A comparative study of the effect of anthropogenic activity on the Palmiet and Sezela Rivers, South Africa

by

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As the candidate's supervisor I have approved this dissertation for submission.

Signature: _____   Name: Dr. Letitia Pillay   Date: _____

Preface

I hereby declare that the work conducted in this research effort and presented in this thesis, is my own work conducted from July 2014 to April 2016, at the Analytical Research laboratories located at the University of KwaZulu-Natal, Westville Campus, Durban, under the supervision of Dr. L. Pillay.

This work has not been submitted at this or any other university or tertiary institution. Where the works of others have been used, it has been duly recognized in this text.

Declaration

I, Shaeen Chetty, declare that:

1.	The research reported in this thesis, except where otherwise indicated, and is my own work

2.	This thesis has not been submitted for any degree or examination at any other university or tertiary institution

3.	This thesis does not contain any other person's data, pictures, graphs, or any other information unless specifically acknowledged as being sourced from other persons

4.	This thesis does not contain other person's writing unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:

	(a)	Their words have been re-written but the general information attributed to them has been referenced

	(b)	Where exact words have been used, then their writing has been placed in italics and inside quotation marks, and referenced

5.	This thesis does not contain text, graphics or tables copied and pasted from the internet, unless specifically acknowledged, and the source being detailed in the thesis and in the Reference sections

Signed

_____

Shaeen Chetty

Conference Contributions

1. Postgraduate Research Day 2015, Pietermaritzburg, South Africa: poster presentation on "A comparative study of the physico-chemical characteristics of the Palmiet and Sezela Rivers, KwaZulu-Natal "

Acknowledgements

"We must find time to stop and thank the people who make a difference in our lives."
— John F. Kennedy

Dedication

॥ माता पिता गुरु दैवम् ॥

mātā pitā guru daivam

I dedicate this work to my parents, my teacher, and God.

Abstract

The Palmiet and Sezela Rivers, both located in KwaZulu-Natal, South Africa, are two rivers that have distinctively different anthropogenic activities. The Palmiet River is typically influenced by industrial activity whereas farming and agriculture are the predominant activities along the Sezela River. Total metal concentrations in water and sediment and bioavailable metal concentration in sediment for the different metals (Cr, Cu, Bi, Pb and Zn) as a function of seasonal variations as well as anions (NO_3^-, SO_4^{2-}, PO_4^{3-}) in water were measured to determine the effect of different anthropogenic activities. Metal concentrations were quantified using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and anions were quantified using ion chromatography (IC).

Seasonal analysis of the total metal concentration in sediment and water showed statistically significant differences for both rivers (ANOVA, $p > 0.05$). The Palmiet and Sezela Rivers had higher concentrations of metal pollutants in sediment in winter and spring, respectively. Seasons with lowest and highest rainfall corresponded with highest and lowest metal concentrations, respectively. Metal concentrations in water were compared to the South African Water Quality Guidelines. The Palmiet River water exceeded permissible limits for Cr, Ni and Pb particularly in the industrial area. Water in the Sezela River exceeded the permissible limits for Pb. Anion concentrations were predominantly higher in winter for both rivers. Nitrate concentration exceeded guideline limits in both rivers while sulfate concentration was higher in the Sezela River. Sezela River had higher overall anion concentrations than the Palmiet River which is attributed to continuous fertiliser use. Both rivers showed no seasonal variation in pH and all pH values were within the permissible limits. The EC and TDS were higher in the Sezela River, particularly in summer and autumn, which was attributed to higher rainfall that lead to nutrient run-off into the river.

Total metal concentrations for both rivers were compared to the Effects Range Median (ERM). All metals were greater than the ERM in the Palmiet River while only Ni exceeded this limit in the Sezela River. Palmiet River had higher overall metal concentrations than Sezela River which is attributed to the industrial input on the Palmiet River. Bioavailable

metals were determined using the BCR sequential extraction method. Sezela River had metals predominantly unavailable for uptake in the sediment. Palmiet River had bioavailable Cu, Ni and Zn within the sediment. Metal toxicity is therefore of concern in Palmiet River due to high metal concentrations and readily bioavailable metals. The data shows the type of anthropogenic pollutants in each river system.

Abbreviations

ANOVA	Analysis of Variance
CRM	Certified Reference Material
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
EC	Electrical Conductivity
ERL	Effects Range Low
ERM	Effects Range Median
SAWQG	South African Water Quality Guidelines
TDS	Total Dissolved Solids
WHO	World Health Organisation

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Chapter 1

Introduction

Water is the crucial element of social and economic infrastructure and is a key component for sustainable development (Abhineet and Dohare 2014).Water resources worldwide have multiple uses; essentially a source of fresh drinking water, agricultural uses, and energy production (Anyanwu 2012). With the rapid increase in population growth and the need for water as a basic requirement, water resources are becoming scarce (Singh *et al.* 2014). Fresh water contributes to 2.50% of the earth's water (UNEP 2008). Only 0.30% of this water is surface water that is consumed and utilised. Rivers are the primary source of fresh water and are created by the melting of mountain snow and ground water.

In many third world countries, including South Africa, locals depend on river water for their daily needs. The consumption is hindered by lack and pollution of the resource. The degradation of fresh water systems is increasing with an influx of pollutants being introduced into the system by anthropogenic activities (Pheiffer *et al.* 2014).

1.1. Problem Statement

Life on earth is dependent on water which is an essential resource. The demand for this resource has been stressed in the urban, industrial and agricultural sectors. Rivers have become disposal sites for pollutants that these sectors discard. Climate change may also have a direct or indirect link to water consumption.

The sub-tropical climate of South Africa affects the temperature and weather of the country. South Africa does not have four distinct seasons but rather two transitional seasons, autumn and spring, which last for about two months each, and two main seasons, summer and winter, which last for four months each. Winter experiences a lower rainfall than the summer

season. The rainfall in KwaZulu-Natal is strongly seasonally driven with the province experiencing 80% of the rainfall between October and March (WRC 2002).

Rainfall can affect a river system by diluting and dispersing constituents in the system (Klerk *et al.* 2012, Rahman *et al.* 2012). This would change the actual concentration of an analyte within the system. Run-off is another process that occurs in river systems. Pollutants are transported into the system *via* run-off and increase the concentration of pollutants. This would primarily occur in agricultural regions where the use of vast amounts of fertilisers on fields is common. Heavy rains would wash these chemicals into the river and increase the concentration within the river system (Klerk *et al.* 2012). Industrial pollution is another type of anthropogenic pollution which generally have a high localised concentration of pollutants near the outlets of factories. Heavy rainfall will dilute and disperse these pollutants downstream of the outlet making concentrations appear low.

Anthropogenic activities contribute to the decline in environmental systems. The analysis of water and surface sediment within river systems would provide an insight into the impact that these activities have on the rivers.

1.2. Aim

This study intends to identify the possible sources of pollution along the Palmiet and Sezela Rivers by assessing the water quality using chromatographic and spectroscopic techniques.

1.3.   Specific objectives of the study

- ➢ To identify and select suitable sampling points along the Palmiet and Sezela Rivers
- ➢ To measure the total concentrations of Cr, Cu, Pb, Ni and Zn in the water using the ICP-OES and anion concentration (NO_3^-, SO_4^{2-}, PO_4^{3-}) using IC.
- ➢ To determine the bioavailability of the metals in sediment and to establish toxicity is of concern using microwave assisted digestion, sequential extraction techniques, and the ICP-OES
- ➢ To determine and compare the impact of industrial and agricultural activity on rivers
- ➢ To determine if the river water quality conforms to the South African Water Quality Guidelines by measuring water guideline parameters including pH, electrical conductivity, and total dissolved solids.
- ➢ To investigate seasonal variation of metals in the river system

1.4. References

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Chapter 2

Study Area

Different sources of anthropogenic influence affect the physico-chemical composition of both rivers. The Palmiet River is noted for the industrial input as well as residential input and is classified as an industrial-residential river. The Sezela River predominantly flows through agricultural fields and through rural areas and is classified as an agricultural river.

Sample sites were selected with consideration to spatial distribution and anthropogenic activity. Both rivers were sampled at the source, upstream and downstream of industrial and/or agricultural activity and upstream and downstream of informal settlements.

2.1. Palmiet River

The Palmiet River, northwest of Durban, Republic of South Africa, is a 26 km tributary of the Umgeni River. The Umgeni River is a major river in KwaZulu-Natal which is monitored by Umgeni Water and eThekwini Municipality. It passes through four of the major dams in KwaZulu-Natal which supplies fresh water to the province. The Palmiet River is a relatively small drainage catchment area of approximately 37 km^2 (du Preez and De Villiers 1986). The sedimentation of the study area is primarily Natal red-brown sandstone, granites and shale (WRC 2002).

The river has been fully urbanised over the years with the exception of a 6 km nature reserve (Naidoo 2005). The river rises from an urbanised area northwest of Durban, Kloof (Sample point 1), and flows through the Pinetown-New Germany industrial area (Sample points 2-5). It is in this region of the river where pollutants enter the river due to the plastic, metal, and chemical manufacturing and processing industries. The river then flows through Westville, a residential area, and into the key drainage system, the Palmiet Nature Reserve. The river flows through the residential suburb of Clare Estate. Along the river, from this point, people

in informal settlements utilise the water for their daily requirements. The Palmiet River finally drains into the Umgeni River.
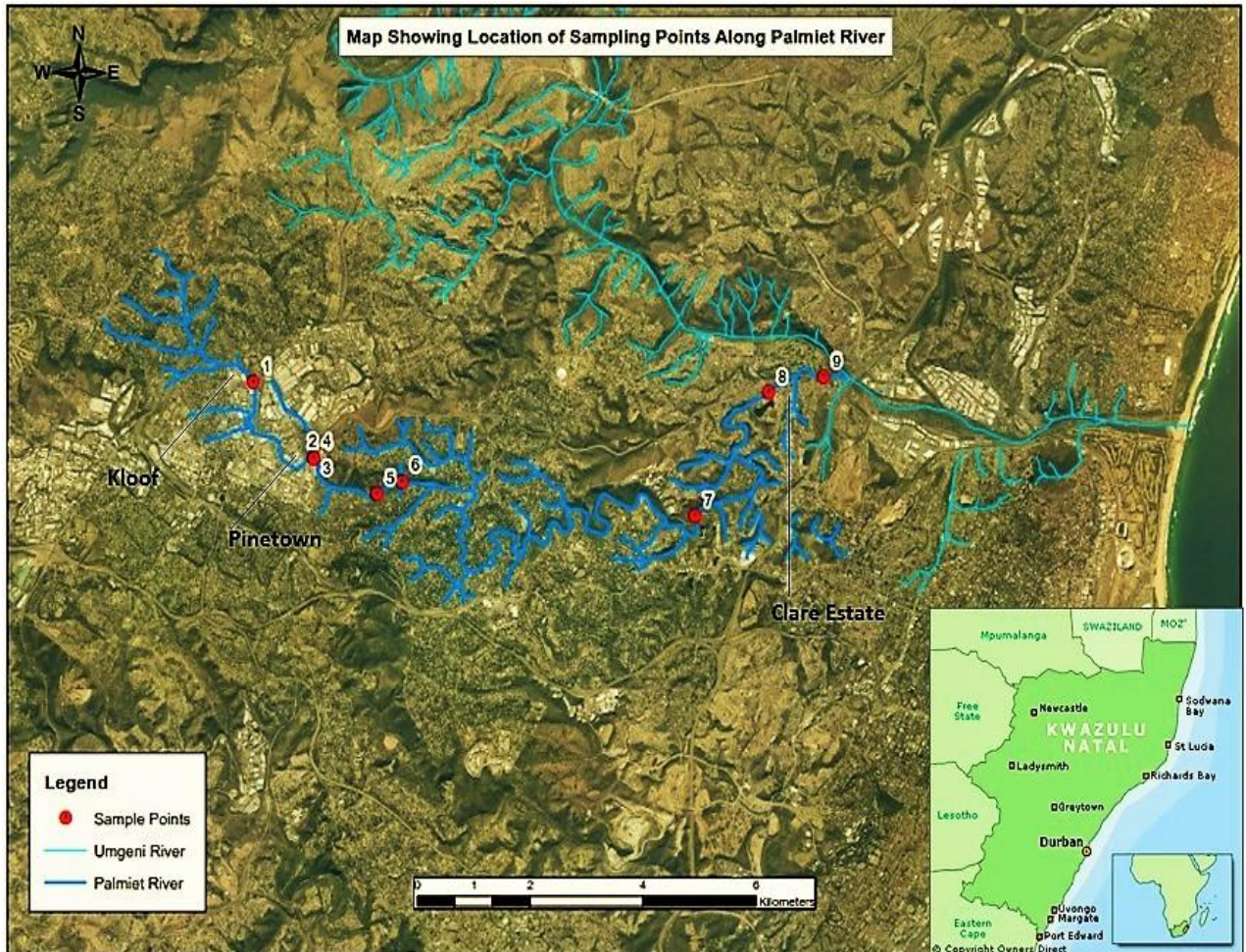
Figure 2.1.: Locations of the sampling stations along the Palmiet River

Table 2.1: Palmiet River sampling sites-description and location in decimal degrees.

Site	Site Description	Latitude	Longitude
1	Dense vegetation is present on the riparian zones with a textile industry on one of the banks.	-29.803933	30.870568
2	An industrial effluent pipe from the Pinetown industrial area that was fed into the river was visible and the sediment in this area was orange in colour. This effluent fed into the river.	-29.814572	30.881639
3	This was a branch of the river that merged with the effluent arm (site 2) of the river. The river bed was mostly covered in gravel and this flow was from the Pinetown industrial district.	-29.814598	30.881624
4	The two branches of sites 2 and 3 merged into one continuous river.	-29.814626	30.881676
5	One of the banks at this site had dense mangroves whilst the other was a residential area of Pinetown.	-29.819762	30.893227
6	This site passes through the Westville residential area and signs of dumping were noted.	-29.817918	30.897869
7	A sample was collected from the residential area of Clare Estate. It was observed that there was dumping of construction material and refuse at this site.	-29.815765	30.961806
8	Dense vegetation covers the banks of this site until it reaches the informal settlement. The river at this point flows under a bridge that leads to the University of KwaZulu-Natal, Westville Campus. This is a high traffic zone.	-29.805474	30.965139
9	Dumping of refuse was noted at this site which is approximately 400 m from the Palmiet River mouth.	-29.803184	30.975161

2.2. Sezela River

The Sezela area is a small town which is located south west of Durban, KwaZulu-Natal. The area is predominantly used for agricultural purposes including sugarcane cultivation and livestock rearing. There are primarily low cost housing and informal dwellings in the area particularly along the river. The Sezela River emerges 13 km from the coast and predominantly flows through sugarcane fields.

The river flows through the cane fields and towards the Sezela Sugar Mill located on the coastline. Invasive water plants including water hyacinth, water lettuce and water ferns were observed in abundance between the start and end of the mill area.
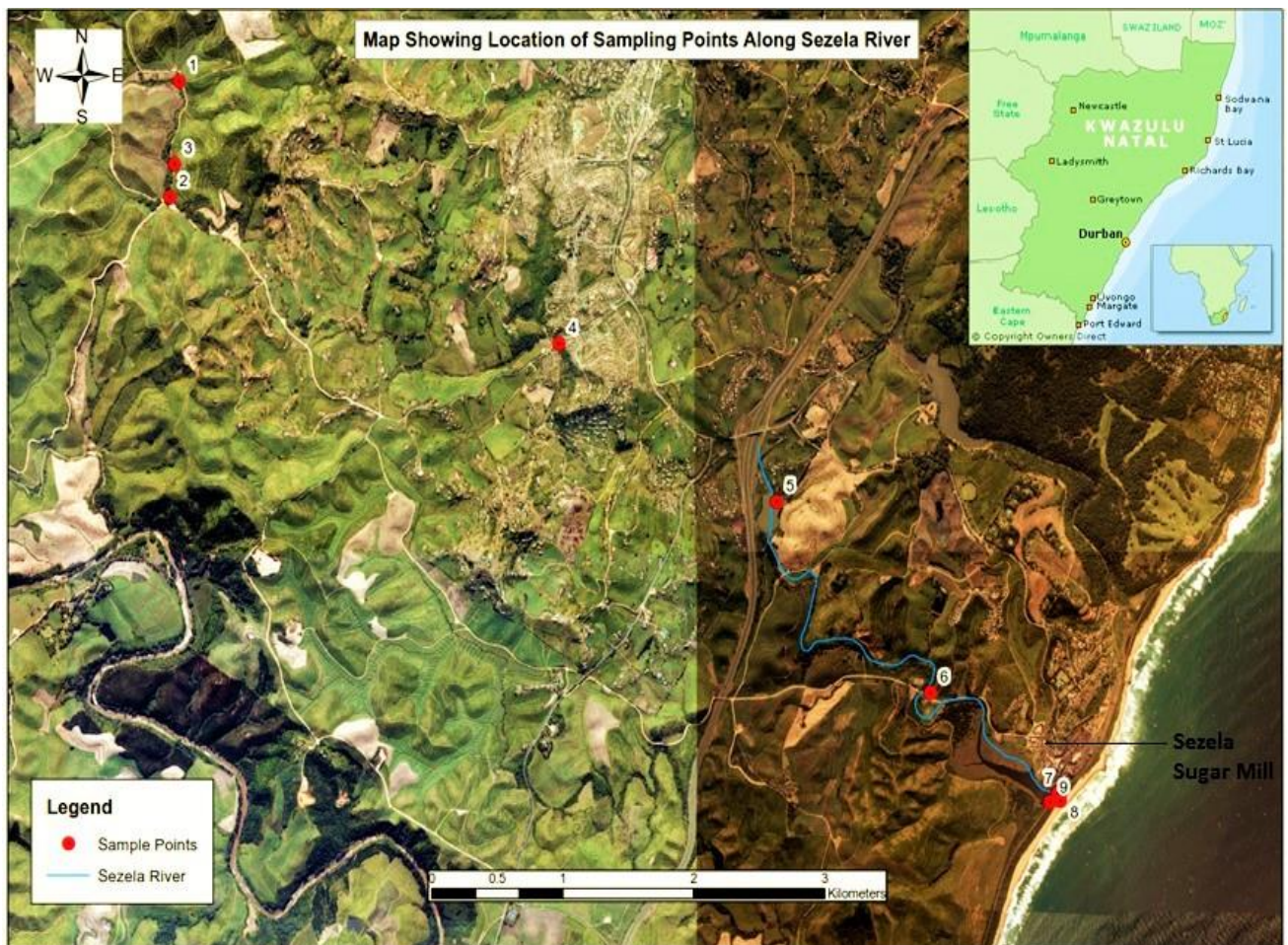
Figure 2.2: Sample station location along the Sezela River.

Table 2.2: Sezela River sampling sites-description and location in decimal degrees.

Site	Site Description	Latitude	Longitude
1	The river source is found in the middle of a sugarcane field and flows through the cane fields amidst dense vegetation.	-30.368395	30.607417
2	This site is on one side of a man-made bridge which is used as an access by heavy vehicles.	-30.375778	30.606641
3	This site had orange sediment with indications of oil or fuel slicks.	-30.37375	30.607024
4	The river then flows through rural area of Uswani where the residents utilise the water for domestic purposes.	-30.385271	30.63757
5	The river runs under the N2 highway into Sezela Dam where the residents use the water for domestic and recreational purposes.	-30.395384	30.654811
6	This site was before the Sezela sugar-mill and the invasive water plants are present.	-30.407615	30.667011
7	Effluent pipes and concrete slabs are present at this site. The concrete slabs stabilize the banks of the estuary. A railway line runs across the estuary area.	-30.414107	30.677112
8	This site opens to the Indian ocean during tide changes. It consists typically of course sea-sand. The water in this area had a brownish colour and the river mouth was closed during sampling.	-30.414596	30.677348
9	Plants including water hyacinth, water lettuce and water ferns were observed at this site.	-30.414668	30.676486

2.3. References

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Chapter 3

Theory

3.1. Pollutants

Pollution is the introduction of foreign substances that cause adverse effects to an ecosystem (Owa 2013). Discharge of pollutants into the atmosphere and water is a daily operation in industry. Although laws and limits of permissible contaminants have been standardised nationally and globally, industrial discharge above permissible levels continue. The manufacturing of processed, consumable goods promotes the increased activity of industries. Pollutants can be organic or inorganic in nature and can either exterminate organisms or accumulate in organisms. Organisms that spend a greater part of their life-cycle along riparian zones tend to accumulate pollutants which have an adverse effect not only on the organisms but to the food chain that they supply.

3.2. Inorganic Pollutants

Inorganic pollutants are defined as substances of a mineral origin and that are not composed of carbon except carbon oxides (Brandon 2012). Inorganic pollutants are classified as metallic or oxides. Heavy metals are common inorganic pollutants however oxides can be included. Oxides of nitrogen, phosphorus and sulfur are of particular concern as anions tend to form and change the chemistry of systems (Mortvedt 1996). Nitrates and ammonium ions are very mobile in systems compared to sulfates and phosphates (Maghanga *et al.* 2013). Nitrates leach heavy metals and increase metal mobility in systems. These anions are primary constituents of inorganic fertilisers. Commercial fertilisers contain heavy metal contaminants which enter the ecosystem upon fertilisation of agricultural soil (Mortvedt 1996).

3.3. Heavy Metals

A heavy metal may be any transition metal or metalloid that has a relatively high density and is associated with contamination, potential toxicity and ecotoxicity even at low concentrations (Street 2008, Lenntech 2016).

The earth's crust is the principal source of heavy metals which have been used for a range of aspects from industrial, commercial to agricultural (Järup 2003). The most common is the motor and mechanics manufacturing industry which uses metals including Zn, Cu, Cr and Ni, amongst other metals, for consumer production (Schneider *et al.* 2014).

The production of heavy metals has increased since the 19th century with a resultant increase in heavy metal emissions (Järup 2003). Climatic and geographical conditions promote the dispersion of the emitted metal species. Once these species settle, plants take up these metals from soil and water and translocate them through the food web. Animals and humans ingest these elements *via* food and water and elevated levels may have negative effects on metabolic activities (Singh *et al.* 2014).

Despite having potential toxicity to humans, heavy metals may be required in trace quantities for structural, physiological, catalytic or hormonal functions (Tchounwou *et al.* 2012). At higher concentrations some are more toxic than others and thus pose a significant problem in that metals do not biodegrade and can cause major damage to internal tissue (Street 2008). Metabolic and physiological pathways are adversely affected by the accumulation of metals in both humans and wildlife (Pheiffer *et al.* 2014). Carcinogenesis is the core effect of heavy metals including Pb, which is toxic and, Cu, Zn, Cr, and Ni which are toxic at elevated levels (Pheiffer *et al.* 2014, Singh *et al.* 2014).

3.3.1. Chromium

Chromium in the metallic form is a white, hard, brittle, lustrous metal that dissolves easily in non-oxidizing mineral acids (Shupack 1991). Chromium in both its metallic and salt form are utilized in the paint and pigment, tanning and textile, wood preservative, catalyst, stainless steel, fungicide, chrome alloy and plating industries (Shupack 1991, WHO 1996, Swarnalatha and Radhakrishnan 2015). Chromium has oxidation states from (II) to (VI), of which the (III) and (VI) states are of particular importance (Shupack 1991, Elangovan *et al.* 2015). Trivalent chromium is the more stable state in water and is generally found in complex form with hydroxyl counter-ions (Shadreck and Mugadza 2013). Chromium(III) and Cr(VI) are contrasted in their functions in the human body; Cr(III) is an essential element in human nutrition involved in glucose metabolism while Cr(VI) has been identified as a carcinogenic substance to both animals and man (WHO 1996). The distribution of trivalent and hexavalent chromium in sediment and water is dependent on the redox potential, pH, solubility of compounds, and kinetics of the compounds (Shupack 1991, Oliveira 2012). Trivalent chromium is predominant in sediment as Cr(VI) (the more toxic form) is easily reduced by organic matter in sediment (Shupack 1991). Chromium concentrations in surface water indicates the extent of anthropogenic activity with most surface waters containing 0.1 ppm Cr, naturally (WHO 1996, Elangovan *et al.* 2015).

3.3.2. Copper

Copper metal is a malleable, ductile and an excellent thermal and electrical conductor which is found in electrical wiring, pipes, building materials, fittings and valves, amongst other materials (WHO 2004). Copper compounds are also used in fungicides, algicides and insecticides, and is added to fertilisers to support plant growth (WHO 2004, Okocha and Adedeji 2012, Nel 2014). Copper has two oxidation states, Cu(I) and Cu(II). In water, Cu(II) is more common (WHO 2004, Giacalone *et al.* 2005). Copper in water adsorbs strongly to clay minerals as well as organic solids but this is pH dependent (WHO 2004, Nel 2014). Copper is an essential element and toxicity increases at concentrations greater than 2 mg L^{-1} (Nel 2014). Ingestion of Cu(II) causes gastrointestinal bleeding and renal failure amongst other complications (WHO 2004). Fate of copper in the environment, like all metals, is

dependent on the pH and presence of oxidising agents (WHO 2004). Copper has a tendency of accumulating in aquatic organisms, particularly bivalves (Nel 2014).

3.3.3. Lead

Lead is present in the earth's crust in relatively small amounts (13 to 16 mg kg^{-1}) as a bluish-grey metal (Botes 2003, Tchounwou *et al.* 2012). Lead is a poor conductor of with only two oxidation states, lead(II) and lead(IV) (Botes 2003). Lead has been used in industrial, agricultural and domestic applications including lead-acid batteries, pipes, paints and pigments, and X-ray shields (Botes 2003, Tchounwou *et al.* 2012). Lead released into the atmosphere settles in soil and water. The mobility of lead in sediment is related to the sediment pH where the higher the pH, the more labile the Pb complex (Botes 2003). Lead is a well-studied carcinogenic, teratogenic and genetic disruptor which is toxic and affects the gastrointestinal tract and other endocrine organs (Botes 2003).

