IDENTIFICATION AND DETERMINATION OF POTENTIALLY TOXIC ELEMENTS IN WATER AND SEDIMENTS FROM BLOOD AND MOKOLO RIVERS IN LIMPOPO PROVINCE, SOUTH AFRICA

By

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[redacted]

DISSERTATION

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DECLARATION BY CANDIDATE

I hereby declare that the full dissertation submitted for the degree of Master of Science in Chemistry at the University of Limpopo titled “Identification and determination of potentially toxic elements in water and sediments from Blood and Mokolo Rivers in Limpopo province, South Africa”, is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited are indicated and acknowledged by means of a comprehensive list of references.

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DITHOBOLONG LOVIA MATABANE **Date**

[redacted]

DEDICATION

In memory of my late close ones, Rakgadi ‘’Ntswaki” Ramadimetja Rebecca Matabane, Malome Kotole Meshack Mamaro, and Rakgolo Disego William Mamaro, Robalang ka kgotso.

This study is dedicated to my mother Margaret Machoey Matabane who always supported me through thick and thin and my son Dimpho for being my source of inspiration at all times.

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ABSTRACT

The increase in industrialisation such as mining and smelting activities is among the main sources of heavy metal pollution in the environment. These potentially toxic elements may be adsorbed directly by sediments of water course and may affect the inhabiting flora and fauna. Potentially toxic elements are not permanently fixed by the sediments since changes in environmental conditions can mobilise these elements from sediments to water. Thus, analysis of sediments could indicate the degree of environmental contamination by potentially toxic elements and therefore are suitable for pollution studies.

The aim of this study was to assess and quantify the levels of selected potentially toxic elements (Mn, Zn, Ni, Cu, Cr, Pb, V, Cd, As, Se and Fe) in sediments and water samples collected from Blood and Mokolo Rivers in Limpopo Province, South Africa. Water and sediments samples were collected from 10 sampling sites from each river. Water samples were filtered and acidified with HNO_3 prior to analysis for preservation of elements. Potentially toxic elements in water samples were analysed by ICP-MS and the results validated by analysing standard reference material of water (SRM 1634f).

Concentration levels of potentially toxic elements in samples from the water of Mokolo and Blood Rivers during low and high flow seasons were determined with ICP-MS. The levels in the water of Mokolo and Blood Rivers followed the order of Mn > Se > Zn > Cr > Pb > V > Cu > As > Ni > Cd > Fe in Mokolo River and Mn > Se > Zn > Cr > V > Ni > Cu > As > Pb > Cd > Fe during high flow season, Mn > Se > Zn > Cr > Cu > V > As > Pb > Ni > Cd > Fe and Mn > Zn > Se > Cr > V > Ni > Cu > As > Pb > Cd >Fe during low flow season, respectively. As expected, the concentrations of selected elements determined during the low flow season exceeded the high flow season for both rivers, which confirms acceptability of the analytical method performance. The analysis of variance (ANOVA) showed significant differences ($P<0.05$) in the concentrations of the elements seasonally.

The concentrations of the toxic metals in the water samples of Mokolo and Blood Rivers were below the maximum permissible levels (MPLs) set by different organisations such as World Health Organisation (WHO) and Department of Water and Sanitation (DWS).

Comparison of concentrations of elements in sediments determined during high flow and low flow seasons was done to establish the variation on the levels of the elements between seasons. Levels of potentially toxic elements in sediments of Mokolo and Blood Rivers followed the order Fe > Cr > Mn > V > Zn > As > Ni > Cu > Pb > Cd > Se and Fe > Mn > Cr > Ni> V > Zn> Cu > Pb > As > Cd> Se during high flow season as well as Fe > Mn > Cr > V > Zn > Ni > Pb > Cu > As > Cd > Se and Fe > Mn > Cr > Zn > V > Ni > Cu > Pb > As > Cd > Se during low flow season, respectively.

The ANOVA results showed significant differences in the concentrations of the elements between two seasons and that concentrations of the elements determined in sediments during low flow season were significantly higher than the high flow season levels. Compared with sediments quality guidelines (SQGs), the concentrations of Fe, Mn, Ni and Cr in the sediments of Mokolo and Blood Rivers were above the SQGs, which could pose the risk to sediments dwelling organisms.

The Community Bureau of Reference (BCR) sequential extraction method was applied to extract selected potentially toxic elements (Cd, Cr, Cu, Fe, Ni, Pb and Zn) in sediments samples.

Microwave-assisted sequential extraction procedure was developed and employed for extraction of the selected potentially toxic elements in sediments samples. This method enables safe and fast extraction of potentially toxic elements in sediments using smaller volume of reagents. The methods employed were validated with the BCR-701 certified reference material of sediments. The Efficiency and reliability of the microwave-assisted sequential extraction method was shown by calculating the percent recoveries. The percent recoveries were within acceptable range for method validation.

Following the sequential extraction analysis, the risk assessment study was done to determine the effect of the potentially toxic elements in the sediments to the water quality. The calculated individual contamination factor (ICF), global contamination factor (GCF) and risk assessment code (RAC) values of Cd, Cu, Ni and Pb indicated that the elements were mobile and may be bioavailable in the aquatic environment.

The assessment of eco-toxicological risk indicated that the elements in sediments samples collected from Blood River are at high toxicity risk level and thus, could pose threat to sediments dwelling organisms and humans through consumption of crops irrigated with the river water.

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ABBREVIATIONS

ANOVA:	Analysis of Variance
APHA:	American Public Health Association
BCR:	Community Bureau of Reference
CCME:	Canadian Council of Ministers of the Environment
CF:	Contamination Factor
CRM:	Certified Reference Material
CSIR:	Council for Scientific and Industrial Research
DWAF:	Department of Water Affairs and Forestry
F-AAS:	Flame-Atomic Absorption Spectrometry
GCF:	Global Contamination Factor
GF-AAS:	Graphite Furnace-Atomic Absorption Spectrometry
HCL:	Hollow Cathode Lamp
HPLC:	High Performance Liquid Chromatography
ICF:	Individual Contamination Factor
ICP-MS:	Inductively Coupled Plasma-Mass Spectrometry
ICP-AES:	Inductively Coupled Plasma-Atomic Emission Spectrometry
IDP:	Integrated Development Planning
LOD:	Limit of Detection
LOQ:	Limit of Quantification
MPL:	Maximum Permissible Level
M/z:	Mass-to-charge ratio

NADP:	National Atmospheric Deposition Program
RAC:	Risk Assessment Code
SD:	Standard Deviation
SM & T:	Standards, Measurements and Testing
SQG:	Sediments Quality Guidelines
SRM:	Standard Reference Material
TDS:	Total Dissolved Solids
USEPA:	United States Environmental Protection Agency
USGS:	United States Geological Survey
UV:	Ultra-violet
WHO:	World Health Organisation

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STRUCTURE OF THE DISSERTATION

This dissertation consists of six chapters. **Chapter One** introduces the context of the research, which comprises of the background and motivation of the study, as well as the problem statement. The aim and objectives of this study are outlined in this section of the dissertation.

