.32±1.78) and SO3 (winter) (8.15±4.36) at site 4 (**Table 8.1-1**). This may be linked to metabolic processes (i.e. growth effect) which may have diluted metals due to algae growth resulting in a decrease in Pb concentrations in summer (SO1) (Villares et al., 2002; Żbikowski et al., 2007). The statistically higher Pb concentrations recorded in winter (SO3) in comparison to summer (SO1) at site 4 may be ascribed to the increase of dissolved metals due to river discharges as a result of elevated rainfall levels (Villares et al., 2002). Also, the decrease in salinity as a result of river discharges can lead to an increase in the levels of metals in algae (Struck et al., 1997; Wang and Dei, 1999; Villares et al., 2001). Statistical differences ($p<0.05$) in Pb concentrations were recorded in the pairwise consecutive comparisons between SO1 (summer) (6.35±4.43) and SO2 (autumn) (4.19±1.27) at site 5 (**Table 8.1-1**). This may potentially be attributed to the existence of higher rates of photosynthesis and respiration during the summer period, which may support the accumulation of metals (Żbikowski et al., 2007).

CHAPTER 9

COPPER RESULTS AND DISCUSSION

9.1. RESULTS

It should be noted that statistical comparisons presented in this chapter were made between consecutive sites and occasions.

Table 9.1-1: Mean concentrations (±SD) of copper in water, sediment and algae during different sampling occasions at five sampling sites (n=5).

	SAMPLING OCCASIONS (SO)			
COPPER	SO1 (**Summer**)	SO2 (**Autumn**)	SO3 (**Winter**)	SO4 (**Spring**)
WATER (mg/ℓ)				
Site 1	^{a}0.1478^{a} (±0.1516)	^{a}0.0047^{b} (±0.0058)	^{a}0.1250^{c} (±0.1717)	ND
Site 2	^{a}0.0461^{a} (±0.0072)	^{a}0.0170^{a} (±0.0202)	^{a}0.0847^{a} (±0.1157)	ND
Site 3	^{a}0.0695^{a} (±0.0436)	^{a}0.0126^{a} (±0.0078)	^{a}0.0335^{a} (±0.0550)	ND
Site 4	^{a}0.0814^{a} (±0.0151)	^{a}0.0076^{b} (±0.0057)	^{a}0.0227^{b} (±0.0347)	ND
Site 5	^{a}0.0860^{a} (±0.0254)	^{a}0.0026^{b} (±0.0027)	^{a}0.0586^{c} (±0.0444)	ND
SEDIMENT (mg/kg)				
Site 1	^{a}1.26^{a} (±1.13)	^{a}101.71^{b} (±211.04)	^{a}7.20^{b} (±5.08)	^{a}0.42^{c} (±0.94)
Site 2	^{b}8.52^{a} (±9.03)	^{a}9.50^{a} (±5.81)	^{a}4.95^{a} (±2.45)	^{b}5.06^{a} (±2.36)
Site 3	^{c}0.63^{a} (±0.69)	^{a}1.97^{a} (±1.01)	^{a}1.59^{a} (±1.72)	^{c}1.20^{a} (±1.40)
Site 4	^{c}0.36^{a} (±0.40)	^{a}13.94^{b} (±15.68)	^{a}2.81^{b} (±1.90)	<LD
Site 5	^{c}0.60^{a} (±0.59)	^{a}2.01^{a} (±1.52)	^{a}1.55^{a} (±1.07)	^{c}0.32^{a} (±0.71)
ALGAE (mg/kg)				
Site 1	^{a}15.73^{a} (±6.18)	^{a}6.12^{a} (±2.26)	^{a}5.63^{a} (±1.32)	^{a}7.28^{a} (±3.38)
Site 2	^{a}10.83^{a} (±4.16)	^{b}2.53^{b} (±0.63)	^{a}10.58^{c} (±5.91)	^{a}6.24^{d} (±1.80)
Site 3	^{a}9.67^{a} (±1.20)	^{b}2.34^{b} (±1.73)	^{a}7.38^{b} (±6.75)	^{a}8.10^{c} (±2.49)
Site 4	^{a}8.02^{a} (±1.26)	^{c}10.37^{a} (±4.16)	^{a}16.69^{a} (±4.69)	^{a}7.14^{a} (±1.31)
Site 5	^{a}12.68^{a} (±10.37)	^{c}19.69^{a} (±20.38)	^{a}16.97^{a} (±11.63)	ND

LD = Limit of Detection
ND = No Data
The statistical differences between sites per sampling occasion are indicated by superscripted letters to the left of mean values. The statistical differences between sampling occasions per site are indicated by superscripted letters to the right of mean values. Water, sediment, and algae samples were not statistically compared with each other.

Table 9.1-2: Bioconcentration factors of algae for copper.

Copper		SO1 (Summer)	SO2 (Autumn)	SO3 (Winter)	SO4 (Spring)
Site 1	A/W	106.43	1302.13	45.04	
	A/S	12.48	0.06	0.78	17.33
Site 2	A/W	234.92	148.82	124.91	
	A/S	1.27	0.27	2.14	1.23
Site 3	A/W	139.14	185.71	220.30	
	A/S	15.35	1.19	4.64	6.75
Site 4	A/W	98.53	1364.47	735.24	
	A/S	22.28	0.74	5.94	
Site 5	A/W	147.44	7573.08	289.59	
	A/S	21.13	9.80	10.95	

Shaded areas represent sites with either no data or where the recorded concentrations were below the level of detection.

$$A/W = \frac{\text{Algae metal concentration } (\frac{mg}{kg})}{\text{Water metal concentration } (\frac{Mg}{L})} \quad A/S = \frac{\text{Algae metal concentration } (\frac{mg}{kg})}{\text{Sediment metal concentration } (\frac{mg}{kg})}$$

9.1.1. Water

9.1.1.1. Comparisons of metal concentrations between sites per occasion

The results showed no statistical differences in copper (Cu) concentrations between the different sampling sites on SO1, SO2 and SO3 ($p>0.05$). No data was recorded for SO4 (**Table 9.1-1**).

9.1.1.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions revealed statistical differences in Cu concentrations between the different sampling occasions at sites 1 and 5 ($p<0.05$). The results showed no statistical differences ($p>0.05$) in Cu concentrations recorded between the different sampling occasions at sites 2 and 3. Pairwise multiple comparison of consecutive sampling occasions revealed the following differences at site 4: SO1 and SO2 ($p<0.05$) as well as SO2 and SO3 ($p>0.05$). No data was recorded for sampling occasion 4 (**Table 9.1-1**).

9.1.2. Sediment

9.1.2.1. Comparisons of metal concentrations between sites per occasion

The results showed statistical differences in Cu concentrations recorded between the different sampling sites on SO2 and SO3 ($p>0.05$). Pairwise multiple comparison of consecutive sampling sites revealed statistical differences ($p<0.05$) in Cu concentrations recorded between sites 1 and 2 as well as sites 2 and 3 on SO1 and SO4. However, there results showed no significant differences ($p>0.05$) in Cu concentrations recorded between sites 3 and 4 as well as sites 4 and 5 (**Table 9.1-1**).

9.1.2.2. Comparisons of metal concentrations between sampling occasions per site

Pairwise multiple comparison of consecutive sampling occasions revealed the following differences at sites 1 and 4: SO1 and SO2 ($p<0.05$), SO2 and SO3 ($p>0.05$), as well as SO3 and SO4 ($p<0.05$). The results showed no statistical differences in Cu concentrations recorded between the different sampling occasions at sites 2, 3 and 5 ($p>0.05$) (**Table 9.1-1**).