3.3.4. Nickel

Nickel is a hard, silver-white metal with five oxidation states ranging from (0) to (V). The most common and stable states are Ni(0) and Ni(II) (Botes 2003). Nickel is utilised in the manufacturing of alloys, batteries and electronic components (Botes 2003, Iyaka 2011). The main sources of Ni pollution comes from Ni refining and phosphate fertilizers (Botes 2003). The mobility of Ni in natural systems increases when the pH of the system decreases (Botes 2003). Nickel is mobile in plants which gives it a high potential to enter the food chain (Iyaka 2011). It has been reported that Ni may accumulate in plants including cabbage, cauliflower, turnip and leguminosae (Iyaka 2011). In humans, Ni is an important micronutrient which functions as a metalloenzyme and aids in the adsorption of Fe in the blood (Avenant-Oldewage and Marx 2000). The toxicity of Ni is dependent on physical parameters but also the route of exposure and the solubility of the compound (Harasim and Filipek 2015). Nickel has the ability to exchange with divalent ions and enables incorporation in physiological functions and can cause cancer (Cempel and Nikel 2006).

3.3.5. Zinc

Zinc is a soft, bluish-white metal which is mainly found in a Zn(II) ionic state (Botes 2003). Zinc is commonly used in galvanising, paint pigments, fertilizers, batteries, and in cement (Botes 2003, Nel 2014, Swarnalatha and Radhakrishnan 2015). It is a highly mobile metal in acidic soils and under alkaline conditions, it is found in the hydroxide form as a precipitate (Botes 2003). Zinc has an affinity for organic matter and forms organo-metallic complexes which are less soluble (Botes 2003). Zinc pollution arises mainly from industrial wastes and over fertilization (Botes 2003, International Zinc Association 2014). It is commonly used in fertilizers as more than 50% of the agricultural soil worldwide is Zn deficient (International Zinc Association 2014). Zinc is an essential element to both plants and animals and is utilized in enzymatic activities (Lu *et al.* 2004, Plum *et al.* 2010). Unlike other metals, Zn has low toxicity and poisoning causes gastrointestinal problems and may induce vomiting and diarrhoea (Botes 2003, Plum *et al.* 2010).

3.4. Water Quality

3.4.1. Water Quality Parameters

Water quality may be defined as the biological, chemical and physical characteristics of water (Abhineet and Dohare 2014, Plessis *et al.* 2014). Physical parameters refer to physical properties of water including odour, taste and temperature. Chemical parameters refer to the chemical composition of solutes in the water. The biological parameters include identification and quantification of pathogens and micro-organisms present in water (Plessis *et al.* 2014). Water quality parameters are dependent on the intended use of the water. Drinking water parameters include low concentrations of dissolved metals, no algal growth or pathogens, no odour or colour whereas industrial water has less stringent guidelines (DWAF 1996).

The key water quality parameters are alkalinity, electrical conductivity, total dissolved solids, odour, anions, chemical and biological oxygen demand. Some key parameters are discussed below:

❖ Alkalinity refers to the pH of the water. A neutral pH is ideal to sustain aquatic life in water (Jayalakshmi *et al.* 2011). Most natural waters are alkaline in nature due to dissolved atmospheric carbon dioxide forming carbonates and bicarbonates (Abhineet and Dohare 2014). Biochemical activities including photosynthesis can deplete the carbon dioxide levels in water and decrease the pH of the system (Abhineet and Dohare 2014). At a lower pH, metal ions are soluble and mobile in water. Most metals at a higher pH precipitate out of solution into the sediment.

❖ Electrical conductivity (EC) is the capacity of water to conduct a current caused by electrolytes (Jayalakshmi *et al.* 2011, Abhineet and Dohare 2014). Dissolved salts from the bedrock can be related to conductivity and can be used to identify the region that sediment has been transferred from particularly in larger river systems (Jayalakshmi *et al.* 2011). The higher the EC, the greater the quantity of dissolved metals and anions in solution.

❖ Anions (nitrates, sulfates and phosphates) may precipitate metals from the water depending on the concentration of the anion, its' affinity to the metal cation and pH of the solution. Sulfates are naturally present in water and vary depending on the mineral content of the area (Abhineet and Dohare 2014). They are usually deficient in natural waters having concentrations lower than 5 mg L^{-1} and tend to accumulate in shallow

ground water in areas of urban and agricultural run-off (DWAF 1996, Abhineet and Dohare 2014). Nitrates are of particular concern, they change haemoglobin in the blood and decrease oxygen carrying capacity (Canadian Council of Resource and Environment Ministers 1987, Abhineet and Dohare 2014). Phosphates, orthophosphates and organically bound phosphates are released by microbial activity in water (Abhineet and Dohare 2014). Phosphates are used as indicators for algae growth which may cause eutrophication (Abhineet and Dohare 2014, Pavan and Benarjee 2015). Permissible limits of anions in drinking water (Table 3.1) have been set by organisations and governments around the world as an indication of pollution. Concentrations exceeding the guideline limits for nitrates, sulfates and phosphates may cause methaemoglobinaemia in infants, laxative effects and kidney damage, respectively (WHO 2006).

Table 3.1: WHO guidelines on selected anions (WHO 2006)

Anion	Phosphate	Nitrate	Sulfate
WHO limit / mg L^{-1}	5	50	500

3.4.2. Water Quality Guidelines

Water quality guidelines determine if the water is fit for use or if it has the potential to spread water borne diseases (Carr and Neary 2006). A water quality guideline is a set of criteria provided for specific water quality constituents. The South African Water Quality Guidelines (SAWQG) compiled by the Department of Water Affairs and Forestry (DWAF) is utilised as a primary tool to judge the fitness of water for use as domestic, agricultural or industrial water (DWAF 1996).

Table 3.2: Water guidelines or standards of selected metals by international organisations or countries (DWAF 1996, Carr and Neary 2006, Mamba *et al.* 2008)

Element	Unit	WHO (Guidelines)	European Union (Standards)	South Africa (Guidelines)
Chromium	$\mu g\,L^{-1}$	50	50	100
Copper	$\mu g\,L^{-1}$	2000	2000	1000
Lead	$\mu g\,L^{-1}$	10	10	20
Nickel	$\mu g\,L^{-1}$	20	20	150
Zinc	$\mu g\,L^{-1}$	3000	#	5000

 # - Not mentioned

The WHO guidelines and European Union (EU) standards are adopted by most countries worldwide (Carr and Neary 2006). The South African guidelines are higher for specific chemical parameters as timeframe provisions are allocated for accumulation of these chemicals in humans before an effect is observed (Mamba *et al.* 2008). The WHO and EU guidelines do not make provisions for timeframe accumulation.

3.4.3. Sediment

Sediment is an essential component of any river system (Islam *et al.* 2015). Sediments are classified as sinks and carriers of pollutants in aquatic environments. The analysis of sediments is therefore employed to evaluate the health of an aquatic system (Bartoli *et al.* 2012). Natural and anthropogenic sources promote the metal content in sediment and also influence the form in which these elements behave in the environment. Changes including pH, temperature, redox potential, and ion exchange affects the fate of the metals in sediment (Filgueiras *et al.* 2002). Carbohydrates and minerals including iron and manganese oxides adsorb trace elements (Bartoli *et al.* 2012). Physical and chemical variations in the environment contribute to the release or binding of potentially toxic elements from anthropogenic sources to the fine particles in the sediment (Bartoli *et al.* 2012).

In addition, particle size distribution of the sediment is directly related to the heavy metal adsorption capacity by the sediment (Bartoli *et al.* 2012, Sadeghi *et al.* 2012). Metal ions bind to finer particles in sediment due to the higher surface area available. These ions partition between the organic matter, oxyhydroxides of Fe, Al and Mn, phyllosilicate materials, carbonates and sulfides (Filgueiras *et al.* 2002).

Heavy metals in sediment can be analysed in two ways *viz.* total and Bioavailable. Total metal content is the total amount of a metal in all fractions of sediment. The sediment is subjected to strong acids to decompose the sediment sample rendering metals aqueous. The total metal concentration in sediment is not sufficient for the assessment of environmental impact since the total concentration is not the driving factor to an elements toxicity (Islam *et al.* 2014). The major concern for heavy metals in sediment is the bioavailability and toxicity to organisms. The availability of metals for uptake by an organism is the determinant of toxicity. In order to determine the bioavailable metal content in sediment, fractionation of the sediment is utilised. Fractionation of sediment is a vital part of understanding the interactions of heavy metals and their concentrations and bioavailability within ecosystems (Islam *et al.* 2014). It is easier to monitor the soil from which plants uptake trace metals and introduce them into the food web indirectly (Ivezić *et al.* 2013).

The single extraction and sequential extraction procedures are the two main approaches utilised in the assessment of bioavailability. Single extractions utilise a single reagent to extract metals from a component in sediment while sequential extraction uses a series of reagents to extract metals from each of the components in sediment (Ivezić *et al.* 2013). The sequential extraction method is suitable for trace metal determination in soil however, it has been criticised for its lack of selectivity resulting from reagents dissolving compounds of little to no toxic effects, metals extracted in previous steps can be reabsorbed and redistributed, and speciation of the metal can change here are notable deviation of results between the single and sequential extraction procedures (Mossop and Davidson 2002, Ivezić *et al.* 2013). Sequential extraction is time consuming but provides vital information about the behaviour of metals in an ecosystem and their potential for ecotoxicity (Filgueiras *et al.* 2002).

Sequential extraction has become a widely used method for fractionation of trace metal concentration in sediment (Mossop and Davidson 2002). Different techniques have been developed using a range of reagents that have similar underlying principles. The Commission of the European Communities, Community Bureau of Reference (BCR) have produced a four step method to fractionate trace metals in sediment (Mossop and Davidson 2002). This sequential extraction method utilises four steps for extracting metals from different components of the sediment.

Fraction 1 (Exchangeable, water and acid-soluble): The first step of the BCR extraction utilises a weak acid *e.g.* acetic acid to release metals bound to carbonates and that are exchangeable with the extracting solution (Filgueiras *et al.* 2002, Mossop and Davidson 2002). These metals are bioavailable for plant uptake and are affected by the ionic strength of the extractant (Filgueiras *et al.* 2002).

Fraction 2 (Reducible iron and manganese oxides): Hydroxylamine hydrochloride adjusted to pH 2 is widely used in this step. The iron and manganese oxyhydroxides are well known structures that encage heavy metals (Filgueiras *et al.* 2002). The reduction of Fe(III) and Mn(IV) under anoxic conditions release adsorbed trace metals (Filgueiras *et al.* 2002).

Fraction 3 (Oxidisable organic matter and sulfides): This fraction of sediment requires oxidation of the organic and sulfide matter with hydrogen peroxide to release the metal ions and then extract the ions with a buffer *e.g.* ammonium acetate. This fraction is generally associated with humic substances and is not very mobile in sediment. This is important in polluted sediment as most pollution is composed of organic matter (Filgueiras *et al.* 2002). Organic substances have a high affinity for divalent ions particularly Cu and Pb in aquatic environments and retain these elements for a longer period of time (Filgueiras *et al.* 2002, Giacalone *et al.* 2005).

Fraction 4 (Residual): The residual fraction is digested with strong acids including nitric and hydrochloric acids. This fraction consists of metals bound to silicates which are mainly clay type minerals, and potentially holds the highest concentration of metals which are not bioavailable for uptake by organisms.

Table 3.3: Summary of the sequential extraction method

Fraction	Extractant	Associated Metal Partitioning	
1	Acetic acid	Exchangeable, water and acid-soluble	Decreasing Availability
2	Hydroxylamine hydrochloride (pH 2)	Reducible iron and manganese oxides	
3	Hydrogen peroxide, Ammonium acetate	Oxidisable organic matter and sulfides	
4	Nitric and hydrochloric acids	Residual	

3.4.4. Sediment Quality Guidelines

All metals are toxic to organisms above a threshold limit. There have been numerous investigations into the effects of elevated metal concentrations on organisms and the environment. Guidelines established by various environmental agencies are based on biological effects exhibited by organisms to differing levels of metal concentrations. Sediment quality guidelines have been developed by many researchers in three main approaches, equilibrium-partitioning modelling, laboratory bioassays, and field studies (Long *et al.* 1995). Data was collated from all major guideline developments to prepare the informal guidelines for the National Oceanic and Atmospheric Administration (NOAA). The observed concentrations of metals in sediment were compared to marine organisms to determine the species responses to the metal concentration (Dong *et al.* 2012). The results were divided into three ranges by two threshold limits; the "effects range low" (ERL) and the "effects range median" (ERM). ERL and ERM are specific values of a chemical at which toxicity effects have been observed in 10% and 50% of a population using the NOAA bioassay, respectively (Long *et al.* 1995, O'Connor 2004, Dong *et al.* 2012). At concentrations below the ERL value, organisms exhibit minimal toxic effects and at concentrations above the ERM value, organisms exhibit toxic effects (Long *et al.* 1995, Binning and Baird 2001). Concentrations between the ERL and ERM values indicate potential infrequent toxicity (Table 3.4). These guideline values correspond closely with guidelines of other methods with the draw-back of only being derived for nine trace metals and nineteen organic pollutants (Long *et al.* 1995). The advantage of this guideline is that it collates different methods and data to produce a unified quantitative value (Long et al. 1995). The Benguela Current Large Marine Ecosystem (BCLME), in 2006, which encompasses Angola, South Africa and Namibia investigated the different sediment quality guidelines in an attempt to define sediment quality guidelines for the African coast and it was proposed that South Africa use the values stipulated by the NOAA (Taljaard 2006).

Table 3.4: ERL and ERM guideline values (ppm, dry weight) for selected trace metals in sediment (Long *et al.* 1995).

Metal	Cr	Cu	Pb	Ni	Zn
ERL value	81	34	46.7	20.9	150
ERM value	370	270	218	51.6	410

3.5. Quality Assurance

Certified reference materials (CRMs) are an important tool for validating analytical methods (Marsland 2007). A CRM is defined as a "reference material, accompanied by a certificate, which has one or more properties whose value is certified by a procedure that establishes traceability to the accurate realisation of the unit in which the values of the properties are expressed, and for which each certified value is accompanied by a stated uncertainty with a given level of confidence" (Ulberth 2006).

Due to an increase in stringent regulations pertaining to analysis, quality assurance is guaranteed by the use of a CRM. CRMs focus on achieving accuracy of the method rather than precision. The precision of the method gives no indication on how close a quantified value is to the actual value (Rasberry 1988). The CRM should be a material that matches closely to the matrix of the sample being analysed to eliminate interferences and provide accurate results (Ulberth 2006).

3.6. Statistical Analysis

Statistical analysis was done using GraphPad Prism 6.01 and Minitab 17. The one-way Analysis of Variance (ANOVA) was done on GraphPad Prism to determine the variability of seasonal metal concentration. The null hypothesis stated that there was no significant difference between the seasons for each metal at different sampling sites. If the *p*-value was greater than 0.05, the null hypothesis was rejected.

Minitab was used to for the Principle Component Analysis (PCA) and Cluster Analysis (CA). PCA is a chemometric method that assumes a bilinear model that reduces the number of components to explain data variance (Terrado *et al.* 2006, Felipe-Sotelo *et al.* 2007). PCA is represented as a score plot of the loadings (original data reduced to fewer components) and similarities between them. CA uses Ward's Method which is an effective method that standardises data and clusters or groups data that are similar to each other but are different from other data sets within the same analysis (Felipe-Sotelo *et al.* 2007). The CA is represented as a dendogram which clearly shows the clusters or similar data.

3.7. Analytical Techniques

3.7.1. Microwave Assisted Digestion

Two methods are used to make metals bound to sediments labile; microwave assisted digestion and hot plate digestion. Microwave assisted digestion has been reported to be more effective. It is the most suitable technique for complex matrices such as soils and sediment (Bettinelli *et al.* 2000). The high temperature, high pressure closed system and type of acid used are critical factors that ensure complete digestion of the sample into a clear liquid (Ghanthimathi *et al.* 2012). The advantages of microwave assisted acid digestion include shorter digestion times, reduction in external contaminants, efficient digestion, low volume and minimal loss of volatile metals (Bettinelli *et al.* 2000, Shirdam *et al.* 2008, Roa *et al.* 2010). It is for these reasons that it has been adopted as an EPA method to extract metals from soil, sludge and sediments.

3.7.2. Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)

ICP-OES is a versatile, sensitive method of elemental analysis (Butcher 2010). ICP-OES is utilised for multi-elemental analysis of analytes in a shorter time and to achieve a wider sample characterisation (Bettinelli *et al.* 2000). ICP-OES is widely utilised due to low costs, reduced interferences and sensitivity for many elements in the parts-per-billion range (ppb) (Butcher 2010).

Components of a typical ICP-OES instrument include:
- a sample introduction system, comprised of a peristaltic pump, nebulizer, spray chamber and a drain assembly
- the plasma, ICP torch and gas supply
- transfer optics and an optical spectrometer
- detectors
- data collection unit and display output (Botes 2003)

Biological, geological and environmental samples are introduced into the ICP in liquid form *via* the peristaltic pump (Botes 2003, Skoog *et al.* 2004). The peristaltic pump controls the sample flow rate and aids in rinsing the system thus minimising analysis time. The sample enters the nebulizer where it is converted into an aerosol or fine mist which is a few microns in diameter (Botes 2003). Argon gas carries the aerosol and the flow rate of the gas determines the size of the droplets. Larger droplets are not carried to the plasma by the argon gas and are condensed in the spray chamber and led to the drainage system.

An ICP torch is made of quartz which does not absorb light in the infrared (IR) region of the electromagnetic spectrum. The quartz material ensures that it withstands extremely high temperatures (Skoog *et al.* 2004). Argon gas interacts with a high energy radio frequency magnetic field which produces plasma (Botes 2003). The magnetic field is generated by coils made of gold or silver. These materials provide minimum resistance to the radio frequencies that travel along the surface and do not form oxides when exposed to plasma (Skoog *et al.* 2004). Plasma is a highly ionisable gas that is electrically neutral and composed of ions, electrons and atoms (Skoog *et al.* 2004). Using radio waves, the argon gas is heated and atomization occurs in a non-reactive, stable environment which minimizes interferences. Infinite number of electrons and cations are present in the plasma which has the ability to conduct electricity. The aerosol is atomised and the metal analyte absorbs energy. The metal ion electrons upon absorbing the energy are excited and elevated to higher energy levels but instantaneously emit the absorbed energy and return to their ground states (Skoog *et al.* 2004).

Two torch configurations are utilised in ICP-OES analysis *viz.* radial and axial. Axial view is horizontal to the optical system and radial is vertical to the optical system (Trevizan and Nóbrega 2007). Axial view has better limits of detection but increased interferences (Trevizan and Nóbrega 2007).

Spectrometers select a specific wavelength in the emission spectrum and isolate it hence the spectrometer must have a high resolution, line throughput, stability, and minimal stray light (Botes and Staden 2004). Emissions are converted into electrical signals and are normally amplified by transducers using photomultipliers which ensures a smaller margin of error and a better, higher output to be generated (Skoog *et al.* 2004). The photomultiplier functions on the principle of the photoelectric effect. The emission of electrons is generated by photons

emitted by the sample that strike the cathode of the tube. The electrons generated strike the dynodes which result in more electrons being generated and this response produces the amplification of the signal. Concentration can be determined by the current which is proportional to the sample emission (Skoog *et al.* 2004). Since concentration of an element is proportional to the energy emitted, ICP-OES can be utilised to detect very low concentrations of trace elements.

ICP-OES is susceptible to errors of matrix and spectral natures. Matrix errors can be physical, chemical or ionisation interferences (Botes 2003, Skoog *et al.* 2004). Physical interferences include changes in density, viscosity and surface tension of the solution which affects the droplet size and influence the sensitivity of the instrument (Botes 2003, Trevizan and Nóbrega 2007). Chemical interferences occur when undesired compounds form and prevents the atomisation of an element (Skoog *et al.* 2004). The ionisation interference is due to the temperature of the plasma, ionisation potential of the element, and concentration of the element (Botes 2003). Spectral overlaps are one of the major problems of the ICP-OES and is directly linked to the high temperatures. The ICP-OES allows for the selection of various emission lines due to an overlap of emission lines either direct or winged is plausible.

3.7.3. Ion Chromatography

Ion chromatography (IC) is a branch of liquid chromatography that is based on the principle of ion exchange (Jackson 2006). The ions are separated on a resin exchange column and then on a second suppressor exchange column before detection (Shpigun 1985). Ion chromatography is widely used in the analysis of inorganic ions in environmental samples due to low detection limits, high sensitivity in complicated matrices, high determination speed, simple sample pre-treatment, and no preliminary concentration (Shpigun 1985, Bolanča *et al.* 2006, Jackson 2006). Cations and anions have different modes of detection although IC is generally more utilised for anions (Shpigun 1985).

Table 3.5: Differences in the key components of IC in analysis of cations and anions (Shpigun 1985).

	Cation	Anion
Separation Column	The column has an acidic cationic exchange resin that is protonated.	The column has a basic anionic exchange resin.
Suppressor Column	The column is a basic anionic exchange resin that is hydroxylated.	The column is a protonated acidic cationic exchange resin.
Eluent	Diluted HCl	$NaHCO_3$

3.8. Comparative Studies

River pollution has always been of major concern globally with much research being conducted. The research provides a state of river health analysis which help governments to implement policies and provide clean-up efforts to affected areas.

Pollutants, in the form of discharge of solid waste, organic waste, industrial waste, heavy metals, oils and tar, are released into fresh water systems on a daily basis. It is found that industrial activities including metal smelting and refining, consumer products as well as burning of waste contributed to the metal levels in the rivers (Singh *et al*., 2015). The metals either adsorbed onto particulate matter, formed free ions or soluble complexes for bioavailable uptake. Industrial and agricultural activities are two of the largest contributors to anthropogenic input in ecosystems.

The heavy metal concentration in the Ganges and Mithi Rivers in India (Table 3.6) exceed the ERM values for all elements. The ERM value indicates the level at which toxic effects would be experienced. Compared to the Sinos and Tembi Rivers, metal concentrations in the Indian rivers are at toxic levels. The Sinos and Tembi Rivers have lower metal concentrations which suggests stringent monitoring of released effluent in these industrial rivers. It is expected that the industrial rivers would exhibit higher metal concentrations.

Agricultural activities have increased over the last decade as human populations across the world increases. The demand for food has increased and supply needs to meet the demand. Crops are grown faster with the aid of fertilisers. Commercial fertilisers have trace elements which contaminate soil. Continual use of fertilisers contaminate soil rendering it toxic. Agricultural rivers are expected to have low metal concentration and a high anion concentration. Anions are introduced into fresh water systems as run-off from fertilised fields. The agricultural rivers (Table 3.6) have metal concentrations lower than the ERM values. Nickel in Korotoa River, Bangladesh, is above the ERM value which is attributed to over-fertilisation or area geology.

South Africa, like much of the world, has many rivers, tributaries and catchments that build the water resources of the country. Intensive research has been done within South Africa on rivers and catchments, investigating pollution and its' effects on humans and the environment. These water bodies often run through industrial or agricultural areas and may become polluted with different waste material.

The Umgeni River is a major river in KwaZulu-Natal which has been documented over the years for high contaminant levels. It has anthropogenic activities including industrial, recreational and agricultural. Anthropogenic activities have been identified as the primary reason for ecosystem collapse. The Palmiet River has been known for metal and anion contamination since 1985 (De Villiers and Malan 1985). The Palmiet River exhibited higher physico-chemical parameters than the major Umgeni River that it feeds into (Dikole, 2014). The Pinetown area has been of concern with elevated sediment metal concentrations (Dikole, 2014). This industrial center was identified as a key contributor to metal contamination in the Palmiet River.

Table 3.6: Comparison of pseudo-total heavy metal concentration (average concentration) from river studies

River	Country	Type of River	Metal Concentration / mg kg^{-1}					Reference
			Cr	**Cu**	**Ni**	**Pb**	**Zn**	
Mithi	India	Industrial	477.33	-	860.33	849	-	(Singare *et al.* 2012)
Tembi	Iran	Industrial	48.75	58.25	101.4	202.5	38	(Shanbehzadeh *et al.* 2014)
Sinos	Brazil	Industrial	252.78	87.45	43.96	36.95	206.11	(Schneider *et al.* 2014)
Ganges	India	Industrial	3740	2240	-	5950	14390	(Gupta *et al.* 2008)
Ebro	Spain	Agricultural	56.25	43.10	31.18	40.68	292.58	(Terrado *et al.* 2006)
Lis	Portugal	Agricultural	68.36	38.5	21.23	27.03	189.38	(Vieira *et al.* 2009)
Korotoa	Bangladesh	Agricultural	108.5	76.5	94.5	58.5	-	(Islam *et al.* 2015)

Values in red represent concentrations greater than the ERM value

3.9. References

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Chapter 4

Methodology

4.1. Sampling

Sampling was conducted over four seasons for both rivers in July 2014, October 2014, January 2015 and April 2015. Sediment and water samples were collected in the field and taken to the laboratory for analysis. Physical parameters (electrical conductivity (EC), pH, and total dissolved solids (TDS)) were measured in the field. Electrode measurements for physical parameters (TDS and EC) were done *in situ* using a WTW™ LF340 conductivity meter that was calibrated with KCl solution. The pH was measured using a Metrohm 827 pH lab meter.

4.1.1. River sediment sampling

Surface sediment samples were collected in plastic beakers. At each site numerous samples were collected in polyethene beakers and stored in plastic bags. A number of locations at each site were sampled to make a composite sample. The samples were taken to the laboratory and stored before sample preparation.

4.1.2. River water sampling

Samples were collected in 50 mL plastic vials that were washed with 10% (v/v) nitric acid, followed by double distilled water before use. The tubes were rinsed twice with river water prior to sample collection where the tubes were submerged and water collected. Samples were stored in a refrigerator.