Chapter Two reviews the existing literature on the factors affecting the mobility and transportation of potentially toxic elements in water and sediments embodiments. Further discussion of sample preparation methods, mainly based on sequential extraction and acid digestion techniques is provided. This chapter also gives an overview of predominantly employed detection techniques for the determination of potentially toxic elements in water and sediments samples.

Chapter Three provides a thorough description of the study areas and materials including apparatus, instruments, reagents, certified reference materials and standards used in experiments performed to reveal the identity and quantity of potentially toxic elements and to confirm the quality of analytical data. The sample collection, preservation and storage procedures are outlined, including the digestion and extraction techniques applied for sediments sample preparation. Evaluation of the analytical figures of merit and the statistical data analysis methods employed are also included.

Chapter Four gives the analytical figures of merit, concentrations and the observed trends along the river course of the analytes in the water and sediments samples from Mokolo and Blood Rivers. Comparison of the pseudo-total concentrations of potentially toxic elements in sediments of the current study with sediments quality guideline values from different organisations was done to indicate the pollution level. Concentrations of potentially toxic elements in water with maximum permissible levels along with their effects are discussed in this chapter.

Chapter five discusses the trends in the levels of potentially toxic elements determined with sequential extraction of elements in sediments from Blood River during the summer of 2016. Further comparison of the modified BCR sequential extraction method with the microwave-assisted sequential extraction method is done to evaluate the relative efficiency and reliability of the two methods.

Chapter six summarises the conclusions drawn based on key findings of this study. It also makes salient recommendations for further research in this field.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

The increase in industrialisation such as mining and smelting activities is among the main sources of potentially toxic elements pollution in the environment. These potentially toxic elements may be adsorbed directly by sediments of water course and may affect flora and fauna (Alloway and Ayres, 1997). Potentially toxic elements are not permanently fixed by the sediments since changes in environmental conditions can cause the mobilisation of these elements from sediments to water (Yuan *et al*., 2004). Such potentially of sediments for being a sink as well as a source of contaminant can make sediments chemistry and toxicity key components of the water quality studies (Sarkar *et al*., 2014).

The risk of contaminated sediments by potentially toxic elements strongly relates with their specific chemical fractions and binding states. However, much attention has been given to the investigation of the total element concentrations in water and sediments (Su *et al*., 2013). Determination of pseudo total elemental concentration cannot provide information about mobility, bioavailability and toxicity of the analyte. These properties depend on the chemical speciation of the different components of the sample (Qjao *et al*., 2013). Thus, fractionation of potentially toxic elements in sediments by sequential extraction is helpful to understand their source, mode of occurrence, mobility, and bioavailability related with the realistic risk assessment (Yang *et al*., 2014). Trace elements present in sediments and water samples include As, Fe, Cr, Cu, Cd, Mn, Ni, Pb, Se, V, and Zn, among others (Fuentes *et al*., 2008). Although Pb is considered as a priority environmental pollutant and highly toxic, other elements such as Cd, Hg, Ni, and Sr have been reported as some of the potentially toxic elements released in the process of burning coal in mining industries and coal-fired power stations (Chang and Foland, 2005).

High levels of potentially toxic elements in river water have been reported whereby they tend to exceed maximum permissible levels (MPLs) according to the water quality guidelines set by the World Health organisation (WHO) (Garbarino *et al*., 1995; Peplow, 1999).

The human health risk assessment identified that Sb and Cr at Flag Boshielo Dam as well as Pb at Phalaborwa Barrage were above acceptable levels for the safe consumption of fish based on a weekly 150 g fish metal diet (Heath *et al*., 2004; AddoBediako *et al*., 2014). Many rural communities in South Africa regularly feed on fish caught from rivers and lakes (Ellender *et al*., 2009; McCafferty *et al*., 2012). According to Ashton and Dabrowski (2011), the Olifants River which is a tributary of Limpopo River has regularly been damaged by disposal of chemicals from industries, agricultural chemicals, municipal and domestic wastes. It is currently known as one of the most polluted river systems in South Africa. In addition, continuous monitoring of the river systems should be conducted by the National Health Authorities to control the risks associated with the exposure of possibly polluted rivers by the potentially toxic elements.

The sediments quality guidelines (SQGs) have been developed by the environmental protection agency under clean water act to identify and single out toxic metals of concern in aquatic ecosystems (McCauley *et al*., 2000; USEPA, *2001)*. The SQGs provide maximum permissible levels of the potentially toxic elements in the sediments systems based on applications, the design of monitoring programs, historical data interpretations, conducting remedial investigations and ecological risk assessments among others (MacDonald *et al*., 2000). The sediments quality guidelines vary from country to country and from states to states based on the geographical and ecological influences and backgrounds of each country and state. Evaluation methods have limitations based on the sediments type because different sediments exhibit different degrees of bioavailability for the same total metal content (Di Toro *et al*., 1990).

Sequential extraction techniques have been widely used to fractionate metals in solid samples (soil, sediments, sludge and solid wastes) based on their leachability (Bacon and Davidson, 2008; Hanay *et al*., 2008). The method depends on a succession of chemical reagents that sequentially extract various targeted phases of the leachable, easily available potentially toxic elements in the sediments samples (Nemati *et al*., 2009). The goal of this method is to divide the total extractable concentration of metals into separate fractions in order to identify the form in which the metals occur in the material under study. A sequence of reagents is usually applied to a sediments sample to increase the strength of the extraction at each fractionation step in order to dissolve the trace elements which are present in different sediments phases.