9.1.3. Algae

9.1.3.1. Comparisons of metal concentrations between sites per occasion

The results showed no statistical differences ($p>0.05$) in Cu concentrations recorded between the different sampling sites on SO1, SO3 and SO4. Pairwise multiple comparison of consecutive sampling sites has revealed the following differences on SO2: sites 1 and 2 ($p<0.05$); sites 2 and 3 ($p>0.05$); sites 3 and 4 ($p<0.05$); and sites 4 and 5 ($p>0.05$) (**Table 9.1-1**).

9.1.3.2. Comparisons of metal concentrations between sampling occasions per site

The results, once again, showed no significant differences ($p>0.05$) in Cu concentrations recorded between the different sampling occasions at sites 1, 4 and 5. Pairwise multiple comparison of consecutive sampling occasions revealed statistical differences ($p<0.05$) in Cu concentrations recorded between the different sampling occasions at site 2. Pairwise multiple comparison of consecutive sampling occasions showed the following differences at site 3: SO1 and SO2 ($p<0.05$); SO2 and SO3 ($p>0.05$); and SO3 vs SO4 ($p<0.05$) (**Table 9.1-1**).

9.1.3.3. Bioconcentration factors of algae for copper

The bioconcentration factors of Cu in *Enteromorpha* spp. are presented in **Table 9.1-2**. The bioconcentration factors of Cu in *Enteromorpha* spp. relative to water concentrations were very high, ranging from 45.04 to 7573.08, when compared to bioconcentration factors relative to sediment, which ranged from 0.06 to 22.28. Bioconcentration factors of <1 for Cu in *Enteromorpha* spp. relative to sediment were recorded at sites 1 (SO2, SO3); 2 (SO2); and 4 (SO2).

9.2. DISCUSSION

9.2.1. Water

The recorded mean Cu concentrations ranged from 0.0026±0.0027 to 0.1478±0.1516 (**Table 9.1-1**). Spatial variations in Cu concentrations (mg/ℓ) show no statistical differences ($p>0.05$) in the pairwise consecutive comparisons between the different sampling sites on each sampling occasion (SO) (**Table 9.1-1**). Due to a technical error, there were no results available for sites on SO4 (spring). Comparison of the recorded mean Cu concentrations show a relatively widespread distribution of Cu across the different sites in each sampling occasion. However, it was noted that most of the recorded mean concentrations exceeded the recommended Target Water Quality Range (TWQR) values stipulated by DWAF (1996) and water quality guideline concentrations recommended by CCME (2001) and ANZECC (2000) (**Table 10.1-2**). This may be an indication of Cu contamination in the Zandvlei. Anthropogenic sources of Cu account for 33 – 60% of the total global input of Cu to the aquatic environment. These include effluents from wastewater treatment plants and industrial activities, including run-off and corrosion of copper pipes (DWAF, 1996). Anthropogenic activities (i.e. industrial, urbanisation, and agricultural practices) from catchment areas are likely to have introduced Cu into the estuary. The toxicity of copper is dependent on several water quality conditions such as water hardness, dissolved oxygen, pH, and the interaction of Cu with other metals (DWAF, 1996). Elevated levels of Cu were reported in the lower Keysers River in 2002 (WCG, 2018).

Seasonal (temporal) variations in mean Cu concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between the different sampling occasions (SO1, SO2 and SO3) at both sites 1 and 5 (**Table 9.1-1).** Seasonal variations show both SO1 (summer) and SO3 (winter) recorded mean Cu concentrations that were statistically significantly higher than SO2 (autumn) at sites 1 and 5. SO2 (autumn) also recorded statistically lower concentrations when compared to SO1 at site 4 (**Table 9.1-1**). The above seasonal variations in the recorded Cu concentrations may be due to seasonal fluctuations or changes in weather conditions between the different seasons as well as physicochemical parameters. Some researchers argue that chemical parameters such as pH and salinity may affect metal concentrations dissolved in the water column (Sany et al., 2013). Increased salinity intensifies competition between cations and metals for binding sites and this moves metals off into the overlying water through a process known as desorption (Nduka and Orisakwe, 2011). A low pH has a similar effect to that of elevated salinity levels in an aquatic system as it also increases competition between metal and hydrogen ions for binding sites which can lead to the release of free metal ions into the water column (Nduka and Orisakwe, 2011). SO2 (site 1) recorded a slightly higher salinity concentration (18.65ppt), including a slightly higher pH (8.81) when compared to SO1 (site 1) which recorded a salinity concentration of 15.15 ppt and pH of 8.63 (**Tables 3.1-1** and **3.1-2**). Based on the above, it is expected that SO2 (site 1) would have higher Cu concentrations than SO1 (site 1) but this was not the case as SO (site 1) recorded concentrations that were statistically lower than SO1 (site 1). This was also noted in the comparison of seasonal variations for the other sites. Both pH and salinity seemed to have no effect on the metal concentrations recorded in the water column. No statistical differences ($p>0.05$) in mean Cu concentrations were recorded between the different sampling occasions at sites 2 and 3. Temporal variations in mean Cu concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO1 and SO2 at site 4 (**Table 9.1-1**). SO2 (site 4) also recorded concentrations that were statistically lower than those recorded in SO1 (summer) and SO3 (winter). The seasonal variations in mean concentrations recorded above are likely due to seasonal changes in weather conditions, anthropogenic load inputs and other environmental factors.

9.2.2. Sediment

Mean Cu concentrations (mg/kg) recorded in sediment samples at the different sampling sites within the study area ranged from 0.32±0.71 to 101.71±211.04 (**Table 9.1-1**). Spatial variations in mean Cu concentrations (mg/kg) show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 and 2 as well as sites 2 and 3 on SO1 (summer) and SO4 (spring) (**Table 9.1-1**). Site 2 recorded statistically higher Cu concentrations when compared to the other sampling sites on both sampling occasions (i.e. SO1 and SO4). Site 2 is located near a stormwater drainage outlet (**Figure 5.2-1**) which may be responsible for the discharge of Cu into the estuary. Site 2 is also located near a parking area and a Yacht club. Furthermore, influent rivers feeding into the estuary may also have been responsible for the elevated levels of Cu at site 2. Cu may emanate from various anthropogenic sources such as industrial activities, wastewater treatment plants as well as run-off and corrosion of copper pipes, among others (DWAF, 1996). The gradient of the estuary comprises of deeper channels in areas where there is dense growth of pondweed (WCG, 2018) which could potentially result in water flow being stagnant. This could also result in contaminants being potentially trapped in the aforementioned areas. The estuary mouth was closed on both occasions (SO1 and SO4) (**Table 3.3-1**) and this could have also contributed in waters being stagnant in some areas, including site 2 which is in the northern parts of the estuary. Sediment and suspended particles play an important role in metal absorption, desorption and dissolution processes particularly since metals are transported in particulate or dissolved form in aquatic system such as estuaries (De Souza Machado et al., 2016). Adsorption removes metals from the water and transports it to the sediment for storage, while desorption transports the metal back to the water column where bio-assimilation and recirculation may take place (Sany et al., 2013).