4.2. Sample Preparation

4.2.1. River sediment

Samples were air-dried, ground using a mortar and pestle and sieved using stainless steel sieves. The sample fraction < 90 μm was collected for analysis and stored in plastic bags.

4.2.2. River water

A sample volume of 15 mL were filtered using a 0.45 μm Whatman ® membrane filter. A portion of the water was stored at 4°C for ion chromatography analysis. The remaining filtered water was preserved with a few drops of concentrated HNO_3 (Analytical Grade, Merck) until ICP-OES analysis.

4.2.3. Certified Reference Material

SRM 2702 (Inorganics in Marine Sediment) was used to determine total metal concentration in the rivers (Appendix A). BCR-701 was used for sequential metal extraction analysis in fresh water systems. It provides certified values for all fractions for Cr, Cu, Ni, Pb and Zn (Appendix A).

4.2.4. Acid digestion: Sediment

Approximately 0.5 g of the sieved sediment or CRM was accurately weighed into a Teflon digestion tube together with 10 mL of concentrated HNO_3 (Analytical grade, Sigma-Aldrich). The sediment or CRM was left to pre-digest for 30 minutes. The digestion vessels were then sealed and placed onto a microwave rotor (following the procedure outlined by the user manual). The rotor was then placed into a MARS 6 CEM microwave unit for digestion.

Table 4.1: CEM microwave digestion unit parameters

Parameter	Setting
Ramp Time / min.	15
Ramp Rate / °C min.$^{-1}$	12
Hold Time / min.	15
Hold Temperature / °C	180
Cool down time / min	15

After complete digestion, the samples were then filtered into a 25 mL volumetric and made to the mark using double distilled water. The solutions were transferred into a plastic vial before being stored in the refrigerator. Three replicate digestions were conducted for each sample.

4.2.5. Sediment sequential extraction procedure

Approximately 0.5 g of sediment samples were accurately weighed and subjected to the procedure (Rauret *et al.* 2001).

Step 1: 20 mL of a 0.11 M acetic acid solution (Merck) was added to the sediment sample in

a 50 mL centrifuge tube, sealed and placed side-on an orbital shaker for 16 hours. The extract was then separated from the solid residue by centrifugation for 20 minutes. The extract was decanted into a vial and stored in the refrigerator prior to analysis. The residue was washed using 20 mL distilled water, shaken for 20 minutes on the orbital shaker and centrifuged for 20 minutes. The supernatant was discarded.

Step 2: A 0.5 M solution of hydroxylammonium chloride was prepared and acidified with

25 mL of 2 M nitric acid. The sediment residue from step 1 was added to 20 mL of the hydroxylammonium chloride solution, sealed and placed side-on an orbital shaker for 16 hours. The extraction procedure was followed as in step 1.

Step 3: The residue from step 2 was placed in a 50 mL centrifuge tube and partially sub-

merged in a water bath. Hydrogen peroxide was added (5 mL) and the vial loosely sealed for an hour. The vial was then uncapped and the solution allowed to evaporate to near dryness. Hydrogen peroxide was then added dropwise for 30 minutes with constant shaking. Another 5 mL of hydrogen peroxide was added and the process repeated. A 1 M ammonium acetate solution was prepared and the pH adjusted to 2 with nitric acid. To the residue in the vial, 25 mL of the ammonium acetate solution was added, sealed and placed side-on an orbital shaker for 16 hours. The extraction procedure was followed as in step 1.

4.3. Analysis

4.3.1. ICP-OES

The Perkin Elmer Optima DV5300 ICP-OES was calibrated with standards prepared from commercial 1000 mg L^{-1} solutions (Sigma-Aldrich). Matrix matching was done with the different sets of standards. Wavelengths (Table 4.2) were selected due to their detection limits and potential interferences.

Table 4.2: Wavelengths and detection limits for axial analysis

Element	Wavelength / nm	Detection Limit / mg L^{-1}
Cr	267.716	0.0071
Cu	327.393	0.0097
Ni	231.604	0.0150
Pb	220.857	0.0042
Zn	213.857	0.0018

Table 4.3: Perkin Elmer Optima DV5300 ICP-OES parameters

Parameter	Setting
Power /W	1500
Auxiliary gas	Nitrogen
Auxillary gas flow / $L\,min^{-1}$	0.2
Nebuliser gas	Argon
Nebuliser gas flow / $L\,min^{-1}$	0.8
Sample introduction rate / $mL\,min^{-1}$	1
Sample replicates	3

4.3.2. Ion Chromatography

A Metrohm 762 Compact Ion Chromatograhy (Leonberg, Germany) with a Metrosep A Supp 5 150 x 40 mm column was used to quantify the nitrate, sulfate and phosphate ions in the filtered water samples.

Table 4.4: IC analysis parameters

Eluent	1.0 mM $NaHCO_3$ and 3.2 mM Na_2CO_3
Flow Rate / $mL\,min^{-1}$	0.7
Suppressor solution	50 nM H_2SO_4 and deionised water

4.3.3. Statistical Analysis

Statistical analysis was done using GraphPad Prism 6.01 and Minitab 17. The one-way Analysis of Variance (ANOVA) was done on GraphPad Prism. Minitab was used to for the Principle Component Analysis (PCA) and Cluster Analysis (CA).

4.4. References

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Chapter 5

Results and Discussion

This chapter provides data on rainfall patterns, physical parameters, pseudo-total and bioavailable metal concentrations in the Palmiet and Sezela Rivers. Both rivers are contrasting in their anthropogenic influence. The aim of this chapter is to investigate the impact of industry on an aquatic environments using rivers subjected to industrial and agricultural activities.

5.1. Quality Assurance

Validation of the sample preparation and the analytical methods were conducted using CRMs. Two CRMs *i.e* Inorganics in marine sediment (SRM 2702, NIST) and Lake Sediment (BCR-701, IRMM) were used for the total and sequential extraction of metals from sediment respectively. The digests/extracts were analysed by ICP-OES and compared to certified values (Tables 5.1 and 5.2).

Table 5.1: Certified and calculated values for SRM 2702, NIST

Element	Certified Value / $mg\,kg^{-1}$	Experimental Value / $mg\,kg^{-1}$
Cr	352 ± 22	349.4 ± 29.5
Cu	117.7 ± 5.6	118.0 ± 11.6
Ni	75.4 ± 1.5	72.7 ± 7.8
Pb	132.8 ± 1.1	123.8 ± 17.1
Zn	485.3 ± 4.2	491.7 ± 81.7

The percent recovery for the total metal CRM analysis ranged from 93.22% to 101.32%, indicating the validity of the method.

Table 5.2: Certified (bold) and experimental values for BCR-701

Element	Step 1 (Exchangeable, water and acid soluble)	Step 2 (Reducible iron and manganese oxides)	Step 3 (Oxidisable organic matter and sulfides)
Cr	2.22 ± 0.47 **2.26 ± 0.16**	45.9 ± 9.1 **45 ± 2**	142.6 ± 28.3 **143 ± 7**
Cu	49.7 ± 0.23 **49.3 ± 1.7**	110.2 ± 18 **124 ± 3**	45.1 ± 1.1 **55.2 ± 4**
Ni	14.6 ± 1.5 **15.4 ± 0.9**	25 ± 7 **26.6 ± 1.3**	14.7 ± 3.2 **15.3 ± 0.9**
Pb	3.14 ± 0.18 **3.18 ± 0.21**	113.4 ± 3.3 **126 ± 3**	9.3 ± 0.46 **9.3 ± 2**
Zn	180 ± 0.8 **205 ± 6**	102.7 ± 5.9 **114 ± 5**	40.5 ± 0.7 **45.7 ± 4**

The experimental values calculated had percent recoveries greater than 85% indicating that the sequential extraction method was valid. Errors in the analysis that may have contributed to lower recovery include loss of analyte during decanting between extractions.

5.2. Seasonal Rainfall

The average rainfall from June 2014 to May 2015 in the Pinetown and Sezela areas were 42.1 mm and 51.6 mm, respectively (Table 5.3). The rainfall pattern in both regions is similar *i.e.* lower rainfall during the winter months and high rainfall in the summer months. During the high rainfall months, October to March, the river flow would have increased.

Table 5.3: Rainfall data collected during June 2014 – May 2015 for the Pinetown (eThekwini-Municipality 2011) and Sezela areas (SASRI).

Season	Average rainfall / mm	
	Palmiet	**Sezela**
Winter 2014	5.6	13
Spring 2014	46.5	80.6
Summer 2014/5	85.7	70.8
Autumn 2015	30.7	42.1
Average Rainfall	*42.1*	*51.6*

5.3. Palmiet River

5.3.1. Physical Parameters

Physical parameters provide information about the studied environment. The physical parameters were measured using electrodes *in situ* (Table 5.4).

Table 5.4: Physical parameter data for the Palmiet River

Sample Site	pH				Conductivity /µS cm^{-1}				TDS / mg L^{-1}			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
1	7.84	7.05	6.89	6.87	340	276	334	359	338	142	174	320
2	7.37	6.74	7.15	7.45	422	355	349	70	420	179	181	73
3	6.56	7.2	7.05	7.07	450	188	360	384	450	95	236	420
4	6.71	5.67	7.06	6.82	412	276	363	347	411	141	187	382
5	7.09	6.99	7.45	7.06	495	327	436	81	495	165	227	76
6	8.68	6.6	7.42	7.61	386	538	343	67	386	278	200	63
7	7.88	5.89	7.35	7.58	381	420	415	75	381	217	216	71
8	7.85	7.18	7.27	7.58	398	131	413	76	398	131	215	73
9	7.91	6.26	7.19	7.53	415	479	414	79	414	249	215	72

Values in red indicate above the permissible limits (Carr and Neary 2006).

The target water pH for SAWQG ranges from 6.5 - 9.0 which can sustain most species of fish in rivers (DWAF 1996). The seasonal pH (7.2 ± 0.4) was comparatively stable with minimal fluctuations. All of the pH values were within the target water pH range. The pH of the water may impact the adsorption capacity of soluble metals to particulates and sediment. Typically, low pH values keep metals in solution.

The EC is above the permissible limits for spring, summer, and winter. Autumn, however, has a high conductivity at the source and industrial area and this decreases downstream. Generally, TDS increases at the industrial area and decreases downstream before increasing at the last sample site. TDS and EC are directly related especially in clean water systems.

The geology of the area affects the EC and factors may be used to correlate the EC to TDS (DWAF 1996). Conductivity and TDS in fresh water have limits of 250 μS cm^{-1} (Carr and Neary 2006) and 1000 mg L^{-1}, respectively (DWAF 1996). The elevated EC is possibly due to the ions (responsible for conductivity) being precipitated out of solution, or an uptake of ions by biota. Generally, high rainfall dilutes the conductivity whilst low flow periods (*e.g.* winter) result in higher conductivity.

5.3.2. Metal Concentration in Water

Metal concentrations in water are predominantly low due to dilution and dispersion and were below the SAWQG (Table 5.5). They fluctuate either due to physico-chemical (e.g. pH or Eh) or anthropogenic factors and are often governed by adsorption/desorption processes. Anthropogenic input often increases the metal concentrations in water since it is often associated with pH changes (Jonnalagadda and Mhere 2001).

Variations along the river can be attributed to bio-accumulation of metals in organisms and vegetation along the river, point source inputs *via* anthropogenic activities or physico-chemical changes.

There is no apparent trend of metal distribution between the seasons. Chromium and Ni indicated a few elevated concentrations. The Cr concentrations were higher than the SAWQG limit of 100 μg L^{-1} at the tail-end of the river. Lead concentrations fluctuate between < 1 ppb and values above the permissible level, with a maximum of 86.58 ppb.

There is an increase of Cr from sites 5 to 9 and Ni is elevated in winter and spring at the industrial area. The increase in TDS and EC at these sites suggest anthropogenic activity. The Ni concentrations in summer and autumn are below the permissible limits but from sites 3 to 6 concentrations are comparatively higher than other sites. The elevated Pb levels in water at specific may be introduced by anthropogenic inputs including Pb-acid batteries and cement products (Thambiran 2002).

Table 5.5: Dissolved metal concentration in the Palmiet River water in µg L^{-1} (ppb)

Sample Site	Cu				Zn			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
1	104.12	< 0.4	< 0.4	< 0.4	1908.1	22.14	13.3	8.83
2	46.16	< 0.4	< 0.4	< 0.4	847.51	119.97	12.25	13.71
3	6.26	17.12	< 0.4	8.23	49.71	107.82	13.99	12.12
4	14.21	15.29	0.78	0.29	144.75	109.59	80.97	136.66
5	30.36	20.09	16.61	8.35	150.69	140.31	251.47	484.25
6	0.17	9.37	3.87	< 0.4	15.82	94.52	93.8	68.36
7	< 0.4	< 0.4	< 0.4	< 0.4	33.64	18.33	20.3	14.32
8	14.24	< 0.4	< 0.4	< 0.4	56.53	20.41	10.94	13.38
9	7.44	< 0.4	6.68	0.07	48.2	7.01	8.14	10.28

Sample Site	Cr				Pb			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
1	21.92	0.2	< 0.2	< 0.2	86.58	< 1	1.8	52.08
2	< 0.2	1.96	1.77	< 0.2	< 1	< 1	< 1	< 1
3	5.51	45.73	< 0.2	< 0.2	< 1	< 1	< 1	41.14
4	2.68	33.72	0.84	0.29	< 1	7.38	< 1	< 1
5	57.74	11.84	6.7	0.74	< 1	48.61	17.46	23.48
6	5.54	4.02	12.02	0.3	< 1	< 1	4.72	< 1
7	69.87	< 0.2	99.93	0.98	47.68	< 1	< 1	< 1
8	107.52	0.97	37.8	0.24	2.14	< 1	35.24	27.03
9	111.81	< 0.2	7.13	1.92	16.33	14.8	< 1	26.96

Sample Site	Ni			
	Winter	Spring	Summer	Autumn
1	52.83	11.7	20.85	13.38
2	23.49	5.33	14.03	16.75
3	514.71	2429.9	55.78	112.73
4	451.21	2269.15	51.31	112.05
5	1371.35	1202.96	101.1	83.22
6	58.84	1043.5	67.39	45.65
7	19.57	76.18	29.86	20
8	14.62	78.8	22.41	22.08
9	24.13	34.32	24.48	18.79

Values in red exceed the South African Guidelines for water

5.3.3. Anion Concentration in Water

Only nitrates and sulfates were detected in the water of the Palmiet River at sites 1, 6 and 8 (Figure 5.1). There is clear seasonal variation (ANOVA, $p > 0.05$), with summer concentrations being significantly lower than winter. Sulfate concentrations are below the guideline limits (500 mg L^{-1}) in all seasons. Nitrates were above guideline limits at sites 6 and 8 in winter and also show an increase in concentration downstream of the source. While low water flow undoubtedly contributes to the higher levels present, there appears to be input of nitrates potentially from metal processing industries to the system (Thambiran 2002).

	1	6	8
Summer	11.04	15.49	10.52
Winter	7.22	204.86	560.90

	1	6	8
Summer	10,35	19,25	19,00
Winter	23,67	30,31	37,30
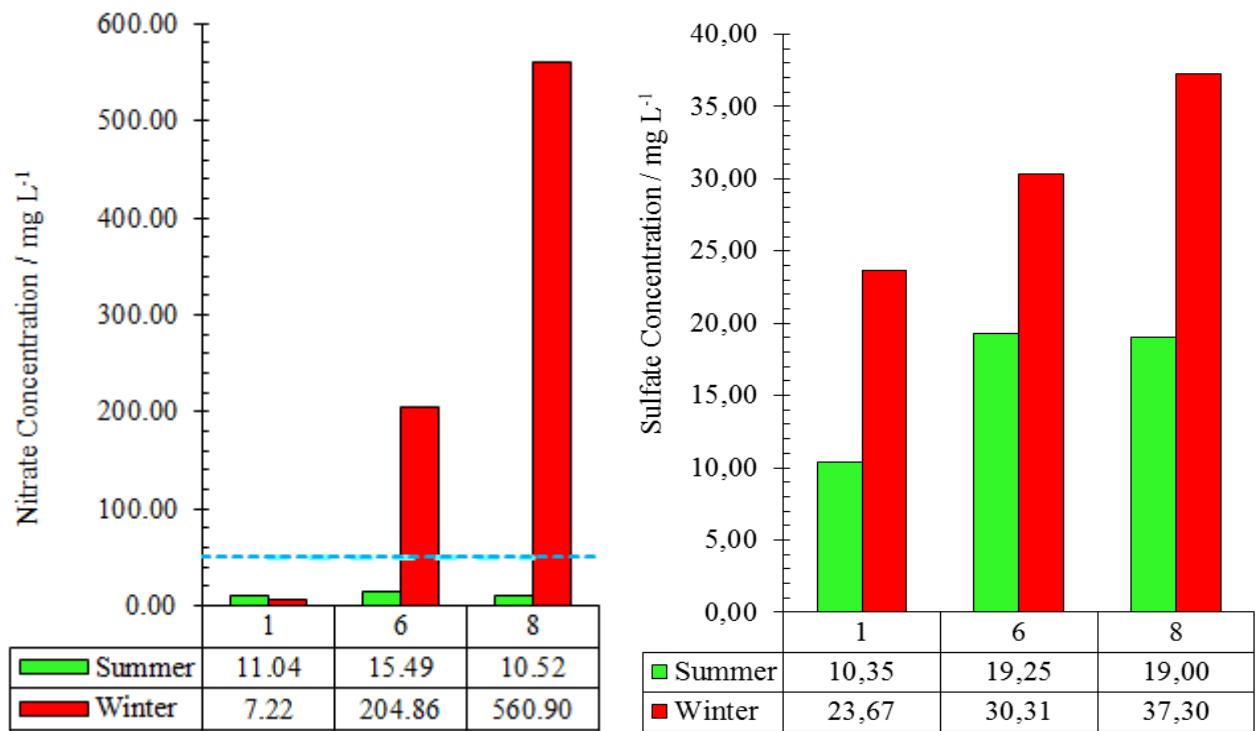
Figure 5.1: Concentration of selected anions in the Palmiet River in summer and winter

5.3.4. Total Metal Concentration in Surface Sediment

Metal distribution along the river clearly indicates the impact of industry to the Palmiet River (Figure 5.2). Typically, metals at the source are lower in concentration than at all other sites. Low concentrations are also apparent at site 2, which represent an effluent stream from alloy industries. It is clear that some attempt to clean up effluent waste prior to their entry into the environment has been made. Site 3, which represents the river flowing past the industrial area, shows the highest concentrations for all metals investigated and is a good indication that illegal dumping of waste by-products does occur. The point of the effluent stream joining the river (site 4) shows a dilution effect in some instances (for Cu and Ni). There is a general decrease of metal concentrations downstream of the industrial area. There are significant differences between metal concentrations between the four seasons (ANOVA, $p > 0.05$). In addition, there are differences in metal concentrations at some individual sites, indicating potential point sources of pollutants. An example of this is the elevated concentrations of all metals at the source in summer.

5.3.4.1. Chromium and Copper

Chromium and Cu exhibit similar trends in their total metal distribution both seasonally and downstream. They vary between the seasons but the general trend observed is autumn > spring > summer > winter (Figure 5.2a). Deviations from the trend are noted at the source in summer and sites 3 and 5 in spring. At the source in summer, the Cr and Cu concentrations are elevated and this may be due to anthropogenic input from a textile industry in the area. Effluent from textile industries have been reported to contain elevated levels of Cr and Cu which accumulate in soil (Manzoor *et al.* 2006).

Cr appears to be problematic in both the sediment and water, with high concentrations also present in water. Winter exhibited high Cr concentrations in water. This would suggest a potential input due to high Cr concentrations in the sediment at the same sampling sites. A probable cause of this may be dumping of refuse in this area.

Chromium sediment concentrations in the Palmiet River range from 10 – 1200 mg kg^{-1} and are above both the ERL and ERM values for most sites and seasons. The potential for mortality of organisms due to Cr toxicity is therefore high. Elevated levels of Cr (> 1.4 mg kg^{-1}) are reported to be carcinogenic, particularly the Cr(VI) species (CCME 1999). Typical sources of Cr that are found in the industrial area of Pinetown are from tool manufacturing and electroplating (Thambiran 2002). Previous studies on the electroplating company in the area reported that a third of the company waste was chromium in terms of mg L^{-1} effluent (Ghebregziabher 2004). The correlation between elevated water and sediment concentrations is an indication that companies of this type contribute to increased Cr levels present in sediment.

Concentrations of Cu range from 0 – 2000 mg kg^{-1} and are above both the ERL and ERM guideline values (Figure 5.2a). Concentrations in the industrial area are significantly higher than the ERM value and therefore toxic effects will be experienced by organisms in this area. Higher organisms may encounter renal failure amongst other complications due to Cu toxicity. In addition, these levels are elevated in comparison to the 23.12 mg kg^{-1} reported in 2004 indicating the increase of waste input into the system as the development of industries in the area continues (Moodley *et al.* 2014).
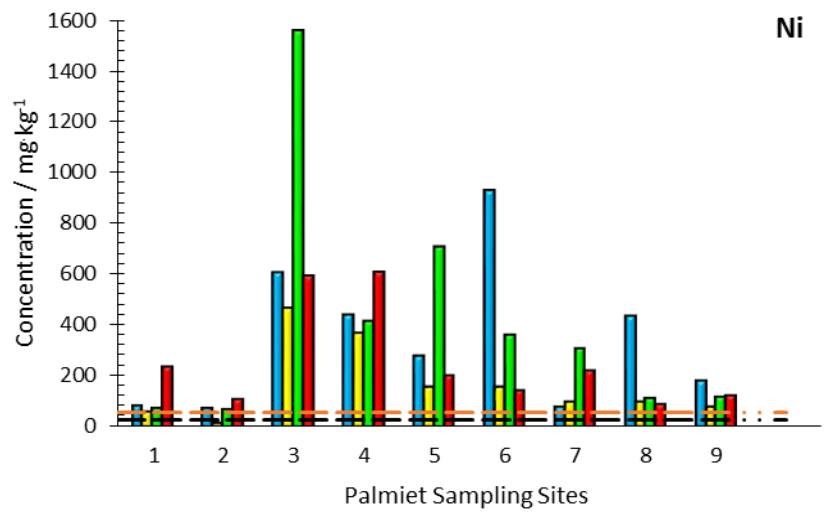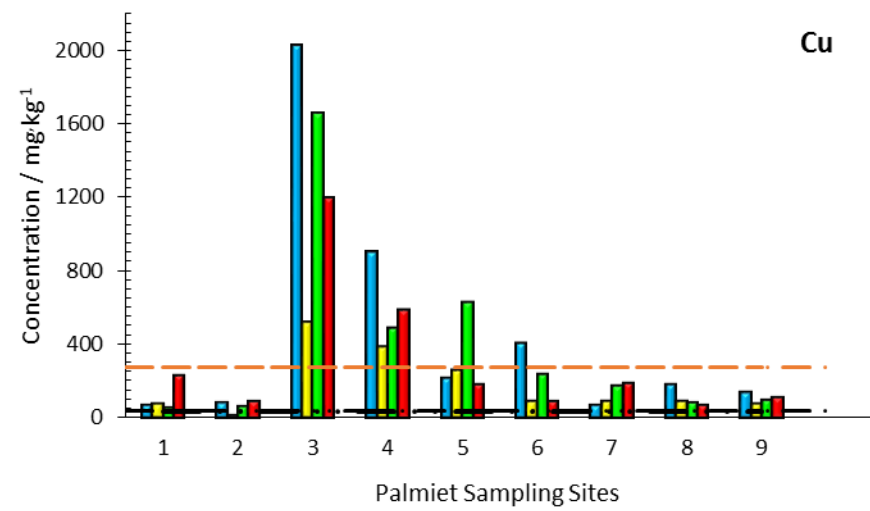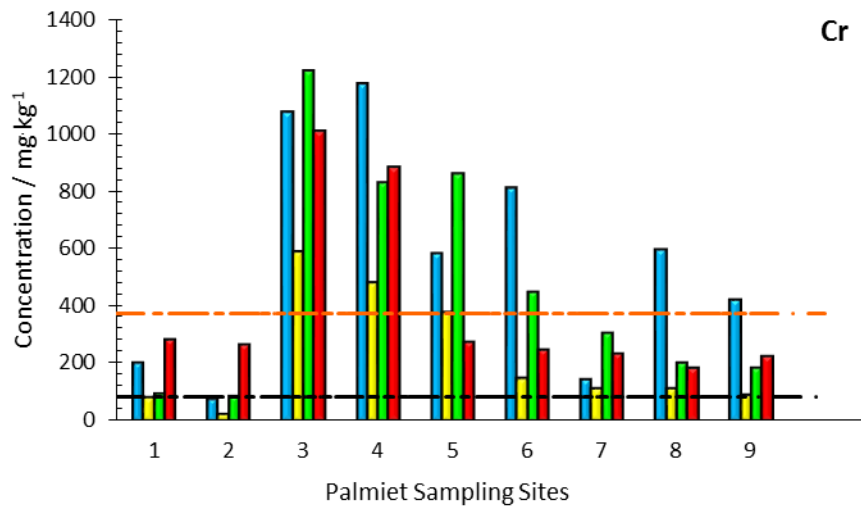
Figure 5.2a: Total surface sediment concentration in the Palmiet River
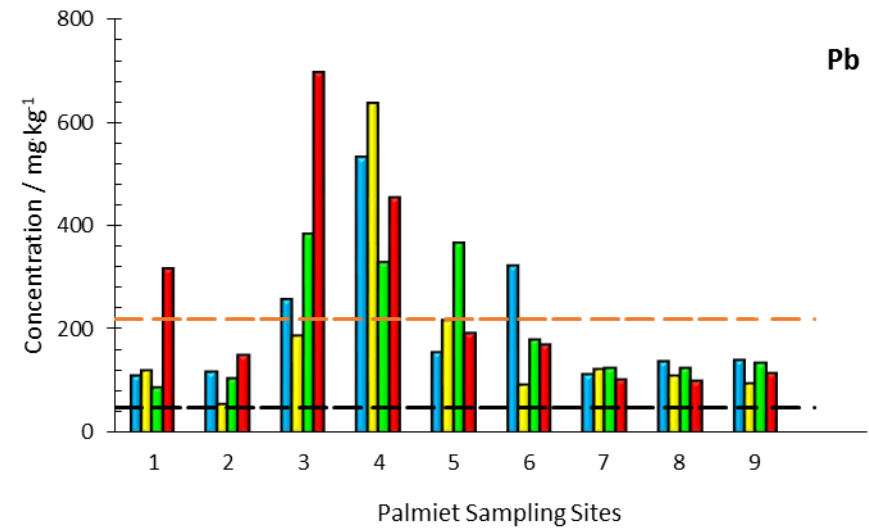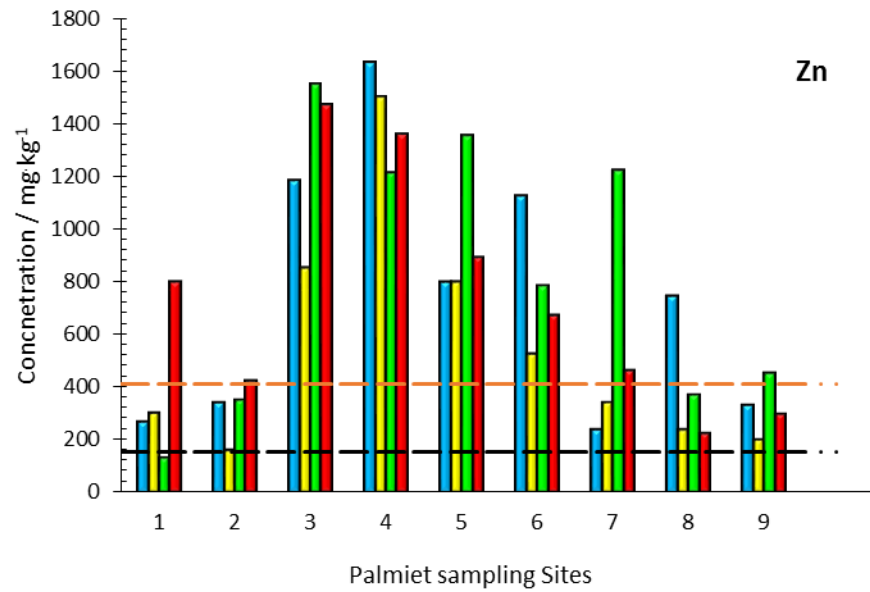
Figure 5.2b: Total surface sediment concentration in the Palmiet River

5.3.4.2. Nickel

Nickel concentrations exhibit the trend spring > summer > autumn > winter (Figure 5.2a). The distribution within the sites is not uniform and there are indications of point sources of Ni in different seasons. Nickel decreases downstream in winter and summer but fluctuates in spring and autumn. It is present at low concentrations before the industrial area however, elevated levels of Ni at the source (in summer) are attributed to the textile industry (Manzoor *et al.* 2006). Concentrations in the industrial area during spring are higher than the other seasons. Industries in the area that may contribute to Ni pollution include metal finishing industries, electroplating industries and ceramic industries (Thambiran 2002, Omolaoye J.A. *et al.* 2010, Moodley *et al.* 2014). The correlation of the winter and spring water data to the sediment data, provides further evidence of anthropogenic input in the industrial area as the dissolved Ni in water also exceed the SAWQS guidelines and the ERM sediment values.