The motive behind the sequential extraction protocol is to successively dissolve the relevant metals at each different component controlled by the strength of the reagent. This provides information about the mobility and bio-availability of an element, or the possible risks posed to the environment by direct or indirect interaction with the aquatic lives and humans (Nemati *et al*., 2009).

A study by Nriagu *et al*. (1996) reported that high Pb levels were detected in children's blood from different provinces in South Africa. The study showed that over 90% of the children in urban and rural communities of the Cape Province have Pb levels ≥10 g/ dL in their blood. Furthermore, an increase in the concentrations of potentially toxic elements has been reported in the groundwater from the mining districts of Johannesburg, South Africa (Naicker *et al*., 2003). This was due to the heavy acidification of the pyrite oxidation obtained from mine tailing dumps. The polluted groundwater was discharged into streams and rivers surrounding the mining and industrial areas, thus contributing up to 20% of the stream flow which lead to an increase in the acidity of the water systems. This effect of the contaminated water from the mines can be transported by the river systems as far as 10 km beyond the possible source of pollution (Naicker *et al*., 2003).

This study assesses the levels of selected potentially toxic elements in sediments and water samples collected from Blood River in Seshego area and Mokolo River in Lephalale area. Both rivers are located in Limpopo province, South Africa. The Blood River is located at Seshego area about 14.5 km away from Polokwane and is located in the west side of the Polokwane municipal area. Based on the findings by the Polokwane municipal report (2012), it was stated that municipal and domestic wastes are the factors that contribute to environmental pollution due to the lack of waste removal services, illegal dumping sites close to the river banks and lack of storm water drainage among others (IDP, 2012-2013/2015). There are visible dumping sites near different parts of the river, sand mines and direct flow of leaking sewage pipes into the river. A report by Munawar *et al*. (1993) pointed out that increased organic matter from sewage effluents reduce the amount of dissolved oxygen in the water body which is essential for fish and other aquatic life to survive in the water. Sewage effluents can also introduce other chemicals and potentially toxic elements into the water column. It may also introduce fine sediments, which have the potentially of bioaccumulation within animal tissues and have long term toxic effects (Osman and Kloas, 2010).

Local farmers utilise the river water for irrigation and livestock farming, as well as ritual ceremonies.

Lephalale local municipality falls within a water-scarce region in which the demand for water is increasingly high and cannot be met by the current water sources in the area (Schachtschneider *et al*., 2010). The majority of people in Lephalale live in scattered rural settlements, which puts a strain on the supply of potable water (IDP, 2009/10). The Lephalale rural residents solely depend on groundwater sources for their drinking water, and at least 23% of the rural population live on average more than 200 m from a water source. In urban areas, 97.9% of households have access to municipal water as reported by the integrated development planning (IDP) for rural development (IDP, 2012/13). The Grootegeluk coal mine, the Matimba coal-fired power station and the Medupi coal-fired power plant which is supplied coal from Grootegeluk coal mine, located north of the site could elevate the levels of potentially toxic elements in water system (IDP, 2012/13; Oosthuizen, 2013). Monitoring the impact of these sources and municipal wastes is necessary to control and improve the quality of water for human consumption and for protection of aquatic organisms and underlying invertebrates.

Supply of potable water is particularly problematic in the rural areas of South Africa where poverty levels are twice as high (70%) as those in urban areas (30%). Lack of adequate potable water supplies often result in poor households having no option but to use unsafe sources of water. In many rural areas, over 75% of poor households have no access to treated tap water and approximately 74% of all rural households need to fetch water from a well, stream or river each day (Oberholster and Ashton, 2008). Therefore, assessment of the levels of potentially toxic elements in water samples collected from rivers is necessary.

1.3 AIM

The aim of the study is to assess and quantify the levels of selected potentially toxic elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, V and Zn) in sediments and water samples collected from Blood and Mokolo Rivers in Limpopo Province, South Africa.

1.2 PROBLEM STATEMENT

The contamination of aquatic environment by potentially toxic elements such as Cd and Pb is a major concern due to their toxicity, persistence and bio-accumulative nature. Rivers are particularly subjected to potentially toxic elements from point and non-point sources such as mining activities, municipal and domestic wastes. While rivers mobilise heavy metal contaminants, the underlying sediments constitute a reservoir of potentially toxic elements and play an important role in geochemical cycles.

South Africa's freshwater resources, including rivers, man-made lakes and groundwater, are under increasing stress due to increased pollution caused by industrialisation, urbanisation, mining, agriculture and power generation (Oberholster and Ashton, 2008). As an example, the Council of Scientific and Industrial Research (CSIR) reported that raw sewage flowing into the Olifants River, irresponsible mining and agricultural fertilisers seeping into the river after heavy rainfalls, have made it more toxic and resulted in death of numerous crocodiles (De Villiers and Mkwelo, 2009).

High levels of Cd and Pb were detected in water and sediments from Tyume River, Eastern Cape Province, South Africa (Awofolu *et al.*, 2005). The evidence shows that the high levels of potentially toxic elements such as Cd and Pb are detrimental to the health of the aquatic ecosystem and the rural communities that utilise river water for domestic purposes without any treatment. Therefore, these reports evoke the need for ongoing assessment of potentially toxic elements in the rivers. This study will focus on Blood River in Seshego, which is heavily affected by municipal and domestic wastes and Mokolo River in Lephalale, which is situated close to Grootegeluk coal mine as well as Medupi and Matimba power stations. The Mokolo River is additionally utilised for irrigation due to agricultural activities existing in Lephalale.

1.4 OBJECTIVES

The specific objectives of this study are to:

- determine the levels of potentially toxic elements in water and sediments samples using flame-atomic absorption spectrometry (F-AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma-mass spectrometry (ICP-MS).

- apply the sequential extraction procedure proposed by the European Standard, Measurements and Testing (SM & T) program, for fractionation of potentially toxic elements in river sediments.

- develop a faster microwave-assisted sequential extraction procedure for fractionation of the elements in sediments in less time, while using smaller volume of reagents.

- validate the developed analytical methods using standard reference materials (SRMs) of water and sediments.

- compare the levels of potentially toxic elements in water and sediments samples with the MPLs for water and sediments SQGs of states, respectively.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

In this chapter, brief literature review focusing on potentially toxic elements in water and sediments is given. Factors such as mining activities, municipal and domestic wastes, which could influence the levels of potentially toxic elements in river water and sediments, are explained. Sample preparation and quantification methods of potentially toxic elements in these samples are reviewed. An overview of the ICP-MS, ICP-AES, GF-AAS and F-AAS is given following the key concepts, principles and applications of the instruments on quantification of potentially toxic elements.