The recorded mean Cu concentrations at sites 3, 4 and 5 were mostly (with some exceptions) lower than those recorded at sites 1 and 2 on each occasion (**Table 9.1-1**). This may be due to sediment grain size (among other factors). As explained in previous chapters, sediment grain size plays an important role in the distribution of contaminants such as metals. Fine-grained sediments are regarded as one of the main parameters which controls the concentration of metals in the aquatic or marine environment (Sany et al., 2013). Metals preferentially adsorb onto fine-grained material, due to the large surface area to volume provided by the fine grains for adsorption and since the surface of silt and clay grains is electrically charged (CSIR, 2015). Some studies show that coarse grained sediment (i.e. sand) was most prominent in the southern parts of the estuary while fine grained sediment (i.e. mud) was mostly found in the northern parts of the estuary and in the Marina (Maurer, 2019). The recorded Cu concentrations appears to be in line with the grain size of the sediment in the estuary as statistically higher mean concentrations were recorded in fine-grained sediment (i.e. mud) and statistically lower concentrations were mostly found within the coarsely grained sediment (i.e. sand). No statistical differences ($p>0.05$) were recorded between the different sampling sites on SO2 (autumn) and SO3 (winter) (**Table 9.1-1**). The sediment Cu concentrations recorded in this study was compared to other studies on estuaries (**Table 9.2-1**).

The Zandvlei estuary recorded the lowest Cu concentration in sediment when compared to the other estuaries as listed in **Table 9.2-1**. The highest mean Cu concentration (46.5±13.23) was recorded at the St. Louis estuary in Senegal. However, the standard deviation (±SD) recorded at the St. Louis estuary was the same as the one recorded in the Zandvlei estuary (8.28±13.23) but the mean Cu concentration recorded in Zandvlei was lower when compared to St. Louis (**Table 9.2-1**). This shows that the Cu concentrations recorded in sediment in the Zandvlei estuary varied significantly as the SD was higher than the mean concentration (**Table 9.2-1**).

Table 9.2-1: Comparison of mean sediment Cu concentrations from the Zandvlei estuary and other estuaries (data extracted from Izegaegbe et al., 2020)

Estuaries	Cu (mg/kg)
Zandvlei, South Africa[a]	8.28±13.23
Mhlathuze, South Africa[b]	10.9±6.2
St Louis Estuary, Senegal[c]	46.5±13.23

[a]present study [b]Izegaegbe et al. (2020) [c]Diop et al. (2015)

Temporal variations in Cu concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between SO1 (summer) (7.20±5.08) and SO2 (autumn) (1.26±1.13), as well as SO3 (winter) (101.71±211.04) and SO4 (spring) (0.42±0.94 mg/kg) at sites 1 and 4 (**Table 9.1-1**). SO2 (autumn) recorded statistically higher mean Cu concentrations when compared to SO1 (summer) and SO4 (spring) at both sites 1 and 4. However, SO2 (autumn) also recorded a high standard deviation value at both sites 1 and 2 (**Table 9.1-1**) and this may reflect a wide variation of Cu concentrations in temporal scales in the Kruskal–Wallis One-Way

ANOVA on Ranks test. Both summer (SO1) and spring (SO4) recorded statistically lower Cu concentrations when compared to autumn (SO2) at both sites 1 and 4. This may be attributed to seasonal fluctuations and changes to anthropogenic load input (Sany et al., 2013). No statistical differences were recorded in the pairwise consecutive comparison of the different sampling occasions at sites 2, 3 and 5 (**Table 9.1-1**). All the recorded mean Cu concentrations (except for site 1, SO2) were well below the sediment quality guidelines recommended by CCME (1999) and ANZECC (2000).

9.2.3. Algae

Mean Cu concentrations (mg/kg) recorded in algae samples (*Enteromorpha* spp.) at the different sampling sites within the study area ranged from 2.34±1.73 to 19.69±20.38 (**Table 9.1-1**). Spatial variations in the recorded mean Cu concentrations show statistical differences ($p<0.05$) in the pairwise consecutive comparisons between sites 1 (6.12±2.26) and 2 (2.53±0.63) as well sites 3 (2.34±1.73) and 4 (10.37±4.16) on SO2 (autumn). A fluctuation in mean Cu concentrations was noted between the abovementioned sites on SO2 (**Table 9.1-1**). Site 1 (SO2) recorded a statistically significantly higher mean concentration (6.12±2.26) than site 2 (SO2), which recorded a mean concentration of 2.53±0.63. This may be due to the proximity of site 1 to the influent rivers, which are responsible for discharging and transporting of stormwater and urban effluents from upstream and neighbouring activities. Mean Cu concentrations recorded at sites 2 and 3 were statistically significantly lower than concentrations at sites 1 and 4 (**Table 9.1-1**). The statistical differences recorded between sites 1 and 2 on SO2 (autumn) shows a significant decline in concentrations while a significant increase in mean Cu concentrations was recorded between sites 3 and 4 on the same SO. High bioconcentration factors of Cu in *Enteromorpha* spp. relative to water can be observed (**Table 9.1-2**). This demonstrates the importance of aquatic plants in absorbing metal concentrations by providing a realistic picture of potential contamination within aquatic environments (Hanaf, 2022). The bioconcentration factors also show Cu deconcentrated in *Enteromorpha* spp. relative to sediment at sites 1 (SO2, SO3), 2 (SO2), and 4 (SO2) (**Table 9.1-2**). The deconcentration occurred mostly in SO2 (autumn) and this could be linked to seasonal conditions which may have affected metal uptake in algae (Żbikowski et al., 2006). Cu concentrations in *Enteromorpha* spp. from sites considered uncontaminated ranged between 6 to 12 mg/kg whereas concentrations from sites known to be contaminated ranged from 20 to 70 mg/kg (Brown et al., 1999). The recorded concentrations in this study seem to suggest that the estuary was, to some extent, contaminated with copper although majority of the recorded concentrations were within the uncontaminated range. The source of metals such as Cu in urban run-off (stormwater) is mainly from industries, buildings, vehicle parts and components, fuel and oil, roofs with metallic elements and other metallic structures from roads, to name a few (Gromaire et al. 2001; Brown and Peake 2006; Barbosa et al. 2012; Sakson et al., 2018). No statistical differences were recorded in the pairwise consecutive comparisons between the different sampling sites on SO1 (summer), SO3 (winter) and SO4 (spring) (**Table 9.1-1**). Spatial variations in mean Cu concentrations show statistical differences only on SO2. The physicochemical parameters recorded on SO2 may have potentially influenced the bioavailability and accumulation of metals in algae (Żbikowski et al., 2006). The recorded salinity levels on SO2 ranged from 18.65 ppt (site 1) to 25.45 ppt (site 5) (**Table 3.1-2).** Dissolved oxygen decreased from site 1 (13.84 mg/ℓ) to site 5 (2.42 mg/ℓ) on SO2 (**Table 3.1-2**). The pH also decreased, ranging from 8.81 (site 1) to 6.8 (site 5) (**Table 3.1-2**). Temperature which also appears to play an important role in the metabolic and growth of algae and which directly affects the accumulation of metals in macroalgae (Sany et al., 2013), ranged between 23.2°C (site 1) to 21.1°C (site 5) (**Table 3.1-3**). During sample collection on SO2, the estuary mouth was closed (**Table 3.2-1**) and this may have also contributed to the accumulation of metals at the sites in the lower/southern parts of the estuary.