As with Cu and Cr, Ni concentrations are all above the ERL and ERM values. This indicates that river biota would experience toxicological effects such as genotoxic and carcinogenic effects, gastrointestinal problems and possibly mortality (Carr and Neary 2006, USEPA 2011, Tchounwou *et al.* 2012).

5.3.4.3. Lead

The seasonal trend in Pb concentrations is different compared to Cr, Cu and Ni, autumn ≈ spring > summer > winter (Figure 5.2b). Lead concentrations are above the ERL values and are primarily below the ERM values except at the industrial area. This clearly indicates the contribution of Pb into the environment by industrial activity. Increased Pb levels would stunt plant growth and accumulate in animal liver resulting in gastrointestinal problems (Gupta *et al.* 2008). Lead concentrations decrease downstream after the industrial zone clearly indicating the influence of industrial activity on the river. Once again, the elevated Pb concentration at the source in summer is attributed to the textile industry in the vicinity of the source (Manzoor *et al.* 2006). Industrial contributions of Pb arise from the electroplating, metal finishing, cement and lead-acid battery industries in Pinetown (Thambiran 2002). Increased Pb concentrations may have accumulated over time from

cement dust and cement products that have been discarded into the river. Lead has a tendency to be mobile particularly in cement dust and is generally present in the surface sediment, less than 20 cm in depth (Gupta and Sharma 2013). The correlation between the water and sediment concentrations are further evidence of anthropogenic influence on the river particularly with elevated levels in spring and autumn in the industrial area. Permissible levels of Pb are exceeded as per the SAWQG and sediment concentrations are above the ERM values.

5.3.4.4. Zinc

There are differences in the seasonal and spatial distribution of Zn compared to other elements. The seasonal trend of Zn concentration is spring > autumn > summer > winter (Figure 5.2b). Concentrations peak at the industrial area and gradually decrease downstream. Zinc concentrations before the industrial area are typically low, however, the elevated Zn concentration at the source in summer is attributed to the textile industry in the area. High Zn concentrations are common in soils where textile effluents are discharged (Manzoor *et al.* 2006). Zinc is above the ERL and ERM values suggesting that the chances of Zn ecotoxicity is high. Zinc toxicity generally induces gastrointestinal problems. Metals finishing industries, electroplating industries as well as scrap metal yards contribute to the load of Zn in the river surface sediment (Thambiran 2002).

5.3.5. Bioavailable Metal Concentration in Surface Sediment

Total metal concentrations provide information on the metal content in sediment but not on the availability for uptake by organisms. Metals in sediment are bound to four fractions *viz.* exchangeable/water/acid soluble fraction, oxidisable fraction, reducible fraction and residual fraction. The fractionation of metals provides more information on the availability for uptake and the potential for toxicity. This section investigates the distribution of Cr, Cu, Ni, Pb and Zn in the sediment. The concentrations of metals are sometimes compared to ERL and ERM values to determine the potential toxicity problems. The bioavailable fractions are considered as the exchangeable/water/acid soluble, oxidisable, and reducible fractions of which the readily available fraction is the exchangeable/water/acid soluble fraction. The residual fraction exhibited the predominant concentration which is in accordance with literature (Zerbe *et al.* 1999, Vieira *et al.* 2009).

5.3.5.1. Chromium

The concentration of bioavailable Cr follows the seasonal trend of winter > autumn > spring > summer (Figure 5.3). The general bioavailability distribution is residual > oxidisable > reducible > exchangeable. Deviations from this pattern typically occur in the industrial area.

Winter and autumn exhibit similar trends and this may be due to the lower rainfall during these two seasons. The distribution of Cr in the bioavailable fraction (fractions 1, 2 and 3) decreases downstream with the last site having a similar distribution to the source. In terms of pollutants in the exchangeable fraction, sites 1, 2, 7, 8 and 9 have less than 19% bioavailable Cr. This is significant as it suggests that very little of the Cr entering the environment will be available for uptake by plants and other organisms unless a change to the environment was recorded. The residual fraction accounts for approximately 85% of the Cr concentration prior to the industrial area. This implies that the Cr is not bioavailable for uptake as it is bound in the silicate material. The metal will become bioavailable when physical changes occurs within a system *e.g.* change in pH or redox potential. At site 3, this pattern changes in autumn and winter, and more than 85% of the Cr is bioavailable according to the BCR procedure. This is significant as the bioavailable Cr concentration at site 3 is greater than even the ERL

and ERM value which is a guideline for total metal concentration. Thus there are serious concerns for ecotoxicity during these two seasons. Site 6 and further downstream have less than 50% of Cr in the bioavailable form. The trend displayed in winter and autumn indicates a high concentration of oxidisable matter which is confirmed by the high TDS values in winter. The highest concentration of bioavailable Cr is found in winter site 3.Spring and summer have higher rainfall patterns which keeps metals soluble (Sinclair-Knight-Merz 2013). Thus the distribution, whilst similar to the other seasons, have majority of the Cr in the residual fraction. However, the change in distribution is still prevalent in site 3 clearly indicating that the input is anthropogenic and released into the environment as aqueous or soluble waste.

Key: F1 - Acid Soluble   F2- Fe/Mn oxyhydroxides
F3- Oxidisable   F4- Residual
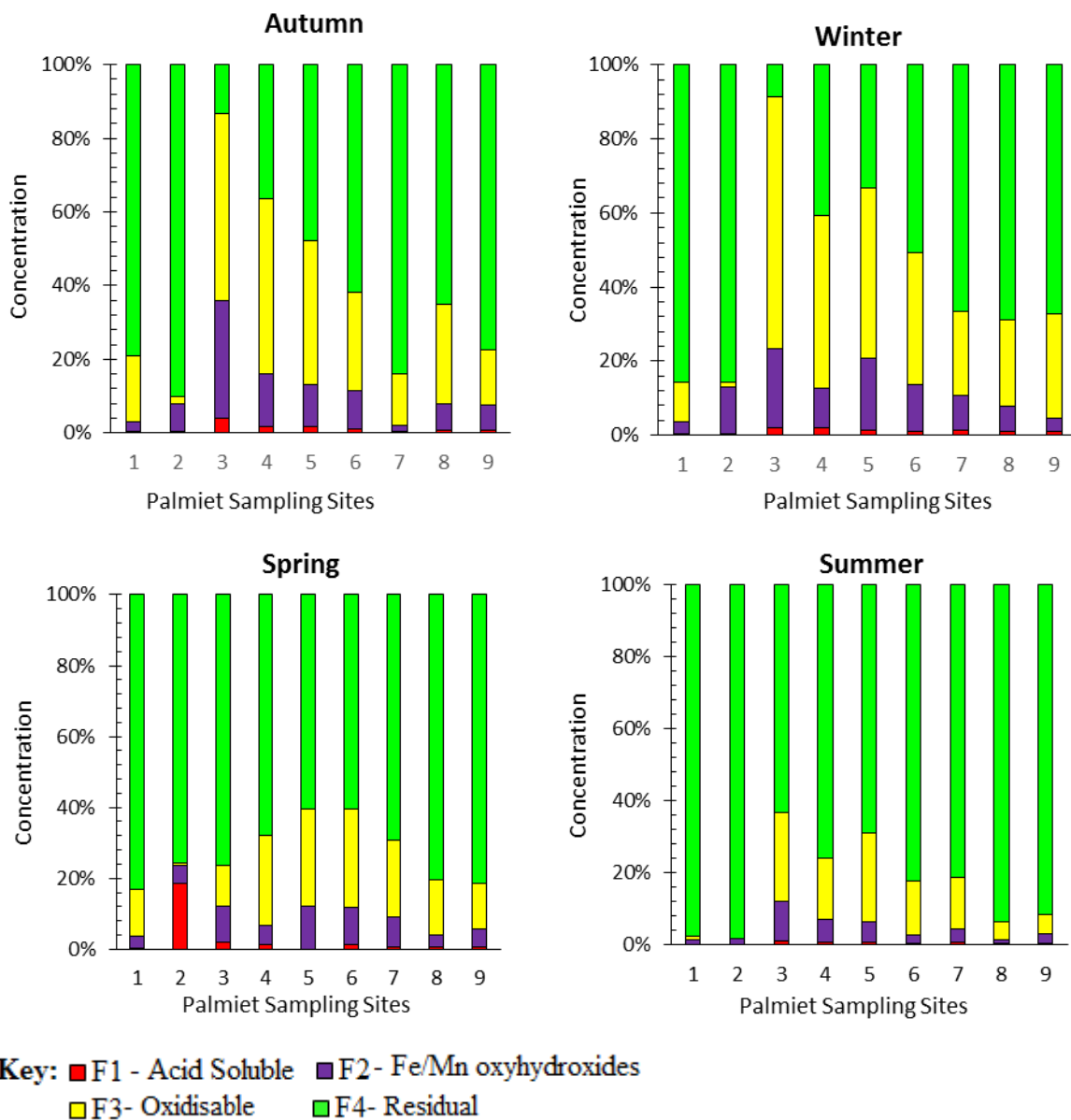
Figure 5.3: Bioavailable Cr in Palmiet River sediment

5.3.5.2. Copper

Copper concentrations are different seasonally however, winter and spring have a similar trend in Cu concentration *i.e* residual > organic > oxyhydroxides > exchangeable (Figure 5.4). Similarly, the trend in autumn and summer is residual > exchangeable > oxyhydroxides > organic. Copper in the residual fraction is predominant in most seasons however there are sites where the distribution is different.

At the industrial sites, the exchangeable/water/acid soluble fraction contributes approximately 20% towards bioavailable Cu. This is a cause for concern as this exceeds the ERL value (for total metal) signifying that Cu is mobile and ready for uptake by organisms. The total metal data supports this theory as the total Cu concentrations are higher than the ERM value at the industrial sites. In spring, the exchangeable fraction is close to the ERM value which may pose a problem. Approximately 30% of Cu has the potential to become bioavailable if pH/Eh changes occur which is of concern as any changes to the system would release Cu into the system. This would have negative effects on the plants and biota as Cu accumulation in organisms cause physiological problems including renal failure (WHO 2004).
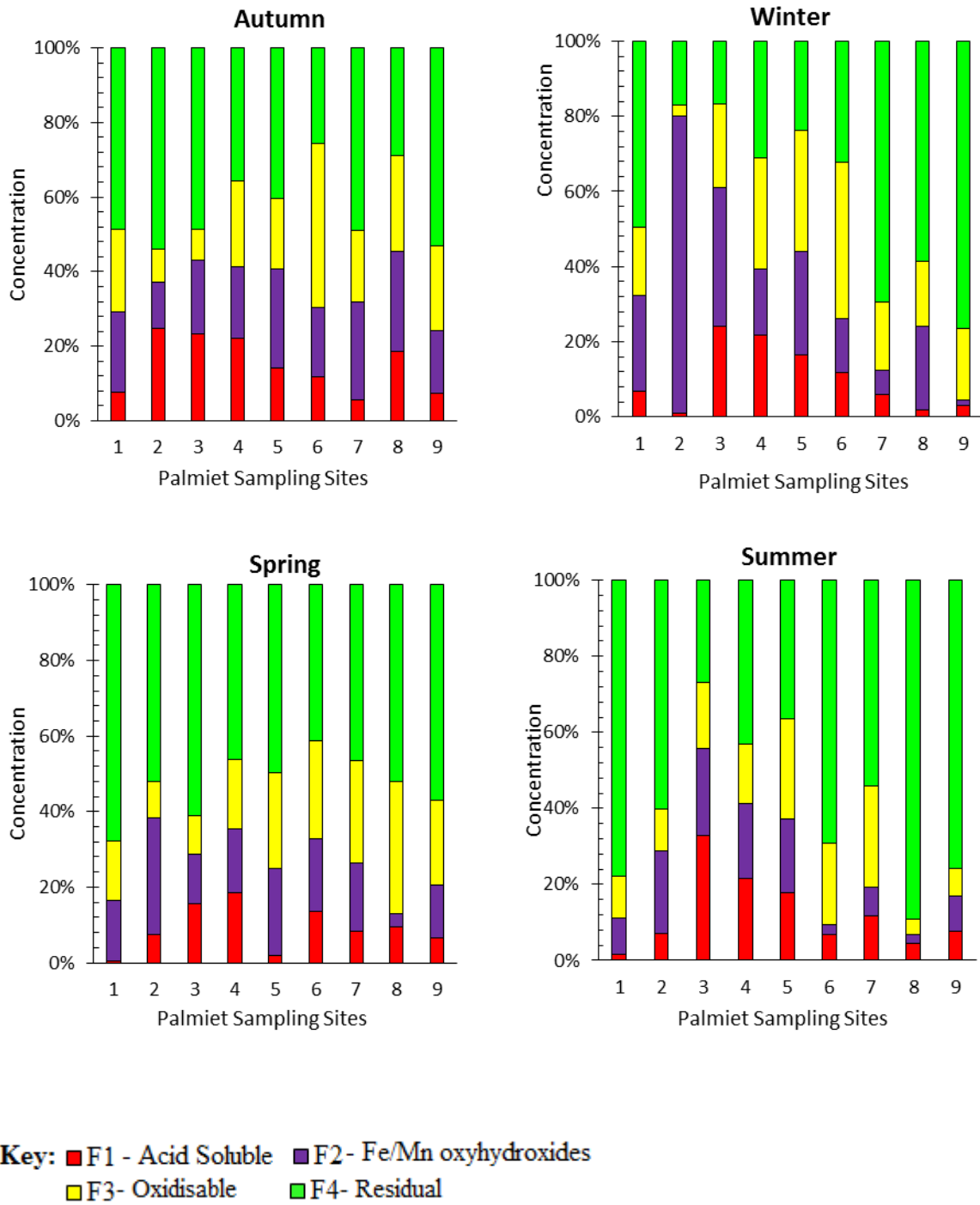
Figure 5.4: Bioavailable Cu in Palmiet River sediment

5.3.5.3. Nickel

There is no apparent distribution pattern in Ni within the seasons however, all the seasons have a predominant trend of residual > exchangeable > oxyhydroxides > organic (Figure 5.5). The total Ni concentration was the highest in spring and the lowest in winter and these two seasons appear to be most similar despite the differences in rainfall (Figure 5.2). As with other metals, the BCR metal distribution is markedly different in the industrial area (Sites 3-5). At the source, the Ni is predominantly in the residual fraction for all seasons. The exchangeable fraction accounts for 20 - 30% of the total Cu concentration in sediment. This is of concern as the ERM value is exceeded ten-fold in the first fraction. Effects including respiratory problems and enzymatic complications (Cempel and Nikel 2006).
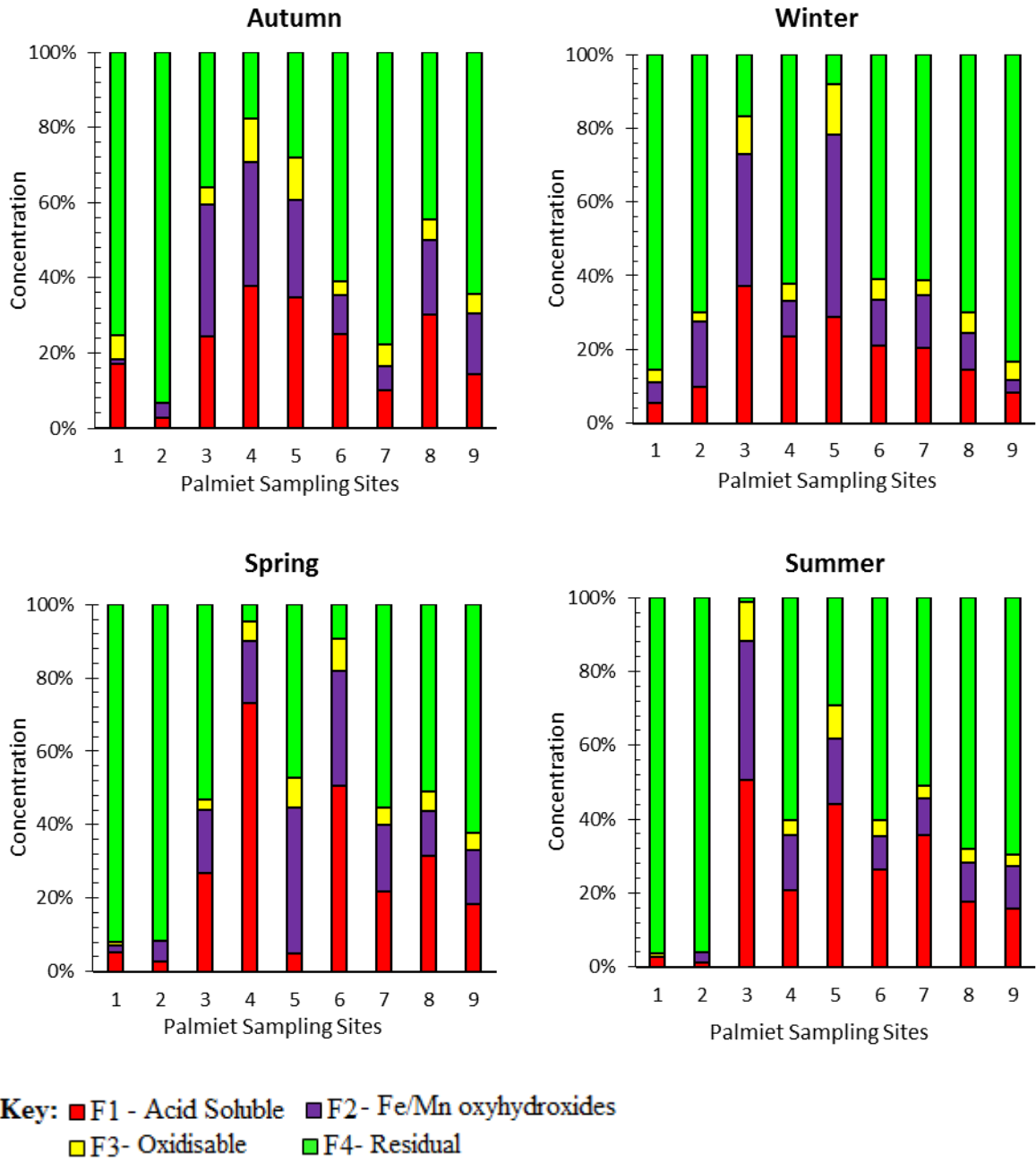
Figure 5.5: Bioavailable Ni in Palmiet River sediment

5.3.5.4. Lead

There is no apparent trend in Pb distribution between seasons however, the predominant trend between spring and summer is residual > oxyhydroxides > organic > exchangeable (Figure 5.6). Autumn and winter exhibit a different trend; oxyhydroxides > residual > organic > exchangeable. Lead exists predominantly in the oxyhydroxide fraction which has been reported in other freshwater systems in literature (Sutherland and Tack 2007, Pillay 2011). Winter and autumn have distributions that are not typical *i.e.* the residual fraction is not the dominant fraction, and may be due to the low flow which may have changed the redox potential. The organic fraction has low concentrations of Pb; this could possibly be due to over extraction of Pb from the reducible fraction. Studies have shown that hydroxylammonium chloride in nitric acid can over extract Pb in the reducible fraction from the organic fraction (Filgueiras *et al.* 2002). The exchangeable fraction accounts for less than 5% of the total Pb concentration. This is below the ERM value therefore immediate toxicity problems would not be experienced by organisms.
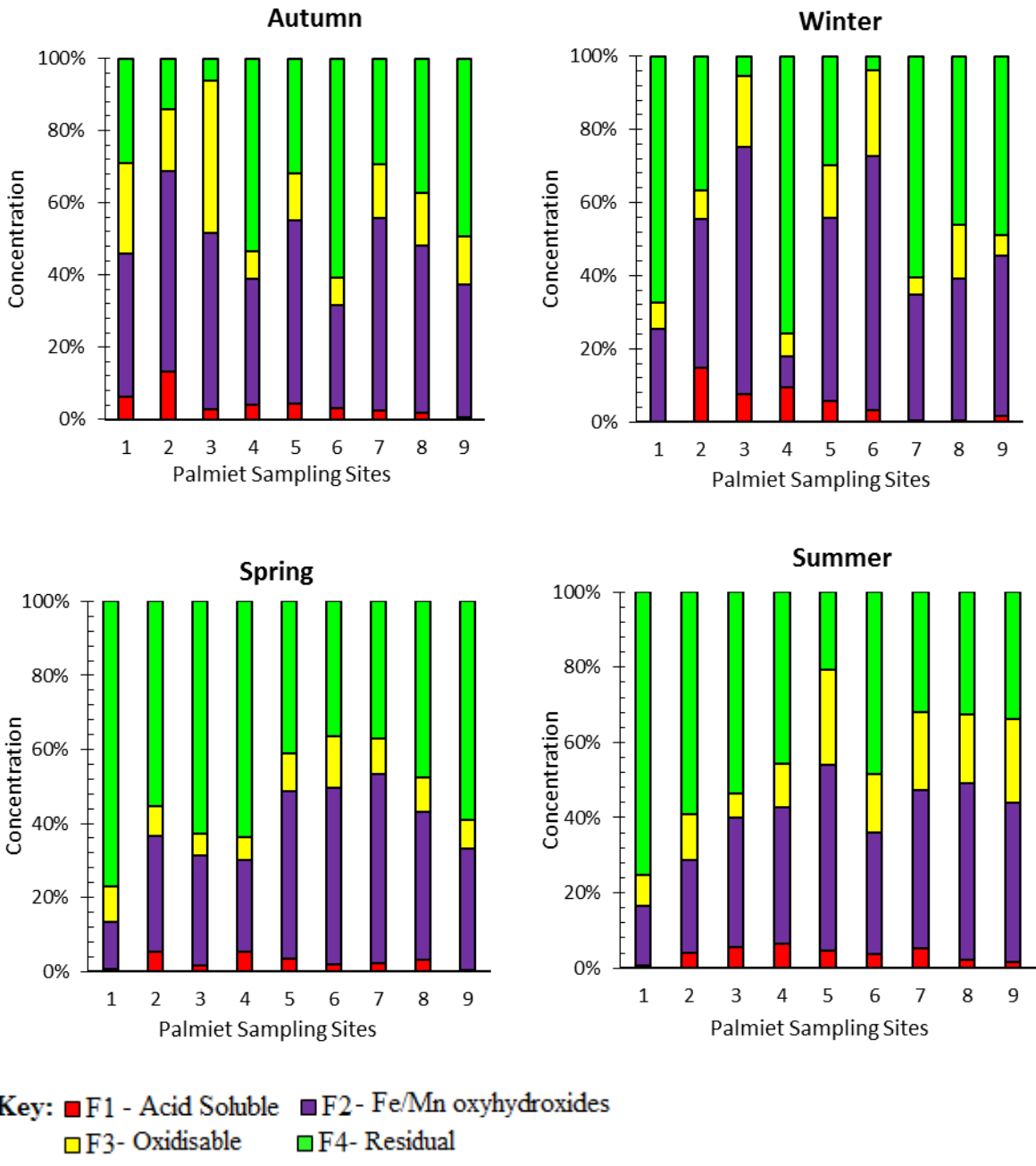
Figure 5.6: Bioavailable Pb in Palmiet River sediment