2.2 POTENTIALLY TOXIC ELEMENTS

Many metals and semi-metals are hazardous to animals including humans, and are pollutants to water and the environment. Most of them have no known benefit for human physiology. The toxic metals can be taken up directly by humans through direct use such as drinking, bathing, and cooking or they may enter the food chain as a result of their uptake by edible plants and aquatic animals such as fish (Gleyzes *et al*., 2002; Islam *et al*., 2014; Martin *et al*., 2015). They may seep down to groundwater and sediments, thus contaminating drinking water sources, and may cause, in both cases, hazards leading to deaths of humans and animals (Senesil *et al*., 1999; Mohiuddin *et al*., 2012).

Toxic wastes generated by mining industries contain high concentrations of metals and metalloids which can be mobilised, leaching into groundwater and surface water. These metals need to be removed from the polluted streams and rivers in order to meet firm environmental quality standards (Ahluwalia and Goyal, 2007). Abandoned mines also play a major role in pollution since they have uncontrollable discharge of decant (Banks *et al*., 1997).

Mining industries have a great impact on aquatic biodiversity through direct and indirect poisoning, posing high risks when contaminants are mobile in the sediments or bioavailable in the water.

Bioaccumulation of potentially toxic elements in the body result when toxic elements are not metabolised in the soft tissues of the human body. This then compromises the health and safety of communities exposed to these metals. One of the most common route of exposure to metal poisoning is ingestion (Lidsky and Schneider, 2003).

Children may develop toxic levels from the normal hand-to-mouth activities. In most cases, they come into contact with contaminated river sediments such as sand and small rocks by licking them or by actually eating objects that are not food such as dirt or paint chips (Dupler, 2001). In teenagers, activities such as swimming in rivers and lakes is common. Hence, they become exposed to these metals by direct contact of the polluted water with their skin and mouth. Young girls fetch water from rivers and do laundry on daily basis at the banks of the rivers. For these reasons, the monitoring of potentially toxic elements in water is essential to minimise the toxic levels in the environment and to possibly provide remediation methods for healthy use of this water. Many elements have been reported to be essential for biochemical processes, however at concentrations above MPLs, they can be toxic leading to accumulation in the kidney, liver and brain (Ferner, 2001; Young, 2005). The toxicity of these potentially toxic elements can result in significant morbidity and mortality if unrecognised or inappropriately treated.

Chromium (VI) (Cr) compounds are toxic and known human carcinogens, whereas Cr (III) is an essential element. Breathing high levels of Cr (VI) can cause irritation to the lining of the nose resulting into nose ulcers and running nose. It can further cause breathing problems such as asthma, cough, shortness of breathing and wheezing. Long term exposure of Cr (VI) can cause damage to the liver and kidney, as well as causing circulatory and nerve disorders (Kabata-Pendias and Pendias, 1992). The MPL of Cr (VI) is 0.1 mg/L (SANS, 2005; DWAF, 1996c) and (WHO, 2011) recommended the MPL at 0.025 mg/L.

Copper (Cu) is an essential substance to human life, but excessive doses can cause anaemia, acne, adrenal hyperactivity and insufficiency, hair loss, arthritis, autism, cancer and depression.

Additionally, it can lead to elevated cholesterol, diabetes, dyslexia, failure to thrive in babies, fatigue, fears, fractures of the bones and headaches.

Heart attacks, hyperactivity, hypertension, infections, inflammation, kidney and liver dysfunction, panic attacks, strokes, tooth decay and other vitamin deficiencies are also formed as a result of over exposure of the element (Kabata-Pendias and Pendias, 1992). The MPL for Cu in water set by (SANS, 2005), is 1.0 mg/L, 0.2 mg/L (DWAF, 1996c), and 2 mg/L by WHO (2011).

Zinc (Zn) is an airborne pollutant. It commonly accumulates in open and above earth crops, whilst root crop plants also soak up great proportion from Zn contaminated soils (Kabata-Pendias and Pendias, 1992). The MPL for Zn in water is 5 mg/L (SANS, 2005), 1 mg/L (DWAF, 1996c), 0.123 mg/L (WHO, 2011) and 0.2 mg/L according to the CCME (1999) standards.

Cadmium (Cd) is very toxic even at ultra-trace levels. Its long-term exposure to lower levels leads to a build-up in the kidneys causing kidney-related diseases. Lung damage, and fragile bones are some of the illness reported to be caused by over exposure of Cd. Hypertension, arthritis, diabetes, anaemia, cancer, cardiovascular disease, cirrhosis, reduced fertility, hypoglycaemia, headaches, osteoporosis, kidney disease, and strokes are some of the odd long-term results of Cd (Divrikli *et al.,* 2003). The MPL level of Cd in water for Cd is of 0.005 mg/L by (SANS, 2005), 0.01 mg/L (DWAF, 1996c), 0.003 mg/L (WHO, 2011) and 0.0051 based on the CCME (1999) standards.

Lead (Pb) enters body system through air, water and food (Divrikli *et al*., 2003). It is a serious cumulative body poison which can affect every organ and system in the body. Exposure to its high levels can severely damage the brain, kidneys and ultimately cause death. The long-term exposure leads to decreased performance in some tests that measure the functions of the nervous system, weakness in fingers, small increases in blood pressure and anaemia. Other effects are abdominal pain, arthritis, attention deficit, back problems, blindness, cancer, constipation, convulsions, depression, diabetes, migraine headaches, thyroid imbalances and tooth decay (Lokeshappa *et al*., 2012).

The presence of Pb in drinking water is limited to 0.02 mg/L and 0.01 mg/L as set by (SANS, 2005) and WHO, (2011) water guidelines, respectively. Tolerance of Pb in water designated for irrigation is limited to a maximum of 0.2 mg/L by DWAF (1996c) and CCME (1999).

Manganese (Mn) is not found as a free element in nature. It is often found in combination with Fe, and in many other minerals. Manganese is a metal with important industrial metal alloy uses, particularly in stainless steel. It plays an important role in human health, being absolutely necessary for development, metabolism, and the antioxidant system.