Seasonal (temporal) variations in mean Cu concentrations show no statistical differences ($p>0.05$) between the pairwise consecutive comparisons of the different sampling occasions at sites 1, 4 and 5 (**Table 9.1-1**). Statistical differences ($p<0.05$) in Cu concentrations were recorded between the different sampling occasions (SO's) at site 2. Copper concentrations fluctuated between the different sampling occasions at site 2. SO1 (summer) recorded the highest concentrations when compared to other sampling occasions at site 2 (**Table 9.1-1**). This may be due to a combination of several factors, such as the concentration of metals in water, salinity, pH, interactions between different metals, light intensity, and metabolic factors such as dilution of metal contents due to algae growth (Żbikowski et al., 2006). Since there are various factors which affect the bioaccumulation of metals in algae, some researchers have found it difficult to determine explicitly the main factors affecting seasonal changes in metal accumulation in algae as this may be caused by the interactions between the various factors (Vasconcelos and Leal, 2001; Szefer, 2002; Żbikowski et al., 2006). Elevated anthropogenic inputs from nearby stormwater drainage outlets and low salinity may result in increased metal concentrations in macroalgae (Munda, 1984, Wang and Dei, 1999) and this may be one of the reasons why SO1 (site 2) recorded a higher concentration than SO2 (site 2). SO1 (summer) recorded a monthly average rainfall of 5.2 mm (**Table 3.2-1**) when compared to SO2 (autumn) which recorded a monthly average rainfall of 24.2 mm (**Table 3.2-1**). The low rainfall recorded on SO1 (summer) may have resulted in water flow being mostly stagnant in the upper and central parts of the study area due to the low gradient of the site which may have resulted in some of contaminants being immobile and readily available for bioaccumulation. Statistical differences ($p<0.05$) in mean Cu concentrations were also recorded in the pairwise consecutive comparisons between SO1 (summer) (9.67±1.20) and SO2 (autumn) (2.34±1.73) as well as between SO3 (winter) (7.38±6.75) and SO4 (spring) (8.10±2.49) at site 3 (**Table**

9.1-1). SO1 (summer) recorded statistically higher concentrations when compared to the other sampling occasions at both sites 2 and 3 (**Table 9.1-1**). The low rainfall (5.2 mm) and slightly high salinities recorded at both sites 2 (15.63 ppt) and 3 (16.30 ppt) on SO1 (summer) (**Table 3.1-1**) may have contributed to the recorded concentrations in summer. However, the slowing down of metabolic processes of algae (i.e. growth) was expected to increase the uptake of metals during the winter period (Hou and Yan, 1998; Villares et al., 2002; Żbikowski et al., 2006). However, the winter period (SO3) in this study (at both sites 2 and 3) recorded lower concentrations when compared to SO1 (summer) (**Table 9.1-1**). Therefore, the above expectation in relation to higher uptake of metals due to slow metabolic processes was not realised in this study and this may have been due to high rainfall which decreases salinity and macroalgae growth in Zandvlei (Muhl, 2003), which ultimately affects bioaccumulation as there is less tissue available or may have been due to a combination (or the interaction) of the different factors that affect the bioaccumulation of metals in macroalgae (Vasconcelos and Leal, 2001; Szefer, 2002; Żbikowski et al., 2006).

CHAPTER 10
CONCLUSION AND RECOMMENDATIONS

10.1 CONCLUSION

The analysis of spatial and temporal variations in mean metal (Al, Mn, Fe, Zn, Pb, and Cu) concentrations found in water, sediment, and algae samples in the Zandvlei estuary revealed a range of variability in metal concentrations due to a combination of natural processes and anthropogenic activities. These variations appeared to be influenced by factors such as land uses, hydrodynamics, geology, climate, and industrial activities.

10.1.1. Water

The results show statistical differences for some of the sampling sites, particularly those located close to, and which are affected by, inter alia, fluvial discharges from the influent rivers, run-off from stormwater drainage outlets, estuary gradient (i.e. deeper channel), closing and opening of the estuary mouth as well as sediment characteristics (i.e. fine-grained sediment). Physicochemical parameters such as inter alia, the acidity level (i.e. pH), temperature, and salinity also influenced the spatial and temporal distribution of metals in the Zandvlei estuary. Weather conditions such as rainfall also played a key role in the transportation of metals from surrounding areas, including the nearby Industrial area. Changes in precipitation patterns and river flows influence the input of metals from catchment areas. Spatial variations in mean metal (Al, Mn, Fe, Zn, Pb, and Cu) concentrations could be attributed to decreased river and land-based discharges due to low rainfall as well as low levels of anthropogenic discharges. Another possible explanation is the process that involves the adsorption of metals (onto sediment) which are transported in dissolved or particulate form in aquatic systems (De Souza Machado et al., 2016). The above process removes metals from the overlying waters and could therefore limit the distribution and variation of metals in the water column. Comparisons of seasonal/temporal variations in mean metal concentrations showed little variation, some of which was due to elevated rainfall in SO2 (autumn) and SO3 (winter) which resulted in land-based discharges and stormwater run-off which increased metal concentration levels. Metal concentrations in water samples decreased in the following sequence: Al > Mn > Fe > Zn >Cu > Pb. The recorded metal (Al, Mn, Fe, Zn, Pb, and Cu) concentrations in water samples were relatively low with a few exceptions, however, mean concentrations recorded for Al, Zn, Fe, Pb, and Cu at some of the sampling sites exceeded one (or more) of the recommended water quality guidelines (**Table 10.1-1**) by institutions such as South Africa’s Department of Water Affairs and Forestry (1996), Canadian Council of Resource and Environment Ministers (1987), Canadian Council of Ministers of the Environment (2001), and Australian and New Zealand

Environment and Conservation Council (2000) (**Table 10.1-1**). The above could be an indication of metal pollution in the Zandvlei estuary as the recorded mean concentrations were above those that are required for the protection and maintenance of the aquatic systems. The source of pollutants appears to be from, inter alia, household products from the Zandvlei recreational area and surroundings, illegal dumping, sewer overflows from malfunctioning pump stations, as well as the upstream industrial area located adjacent to one of the influent rivers (i.e. Keysers River). The recorded Mn concentrations were well below the recommended water quality guideline concentrations by one (or more) of the abovementioned institutions (**Table 10.1-1**).

Table 10.1-1: Zandvlei estuary sites (in the current study) that have exceeded the recommended safe metal concentrations in freshwater as stipulated by the Department of Water Affairs and Forestry (DWAF, 1996), Australian and New Zealand Environment and Conservation Council (ANZECC, 2000), Canadian Council of Resource and Environment Ministers (CCREM, 1987), and Canadian Council of Ministers of the Environment (CCME, 2001).