5.3.5.5. Zinc

Autumn, spring and summer have a similar distribution trend *i.e.* residual > exchangeable > oxyhydroxides > organic (Figure 5.7). Winter exhibits a different trend, exchangeable > oxyhydroxides > residual > organic. The first fraction in all seasons is of particular concern. The Zn in the first fraction is exchangeable and bioavailable. The typical sediment metal association of Zn is bound to organic matter and/or sulfur (Botes 2003). Zinc had elevated total concentrations along the river (Figure 5.2) and it would be expected for a large quantity of Zn to be present in the exchangeable fraction as it was also detected in the water (Table 5.5).
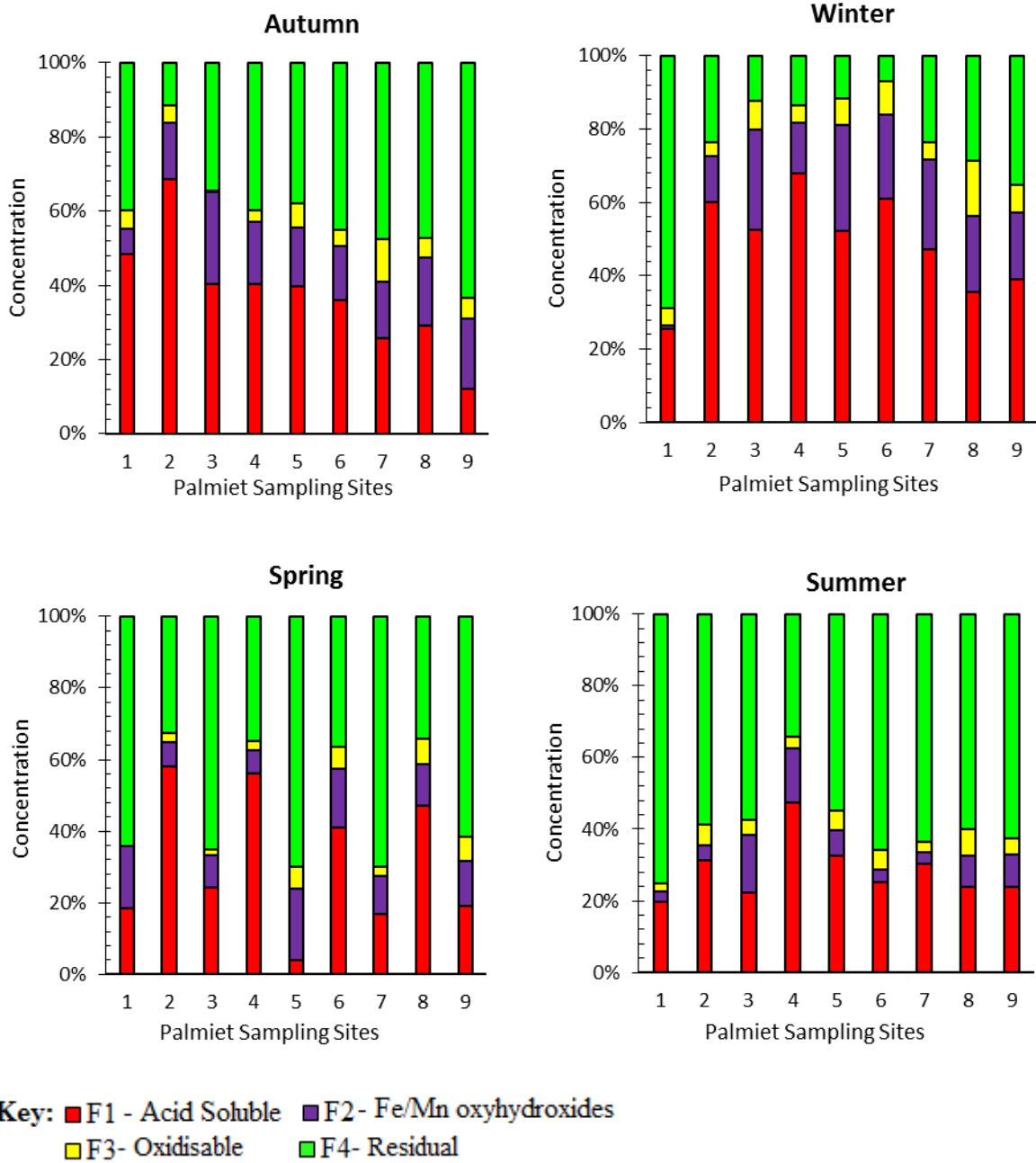
Figure 5.7: Bioavailable Zn in Palmiet River sediment

5.3.6. Statistical Analysis of the Palmiet River

The cluster analysis (CA) clearly indicates that sites 3-4 are not associated with the other sites sampled (Figure 5.8). This is a significant result since these two sites represent the industrial area, and is an indication that the metal input is different. Sites 5-6 exhibit a similar association which is expected since these two sites are of the residential area. Sites 2 and 9 exhibit similar association which is incorporated into a larger cluster of sites 1, 2, 9, 6 and 7. This cluster has a 50% association with sites 5 and 6.

The score plot for Ni in the Palmiet River (Figure 5.9) is similar to the other metals studied. The score plot confirms the association of the Palmiet River sampling sites and their metal distribution associations. Sites 3 and 4 have similar distribution. Sites 1, 2, 7, 8 and 9 have a similar distribution whereas sites 5 and 6 do not correspond to similar distribution patterns.
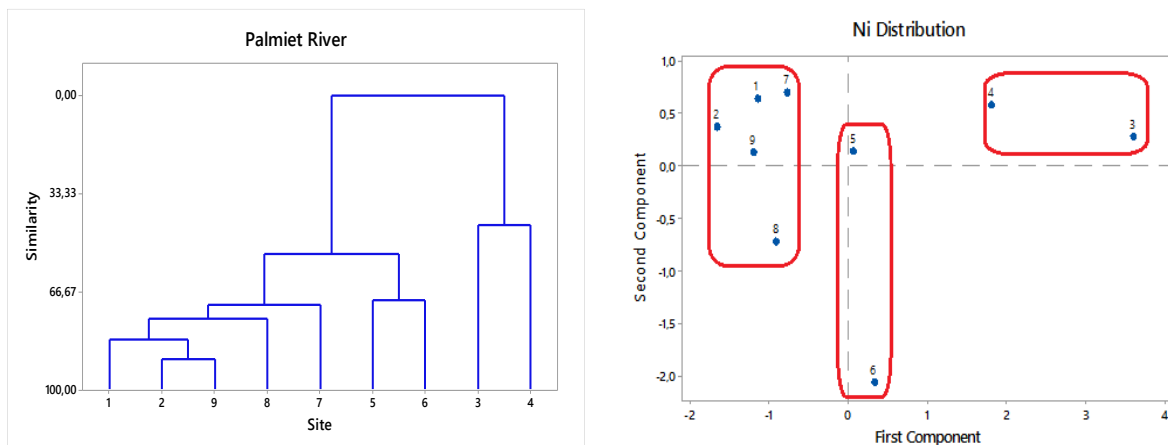
Figure 5.8: Dendogram (left) of the sampling sites and their associations in the Palmiet River and score plot (right) of the Ni distribution in the Palmiet River

5.3.7. Synopsis

There were seasonal variations in the metal concentration in the Palmiet River. Metal concentrations in water and sediment exceeded the guideline limits. The nitrate concentrations exceed the guideline values while sulfate concentrations were comparatively low and phosphates were below detection limits. The bioavailable metal data illustrates that Cu, Ni and Zn are of particular concern as these elements are readily available for biological uptake. Statistical data demonstrates that certain sites along the river are similar in metal distribution and confirms the source of metals to be similar at these sites.

5.4. Sezela River

The Sezela River is predominantly a sugarcane farming and processing region. The physical parameters were measured as an indicator to the water quality. No water data was obtained for winter site 3 and summer sites 1-2 as the river flow had reduced and the river bed was drying up. The total metal concentrations of Cr, Cu, Ni, Pb and Zn in water and surface sediment were quantified to identify pollutants in the river. Partitioning of the selected heavy metals were measured to identify the bioavailability of the metals.

5.4.1. Physical Parameters

The pH of river systems is essential in sustaining life as most species of fish and plants are tolerable to threshold concentrations of hydronium and hydroxide ions. The target water pH has been established to be in a range of 6.5-9.0 to accommodate all species which are pH dependent (DWAF 1996). The seasonal pH was stable at 7.6 ± 0.4 and fell within the normal pH range (Table 5.6). The pH exhibited a trend of increasing from the source to the river mouth in all seasons.

TDS and EC have a direct relationship which is correlated using numerical constants (DWAF 1996). The TDS and EC increase downstream to the estuary and ranged from 81 $\mu S\,cm^{-1}$ to 2140 $\mu S\,cm^{-1}$. Conductivity and TDS in fresh water have SAWQG limits of 250 $\mu S\,cm^{-1}$ (Carr and Neary 2006) and 1000 mg L^{-1} (DWAF 1996). The river has EC values greater predominantly greater than this limit. The activities in Sezela is farming, particularly sugarcane. Fertilisers, commonly used in crop farming have been reported to increase the conductivity of water (Kambwiri *et al.* 2014). Additionally, the sugar milling process may also result in the elevated TDS levels. Sugar milling effluent consists of high levels of TDS which would contribute to the elevated levels found in the Sezela estuary (Saranraj and Stella 2014).

Table 5.6: Physical parameter data for the Sezela River

Sample Site	pH				Conductivity /μS cm^{-1}				TDS / mg L^{-1}			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
1	7.84	7.21	7.51	ND	225	81	85	ND	218	42	44	ND
2	7.01	6.7	7.71	ND	447	86	441	ND	227	45	230	ND
3	7.24	ND	7.41	6.84	523	ND	452	87	263	ND	236	51
4	6.8	6.54	7.49	7.3	585	110	301	539	300	57	278	281
5	7.36	6.61	7.45	7.41	355	87	224	431	181	45	272	228
6	7.79	7.5	7.68	8.03	969	317	1126	1691	497	165	584	885
7	7.93	7.51	7.62	7.89	3650	762	1200	2130	1882	396	1284	1121
8	8.24	7.54	7.58	7.89	3490	764	1275	2140	1799	396	1285	1125
9	8.24	7.62	7.67	7.72	103	761	1286	2140	1804	395	1285	1124

ND – No data available; Values in red exceed guideline limits

5.4.2. Metal Concentration in Water

Metals are found in minerals within sediment. Chemical and physical changes including redox potential changes and desorption processes render these metals mobile in water. The metals generally exist in water as ions or complexes and are found in low concentrations due to dilution and dispersion. The Sezela area does not have many activities which would release metals into the environment and it is expected that metal concentrations would be predominantly low.

Copper and Cr have concentrations generally below the detection limits (Table 5.7). Zinc and Ni have low concentrations, all below the SAWQG. However, the source has elevated levels of Zn and Ni. This may be due to the use of fertilisers around the source area. Studies have shown that the use of fertilisers increase the Zn and Ni content in soil and inadvertently in the water (Thomas *et al.* 2012).

Lead concentrations varied and were < 1 ppb or they exceeded permissible limits. The Sezela sugar cane industry relies solely on heavy machinery which run on fossil fuels. The combustion of these fuels release Pb as particulate matter that settle on sediment and in water (Ramessur and Ramjeawon 2002). This may account for the sporadic appearance of Pb along the river.

Table 5.7: Dissolved metal concentration in the Sezela River water in µg L^{-1} (ppb)

Site	Cu				Zn			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
1	3.93	< 0.4	ND	43.57	45.37	0.51	ND	229.57
2	< 0.4	< 0.4	ND	< 0.4	0.51	0.49	ND	3.13
3	ND	< 0.4	< 0.4	< 0.4	ND	0.79	0.67	0.15
4	< 0.4	< 0.4	0.72	< 0.4	3.29	< 0.2	0.97	6.84
5	< 0.4	< 0.4	< 0.4	< 0.4	1.97	0.26	0.16	5.36
6	< 0.4	< 0.4	< 0.4	< 0.4	0.19	0.16	1.19	0.93
7	< 0.4	< 0.4	< 0.4	< 0.4	0.02	0.021	1.08	0.62
8	< 0.4	< 0.4	8.74	< 0.4	0.71	1.43	< 0.2	0.3
9	< 0.4	< 0.4	1.5	0.93	0.88	0.75	0.5	0.84

Site	Cr				Pb			
	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn
1	10.23	0.12	ND	4.26	35.06	< 1	ND	< 1
2	0.11	0.01	ND	< 0.2	15.54	< 1	ND	< 1
3	ND	< 0.2	< 0.2	1.17	ND	1.46	< 1	10.42
4	< 0.2	< 0.2	< 0.2	< 0.2	< 1	< 1	22.64	5.76
5	< 0.2	< 0.2	0.72	< 0.2	< 1	18.15	< 1	8.09
6	0.47	0.7	0.13	< 0.2	80.87	< 1	33.6	10.68
7	< 0.2	< 0.2	< 0.2	0.71	< 1	< 1	< 1	4.86
8	< 0.2	< 0.2	< 0.2	< 0.2	17.14	41.56	27.32	12.59
9	< 0.2	< 0.2	< 0.2	1.11	14.29	30.58	24.68	< 1

Site	Ni			
	Winter	Spring	Summer	Autumn
1	35.75	28.94	ND	41.19
2	25.92	25.67	ND	18
3	ND	23.46	18.91	18.41
4	16.49	16.77	13.61	13.49
5	4.66	10.23	11.21	11.62
6	37.03	19.22	40.3	25.58
7	24.41	30.61	36.59	30.22
8	23.44	30.39	28.95	25.76
9	16.88	31.67	28	27.01

ND – No data obtained; Values in red exceed the South African Guidelines for water

5.4.3. Anion Concentration in Water

Phosphates, sulfates and nitrates were analysed in the Sezela River for summer and winter (Figure 5.10). The effect of seasonal variation (ANOVA, $p > 0.05$) on anion concentration was evident for all anions. No phosphate was detected at the source however the estuary had phosphate concentrations exceeding the permissible limits (Figure 5.10). Phosphate concentrations were higher in summer than winter. An increasing trend was observed in both seasons from the source to the river mouth. Nitrates and sulfates typically have a higher concentration in winter than summer. In winter, the source (Site 1) exceeded the permissible limit for nitrates while all sulfate concentrations were below the permissible limit. The high levels of anions could be due to the excessive use of fertilisers on crop.
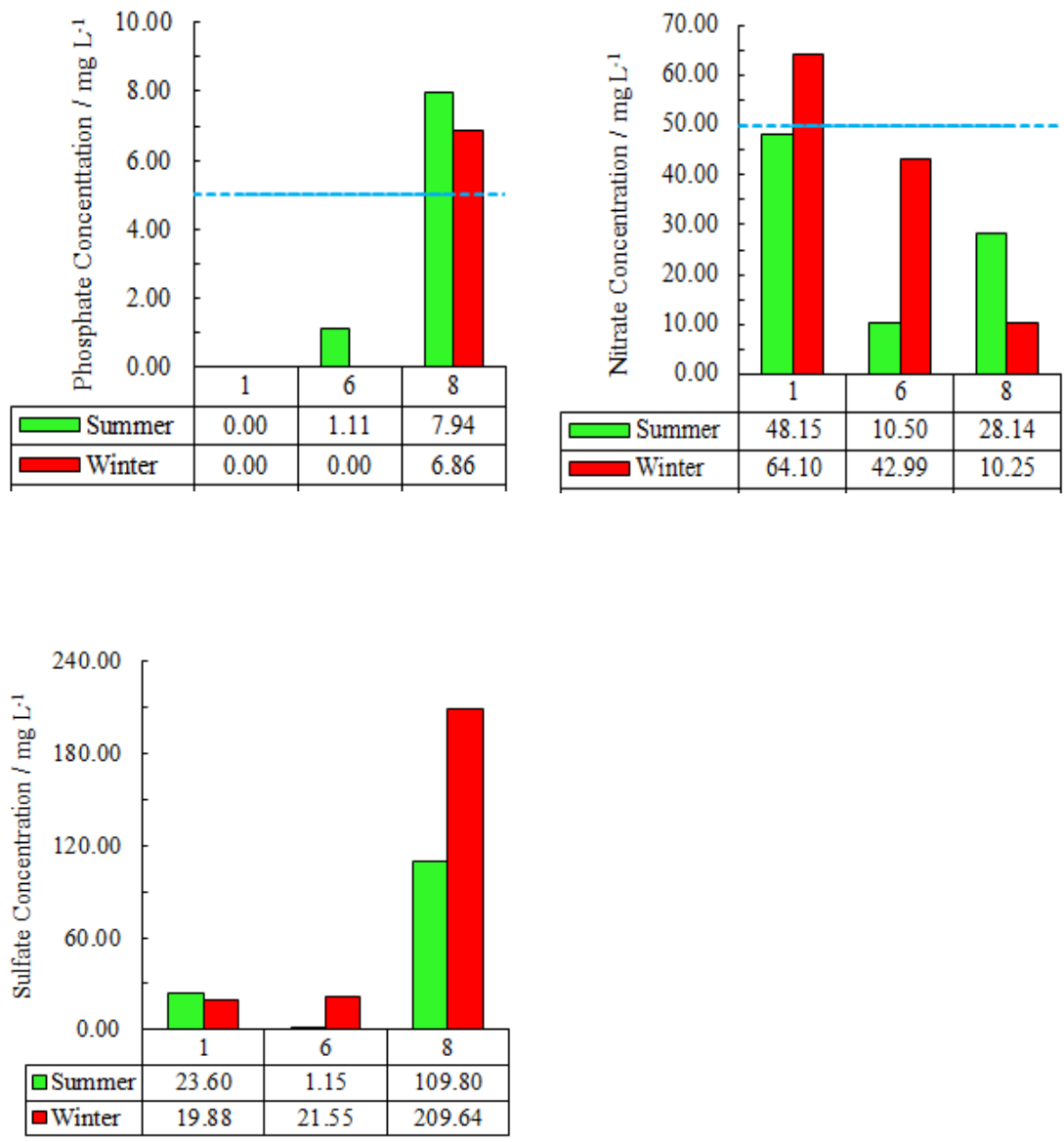
Figure 5.9: Anion concentration in the Sezela River in summer and winter

5.4.4. Total Metal Concentration in Surface Sediment

Total metal concentrations in surface sediment for Cr, Cu, Ni, Pb and Zn at the different sites for the four different seasons are represented in Figure 5.11. There are significant differences in metal concentrations between the four seasons (ANOVA, $p > 0.05$). In addition, there are differences in metal concentrations at different sites, indicating potential point sources of pollutants. Metal distribution along the river indicates an introduction of metals into the system *via* the agricultural and sugar milling industry on the Sezela River, at the tail end of the river. In literature, sugar milling industries and plantations have shown elevated levels of Cr and Cu in both the water and sediment (Molisani *et al.* 1999, Saranraj and Stella 2014). Typically, metal concentration is highest at the estuary which is in close proximity to Sezela Sugar Mill. The metal concentration decreases from the source downstream and then increases at the estuary.
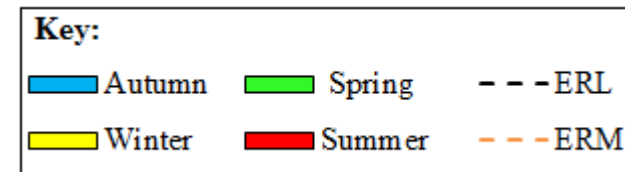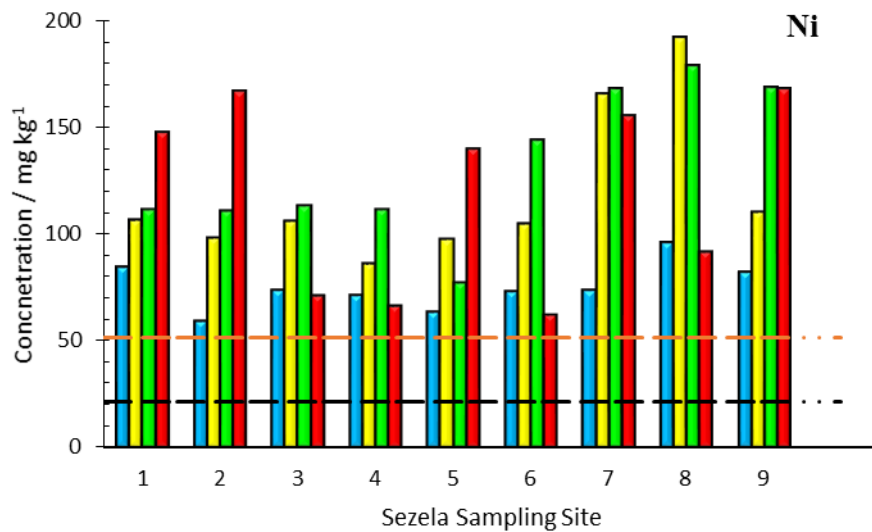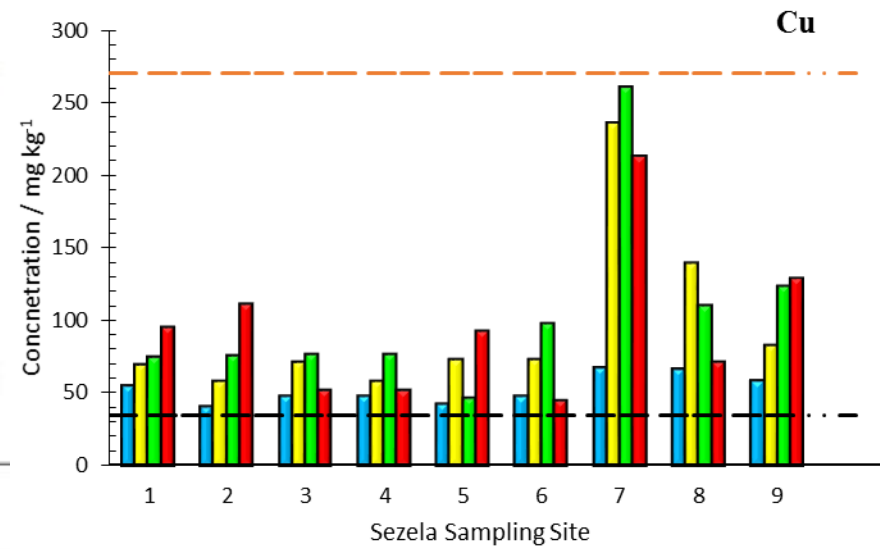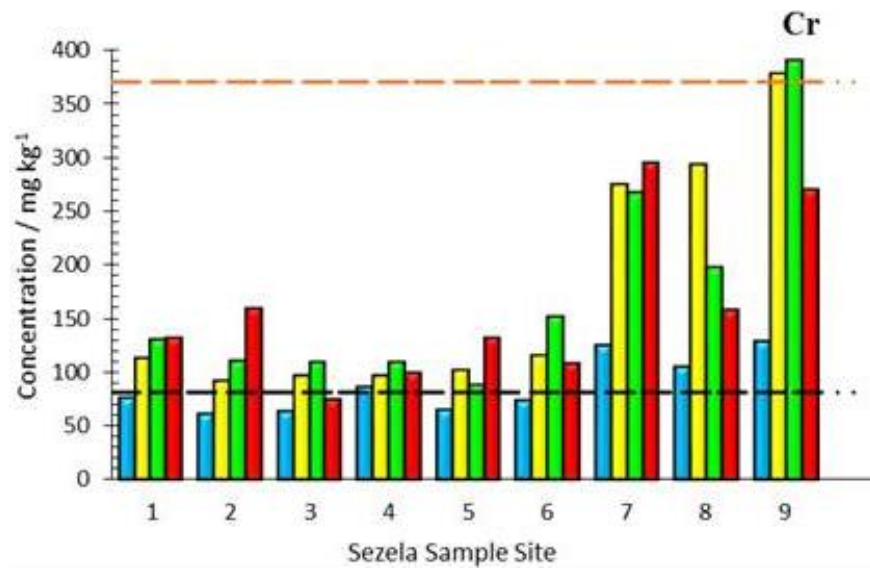
Figure 5.10a: Total surface sediment concentration in the Sezela River
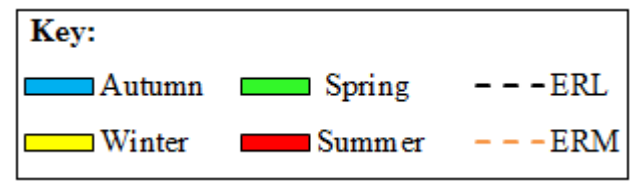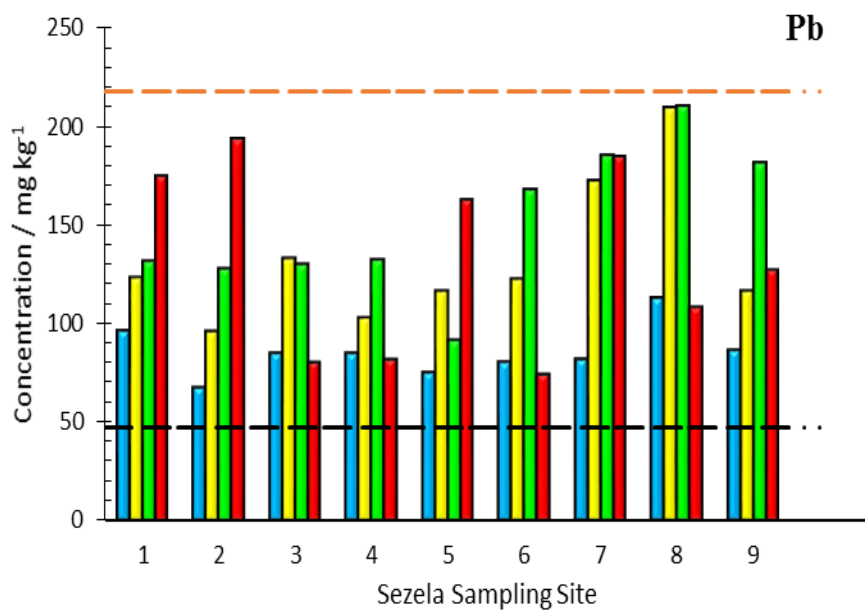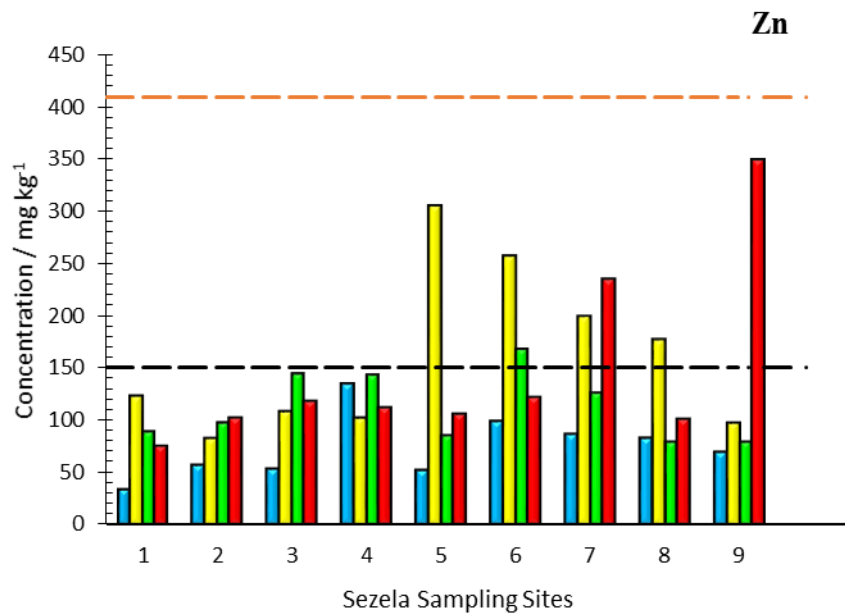
Figure 5.10b: Total surface sediment concentration in the Sezela River

5.4.4.1. Chromium and Copper

Chromium and Cu exhibit similar trends in their total metal distribution both seasonally and spatially. Concentrations vary between the seasons but the general trend observed is spring > summer > winter > autumn (Figure 5.10a).