Nevertheless, excessive exposure or intake may lead to a condition known as Manganism which is a neurodegenerative disorder that causes dopaminergic neuronal death and parkinsonian-like symptoms (Daiana *et al*., 2013). The MPL of Mn in water is 0.1 mg/L according to SANS (2005), 0.02 mg/L (DWAF, 1996c), 0.5 mg/L (WHO, 2011) and 0.2 mg/L CCME (1999).

Iron (Fe) is the most common element on Earth, forming much of Earth's outer and inner core. It is the fourth most common element in the Earth's crust. Large amounts of ingested Fe can cause excessive levels of Fe in the blood and it typically damages cells in the heart, liver and elsewhere. This can cause significant adverse effects, including coma, metabolic acidosis, shock, liver failure, coagulopathy, adult respiratory distress syndrome, long-term organ damage, and even death (Cheney *et al*., 1995). The recommended guideline level of Fe in water is 0.2 mg/L (SANS, 2005) and 5 mg/L by DWAF (1996c) and CCME (1999) under irrigation guidelines.

2.3 ENVIRONMENTAL POLLUTION BY POTENTIALLY TOXIC ELEMENTS

The Mining revolution in South Africa results from the discovery of diamonds in 1867 in the small town of Kimberly, Northern Cape Province. This discovery resulted in what was termed the diamond rush whereby potentially miners where flocking to the town in search of fortune (Worger, 1987; Meredith, 2007).

Mining exists in different parts of the world as a primary resource to strengthen the economy and reduce poverty through employment generation. However, acid mine drainage, heavy metal contamination, erosion and land degradation are some of the negative impacts caused by mining. Irresponsible discharges from the mining, municipal and other industries lead to detrimental damages to the environment and its living organisms (USGS, 2000; Lee, 2003).

Industrial tailings are disposed for the duration of an operation's design life. These operation sites are unstable leading to chemical spills, which can be carried into river systems during rainy seasons contaminating ground water. This in turn endangers aquatic life and humans through food chains or direct use of river water (Ochieng *et al*., 2010).

Prolonged accumulation of the potentially toxic elements in the body by indirect and direct use of possibly polluted water leads to toxic effects that tampers with the body's normal functionality (Jaishankar *et al*., 2014). Various medical conditions have been reported to be the results of over exposure of potentially toxic elements in the body and the common ones are caused by chronic inhalation of high levels of Cd. It also causes both fibrotic and emphysematous lung damage, but it also has major effects on bones and the kidneys (Irfan *et al*., 2013). People who reside in the close proximity of the mining industries are highly at risk of suffering from heavy metal poisoning by the direct inhalation of metal dusts and ingestion of this potentially toxic elements through food chains (Satarug *et al*., 2011).

Pollution is generally regarded as a huge global threat which arises as a direct and indirect source of potential harm to human life and other living organisms (Mayntz, 2014). Due to high demands concerned with the growing human population and activities which expose humans to keep up with the developing world, this promotes anthropogenic activities that are regarded as the biggest source of pollution worldwide. Upon the continuous exhaustion and improper use of their natural resources, South Africa is at a point where they are only causing distress to their environment (Brown and William, 1994). Pollution can be defined based on the main ecosystem which it affects. One pollutant often affects more than one ecosystem in a form of a chain such as solid waste classified as hazardous (radioactive, medical, poisons) which is health and life threatening, or non-hazardous, which is classified by mining, industrial, domestic and scrap metals, among others (Suruchi and Pankaj, 2011).

Pollution of the environment by potentially toxic elements is a universal problem because these elements are considered to be toxic and their bioavailability causes potential hazards to the living organisms.

Potentially toxic elements, which are frequently reported in literature are Cd, Cu, Zn, Pb, Fe and Cr (Alloway, 1995; Akoto *et al*., 2008; Islam *et al*., 2015a).

Trace elements such as Fe, Zn, Cu and Cr have been acknowledged for their biological importance in the human system, but Pb and Cd have no indication of reasonable importance in the human body, hence they are toxic even at low concentrations (Duruibe *et al*., 2007).

2.3.1 Potentially toxic elements in river water

Water is important for the existence of life on earth. All organisms require it and aquatic plants and animals live their life in water hence making water their direct source of life. The contamination of water bodies such as rivers, oceans and lakes in which pollutants are directly or indirectly discharged into water bodies without sufficient treatment to remove harmful particles or substances is termed water pollution (Agrawal *et al*., 2010). Human development has partially contributed to the deterioration of water quality. Water quality is referred by the physical, chemical and biological characteristics of water in relation to their existence of life and especially human activities (Lamb, 1985).

The effect of water pollution damages not only individual species and population, but also the natural biological communities. Water pollution is a major global problem, which requires continuous assessment and modifications of water resource policies at all levels from international to individual aquifers and wells. It has been suggested that water pollution is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than 14,000 people daily as reported by West (2006).

Water pollution is often linked to heart diseases, poor blood circulation, nervous system and skin lesion. Cholera and diarrhoea are among the common problems caused by the accumulation of bacteria and metal poisoning in water (Khan and Ghouri, 2011). The future human and aquatic generation face a threat of alteration in the chromosomal makeup, as a result of uncontrollable water pollution (Nazir *et al.*, 2015). This is caused by toxic effluents from different sources which reduce the availability of oxygen in the water body, when they are directly disposed as wastes in the water system.

As such, the flora and fauna of rivers, seas and oceans is adversely affected by water pollution. Thus, poses as a direct threat to the human health *via* the food chain (Asfaw *et al*., 2013).

The water quality guidelines where classified on their usage such as agricultural, aquatic, domestic and industrial use. Water quality guideline values are used for the relevant and specified activities associated with the water use. Industrial water refers to the different uses of water in industrial under process types, such as cooling water, steam generation, process water, production water and utility water (DWAF, 1996b).

Industries have been popularly identified as the main contributors to elevated levels of chemical deposits in the environment and water bodies (DWAF, 1996b). The untreated water disposed into rivers and streams after industrial productions, may cause corrosions, abrasions and aquatic deaths which through a food chain may cause irreversible damages to human health over long exposure (DWAF, 1996b).

Domestic water refers to water that is used in a domestic environment and also refers to all uses water can be put to in this environment. These include water for drinking, cleaning, cooking and handling food and beverage preparations, bathing and personal hygiene, recreational activities such as swimming, laundry and gardening. Commonly, domestic water users experience impacts such as bad health due to the poor water quality (DWAF, 1996a). The presence of potentially toxic elements in the water systems and water bodies, lead to detrimental health effects due to both short and long-term exposure based on the type of constituents found in the water course (Bernhoft, 2013).