Metals		DWAF (1996) Target Water Quality Range (TWQR) (mg/ℓ)	ANZECC (2000) Water quality guideline (mg/ℓ)	CCREM (1987) Water quality guideline (mg/ℓ)[1]	CCME (2001) Water quality guideline for the protection of aquatic life (mg/ℓ)	Zandvlei estuary sites that exceeded water quality guideline concentrations recommended by DWAF (1996)	Zandvlei estuary sites that exceeded water quality guideline concentrations recommended by ANZECC (2000)	Zandvlei estuary sites that exceeded water quality guideline concentrations recommended by CCREM (1987)	Zandvlei estuary sites that exceeded water quality guideline concentrations recommended by CCME (2001)
Al	pH <6.5	0.005	**NGV**	0.005	**NGV**	✓ Site 4 (SO2)	**N**	✓ Site 4 (SO2)	**N**
	pH >6.5	0.01	0.027[i]	0.1	**NGV**	✓ Sites 1 & 2 (SO1) ✓ Sites 1, 3 & 5 (SO2) ✓ Sites 1, 2, 3, 4 & 5 (SO3)	✓ Sites 1 & 2 (SO1) ✓ Sites 3, 4 & 5 (SO2) ✓ Sites 1, 2, 3 aNGV 4 (SO3)	✓ Sites 3 & 4 (SO2) ✓ Sites 1, 2 & 4 (SO3)	**N**
			0.055[ii]				✓ Sites 3 & 4 (SO2) ✓ Sites 1, 2, 3 & 4 (SO3)		
			0.08[iii]				✓ Sites 3 & 4 (SO2) ✓ Sites 1, 2 & 4 (SO3)		
			0.15[iv]				✓ Sites 3 & 4 (SO2) ✓ Sites 1, 2 & 4 (SO3)		
Mn		0.18	1.2	**NGV**	3.6[a]	**N**	**N**	**N**	**N**
					0.43[b]				**N**
Fe		**NGV**	0.3	0.3	**NGV**	**N**	✓ Sites 2 & 4 (SO3)	✓ Sites 2 & 4 (SO3)	**N**
Zn		0.036	0.0024[i]	**NGV**	0.037[c]	✓ Sites 1, 2, 3, 4 & 5 (SO1) ✓ Sites 1, 2, 3, 4 & 5 (SO2)	✓ Sites 1, 2, 3, 4 & 5 (SO1) ✓ Sites 1, 2, 3, 4 & 5 (SO2)	N	✓ Sites 1, 2, 3, 4 & 5 (SO1) ✓ Sites 1, 2, 3, 4 & 5 (SO2)

					✓Sites 1, 3, 4 & 5 (SO3)	✓Sites 1, 2, 3, 4 & 5 (SO3)		✓Sites 1, 3, 4 & 5 (SO3)
		0.008[ii]				✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 2, 3, 4 & 5 (SO3)		
		0.015[iii]		0.007[d]		✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 2, 3, 4 & 5 (SO3)		✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 2, 3, 4 & 5 (SO3)
		0.031[iv]				✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 3, 4 & 5 (SO3)		
Pb (A)	0.0002 – 0.0012	0.001[i]	0.001 – 0.007	**NGV**	✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 2, 4 & 5 (SO3)	✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 2, 4 & 5 (SO3)	✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 2, 4 & 5 (SO3)	**N**
		0.0034[ii]				**N**		
		0.0056[iii]				✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1 (SO2) ✓Sites 4 (SO3)		
		0.0094[iv]				✓Sites 1, 3, 4 & 5 (SO1) ✓Sites 1 (SO2) ✓Sites 4 (SO3)		
Cu (A)	0.0003 – 0.0014	0.001 – 0.0025	0.002 - 0.004	**NGV**	✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 2, 3, 4 & 5 (SO3)	✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 2, 3, 4 & 5 (SO3)	✓Sites 1, 2, 3, 4 & 5 (SO1) ✓Sites 1, 2, 3, 4 & 5 (SO2) ✓Sites 1, 2, 3, 4 & 5 (SO3)	**N**

[1] The Canadian Council of Ministers of the Environment (1999) water quality guidelines have been withdrawn
[i] 99% Level of species protection
[ii] 95% Level of species protection
[iii] 90% Level of species protection
[iv] 80% Level of species protection
[a] Short-term benchmark (CCME, 2019)
[b] Long-term guideline (CCME, 2019)
[c] Short-term exposure value (CCME, 2018)
[d] Long-term exposure value (CCME, 2018)
N= None (of the sites exceeded the guidelines)
NGV= No Guideline Value
(A) = subject to water hardness (as $CaCO_3$). If the water hardness is unknown, the lowest value is applicable.

10.1.2. Sediment

As expected, sediment samples recorded higher metal concentrations when compared to concentrations recorded in water samples. This is because most metals that are discharged into aquatic systems rapidly become associated with particulate matter and incorporated into sediments (Hanson et al., 1993; Mountouris et al., 2002; Ryan et al., 2012; Binning and Baird, 2001; Sodhi et al., 2022). Comparisons of spatial variations in mean metal concentrations showed fluctuating concentrations between the different sampling sites in each sampling occasion and this could be attributed to several factors such as, inter alia, sediment grain size, salinity, anthropogenic input, acidity level (pH), redox potential (Eh), and ionic strength (Davies et al., 1991). Elevated metal concentrations were largely found in fine-grained sediments between sites 1 and 2 (upstream) in almost all sampling occasions with a few exceptions. This is because fine sediments have a significant capacity to retain metals and as a result can adsorb an increased concentration of metals (Nduka and Orisakwe, 2011). Also, the elevated concentrations recorded at site 2 for some of the metals in sediment may be an indication of a local pollution source as the site is located next to a stormwater drainage outlet, a yacht club, and a parking area. Mean Zn, Pb, and Cu concentrations from one or more of the sampling sites exceeded the sediment quality guidelines by CCME (1999) and ANZEC (2000) (**Table 10.1-2**), while the majority of the sampling sites within the different occasions recorded concentrations were well below the abovementioned sediment quality guidelines (**Table 10.1-2**). Overall, the recorded concentrations in sediment were well below the recommended sediment quality guidelines (**Table 10.1-2**) with some exceptions and this could potentially be an indication of some level of contamination of the sediment and may require further analysis and mitigation to ensure that concentrations remain below the recommended safe quality guidelines. Metal concentrations in sediment decreased in the following sequence: Fe > Al > Zn > Pb > Mn >Cu. Interestingly, Zandvlei recorded the lowest mean concentration for Al, Fe, Zn, Pb, Mn, and Cu when compared to estuaries such as Mhlathuze and other estuaries. This may be because the other estuaries used for comparison are located around various industries. The contamination levels at the Zandvlei estuary require further monitoring and source identification to manage pollution and protect the estuary.

Table 10.1-2: Zandvlei estuary sites (in the current study) that have exceeded the recommended safe metal concentrations in sediment as stipulated by the Australian and New Zealand Environment and Conservation Council (ANZECC, 2000), and Canadian Council of Ministers of the Environment (CCME, 1999).

Metals	ANZECC (2000) sediment quality guidelines (mg/kg)	CCME (1999) sediment quality guidelines for the protection of aquatic life (mg/kg dry weight)	Zandvlei estuary sites that exceeded sediment quality guideline concentrations recommended by ANZECC (2000)	Zandvlei estuary sites that exceeded sediment quality guideline concentrations recommended by CCME (1999)
Al	**NGV**	**NGV**	**N**	**N**
Mn	**NGV**	**NGV**	**N**	**N**
Fe	**NGV**	**NGV**	**N**	**N**
Zn	200[a]	124[i]	✓ Sites 1, 2, 3, 4 & 5 (SO1)	✓ Sites 1, 2, 3, 4 & 5 (SO1)
	410[b]	271[ii]	✓ Sites 1, 2, 3 & 4 (SO1)	✓ Sites 1, 2, 3, 4 & 5 (SO1)
Pb	50[a]	30.2[i]	**N**	✓ Site 2 (SO2) ✓ Site 5 (SO4)
	220[b]	112[ii]	**N**	**N**
Cu	65[a]	18.7[i]	✓ Sites 1 (SO2)	✓ Sites 1 (SO2)
	270[b]	108[ii]	**N**	**N**

[i] Interim sediment quality guidelines (ISQGs) for metal in estuarine/marine environment

[ii] Probable effect levels (PELs) for metal in estuarine/marine environment

[a] Default guideline values (DGVs)

[b] Upper / higher guideline values (GV-high)

N= None (of the sites exceeded the guidelines)