Deviations from the trend are noted at the estuary in summer where the Cr and Cu concentrations are elevated. This may be due to anthropogenic input from the sugar milling industry in the area. Typical sugarcane treated effluent has been found to contain up to 40 μg L^{-1} Cr and 60 μg L^{-1} Cu (Shiva Kumar and Srikantaswamy 2015). Metals in the effluent adsorb to surface sediment or precipitate out of solution increasing the total metal concentration in the sediment.

Chromium concentrations in the Sezela River range from 60 – 390 mg kg^{-1} and are typically above ERL but below ERM values for most sites and seasons. The estuary has a high concentration of Cr. The estuary opens to the ocean during high tide and Cr may change its species to Cr(VI) which is carcinogenic and predominant in seawater (Bonnand *et al.* 2013). The Cr(VI) form would also be prevalent if the redox potential of the system increased or the concentration of dissolved oxygen increased (Bonnand *et al.* 2013, Shadreck and Mugadza 2013). Chromium(VI) is highly mobile and if absorbed by marine organisms, it can cause mutations and mortality (Shadreck and Mugadza 2013).

Concentrations of Cu ranged from 40 – 260 mg kg^{-1} in the Sezela River. These concentrations are below the ERM guideline value. The Sezela River flows primarily through sugarcane fields and it is expected that sugarcane soil would have Cu concentrations that range from 7 – 725 mg kg^{-1} (Rahman *et al.* 2012). The lower Cu concentrations from sites 1 to 6 may be due to uptake of Cu by sugarcane which assimilates and may have concentrations up to 0.4 mg L^{-1} in the sugarcane juice (Damodharan and Reddy 2014). At neutral pH conditions, Cu generally forms precipitates with sulfur and oxides. The precipitation of Cu is evident from the low concentrations in water.

5.4.4.2. Nickel

Ni concentrations exhibit a different seasonal trend compared to Cr and Cu; spring > winter > summer > autumn (Figure 10a). The distribution within the sites is not uniform and there are indications of an external input to the system as seen by elevated concentrations of Ni at all sites. Nickel concentrations exceed both the ERL and ERM values. This suggests that organisms within the river would experience toxicological effects such as genotoxic effects, carcinogenic effects, gastrointestinal problems and possibly mortality (Carr and Neary 2006, USEPA 2011, Tchounwou *et al.* 2012). The elevated Ni concentration in soil is predominantly from fertiliser which may contain up to 396 mg kg^{-1} d.m (Mohiuddin *et al.* 2011, Harasim and Filipek 2015).

5.4.4.3. Lead

The seasonal trend for Pb concentration is spring > winter > summer > autumn (Figure 10b). Total Pb concentrations along the river have values higher than the ERL but lower than the ERM. A potential source of the Pb is the machinery used for the planting, cultivation and transportation of the sugarcane. These machines rely on the combustion of fossil fuels which promote the release of heavy metals into the environment. Lead released by fossil fuel combustion accumulates in the environment over time (Callender and Rice 2000, Nomngongo and Ngila 2014).

5.4.4.4. Zinc

Zinc concentrations follow the seasonal trend winter > summer > spring > autumn (Figure 10b). Concentrations in the sediment are predominantly below the ERL and ERM values hence minimal toxic effects by Zn would be experienced by organisms.

5.4.5. Bioavailable Metal Concentration in Surface Sediment

Collectively, the exchangeable/water/acid soluble fraction, oxyhydroxide fraction and oxidisable fraction is considered to be the bioavailable fraction. The first fraction (exchangeable/water/acid soluble) is the readily available fraction.

5.4.5.1. Chromium

The concentration of bioavailable Cr follows the seasonal trend of trend winter > autumn > spring > summer (Figure 5.11). The general bioavailability distribution is residual > oxidisable > reducible > exchangeable. Deviations from this pattern typically occur in the industrial area.

Winter and autumn exhibit similar trends and this may be due to the lower rainfall during these two seasons. The distribution of Cr in the bioavailable fraction (fractions 1, 2 and 3) decreases downstream with the last sites having a similar distribution to the source. In terms of pollutants in the exchangeable fraction, these sites (1, 2, 7, 8 and 9) have less than 19 % bioavailable. This is significant as it suggests that very little of the Cr entering the environment will be available for uptake by plants and other organisms unless a change to the environment was recorded. The residual fraction accounts for approximately 85 % of the Cr concentration prior to the industrial area. , This implies that the Cr is not bioavailable for uptake as it is bound in the silicate material. The metal will become bioavailable when physical changes occurs within a system e.g. change in pH or redox potential. At site 3, this pattern changes and more than   85 % of the Cr is bioavailable according to the BCR procedure. This is significant as the bioavailable Cr concentration at site 3 is greater than even the ERL and ERM value which is a guideline for total metals concentration. Thus there are serious concerns for ecotoxicity. Site 6 and further downstream have less than 50 % of Cr in the bioavailable form. The trend displayed in winter and autumn indicates a high concentration of oxidisable matter which is confirmed by the high TDS values in winter.

Spring and summer have higher rainfall patterns which keep metal soluble (Sinclair-Knight-Merz 2013). Thus the distribution, while similar to the other seasons, has the majority of Cr in the residual fraction. However, the change in distribution is still prevalent in site 3 clearly indicating that the input is anthropogenic, released into the environment as aqueous or soluble waste. The highest concentration of Cr is in autumn.
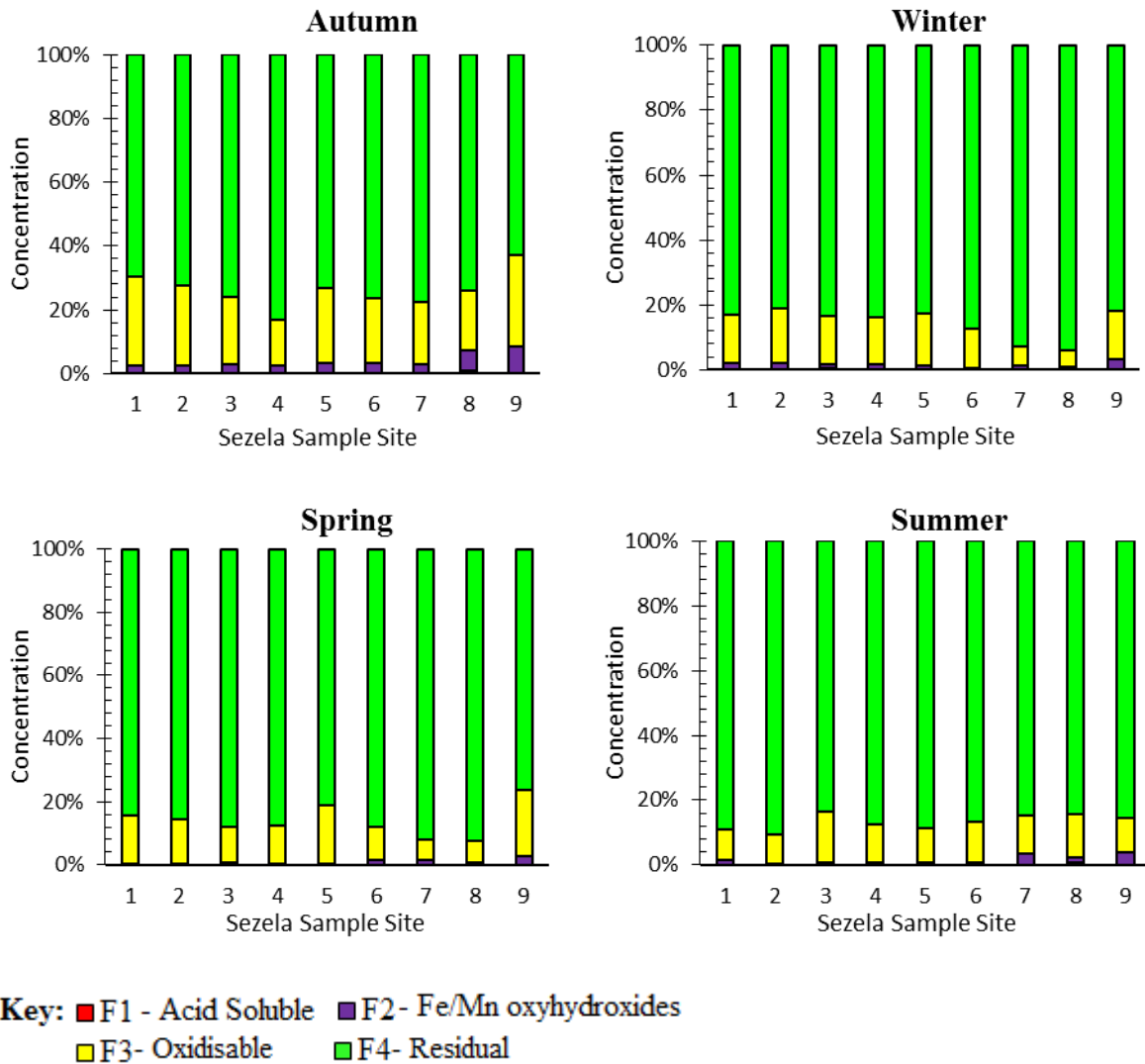
Figure 5.11: Bioavailable Cr in Sezela River sediment

5.4.5.2. Copper

Copper concentrations are different seasonally however, winter and spring have a similar trend in Cu concentration *i.e.* residual > organic > oxyhydroxides > exchangeable (Figure 5.12). Similarly, the trend in autumn and summer is residual > exchangeable > oxyhydroxides > organic. Copper in the residual fraction is predominant in most seasons however there are sites where the distribution is different.

At the industrial sites, the exchangeable/water/acid soluble fraction contributes approximately 20 % of the total Cu. This is a cause for concern as this exceeds the ERL value (for total metal) signifying that Cu is mobile and ready for uptake by organisms. The total metal data supports this theory as the total Cu concentrations are higher than the ERM value at the industrial sites. In spring, the exchangeable fraction is close to the ERM value which may pose a problem. Approximately 30 % of Cu has the potential to become bioavailable if pH/Eh changes occur which is of concern as any changes to the system would release Cu into the system. This would have negative effect on the plants and biota as Cu accumulation in organisms cause physiological problems including renal failure (WHO 2004).
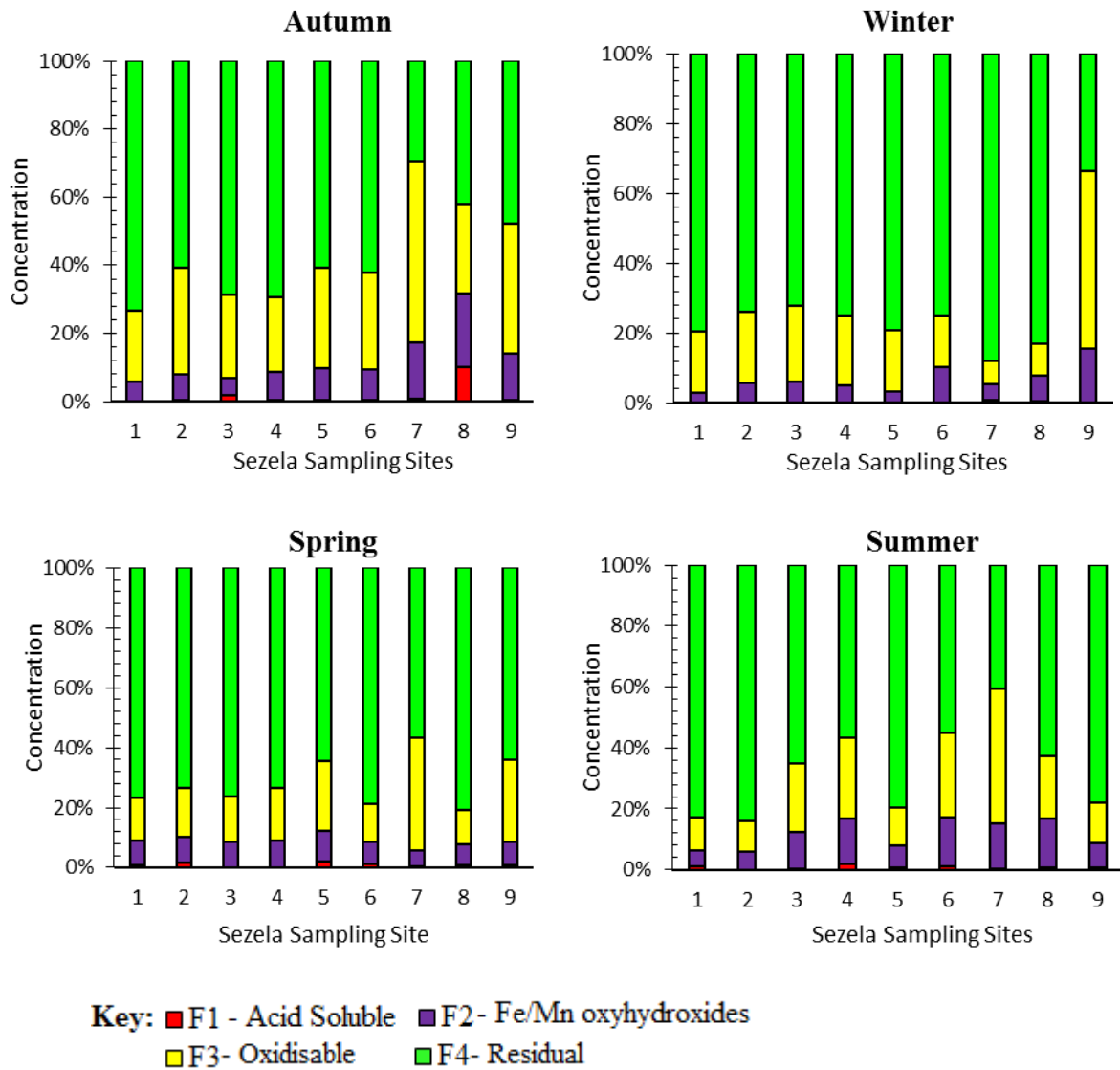
Figure 5.12: Bioavailable Cu in Sezela River sediment

5.4.5.3. Nickel

All seasons have a predominant trend of residual > organic > oxyhydroxides > exchangeable which is similar to Cr and Cu (Figure 5.13). This distribution of Ni is observed in both clean and polluted rivers (Liu et al. 2016). Nickel concentrations in the bioavailable fraction increase downstream to the estuary. Nickel is predominantly in the residual fraction for all seasons. This distribution of Ni is observed in both clean and polluted rivers (Liu et al. 2016). Less than 1 % of the Ni is present in the exchangeable fraction. The bioavailable fractions have a lower concentration of Ni than the ERM. Fertilisers play a role in the introduction of Ni to an ecosystem however the bioavailable Ni concentrations are low (Harasim and Filipek 2015).
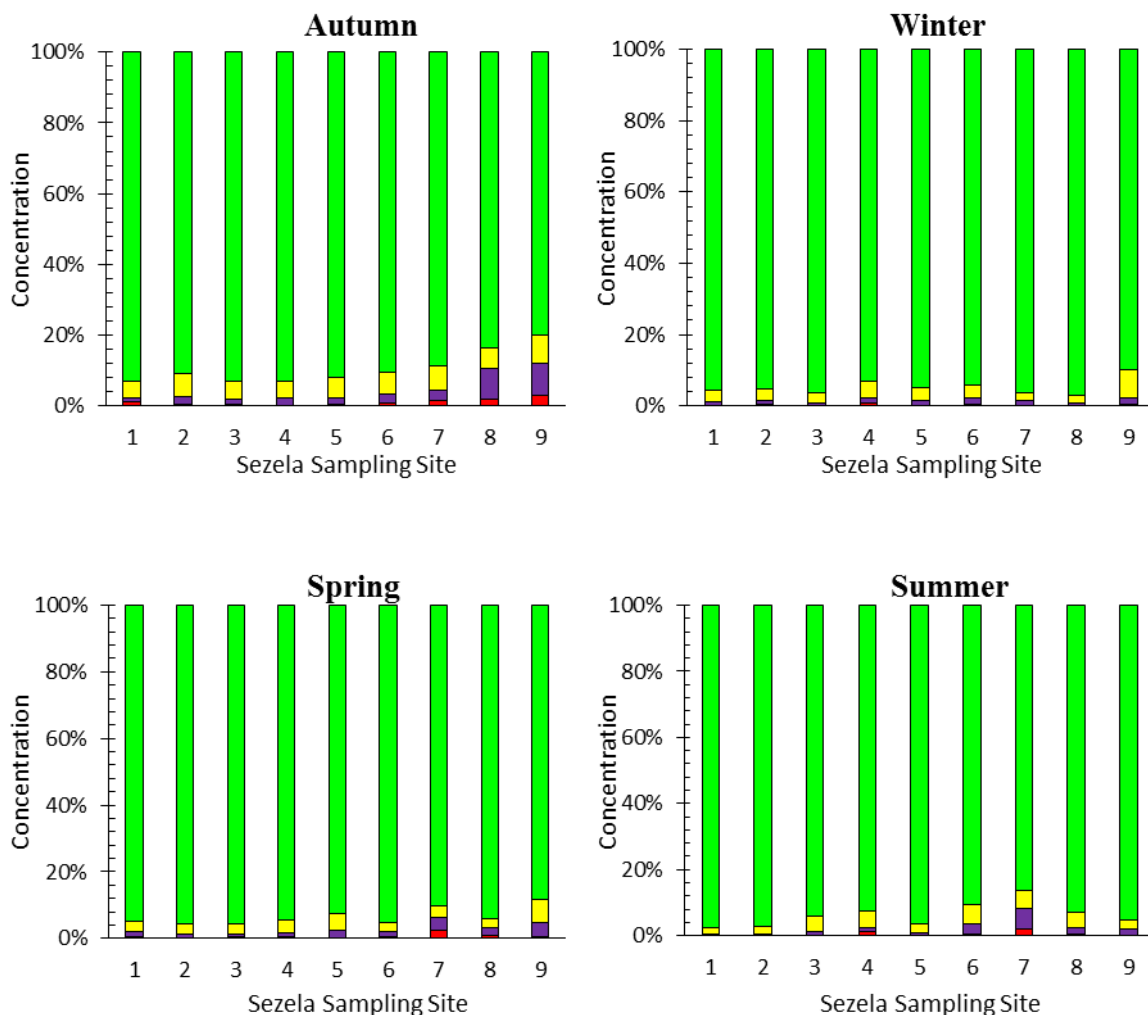
Key: ■ F1 - Acid Soluble   ■ F2 - Fe/Mn oxyhydroxides
     □ F3 - Oxidisable   ■ F4 - Residual

Figure 5.13: Bioavailable Ni in Sezela River sediment

5.4.5.4. Lead

Autumn, spring and summer have the same distribution trend for Pb *viz.* residual > oxyhydroxides > organic > exchangeable while winter follows the trend residual > organic > oxyhydroxides > exchangeable (Figure 5.14). Typically, in freshwater sediment, the highest concentration of Pb is found in the oxyhydroxide fraction which is not seen predominantly in this river profile (Mossop and Davidson 2002). Summer site 3 is an anomaly to the distribution pattern since oxyhydroxides > organic > residual > exchangeable which fits the typical distribution trend. The predominant fraction is the residual fraction indicating that the Pb is unavailable for uptake. The exchangeable/water/acid soluble fraction has approximately 0.5 % and will not have an impact on the environment.
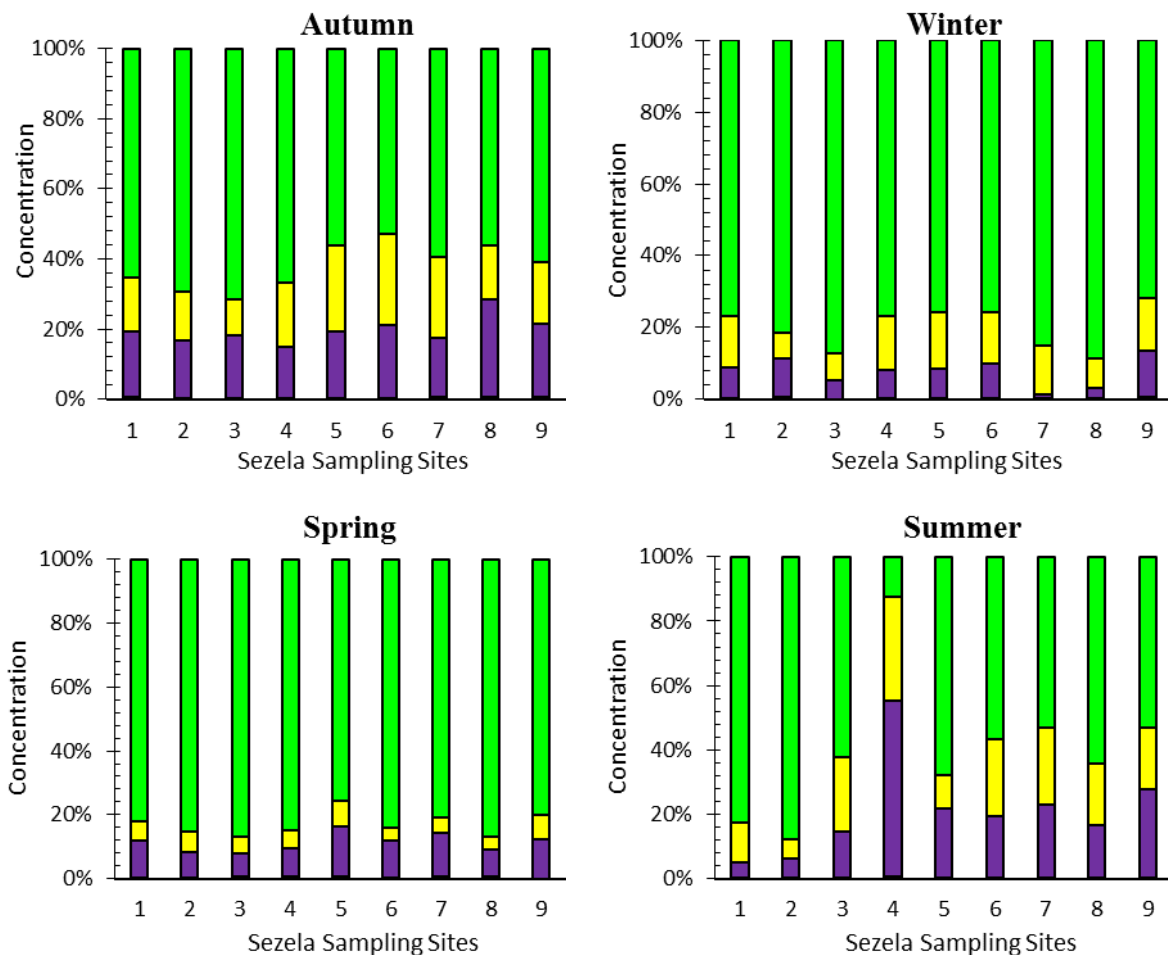
Figure 5.14: Bioavailable Pb in Sezela River sediment

5.4.5.5. Zinc

The predominant distribution of Zn in autumn, winter and summer is residual > organic > oxyhydroxides > exchangeable (Figure 5.15). The distribution trend in spring differs and is residual > organic > exchangeable > oxyhydroxides. The predominant distribution observed does not fit the distribution trends in other studies of freshwater systems which was exchangeable > oxyhydroxides > organic > residual (Segura *et al.* 2006, Vasile *et al.* 2008). However, in autumn site 3 follows the typical Zn distribution trend (Vasile *et al.* 2008).