Irrigation water refers to water which is used to supply the water requirements of crops and plants which are not provided by rain and refers to all uses water may be put to in this environment. This include water for the plantation of commercial crop irrigation water application and distribution systems, small scale home gardening and potted plants sold in flea markets (DWAF, 1996c). Equally important, untreated water used for irrigation can leave detrimental impacts such as the reduction in the yield of crops due to salinity or constituents that are harmful to the proper growth of the plants. Accumulation of potentially toxic elements in the crops lead to corrosion of the planting equipment and toxicity in humans through food chains (DWAF, 1996c).

Although South Africa has made substantial progress in shifting policy bodies to address mine closure and mine water management, and the mining industries have changed their practices to adhere to new laws and regulations, irregularities in the system still remain.

South Africa is one of the few countries in the world that cherish the basic right to sufficient and clean water in its constitution. This is implemented by the existence of water bodies, which are bulk water supply agencies that operate pipelines, dams, waste water systems and sell water from reservoirs to municipalities. Despite the efforts, availability of water in South Africa varies with space and time commonly favouring urban places and excluding rural settlements.

Activities in the mining industries are one of the setbacks that environmental policy makers need to address as an ongoing assessment to minimise the deterioration of water quality.

Disturbance to the food chain is one of the major effects of water pollution. When toxins are present in water they are conveyed to higher level organisms through the food chain. As such, these hazardous wastes are passed on to humans and animals. Potentially toxic elements like Pb, Hg, Fe, Cd, Al and Mg are mostly present in water. If these metals are present in the sediments, they reach the food chain through plants and aquatic animals (Cheng and Hu, 2010; Hosono *et al*., 2011). This then causes accumulation of heavy metal poisoning in the human bodies and aquatic animals.

Poor blood circulation, vomiting, skin lesions and damage to the nervous system are few of the many other health issues related to polluted or untreated water (Ashraf *et al*., 2011). Water pollution is considered to be a major cause of death of the population across the world. Water pollutants modify the overall chemistry of the water, causing changes in acidity, conductivity and temperature. These in turn have an effect on the marine life (Ashton and Dabrowski, 2011).

Marine food sources are contaminated or eliminated by water pollution and disturb the vulnerable ecological balance of marine life. Dead fish, birds, dolphins, and many other animals often wind up on beaches, killed by pollutants in their habitat. Heavy rains carry up dirt and silt, and then deposit them into the water. If the dirt and silt settle in the water body, then the sediments prevent sunlight from reaching aquatic plants.

If the sunlight can’t reach the plants, they will die. The sediments also clog up fish gills and suffocate organisms that live on the bottom body of the water (Hosono *et al*., 2010).

Additionally, pollution affects and modifies the chemistry of water. The pollutants, including toxic chemicals, can alter the acidity, conductivity and temperature of water. Drastic killing of fish and aquatic plants in rivers, oceans and seas is an after-effect of water contamination. Addo-Bediako *et al*. (2014) reported that the metal concentrations in the muscle tissue of fish (*Schilbe intermedius*) appear to have increased at Flag Boshielo Dam and the Phalaborwa Barrage over the past 20 years.

They further reported that the concentrations of Al, Cu, Fe and Cr were high in the muscle tissues of the fish. Studies by Addo-Bediako *et al*. (2014) have been done at the Phalaborwa Barrage where it was reported that almost all fish sample analysed from the river exceeded the recommended level for Pb and more than 50% exceeded the permissible level of As.

2.3.1.1 The effect of pH on the environment and water

There are many contributing factors that can affect the pH of water. These factors are both natural and man-made. Natural changes in pH can result from the existence of carbonate materials and limestone. When carbonate minerals are present in the soil and sediments at the bottom of streams, rivers and seas, the alkalinity of water increases, keeping the pH of water close to neutral even when acids or bases are added (Utah State University, 2013).

Carbonate materials beyond the neutral limits can increase the basicity of water. Volcanic ash, sulphate-reducing bacteria in wetlands, airborne particulates from wildfires and even lightning are some of the additional natural modifications of pH levels in water bodies (NADP, 2013). Low pH levels can influence the solubility of potentially toxic elements. The elevated levels of hydrogen ions influence metal cations such as Al, Pb, Cu and Cd to be easily discharged into the water instead of being absorbed into the sediments (USGS, 2013).

Pollution is one of the anthropogenic activities that can also change a water's pH, which in turn can harm animals and plants living in the water (USGS, 2013).

According to the APHA (1998) the pH range of 6.0-9.0 appears to provide a good protection for aquatic organisms such as fish and the bottom dwelling invertebrates like corals and snails.

Susceptibility of aquatic species determines the degree of damage caused by the changes in pH values. Apart from aquatic species being at risk of being affected by the changes in water pH, humans are also at risk due to the direct and indirect use of river and stream water. In a thesis submitted by borosilicate (2005), he emphasised that although humans have higher pH level tolerance ranging from 4-11 with minimal gastrointestinal irritation, values greater than 11 can cause skin and eye irritations. Furthermore, pH values below 2.5 will cause irreversible damage to skin and organ linings.

Lower pH levels increase the risk of mobilised toxic metals that can be absorbed, even by humans while levels above 8.0 cannot be effectively disinfected with chlorine, causing other indirect dangers to the human health (USEPA, 2012).

The pH levels below and above 6.5-9.5 range can cause damage and corrosion to pipes and other water systems, hence increasing heavy metal toxicity in the environment (WHO, 2003).

Active and abandoned mining and power stations have an equal share in the contributions towards the acidity of the surrounding environment which also affect the pH of water. These industries release chemical emissions during coal burning for coal-fired powered stations and automobiles during transportations (Osmond *et al*., 1995). Mine acid runoffs lower pH values below the accepted levels. This may be tolerable for some aquatic species such as frogs but not for most fish. Potentially toxic elements can accumulate on the gills of fish or cause deformities in young fish, reducing their chance of survival (Pandey and Madhuri, 2014). Agricultural activities also release chemicals such as pesticides and herbicides, which alter the pH levels of water (Morakinyo *et al*., 2013).