NGV= No Guideline Value

10.1.3. Algae

The analysis of metal concentrations in macroalgae (*Enteromorpha* spp.) revealed relatively higher metal concentrations at many of the sampling sites when compared to concentrations recorded in sediment and water and this could be attributed to the fact that biomonitors such as *Enteromorpha* spp. reflect metal concentrations in surrounding waters as well as time-integrated bioavailable concentrations in both dissolved and solute phases (Żbikowski et al., 2006). This was further proven by the bioconcentration factors recorded for each metal in *Enteromorpha* spp. which showed widespread occurrence of microconcentration (1-2) and macroconcentration (>2) of each metal (i.e. in *Enteromorpha* spp. relative to both water and sediment concentrations). However, there were some instances of deconcentration (<1) for most metals (except Mn) in *Enteromorpha* spp. relative to sediment concentrations. No significant differences in mean Al, Fe, Pb, and Cu, concentrations were recorded between the different sampling sites in the winter and spring sampling occasions. The same were found for Zn in the spring sampling occasion. This can be attributed to the metabolic processes of macroalgae which decreases in winter (SO3) (i.e. low growth) and is enhanced in spring (SO4) to reach maximum biomass in summer (SO1) (Żbikowski et al., 2007). Therefore, limited algae biomass and a decrease in salinity due to high rainfall, particularly in winter resulted in a decrease in metal concentrations in some of the sampling sites in the study area. It is important to note that spatial and seasonal variations in metal concentrations found in macroalgae were affected by a range of factors (i.e. salinity, pH, metabolic processes, metals concentrations in water, the interaction between different metals, light intensity, etc.) and it is, therefore, difficult to indicate the main factor as there could be an interaction of different factors (Vasconcelos and Leal, 2001; Szefer, 2002; Żbikowski et al., 2006; Żbikowski et al., 2007), hence the high variability between the different sampling sites in each occasion from this study. Since there are no metal concentration guidelines for algae, the recorded metal concentrations were compared to other studies. Lead concentrations recorded between the different sampling sites in summer, autumn, and winter showed little variation and were below the moderate contamination range (20 – 60 mg/kg) established by Say et al. (1990). This therefore seems to suggest that the Zandvlei estuary was relatively uncontaminated with Pb during the time of data collection. The recorded mean Pb concentrations during the spring sampling were within the moderate contamination range (20 – 60 mg/kg) as defined by Say et al. (1990). This could be an indication of some pollution and may therefore require further monitoring and assessment. Mean Cu concentrations in algae were mostly within the uncontaminated range of between 6 to 12 mg/kg as recommended by Brown et al. (1999). Aluminium and Fe concentrations were relatively high and may need further monitoring and assessment. Metal concentrations in *Enteromorpha* spp. decreased in the following sequence: Fe > Al > Mn > Zn > Pb > Cu.

The results of this study show that spring and summer recorded some of the highest metal concentrations in algae. Some of these elevated metal concentrations in algae were recorded at sampling sites located towards the estuary mouth (sites 3 to 5) where there are relatively higher salinities, whilst some were recorded in sampling sites located towards the influent rivers upstream (sites 1 to 2), where there are relatively lower salinities and possibly higher anthropogenic inputs due to stormwater outlets and river flow. Water circulation, salinity, and algae biomass, among other factors in the Zandvlei estuary appear to have played a significant role in the bioaccumulation of metals by *Enteromorpha* spp.

10.2. RECOMMENDATIONS

- Additional monitoring and analysis of the bioavailable metal contaminants in macroalgae and other suitable biomonitor organisms in the Zandvlei estuary are required to further assess the extent and impact of metal pollution within the system. This will also assist with managing urban pollution and possibly further identify sources of metal contamination.
- Monitoring and assessment of metal contaminants in the influent rivers (i.e. Keysers, Westlake, and Sand/Diep Rivers) is also required to determine the extent of metal contamination and provide mitigation measures for the protection of the estuary.
- The assessment and analysis of metal contaminants in stormwater run-off entering the estuary should also be taken into account as this seems to be a problem area that requires attention.

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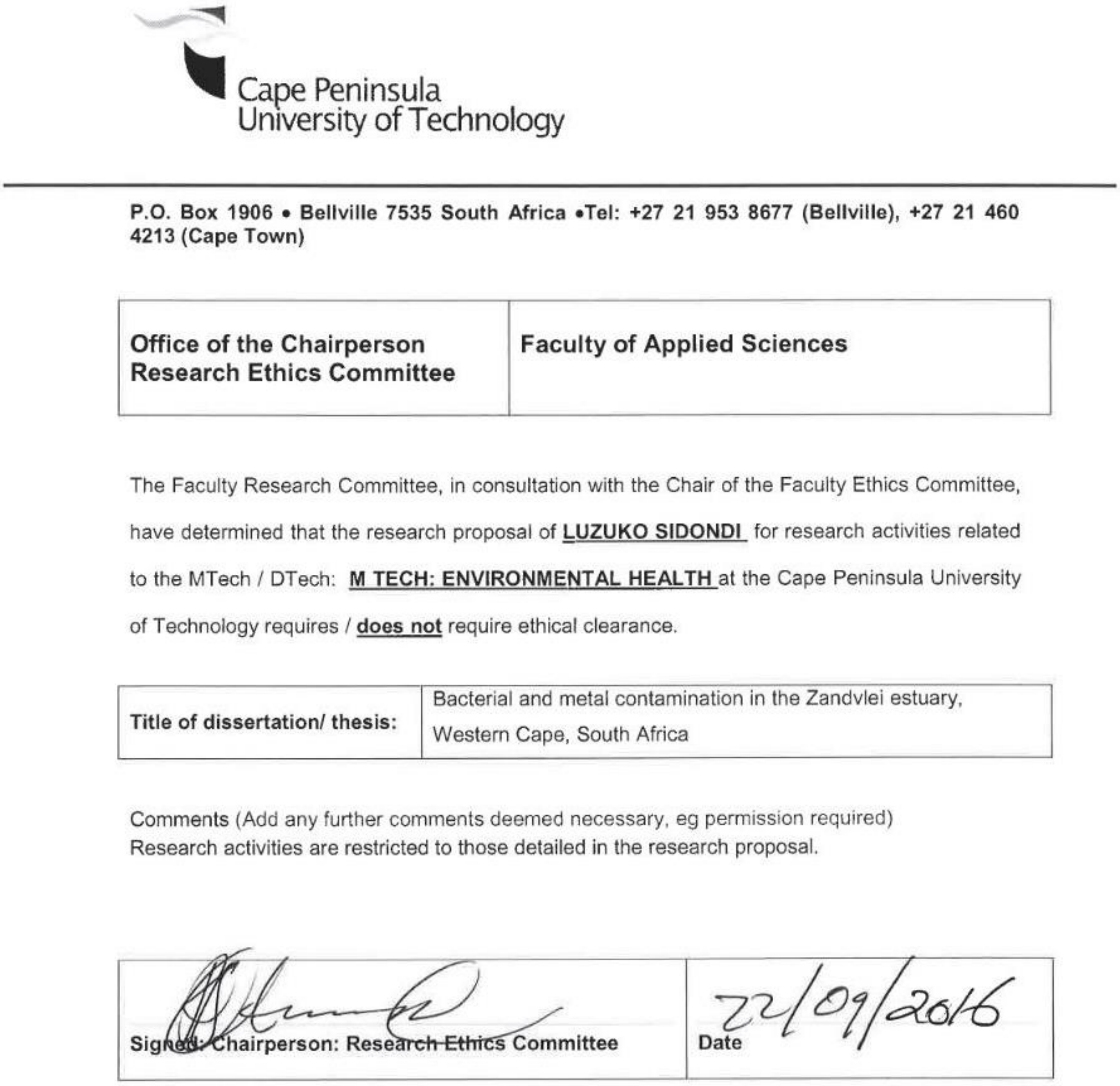
APPENDIX A:

ETHICS APPROVAL LETTER



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 **LUZUKO SIDONDI** for research activities related to the MTech / DTech: **M TECH: ENVIRONMENTAL HEALTH** at the Cape Peninsula University of Technology requires / **does not** require ethical clearance.