Key: ■ F1 - Acid Soluble ■ F2 - Fe/Mn oxyhydroxides
     □ F3 - Oxidisable ■ F4 - Residual
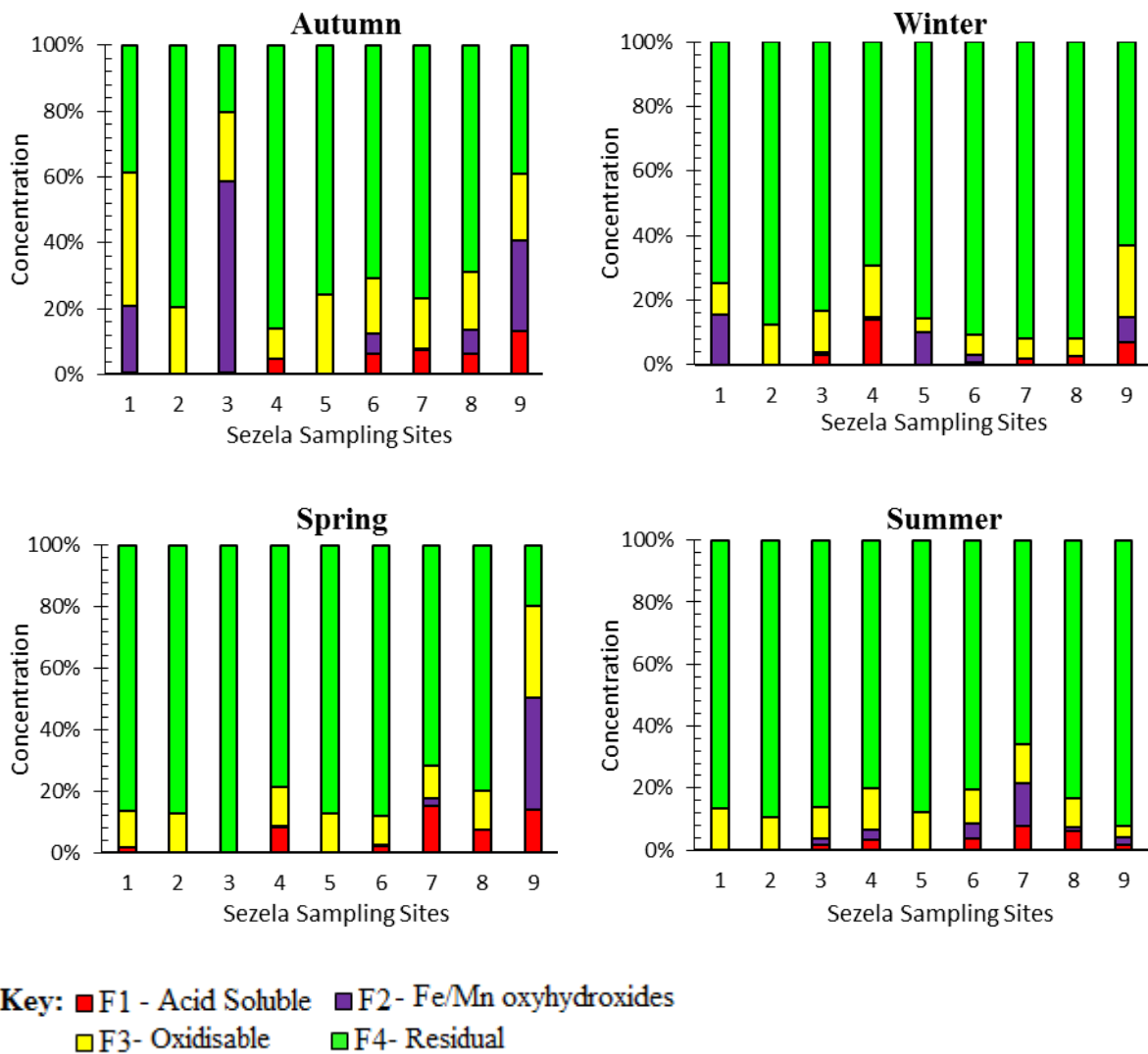
Figure 5.15: Bioavailable Zn in Sezela River sediment

5.4.6. Statistical Analysis of the Sezela River

The CA for the Sezela River (Figure 5.16) show some important associations. Site 1-6 are associated with each other indicating similar contaminant or metal inputs into the system. Site 7 which showed the most variability in terms of metal concentrations, and bioavailable distribution is not associated with other sites. It is the site at which the mill effluent enters the Sezela River. The effluent is not considered to be a contaminant since levels are below the guideline values. The change in the BCR associations from the other sites clearly show that industrial input has the potential to change metal mobility. The closest association to site 7 are sites 8-9 which is expected as these are the sites that follow.

The score plot for Cr distribution in the Sezela River (Figure 5.16) is similar to other heavy metals studied. The score plot provides further evidence of the site associations to metal distribution in the Sezela River. Sites 1, 2 and 5 have similar distribution while sites 3, 4 and 6 are closely related. Sites 8-9 are similar and site 7 has no association with other sites.
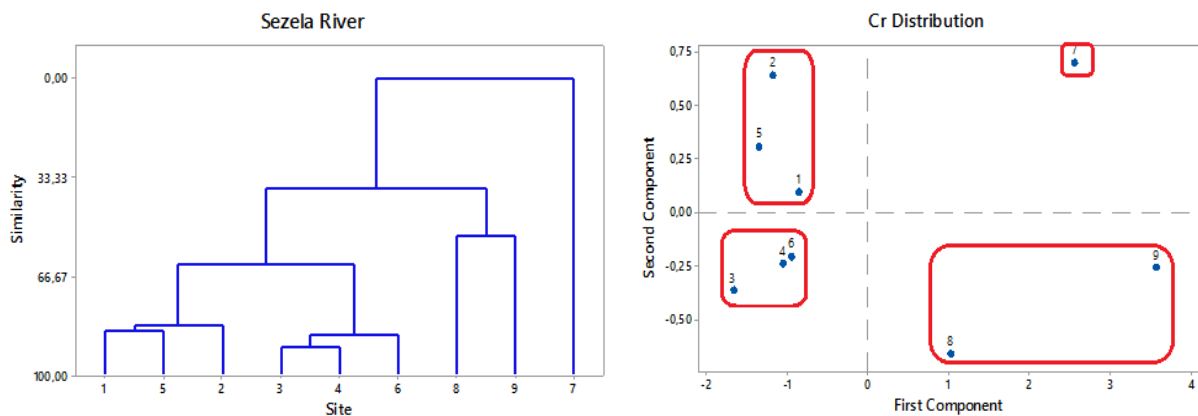
Figure 5.16: Dendogram (left) of the sampling sites and their associations and score plot (right) distribution of Cr in the Sezela River.

Figure: Score plot distribution of Cr in the Sezela River

5.4.7. Synopsis

The Sezela River has Pb water concentrations exceeding the guideline which is of concern. The sediment metal concentrations are below ERM values with the exception of Ni. The bioavailable metal distribution illustrates metals to be predominantly in the residual fraction hence unavailable for uptake. The phosphate and sulfate concentrations exceed guideline values which would be expected as agricultural rivers have elevated fertiliser components. The PCA and CA illustrates that the metal distribution is different at the estuary compared to the river.

5.5. Comparison of the Palmiet and Sezela Rivers

A comparison between the two rivers is made to determine the activities which place a stress on the ecosystem. The Palmiet River is defined as an industrial-residential river whereas the Sezela River is defined as an agricultural-agricultural processing river. The Palmiet River is therefore expected to exhibit higher heavy metal concentrations while the Sezela River may be more prone to inorganic anion pollutants due to the nature of agricultural activities.

5.5.1. Anion Pollutants

A comparison of the average phosphate, nitrate and sulfate concentrations in both rivers was done (Table 5.8). All three anions are different to each other in the rivers. Phosphates and sulfates in the Palmiet River are lower than the Sezela River. These anions are deposited in the river *via* run-off from the fertilised sugarcane fields. However, the levels present in Sezela are not above the listed guideline values. Nitrate levels in the Palmiet River are higher than the Sezela River. Additionally, the levels exceed the stipulated guideline value. The influx of nitrates may be due to the use of nitric acid in industrial processing in the Pinetown area (Thambiran 2002).

Table 5.8: Average metal (sediment) and anion (water) concentrations of the Palmiet and Sezela Rivers.

River	Inorganic Element / mg kg^{-1}					Anion / mg L^{-1}		
	Cr	Cu	Ni	Pb	Zn	PO_4^{3-}	SO_4^{2-}	NO_3^-
Palmiet	415.90	334.27	296.35	212.82	713.96	< DL	23.31	135.00
Sezela	147.48	87.80	110.83	124.79	123.04	2.65	64.27	34.02

Values in red indicate concentrations exceeding the ERM values (metals) and guideline values (anions)

5.5.2. Metal Pollutants

The Palmiet River had higher average metal concentrations in sediment than the Sezela River (Table 5.8). The Palmiet River has metal, metal processing, paint and chemical manufacturing industries in the Pinetown area which contribute significantly to the metal concentrations in the river. Chromium, Cu, Ni and Zn exceed the ERM values which indicates that the risk of metal toxicity is greater. In comparison, metal concentrations in the Sezela River are significantly different to Palmiet (ANOVA, *p >0.05*) and are considerably lower. The effect of industrial activities on metal introduction into the environment can be clearly seen in these two river systems.

5.5.3. Comparison of Palmiet and Sezela Rivers to other South African (SA) Rivers

The two rivers studied were compared to a number of other SA rivers where significant research has been carried out. The Palmiet River is a tributary of the Umgeni River.

5.5.3.1. Anions

Other industrial rivers are compared with the Palmiet River (Table 5.9). No phosphates were detected in the Palmiet and Umgeni Rivers. The nitrate concentration in both these rivers are above the guideline value which suggests that nitrate contamination is a problem since the Jukskei River has comparatively lower concentrations. Sulfates are of concern with concentrations greater than 500 mg L^{-1} (WHO 2006). Sulfates are generally precipitates due to its solubility with cations particularly of Ba, Sr, Pb and Ca.

Sezela River has average anion concentrations below guideline limits (Table 5.9). Compared to other agricultural rivers, the Sezela River has elevated levels of anions. Fertilisers are primary input sources of anions into freshwater systems. The Sezela River flows through sugarcane plantations similar to the Umdloti River (Olaniran *et al.* 2013). This suggests that

commercial fertilisers are being used excessively in these regions as other agricultural rivers have much lower concentrations.

Table 5.9: Comparison of average anion concentrations in this study and other South African rivers.

River	Type of River	Anion / mg·L^{-1}			Reference
		PO$_4^{3-}$	NO$_3^-$	SO$_4^{2-}$	
Palmiet	Industrial	0.00	135.00	23.31	
Umgeni	Industrial	-	66.97	193.57	(Dikole 2014)
Jukskei	Industrial	0.4	22.3	195	(Huizenga and Harmse 2005)
Sezela	Agricultural	2.65	34.02	64.27	
Umdloti	Agricultural	1.06	0.13	27.86	(Olaniran *et al.* 2013)
Crocodile	Agricultural	0.043	0.49	-	(van der Laan *et al.* 2012)
Pongola	Agricultural	0.034	0.45	-	(van der Laan *et al.* 2012)

Values in red indicate concentrations exceeding the guideline values

5.5.3.2. Metal Contaminants

Rivers with similar anthropogenic activities in South Africa were compared to the Palmiet and Sezela Rivers (Table 5.10). The Umgeni and Isipingo Rivers are well documented rivers for heavy metal pollution (Carnie 2014). Industrial effluent containing dissolved metals are released into rivers and waterways. These metals accumulate in sediment and begin to affect the environment. The Palmiet River, a tributary river, has higher concentrations in sediment than the major rivers which is of concern. Industrial rivers have higher concentrations of metals than the agricultural rivers. This suggests that toxicity is of concern particularly in industrial rivers.

The Sezela River has higher average metal concentrations in sediment than similar agricultural rivers (Table 5.10). Metal accumulation in the sediment may be due to the use of fertilisers and additives to the sugarcane fields. The geology of the region contributes to the total metal concentration of the area. The agricultural activity around the Sezela River is a larger scale activity compared to the Tyume and Mvudi regions which rationalises the higher concentrations as more fertiliser and resources are utilised.

Table 5.10: Comparison of total heavy metal concentration (average concentration) in sediment from South African river studies

River	Type of River	Cr / mg kg^{-1}	Cu / mg kg^{-1}	Ni / mg kg^{-1}	Pb / mg kg^{-1}	Zn / mg kg^{-1}	Reference
Palmiet	Industrial	415.9	334.27	296.35	212.82	713.96	
Umgeni	Industrial	82	85	-	172	145	(Dikole 2014)
Isipingo	Industrial	248.44	195.75	32.76	228.75	1796	(Pillay 2011)
Vaal	Industrial	68.77	32.27	71.27	9.4	46.27	(Pheiffer *et al.* 2014)
Orange	Industrial	16.8	20.1	33.8	4.6	31.3	(Pheiffer *et al.* 2014)
Swartkops	Industrial	20.3	6.8	-	33	36	(Binning and Baird 2001)
Sezela	Agricultural	147.48	87.8	110.83	124.79	123.04	
Tyume	Agricultural	-	0.279	0.6	0.052	0.329	(Awofolu *et al.* 2005)
Mvudi	Agricultural	97.8	30.24	-	4.28	26.56	(Edokpayi *et al.* 2016)

Values in red represent concentrations greater than the ERM value

5.6. References

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Chapter 6

Conclusion

The chemical composition of Palmiet and Sezela Rivers were analysed to highlight the effect that different types of anthropogenic input *viz.* industrial and agricultural, have on the river health.

The Palmiet River has a higher concentration of metals than the Sezela River. The spatial distribution of sampling sites within each river indicated that there were direct inputs and anthropogenic hubs along the river. Industries in Pinetown are key enterprises in metal processing, metal manufacturing, chemical manufacturing, and motor mechanics in the municipality which impacts the water quality of the river. These industries utilise Cr, Cu, Ni, Pb and Zn on a regular basis and generally discard these metals in effluent. The partitioning of metals in sediment using the BCR sequential extraction method was performed. The Palmiet River had Cu, Ni, Pb and Zn predominantly in the bioavailable fraction. This is a potential problem due to metal toxicity on aquatic and plant life in the river and riparian zones. PCA and CA were utilised to confirm this as distinct regions of input were illustrated. Industrial input is clearly seen to affect the metal concentrations more severely than agricultural input. Deeper management is required on the Palmiet River to prevent the system from degrading further.

There was no statistical differences in pH seasonally in both rivers. The average pH over all seasons were 7.2 and 7.6 for the Palmiet and Sezela Rivers, respectively. Electrical conductivity and TDS varied seasonally in both rivers. Electrical conductivity is higher in Sezela River particularly in summer and autumn. Conversely, the EC and TDS values are the lowest in the Palmiet River. Sezela River had experienced a higher rainfall than the Palmiet River which may have contributed to the run-off of nutrients from fertile soil into the river. This would provide a plausible explanation for the elevated EC and TDS levels in the Sezela River. The Sezela River had higher concentrations of sulfates and phosphates. Metal concentrations were below the ERM value and metals were predominantly in the residual

fraction. The system would require a harsh event to mobilise the metals from the residual fraction which is highly unlikely. Agricultural activities may not introduce a metal contamination stress on the system over a short period of time but, has the potential to accumulate the contaminants and introduced toxicity problems over a period of time.

A recommendation for the Palmiet and Sezela Rivers would be the implementation of regular monitoring of nutrient and metal concentrations with the addition of more stringent protocols in place. Nutrient analysis would be important, particularly in agricultural systems, as an influx in nutrients enhance the effects of algal bloom.

Future Work

The inorganic contaminant analysis leads to several other avenues of research.

- Nutrient Analysis

  Analysis of agricultural rivers for nutrients which would provide information on water quality and suitability of use. It would also provide information on fertilisers and the capacity of crop to absorb nutrients.

- Geological deposition

  Analysis of sediment cores in industrial rivers to determine the time period of pollutant deposition. This would help identify if pollution in a river has been a long standing problem or a current problem.

- Pesticide Analysis

  Analyses of water and sediment for pesticides to determine if organic pollution is a problem in rivers.

- Metal speciation of Cr

  Speciation of Cr in the rivers to identify and quantify Cr(VI).

A comparative study of the effect of anthropogenic activity on the Palmiet and Sezela Rivers, South Africa

Appendix

Appendix A: Certified Reference Material Information Sheets

Appendix B: Total Metal Data

Appendix C: Bioavailable Metal Data

Appendix A

CERTIFIED REFERENCE MATERIAL
BCR® – 701

CERTIFICATE OF ANALYSIS

SEDIMENT				
		Extractable mass fraction based on dry mass		Number of accepted sets of data p
		Certified value [1] [mg/kg]	Uncertainty [2] [mg/kg]	
Step 1:	Cd	7.34	0.35	14
	Cr	2.26	0.16	14
	Cu	49.3	1.7	14
	Ni	15.4	0.9	13
	Pb	3.18	0.21	14
	Zn	205	6	14
Step 2:	Cd	3.77	0.28	14
	Cr	45.7	2.0	12
	Cu	124	3	14
	Ni	26.6	1.3	12
	Pb	126	3	11
	Zn	114	5	12
Step 3:	Cd	0.27	0.06	13
	Cr	143	7	12
	Cu	55.2	4.0	13
	Ni	15.3	0.9	13
	Pb	9.3	2.0	12
	Zn	45.7	4.0	11

[1] Unweighted mean value of the means of p accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The certified value is traceable to sequential extraction procedure as described in the certification report.

[2] Half-width of the 95 % confidence interval of the mean defined in [1].

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is 1 g.

NOTE

This material has been certified by BCR (Community Bureau of Reference, the former reference materials programme of the European Commission). The certificate has been revised under the responsibility of IRMM.

Brussels, January 2001

Signed:

Latest revision: May 2007
Prof. Dr. Hendrik Emons Unit for Reference Materials EC-JRC-IRMM
Retieseweg 1112440 Geel, Belgium

Indicative Values

	Mass fraction based on dry mass	
	Indicative value [1] [mg/kg]	Uncertainty [2] [mg/kg]
Cd	0.13	0.08
Cr	62.5	7.4
Cu	38.5	11.2
Ni	41.4	4.0
Pb	11.0	5.2
Zn	95	13

[1] Unweighted mean value of the means of p accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The indicative value is traceable to determinations following ISO 11466.

[2] Half-width of the 95 % confidence interval of the mean defined in [1].

DESCRIPTION OF THE SAMPLE

The material consists of a sediment sample in a glass bottle containing about 20 g of powder.

ANALYTICAL METHOD USED FOR CERTIFICATION

- Electrothermal atomic absorption spectrometry
- Flame atomic absorption spectrometry
- Inductively coupled plasma emission spectrometry
- Inductively coupled plasma mass spectrometry

PARTICIPANTS

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- University of Reading, Dept. of Soil Science, Reading (GB)

SAFETY INFORMATION

The usual laboratory safety precautions apply.

INSTRUCTIONS FOR USE

The material is intended for the verification or validation of an analytical procedure. This material is not intended for use as a calibrant.

Before a bottle is opened, it should be shaken manually so that the material is re-homogenised. The sample must be used as it is from the bottle. The correction to dry mass should be made on a separate portion of 1 g which should be dried in an oven at (105 ± 2) °C for 2-3 h until constant mass is attained. The analysis must be carried out following strictly the sequential extraction protocol given in the certification report.

STORAGE

The tightly closed bottles should be kept at 4 °C in the dark.

However, the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples.

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NOTE

A technical report on the production of BCR-701 is supplied on the internet (http://www.irmm.jrc.be/). A paper copy can be obtained from IRMM on request.

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2702

Inorganics in Marine Sediment

This Standard Reference Material (SRM) is marine sediment collected at the mouth of the Baltimore Harbor, Baltimore, MD and is intended for use in evaluating analytical methods for the determination of selected elements in marine or fresh water sediment and similar matrices. All of the constituents in SRM 2702, for which certified, reference, and information values are provided, were naturally present in the sediment material before processing. A unit of SRM 2702 consists of a bottle containing 50 g of radiation-sterilized, freeze-dried sediment material.

Certified Concentration Values: Certified values for concentrations, expressed as mass fractions, for 25 elements are provided in Table 1. The certified values are based on the agreement of results from two or more chemically independent analytical techniques obtained at NIST and collaborating laboratories [1]. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into accounted.

Reference Concentration Values: Reference values for concentrations, expressed as mass fractions, are provided for eight additional elements in Table 2. Reference values are non-certified values that are the best estimate of the true value. However, the values do not meet the NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods [1].

Information Concentration Values: Information values for concentrations, expressed as mass fractions, are provided in Table 3 for 11 elements. An information value is considered to be a value that will be of interest and use to the SRM user, but for which insufficient information is available to assess adequately the uncertainty associated with the value, or only a limited number of analyses were performed [1].

Expiration of Certification: The certification of SRM 2702 is valid, within the measurement uncertainty specified, until 01 October 2022, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Handling, Storage and Use"). However, the certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

The coordination of the technical measurements leading to the certification of this SRM was performed by R.L. Zeisler of the NIST Analytical Chemistry Division.

The preparation and collection of the sediment material was completed by M.P. Cronise and C.N. Fales of the NIST Measurement Services Division and B.J. Porter and M.M. Schantz of the NIST Analytical Chemistry Division. Assistance in collecting the sediment material was provided by G.G. Lauenstein, J. Collier, and J. Lewis of the National Oceanic and Atmospheric Administration (NOAA).

Statistical analysis was provided by M. Vangel and W.S. Liggett, Jr. formerly of the NIST Statistical Engineering Division.

Stephen A. Wise, Chief
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Gaithersburg, MD 20899
Certificate Issue Date: 16 July 2012
Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
Measurement Services Division

Analytical measurements at NIST were performed by C.M. Beck II, T.A. Butler, R. Demiralp Oflaz, M.E. Howard, R.M. Lindstrom, S.E. Long, E.A. Mackey, J.L. Mann, K.E. Murphy, R.L. Paul, J.R. Sieber, R.D. Vocke, L.J. Wood, L.L. Yu, and R.L. Zeisler of the NIST Analytical Chemistry Division.

Certification of SRM 2702 was done in collaboration with S. Willie, J.W.H. Lam, C. Scriver, and L. Yang of the National Research Council Canada, Institute for National Measurement Standards, Chemical Metrology and S.A. Wilson, M. Adams, P.H. Briggs, P. Lamothe, D. Siems, and J. Taggart of the U.S. Geological Survey, Branch of Geochemistry, Denver, CO.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

INSTRUCTIONS FOR HANDLING, STORAGE AND USE

Handling: This material is a naturally occurring marine sediment from an urban area and may contain constituents of unknown toxicity; therefore, caution and care should be exercised during its handling and use.

Storage: SRM 2702 must be stored in its original bottle at temperatures less than 30 °C away from direct sunlight.

Use: Prior to removal of subsamples for analysis, the contents of the bottle should be mixed. The concentrations of constituents in SRM 2702 are reported on a dry-mass basis. The SRM, as received, contains approximately 2.3 % moisture. The sediment sample should be dried to a constant mass before weighing for analysis or a separate subsample of the sediment should be removed from the bottle at the time of analysis and dried to determine the concentration on a dry-mass basis. If the constituents of interest are volatile, then the moisture must be determined with a separate subsample. The drying procedures described below, or equivalent, are recommended; the temperature of 90 °C shall **NOT** be exceeded.

PREPARATION AND ANALYSIS[1]

Sample Collection and Preparation: The sediment used to prepare this SRM was collected from the Chesapeake Bay at the mouth of the Baltimore Harbor, Baltimore, MD near the Francis Scott Key Bridge (39°12.3'N and 76°31.4'W). This location is near the site where SRM 1941 and SRM 1941a were collected. The sediment was collected using a Kynar-coated modified Van Veen-type grab sampler. A total of approximately 3300 kg of wet sediment was collected from the site. The sediment was freeze-dried, sieved at 70 μm (100 % passing), homogenized in a cone blender, radiation sterilized at 33 kGy to 45 kGy (^{60}Co) dose, and then packaged in screw-capped amber glass bottles each containing approximately 50 g.

Conversion to Dry-Mass Basis: The results for the constituents in SRM 2702 are reported on a dry-mass basis; however, the material "as received" contains residual moisture. The amount of moisture in SRM 2702 was determined by measuring the mass loss after freeze drying subsamples of 1.1 g to 1.3 g for four days at 1 Pa with a −10 °C shelf temperature and a −50 °C condenser temperature. The moisture content in SRM 2702 was 2.39 % ± 0.08 % (95 % confidence level). Alternately, drying in a conventional oven was studied. One gram portions were dried at 90 °C for 12 h and 18 h; a stable weight was achieved after 18 h. The measured moisture content was 2.27 % ± 0.09 % (95 % confidence level).

Homogeneity Assessment: The homogeneity of SRM 2702 was assessed by analyzing duplicate samples of approximately 0.1 g from fifteen bottles selected by stratified random sampling. Results from direct determinations by instrumental neutron activation analysis (INAA) and energy-dispersive X-ray fluorescence analysis (ED-XRF) showed observed standard deviations for all reported elements within the precision of the analytical techniques. No statistically significant differences among bottles were observed for the elements at this sample size.