2.3.2 Potentially toxic elements in sediments

Sediments are regarded as the gathering of sand and dirt that settles in the bottom of lakes, rivers and seas and occur through natural processes such as weathering and erosion (Singh, 2006). Eventually they become transported by actions such as rain water and wind (Qiu *et al*., 2011). Sediments are effectively defined as the sink for trace metals in marine and estuarine ecosystems and are also classified as the source of metals for aquatic organisms (Dural *et al*., 2007).

Mohammad-Salah *et al*. (2012) reported the correlation of potentially toxic elements in sediments with water quality. Their study highlighted that sediments serve as the primary source of toxic metals through chemical leaching of bedrocks, water drainage basins and runoffs from river banks.

Sediments pollution by potentially toxic elements has been regarded as a critical problem in marine environments and has received extensive attention because of the toxicity, easy accumulation and non-degradability (Islam and Tanaka, 2004; Todd *et al*., 2010).

Potentially toxic elements, which are fixed to sediments, can be recycled back to water columns through chemical and biological processes, thus serving as a possible future source of potentially toxic elements in marine and estuary systems (Tessier and Campbell, 1988; Soylak and Yilmaz, 2006). The study of sediments plays a major role in the measurement of pollutants since they have a long residence time (Batley, 1989). As such, sediments quality guidelines are set to protect aquatic ecosystems by setting acceptable levels for chemical substances that are prone to be found in the aquatic environment.

Methods of evaluating toxicity in sediments have limitations based on the use of total metal extraction techniques. This is caused by different sediments exhibiting different degrees of bioavailability and concentrations for the same metal content (Di Toro *et al*., 1990). Extraction and digestion techniques have been applied widely for the determination of potentially toxic elements in sediments for which further analysis such as speciation can be done.

2.4 REPORTED CASES OF POTENTIALLY TOXIC ELEMENTS IN SOUTH AFRICA

Irresponsible disposals of chemicals by mining industries and wastewater treatment plants have led to the detrimental damages in the water bodies. This occurs through seepage whereby during heavy rainfalls and windy seasons, harmful chemicals are transported into river streams.

They then deposit toxic elements in the river bodies and in extreme cases, killing the aquatic lives such as flora and fauna (Islam *et al*., 2014; Islam *et al*., 2015b). Many cases surrounding trace elements toxicity have been reported based on the findings investigated following fatality and morbidity cases (Pandey and Madhuri, 2014).

Additionally, livestock farmers suffer the setbacks whereby reduction in livestock product quality such as consumer health hazards and product quality problems arise. Based on a study carried by Chirwa and Lebitso (2014) they emphasised that chickens raised on 100 percent sludge gained weight faster than a control group of chickens raised on conventional feeds such as fishmeal. However, they noted that sludge from city sewage works seldom contain potentially toxic metals from industrial wastewater such as Pb, Mn, Cu, Cd and Zn. The quality of water for livestock may be defined according to the livestock water quality guidelines based on the toxicity effects that may hinder livestock production (DWAF, 1996d).

Silver was detected in high concentrations in municipal sewage sludge in Luis Trichardt, Limpopo Province, in a study carried by Shamuyarira and Gumbo (2014). They reported the highest concentration value of 21.93 mg/kg dry mass in Louis Trichardt while the lowest concentration was detected in Thohoyandou at 6.13 mg/kg dry mass. These concentration values were detected in wastewater sewage sludge and there is no DWAF guideline standard of Ag. Cadmium concentration of 3.10 mg/kg dry mass was detected in Polokwane municipality wastewater sludge, while 102.83 and 171.87 mg/kg of Pb were detected in Polokwane and Louis Trichardt municipalities, respectively. Complementary to this, other elements have been studied. Among other elements, high As concentrations in sediments are generally associated with the anthropogenic activities such as treatment of plants from the fertilisers and arsenical pesticides industries (Fu *et al*., 2014; Ahmed *et al*., 2016).

In the Western Cape, the highest Fe concentrations recorded in the Plankenburg River were 48 mg/L in water and 14 363.8 mg/kg in sediments. The highest Fe concentrations recorded in the Diep River were 513 mg/L and 106 379.5 mg/kg in water and sediments, respectively. Other elements were measured, and the findings stipulated that the concentrations were higher than the recommended water quality guidelines as set by the Department of Water Affairs and Forestry and the Canadian Council for the Ministers of the Environment (Jackson, 2008).

Moyo and Mtetwa (2002) identified Hartebeespoort Dam as one of the pollution hot spots in the Southern Africa. Their study was based on point source pollution, particularly municipal wastewater.

Studies regarding many rivers in South Africa such as the Mooi, Diep, Tyume, Buffalo and Plankenburg Rivers have been undertaken and many were reported to be contaminated with potentially toxic elements such as Cu, Zn and Pb (Moyo and Mtetwa, 2002).

The concentrations of these potentially toxic elements in the river systems were ranging from 1.6 to 9.3 µg/L, 2.0 to 3.0 µg/L, 10.5 to 20.1 µg/L, and 2.1 to 2.5 µg/L for Cd, Cu, Pb and Zn, respectively. The elevated levels of Cd and Pb were associated with sewage effluent, mining and industrial activities (Awofolu, 2004; Okonkwo and Mothiba, 2005; Jackson *et al*., 2009).

The Vaal River in the Gauteng area has been reported as one of the most polluted rivers due to the overpopulation in the province prompted by job opportunities in industries. High levels of Zn, Pb and Mn concentrations were detected in water samples from Vaal River. The measured concentrations exceeded the MPLs based on the guidelines provided by South African water standards (Wepener *et al*., 2011).

The research by Friends of the Earth International/Groundwork discovered that approximately 2200 death cases have been reported in South Africa per year, caused by coal-powered electricity stations, due to exposure to fine particulate matter (Myllyvirta, 2014). According to the WHO (2011), these coal-fired power stations produce pollutants which have been found to cause respiratory illnesses such as bronchitis, asthma and lung cancer. In extreme situations, cardiac conditions occur due to over exposure of power station emissions.

A study by Dahms *et al*. (2017) reported that the concentration of Ni in the sediments samples analysed from Nyl River in Limpopo Province exceeded the Canadian SQGs by a factor of between 1.36 and 1.83 times. Many rivers and streams in South Africa have been reported to contain potentially toxic elements and have been concluded to be toxic to aquatic organisms. Thus, there is a need for constant monitoring and assessment of rivers to maintain good water quality.