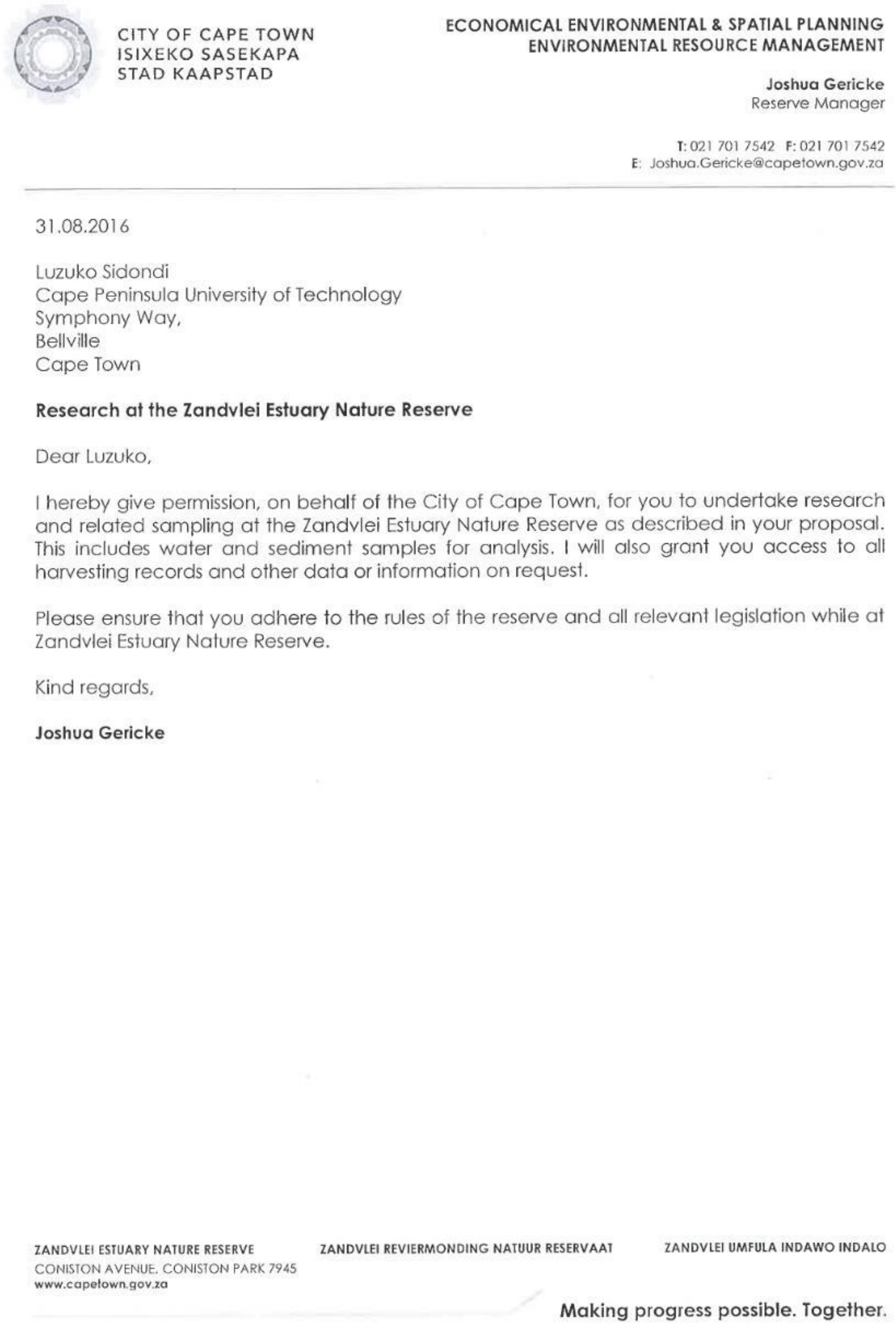
Title of dissertation/ thesis:	Bacterial and metal contamination in the Zandvlei estuary, Western Cape, South Africa

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	**Date** 22/09/2016

APPENDIX B:

SAMPLING PERMIT FROM COCT



CITY OF CAPE TOWN
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ECONOMICAL ENVIRONMENTAL & SPATIAL PLANNING
ENVIRONMENTAL RESOURCE MANAGEMENT

Joshua Gericke
Reserve Manager

T: 021 701 7542 F: 021 701 7542
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31.08.2016

Luzuko Sidondi
Cape Peninsula University of Technology
Symphony Way,
Bellville
Cape Town

Research at the Zandvlei Estuary Nature Reserve

Dear Luzuko,

I hereby give permission, on behalf of the City of Cape Town, for you to undertake research and related sampling at the Zandvlei Estuary Nature Reserve as described in your proposal. This includes water and sediment samples for analysis. I will also grant you access to all harvesting records and other data or information on request.

Please ensure that you adhere to the rules of the reserve and all relevant legislation while at Zandvlei Estuary Nature Reserve.

Kind regards,

Joshua Gericke

ZANDVLEI ESTUARY NATURE RESERVE **ZANDVLEI REVIERMONDING NATUUR RESERVAAT** **ZANDVLEI UMFULA INDAWO INDALO**
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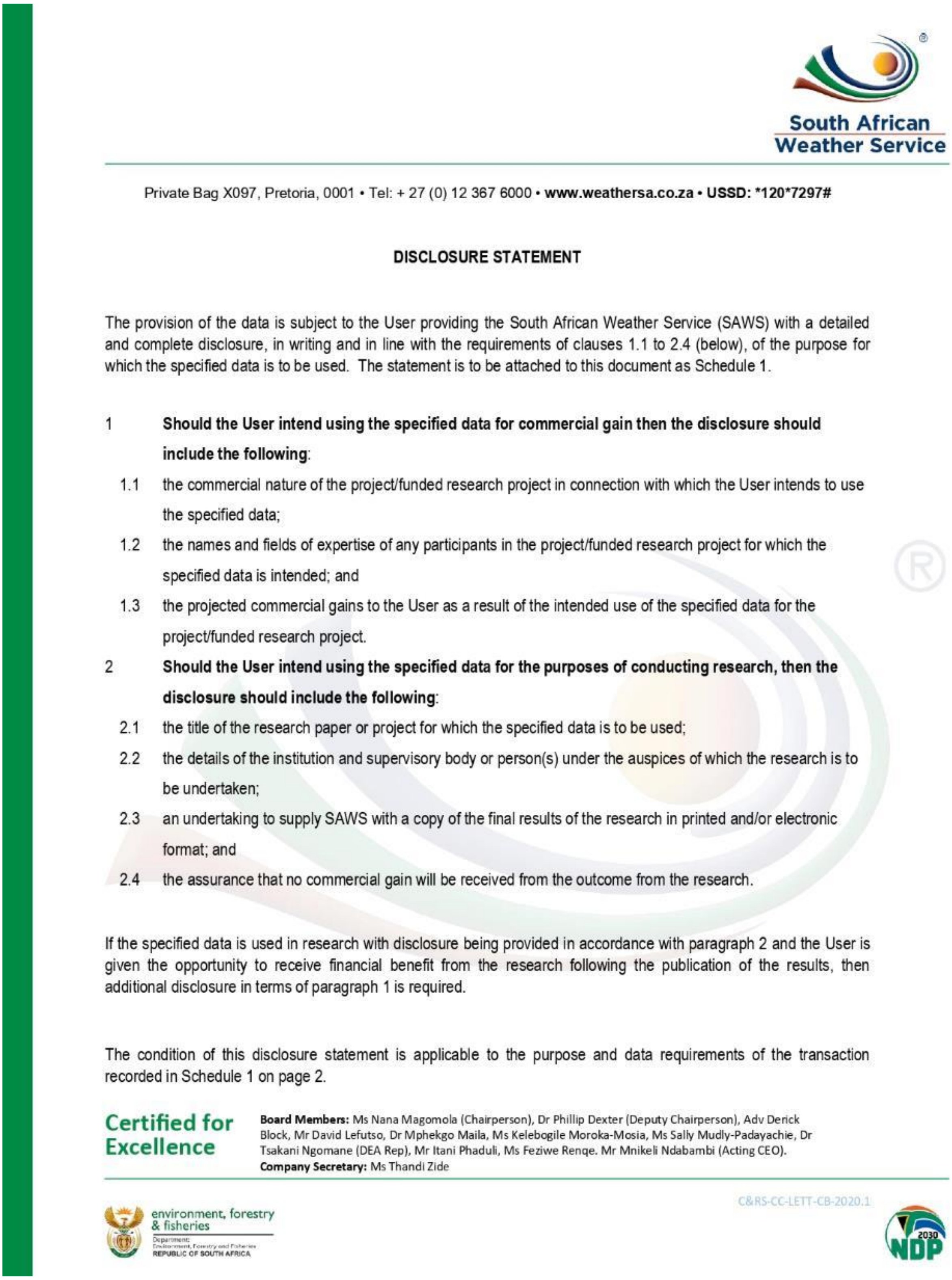
APPENDIX C:

SAWS DISCLOSURE STATEMENT FOR RAINFALL DATA



South African
Weather Service

Private Bag X097, Pretoria, 0001 • Tel: + 27 (0) 12 367 6000 • **www.weathersa.co.za • USSD: *120*7297#**

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.

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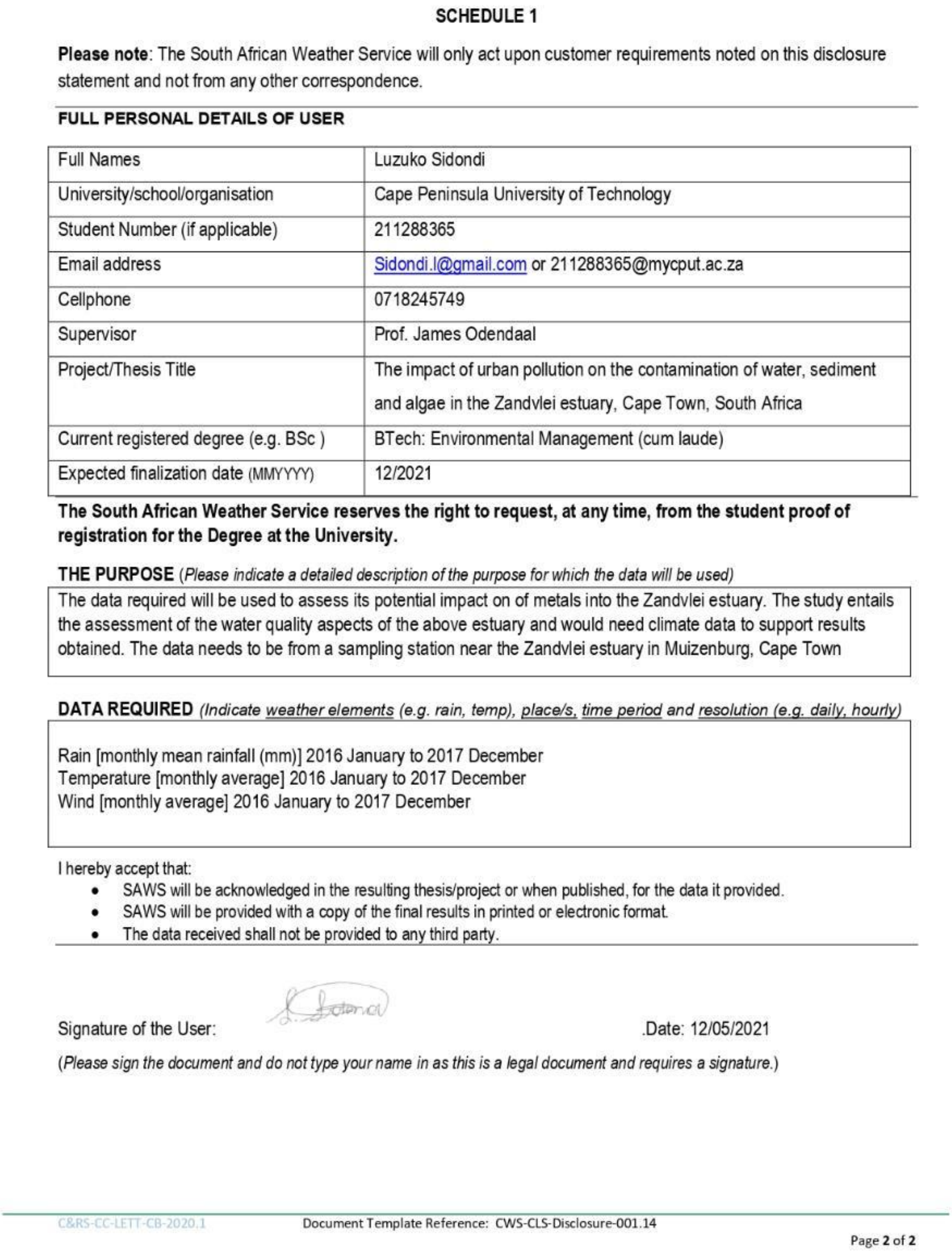

SCHEDULE 1

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	Luzuko Sidondi
University/school/organisation	Cape Peninsula University of Technology
Student Number (if applicable)	211288365
Email address	Sidondi.l@gmail.com or 211288365@mycput.ac.za
Cellphone	0718245749
Supervisor	Prof. James Odendaal
Project/Thesis Title	The impact of urban pollution on the contamination of water, sediment and algae in the Zandvlei estuary, Cape Town, South Africa
Current registered degree (e.g. BSc)	BTech: Environmental Management (cum laude)
Expected finalization date (MMYYYY)	12/2021

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 required will be used to assess its potential impact on of metals into the Zandvlei estuary. The study entails the assessment of the water quality aspects of the above estuary and would need climate data to support results obtained. The data needs to be from a sampling station near the Zandvlei estuary in Muizenburg, Cape Town

DATA REQUIRED *(Indicate weather elements (e.g. rain, temp), place/s, time period and resolution (e.g. daily, hourly)*

Rain [monthly mean rainfall (mm)] 2016 January to 2017 December
Temperature [monthly average] 2016 January to 2017 December
Wind [monthly average] 2016 January to 2017 December

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.

Signature of the User: .Date: 12/05/2021

(Please sign the document and do not type your name in as this is a legal document and requires a signature.)

C&RS-CC-LETT-CB-2020.1 Document Template Reference: CWS-CLS-Disclosure-001.14

Page **2** of **2**