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[1] Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Analytical Approach: NIST has a number of analytical techniques available for value assignment. The ones applied to this SRM include dissolution based techniques such as thermal or inductively coupled plasma isotope dilution mass spectrometry (TID-MS or ICP-IDMS) and direct analysis techniques such as INAA, neutron capture prompt gamma activation analysis (PGAA), and radiochemical neutron activation analysis (RNAA). Several of these techniques were also used by collaborating laboratories and complemented by additional dissolution techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) and direct analysis techniques such as wavelength dispersive X-ray fluorescence (WD-XRF) and ED-XRF. Care has been taken to obtain results from both chemically destructive techniques and non-destructive or direct analysis techniques.

Certified Values and Uncertainties: Certified values, derived from the results of several analytical methods performed by NIST and collaborating laboratories and whose combinations differ from element to element, are provided in Table 1. A complete description of the modes of value assignment is given in reference 1. For consistency across elements, a Bayesian statistical model was chosen for computation of certified values and uncertainties [2]. The uncertainty listed with each value is an expanded uncertainty, with coverage factor 2 (approximately 95 % confidence). The reporting follows the ISO Guide [3].

For each element, there is a NIST result with an uncertainty that is complete in coverage of recognized sources of uncertainties, complemented by results from collaborating laboratories with similarly complete uncertainties, and usually several results without complete uncertainties. The uncertainties of the latter results were augmented on the basis of the differences among the results obtained by different methods [4]. The Bayesian methods combine results by different methods from different laboratories according to the results provided and the uncertainties provided with them. Thus, a consistent methodology was used for all elements.

Table 1. Certified Concentrations for Selected Elements

	Mass Fraction mg/kg (unless noted as %)				Mass Fraction mg/kg (unless noted as %)		
Al[a,b,c,d]	8.41 %	±	0.22 %	Ni[a,b,e,f,g,h]	75.4	±	1.5
As[a,b,d,e]	45.3	±	1.8	P[a,b,d,j]	0.1552 %	±	0.0066 %
Ba[a,f,g]	397.4	±	3.2	Pb[a,b,f,g,h]	132.8	±	1.1
Ce[a,b,d,g]	123.4	±	5.8	Rb[a,d,g]	127.7	±	8.8
Cd[a,e,f,h,i]	0.817	±	0.011	Sb[a,d,g,h]	5.60	±	0.24
Co[a,b,d]	27.76	±	0.58	Sc[a,b,d]	25.9	±	1.1
Cr[a,b,d,g,h]	352	±	22	Sr[a,b,f,g]	119.7	±	3.0
Fe[a,b,c,d]	7.91 %	±	0.24 %	Th[a,b,d,g]	20.51	±	0.96
Hg[e,j,k]	0.4474	±	0.0069	Ti[a,b,c,d]	0.884 %	±	0.082 %
K[a,b,c,d]	2.054 %	±	0.072 %	Tl[f,h]	0.8267	±	0.0060
La[a,b,d,g]	73.5	±	4.2	V[a,b,d]	357.6	±	9.2
Mn[a,b,d]	1757	±	58	Zn[a,b,d,g,h]	485.3	±	4.2
Na[a,b,c,d]	0.681 %	±	0.020 %				

[a] Inductively coupled plasma mass spectrometry (ICP-MS)
[b] Inductively coupled plasma atomic emission spectrometry (ICP-AES)
[c] Wavelength dispersive X-ray fluorescence (WD-XRF)
[d] NIST method - Instrumental neutron activation analysis (INAA)
[e] Atomic absorption spectrometry including hydride generation and cold vapor techniques (AAS)
[f] NIST method - Inductively coupled plasma isotope dilution mass spectrometry (ICP-IDMS)
[g] Energy dispersive X-ray fluorescence (ED-XRF)
[h] Inductively coupled plasma isotope dilution mass spectrometry (ICP-IDMS)
[i] Prompt gamma activation analysis (PGAA)
[j] NIST method – Radiochemical neutron activation analysis (RNAA)
[k] NIST method - Cold vapor ICP-IDMS

Reference Values and Uncertainties: Reference values are based on results from one method carried out in several laboratories or from two or more analytical methods without NIST results. The Bayesian methods of combining the results by different methods from different laboratories were applied as above. These results do not fulfill the criteria for certification since a full estimate of method bias or results from NIST methods have not been available. The reporting follows the ISO Guide [3].

Table 2. Reference Values for Concentrations of Selected Elements

	Mass Fraction mg/kg (unless noted as %)				Mass Fraction mg/kg (unless noted as %)		
Ag[(a,b)]	0.622	±	0.078	Mg[(c,d,e)]	0.990 %	±	0.074 %
Ca[(c,d,e)]	0.343 %	±	0.024 %	Mo[(c,d,f)]	10.8	±	1.6
Cu[(a,b,c,d,f)]	117.7	±	5.6	Se[(c,d,f)]	4.95	±	0.46
Ga[(c,d,f)]	24.3	±	1.9	Sn[(a,d,f)]	31.6	±	2.4

[(a)] Inductively coupled plasma isotope dilution mass spectrometry (ICP-IDMS)
[(b)] Atomic absorption spectrometry including hydride generation and cold vapor techniques (AAS)
[(c)] Inductively coupled plasma mass spectrometry (ICP-MS)
[(d)] Inductively coupled plasma atomic emission spectrometry (ICP-AES)
[(e)] Wavelength dispersive X-ray fluorescence (WD-XRF)
[(f)] Energy dispersive X-ray fluorescence (ED-XRF)

Information Values: Information values are given in SRM 2702 to assist users in the assays of non-certified elements. Information values are based on results that did not allow complete assessment of all sources of uncertainty, hence, only estimated means without uncertainties are given.

Table 3. Information Values for Selected Elements

	Mass Fraction mg/kg (unless noted as %)		Mass Fraction mg/kg (unless noted as %)
Be[(a,b)]	3.0	Nb[(b,e)]	63
C (total) [(c)]	3.36 %	Nd[(d)]	56
C (organic)[(c)]	3.27 %	S[(c)]	1.5 %
Cs[(a,d)]	7.1	Sm[(d)]	10.8
Hf[(d)]	12.6	U[(a,e)]	10.4
Li[(a,b)]	78.2	W[(d)]	6.2

[(a)] Inductively coupled plasma mass spectrometry (ICP-MS)
[(b)] Inductively coupled plasma atomic emission spectrometry (ICP-AES)
[(c)] Combustion techniques
[(d)] NIST method - Instrumental neutron activation analysis (INAA)
[(e)] Energy dispersive X-ray fluorescence (ED-XRF)

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[4]   Levenson, M.S.; Banks, D.L.; Eberhardt, K.R.; Gill, L.M.; Guthrie, W.F.; Liu, H.-k.; Vangel, M.G.; Yen, J.H.; Zhang N.F.; *An Approach to Combining Results from Multiple Methods Motivated by the ISO GUM*; J. Res. Natl. Inst. Stand. Technol., Vol. 105, pp. 571–579 (2000).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

Appendix B

Total Metal Concentrations (mg kg^{-1}) Palmiet River

Site	Pb Autumn	Pb Winter	Pb Spring	Pb Summer	Cr Autumn	Cr Winter	Cr Spring	Cr Summer	Ni Autumn	Ni Winter	Ni Spring	Ni Summer
1	111.32	120.04	87.21	317.13	202.65	82.12	93.52	281.43	83.33	58.25	73.81	234.12
2	117.09	56.48	104.86	149.85	75.12	24.43	85.33	264.51	75.79	12.89	70.02	109.22
3	258.34	187.11	384.01	695.79	1079.92	588.33	1224.16	1012.85	608.18	467.46	1564	593.34
4	530.37	634.85	329.05	454.52	1178.66	479.93	833.5	887.31	441.46	368.44	417.17	606.36
5	155.39	216.36	367.9	191.23	586.55	376.36	863.91	275.59	278.17	154.92	708.28	201.89
6	321.74	92.32	181.21	170.57	814.97	150.57	449.37	246.72	932.1	154.73	364.69	143.6
7	113.11	122.36	126.11	102.77	144.71	111.11	306.53	233.69	79.08	98.98	311.14	221.86
8	138.51	111.29	125.83	99.64	596.56	111.73	204.41	182.5	438.41	96.09	111.36	90.2
9	139.21	95.7	136.29	115.8	422.27	91.17	184.56	225.3	179.73	76.82	118.28	124.31

Site	Cu Autumn	Cu Winter	Cu Spring	Cu Summer	Zn Autumn	Zn Winter	Zn Spring	Zn Summer
1	72.45	79.17	60.79	236.57	267.65	302.86	131.39	803.7
2	85.57	16.63	64	92.11	342.27	161.65	351.55	424.56
3	2024.72	519.71	1659.52	1196.22	1185.32	856.3	1554.53	1476.32
4	903.66	387.52	489.63	593.19	1637.8	1505.42	1215.7	1363.7
5	220.7	258.89	629.28	186.98	799.42	803.11	1359.73	891.84
6	408.74	95.02	241.59	95.58	1130.79	527.19	787.05	674.58
7	70.92	93.28	181.38	194.54	241.75	342.15	1223.76	466.93
8	182.12	94.54	84.71	71.7	747.32	241.28	371.91	223.61
9	143.07	76.82	104.14	118.37	334.74	199.6	455.21	299.77

Total Metal Concentrations (mg kg⁻¹) Sezela River

Site	Pb Autumn	Pb Winter	Pb Spring	Pb Summer	Cr Autumn	Cr Winter	Cr Spring	Cr Summer	Ni Autumn	Ni Winter	Ni Spring	Ni Summer
1	95.85	122.91	131.14	174.58	75.93	112.22	130.08	131.38	84.23	106.62	110.97	147.48
2	67.1	95.94	127.29	193.25	60.32	91.59	110.57	158.95	59.12	98.03	110.84	166.85
3	84.5	132.48	129.62	79.57	62.58	96.46	108.54	73.81	73.09	106.07	112.85	71.19
4	84.38	102.68	131.8	80.98	85.83	96.87	108.84	99.5	70.83	85.78	111.27	66.21
5	74.65	116.29	91.03	162.52	64.01	101.76	88.2	131.68	63.29	97.24	76.94	139.68
6	80.06	122.45	167.5	73.86	72.73	114.97	151.59	107.77	72.45	104.79	143.98	61.79
7	81.55	171.97	184.61	183.82	124.3	273.98	266.52	293.79	73.46	165.27	167.91	155.24
8	111.95	209.5	209.94	107.5	103.86	292.99	197.62	156.96	95.8	191.99	178.94	91.12
9	85.51	115.76	181.48	126.54	127.11	376.93	389.79	269.11	81.7	110.02	168.75	167.96

Site	Cu Autumn	Cu Winter	Cu Spring	Cu Summer	Zn Autumn	Zn Winter	Zn Spring	Zn Summer
1	55.05	69.85	75.47	96.15	32.43	122.08	88.62	74.92
2	40.88	58.93	76.44	112.2	56.45	82.05	96.81	102.33
3	48.38	71.9	77.05	52.68	52.22	108.27	143.27	118.28
4	48.31	59.03	77.69	52.47	133.89	102.28	142.77	111.01
5	42.49	73.38	47.24	92.96	51.06	305	85.17	105.26
6	48.36	74	98.75	45.34	97.79	256.05	167.46	120.96
7	67.63	236.36	261.44	213.43	86.36	198.46	125.64	233.99
8	66.87	140.21	111.27	72.14	82.07	176.56	78.14	100.49
9	58.83	83.28	124.38	129.89	68.53	96.57	77.96	348.15

Appendix C

BCR Metal Concentration (mg kg^{-1}) - Palmiet River

Cr	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	0.62	5.16	36.72	66.65	0.29	2.67	8.79	70.37	0.23	3.18	12.29	31.73	0.15	3.77	2.74	102.64
2	0.41	5.47	1.55	61.07	0.11	3.06	0.34	20.91	15.94	4.27	0.63	22.74	0.23	4.49	0.00	109.42
3	41.32	345.42	548.66	1466.87	12.47	124.99	400.02	97.35	22.32	127.68	137.44	1091.74	12.59	109.48	250.62	350.10
4	18.86	168.53	561.77	567.31	9.07	51.07	224.04	195.76	10.39	46.93	210.46	154.70	7.04	55.49	151.70	356.55
5	9.11	68.56	227.68	345.35	5.23	72.40	172.97	162.52	0.63	104.55	235.18	114.61	2.09	15.85	67.69	60.49
6	9.18	83.81	217.82	608.14	1.75	18.83	53.81	76.17	5.79	46.97	124.33	48.21	1.42	5.17	37.30	134.78
7	0.67	2.42	20.14	117.55	1.56	10.50	25.12	76.34	1.94	26.35	66.44	61.02	1.77	8.88	33.46	55.09
8	4.22	42.57	161.48	134.65	1.14	7.49	26.28	76.70	1.50	6.85	31.94	63.44	0.71	2.05	8.87	68.21
9	2.50	29.27	62.96	139.82	0.83	3.21	25.91	73.06	0.95	9.51	23.55	59.56	0.92	6.27	11.76	91.38

Cu	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	5.56	15.60	16.05	13.51	5.34	20.32	14.30	29.02	0.24	9.78	9.59	9.36	3.68	22.65	25.94	87.12
2	21.10	10.61	7.75	28.44	0.17	13.15	0.50	2.81	4.74	19.76	6.18	4.71	6.45	19.97	10.32	2.72
3	474.09	400.51	166.76	712.26	125.61	191.17	115.68	22.05	258.39	217.93	167.32	1653.09	393.01	275.16	208.40	107.38
4	200.55	172.86	208.41	100.54	84.78	68.23	114.06	120.45	90.86	82.82	90.08	96.76	127.39	117.55	92.74	48.80
5	31.20	58.84	41.46	66.89	43.02	71.24	83.28	31.90	12.19	145.06	159.10	157.23	33.21	36.49	49.30	24.46
6	48.57	75.09	180.09	9.45	11.20	13.52	39.58	30.72	32.93	46.46	62.32	41.82	6.49	2.39	20.72	42.97
7	4.04	18.62	13.58	19.71	5.50	5.94	16.99	45.63	15.12	32.90	48.79	43.70	22.78	14.92	51.57	34.79
8	33.76	48.81	47.08	38.95	1.77	20.98	16.48	31.27	8.06	2.94	29.59	23.93	3.32	1.58	2.82	40.27
9	10.75	23.66	32.52	47.14	2.37	1.01	14.77	40.31	6.90	14.38	23.51	32.48	9.15	10.76	8.75	59.81

Ni	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	14.35	0.97	5.16	9.07	3.26	3.26	1.88	13.75	3.87	1.30	0.70	14.86	6.03	0.67	1.88	34.18
2	2.05	3.13	0.00	4.55	1.25	2.30	0.31	2.59	1.80	3.98	0.00	4.97	1.33	2.86	0.00	6.04
3	148.03	213.59	28.79	140.19	268.04	166.41	47.72	0.00	420.09	269.51	44.41	959.64	300.26	222.41	62.79	8.34
4	167.16	145.03	50.97	53.22	86.60	36.05	16.48	45.09	305.52	69.84	23.03	21.99	126.39	89.51	24.74	0.00
5	96.45	72.80	30.96	44.00	121.89	76.95	20.84	0.00	34.79	282.41	56.57	357.35	88.98	35.77	18.12	0.00
6	232.38	98.11	32.88	44.09	32.29	19.23	8.68	17.16	238.86	114.87	31.17	0.00	37.88	12.75	6.37	17.26
7	8.07	5.08	4.42	9.70	20.27	14.18	3.91	5.87	67.53	57.25	14.42	1.63	79.30	21.97	7.54	0.00
8	132.63	86.31	24.58	0.00	14.06	9.46	5.39	11.05	35.19	13.61	5.71	0.00	15.88	9.50	3.48	7.48
9	25.95	28.96	9.15	19.45	6.37	2.69	3.70	9.91	21.61	17.46	5.72	2.41	19.64	14.36	3.73	28.50

Pb	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	7.04	44.14	27.96	0.00	0.16	30.44	8.68	12.90	0.44	11.10	8.39	0.00	1.91	50.25	26.71	10.27
2	15.64	82.54	22.59	0.00	8.41	22.95	4.41	0.00	5.57	32.94	8.48	1.97	6.00	36.84	18.29	0.00
3	7.17	164.92	109.04	0.00	14.09	154.57	36.35	0.00	5.70	114.60	23.07	40.53	38.93	238.53	44.60	0.00
4	21.41	184.49	40.29	11.50	59.69	53.94	40.02	163.77	17.74	81.14	20.18	38.96	29.66	165.22	51.55	0.00
5	6.84	78.89	19.95	0.00	12.68	231.65	30.76	0.00	12.91	165.92	38.07	0.00	8.71	94.81	48.21	0.00
6	9.61	92.29	23.96	26.24	2.96	82.54	21.69	0.00	3.40	86.73	25.21	0.00	6.46	54.85	26.60	0.00
7	2.68	60.36	16.89	0.00	0.60	42.07	5.84	0.63	2.75	64.34	12.47	0.00	5.25	43.43	21.32	0.00
8	2.51	64.35	19.82	0.00	0.54	43.12	16.20	0.00	4.11	50.18	11.48	0.00	2.30	46.62	18.37	0.00
9	0.68	51.36	18.46	0.00	1.61	41.75	5.53	0.00	0.35	44.72	10.53	0.13	1.89	48.87	25.97	0.00

Zn	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	129.34	18.82	13.30	48.83	77.60	2.83	14.06	208.36	24.16	22.84	0.00	4.59	158.59	22.18	19.67	302.59
2	234.62	52.66	15.19	0.00	129.49	20.30	5.96	5.90	204.73	23.77	9.03	40.87	132.60	17.52	25.23	9.12
3	478.49	293.85	4.93	353.72	554.78	233.73	67.30	0.00	376.71	140.68	27.21	688.23	328.52	237.34	61.08	147.25
4	661.19	272.43	53.66	335.17	1022.23	208.34	68.28	206.57	681.84	79.36	30.04	188.22	647.00	205.37	45.05	29.37
5	317.73	127.27	52.10	258.28	522.85	232.39	57.79	0.00	52.63	273.94	81.28	688.57	291.18	63.11	47.37	30.23
6	407.10	163.41	51.87	262.51	321.42	120.29	47.95	37.53	321.64	130.87	47.68	55.72	170.44	23.27	36.66	118.13
7	62.49	36.82	27.75	76.51	161.45	83.46	15.84	0.00	207.74	127.09	34.06	614.60	141.30	15.53	12.73	46.08
8	218.40	136.62	40.04	69.81	86.12	50.00	36.33	68.06	175.67	43.29	26.30	56.41	53.22	19.34	17.19	50.48
9	40.04	63.99	18.46	145.25	77.72	36.58	14.64	33.83	86.39	57.74	30.67	35.94	71.32	27.01	13.45	154.88

BCR Metal Concentration (mg kg⁻¹) - Sezela River

Cr	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	0.20	1.70	21.04	39.57	0.04	2.08	16.87	12.68	0.06	0.41	19.83	22.11	0.00	1.99	12.14	1.44
2	0.02	1.54	15.08	18.32	0.20	1.61	15.49	13.16	0.02	0.54	15.18	10.41	0.06	0.51	14.34	9.51
3	0.18	1.54	13.32	13.66	0.34	1.35	14.12	3.90	0.05	0.58	12.36	17.64	0.01	0.46	11.67	16.42
4	0.05	2.04	12.20	23.40	0.13	1.43	13.98	18.73	0.00	0.54	12.81	9.26	0.24	0.53	11.57	31.18
5	0.08	1.92	15.05	13.19	0.20	1.02	16.43	12.78	0.01	0.15	16.61	20.08	0.07	1.07	13.61	7.16
6	0.08	2.20	14.87	34.26	0.16	0.56	13.95	22.62	0.09	1.96	16.45	25.05	0.07	0.81	13.25	37.50
7	0.19	3.50	24.15	43.54	0.29	2.80	16.85	95.27	0.11	4.37	17.26	91.64	0.26	10.26	34.60	210.77
8	0.78	6.74	19.44	144.69	0.15	2.45	14.68	103.28	0.11	1.48	13.55	145.16	0.94	2.46	20.90	122.44
9	0.21	10.41	36.80	97.04	0.16	12.51	55.50	120.72	0.09	10.49	81.84	76.83	0.04	10.58	28.69	156.17

Cu	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	0.03	3.15	11.46	0.00	0.00	2.08	12.18	0.00	0.55	6.10	11.00	0.00	0.83	5.19	10.58	0.00
2	0.10	3.17	12.74	0.00	0.00	3.29	12.05	0.00	1.20	6.63	12.33	0.00	0.04	6.57	11.46	0.00
3	0.80	2.44	11.91	0.00	0.00	4.42	15.61	0.00	0.10	6.47	11.58	0.98	0.23	6.19	12.05	0.00
4	0.17	4.08	10.54	0.00	0.02	2.97	11.79	0.00	0.06	6.86	13.62	0.00	1.04	7.75	13.88	0.00
5	0.15	3.95	12.53	0.00	0.00	2.34	12.93	6.78	0.86	4.87	11.10	0.00	0.68	6.51	11.83	0.00
6	0.13	4.36	13.72	0.00	0.00	7.64	10.84	0.00	1.00	7.63	12.42	0.00	0.57	7.29	12.44	0.00
7	0.41	11.35	36.07	0.00	1.78	11.02	15.37	146.37	0.75	14.22	97.97	91.28	0.64	31.97	93.84	0.00
8	6.80	14.38	17.58	0.00	0.36	10.56	13.02	12.28	1.09	7.40	13.01	0.00	0.51	11.53	14.93	0.00
9	0.22	8.01	22.39	3.35	0.00	12.91	42.45	0.00	0.87	9.57	34.40	0.00	0.67	10.46	17.63	0.00

Ni	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	0.85	1.06	3.81	2.44	0.17	1.12	3.46	3.00	0.68	1.49	3.46	3.84	0.32	0.33	3.01	0.00
2	0.36	1.19	3.81	1.99	0.37	1.13	3.13	16.85	0.20	1.09	3.62	3.93	0.26	0.86	3.44	1.98
3	0.37	1.05	3.72	0.00	0.12	0.57	3.21	0.00	0.44	1.00	3.46	4.36	0.07	0.84	3.26	4.08
4	0.03	1.66	3.34	3.68	0.55	1.32	4.12	1.50	0.62	1.25	4.10	1.27	0.73	0.83	3.34	2.94
5	0.39	1.06	3.54	0.00	0.02	1.33	3.68	2.38	0.17	1.58	4.10	6.37	0.20	1.06	3.69	0.83
6	0.53	2.02	4.39	5.59	0.42	1.80	3.71	4.37	0.89	2.10	3.99	5.12	0.38	1.86	3.57	6.58
7	1.13	2.13	5.11	7.27	0.37	2.10	3.66	15.51	3.94	6.77	5.54	12.46	3.39	9.34	8.45	0.00
8	1.69	8.33	5.54	0.15	0.06	1.72	3.56	10.89	1.71	4.20	4.56	5.10	0.47	1.77	4.38	5.70
9	2.48	7.42	6.33	13.58	0.67	1.96	8.35	4.99	0.90	6.87	12.00	4.16	0.40	2.78	5.13	54.10
Pb	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	0.55	18.06	14.52	0.00	0.24	10.66	17.65	0.00	0.55	14.88	8.09	0.00	0.24	8.48	21.91	0.00
2	0.13	11.13	9.25	0.00	0.53	10.36	6.90	0.00	0.40	10.19	8.31	0.00	0.63	11.66	11.61	0.00
3	0.27	15.13	8.59	0.00	0.02	6.99	10.09	0.00	0.92	9.09	6.86	0.00	0.28	11.40	18.46	0.00
4	0.05	12.43	15.66	0.00	0.07	8.24	15.55	0.00	0.57	11.67	7.51	0.00	0.37	44.43	26.13	0.00
5	0.16	14.19	18.34	0.00	0.30	9.33	18.73	0.00	0.41	14.49	7.40	0.00	0.11	34.93	17.49	0.00
6	0.15	16.81	20.64	0.00	0.19	11.86	17.79	0.00	0.40	19.45	6.54	0.00	0.07	14.38	17.44	0.00
7	0.59	13.75	18.69	0.00	0.40	1.97	23.14	0.00	0.80	25.86	8.66	0.00	0.07	42.28	43.53	0.00
8	0.82	31.16	17.26	0.00	0.34	5.81	17.90	0.00	0.95	18.22	8.38	0.44	0.10	17.52	20.77	0.00
9	0.57	17.94	15.00	0.00	0.69	14.82	16.90	0.00	0.00	22.54	13.71	0.00	0.15	35.15	24.04	0.00

Zn	Autumn				Winter				Spring				Summer			
Site	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	0.20	6.54	13.15	32.25	0.00	18.96	11.86	12.11	1.59	0.00	10.62	21.31	0.00	0.00	10.13	14.10
2	0.00	0.00	11.61	25.21	0.00	0.00	10.22	42.71	0.00	0.00	12.22	16.43	0.00	0.00	10.93	20.07
3	0.21	30.48	10.84	18.82	3.11	0.89	13.92	46.69	0.00	0.00	0.00	42.20	1.83	2.60	12.01	41.26
4	6.41	0.15	11.99	59.20	14.19	0.73	16.65	25.22	11.61	1.04	18.11	11.37	3.61	3.78	14.62	41.13
5	0.00	0.00	12.40	57.39	0.00	31.06	12.22	135.04	0.00	0.00	11.03	15.44	0.00	0.00	12.71	18.44
6	6.30	5.98	16.25	51.33	2.11	6.09	15.25	51.27	3.47	0.90	15.85	33.65	4.26	6.09	13.10	46.14
7	6.54	0.21	13.04	29.49	3.74	0.00	12.50	41.62	19.35	2.87	13.22	36.98	18.55	31.54	29.63	19.13
8	5.18	5.98	14.37	30.97	4.53	0.00	9.47	87.21	5.86	0.00	9.97	11.35	6.17	0.99	9.78	21.17
9	9.10	18.66	13.87	33.19	6.54	7.53	21.71	24.85	10.95	28.27	23.53	0.00	5.71	8.53	13.26	192.84