Despite the numerous reports of contaminated rivers, Dzindi, Madanzhe and Mvudi are some of the rivers that have been reported to be clear of potentially toxic element contaminations (Okonkwo and Mothiba, 2005).

2.5 ENVIRONMENTAL SAMPLE PREPARATION METHODS

The choice of the sample preparation technique depends on the analyte of interest, expected levels (in ppt, ppm and ppb), sample matrix, instrumental measurement conditions and the amount of sample available. In most Spectrometry analysis, solid samples are dissolved by mineralisation to form liquid solution that can be introduced into the flame or plasma depending on the instrument of choice (Badera and Zimmermann, 2012). In this section, sample preparation techniques employed in this study are reviewed.

2.5.1 Microwave-assisted acid digestion

The microwave technology evolved in the late 1980s for the development of sample preparation strategies. This technology is currently applied in the four major fields of chemistry, namely analytical chemistry, organic synthesis, preparation of catalysts in inorganic reactions and physical chemistry (Nóbrega *et al*., 2002). The microwave technique was developed to enhance the sample preparation quality by basing its improvement on the shorter acid digestion time, better recovery of volatile trace elements in samples and reduced airborne contamination levels. Additionally, more reproducible procedures and a safer working environment can be obtained due to the enclosed vessels during sample throughput (Agazzi and Pirola, 2000).

The closed microwave vessels yield complete digestion due to the increase in the higher temperatures and high pressure produced in the vessels during sample digestion.

The reduction in digestion time and reagents volume serve as some of the advantages of the closed microwave vessel system. Additionally, elimination of volatile elements loss during digestion and also the digestion of more difficult samples is made easier (Agazzi and Pirola, 2000).

One of the setbacks of the instrument is that at each of the digestion temperatures, the sample analyst is bound to know about the relative proportions of constituents in a sample matrix (Hiefje, 2000). Correlation between the increment in temperature and pressure is observed graphically based on the instrument in use.

Considering the limitations of current closed-vessel microwave digestion technologies, the most limiting factor is the amount of sample that can be digested. This is highly disadvantageous with organic matrices and less complications with inorganic samples (Agazzi and Pirola, 2000).

Microwave-assisted acid digestion is a widely used technique for the dissolution of food samples with HNO_3 and HCl, and this acid combination is called aqua regia (Melŕ *et al*., 2008; Nardi *et al*., 2009). Nitric acid is classified as an oxidising acid which is frequently mixed with HCl. It forms insoluble nitrates with all elements except Au, Pt, Al, B, Cr, Ti and Zr (Sastre *et al*., 2002; Uddin *et al*., 2016). Hydrochloric acid is a strong non-oxidising acid which occasionally forms soluble chlorides with many elements except Ag, Hg and Ti.

Hydrochloric acid and H_2SO_4 are commonly termed harsh acids which are prone to be highly destructive to the sample organic matter, however, they are generally excluded to avoid chloride and sulphur interferences (Cubadda, 2004).

Acid digestion procedures are typically employed for the determination of elements in solid matrices to completely transfer the analyte into a solution that can be introduced into analytical systems. Acid combinations are chosen on the basis of complete digestion and the target element with respect to different sample matrices. Kotz *et al*. (1972) discovered that the methods of digestion that involve a mixture of HNO_3, HCl, H_2SO_4 or $HClO_3$ were satisfactory for digesting mineral elements in organic and biological materials. In this context, HF acid is commonly chosen based on its acidic strength to break down the borosilicate sediments samples and leach out the silicate matrices (Makishima *et al*., 2009).

Complexing of HF is required to mask fluorides before further use, thus H_3BO_3 is introduced (Wilson *et al*., 2006). Hydrochloric acid and HNO_3 are used in combination with HF due to their combined mineralisation strength (Potts, 1987).

2.5.2 Sequential Extraction

Sequential extraction is an analytical process that chemically leaches metals or elements out of soil, sediments and sludge samples with the most mobile metals removed in the first fraction and continually in the order of mobility decrement (Ahnstrom and Parker, 1991; Uduma and Jimoh, 2013).

The main idea behind sequential extraction is to mimic the selective release of metals (potentially toxic elements and trace elements) into solutions from solid samples under controlled environmental conditions with the help of selective reagents relative to the chemical nature of the metals (Rodgers *et al*., 2015). Hence, sequential extraction method provides fractionation stages which characterise the chemical forms in which trace elements exist in sediments and sewage sludge among other samples (Fuentes *et al*., 2008).

The classical sequential extraction method introduced by Tessier *et al*. (1979), reported five stages to fractionate potentially toxic elements in river sediments. The five stages extraction method was selected to fractionate Cd, Co, Cu, Fe, Pb, Mn, Ni and Zn in river sediments at low concentrations.

In the first fractionation stage, also termed the exchangeable stage, either 1 M $MgCl_2$ or 1 M NaOAc solution at a pH of 8.2 was used for continuous agitation and extraction of selected analytes. Analytes bound to carbonates in the residue from first stage of extraction are leached with a solution of NaOAc adjusted to pH 5.0.

In the third fractionation stage, analyte bound to Fe-Mn oxides in the residue from the 2nd fraction is extracted using either a mixture of 0.3 M $Na_2S_2O_4$, 0.175 M Na-citrate and 0.025 M H-citrate (Anderson and Jenne, 1970) or 0.04 M NH_2OH-HCl in 25% (v/v) HOAc. Analytes bound to organic matter found in the residue obtained from stage 3 are extracted by continuously agitating with a mixture of 0.02 M HNO_3 and H_2O_2 adjusted to pH 2 and heated at 85 ± 2 °C. In this stage, adsorption of the dissolved metals on to the oxidized sediments was prevented by further agitation with 3.2 M NH_4OAc in 20% (v/v) HNO_3 (Gupta and Chen, 1975).

Trace metals in their crystal structures held in the residue from the fourth stage of extraction were dissolved by digesting with HF-$HClO_4$ mixture, the resulting solution diluted and analysed by atomic spectrometry.

2.5.2.1 Scope and Limitations of Sequential Extraction

The sequential extraction procedure has been developed and currently used for the reduction of the multi-step procedure, which requires the use of more reagent volumes and increased extraction time. The method has been adopted for its complete extraction of the metals of concern from samples.

Results from all the different steps are calculated and used to determine the accurate concentrations of the metals under different conditions. The use of the sequential extraction procedure can provide information about the bioavailability and leachability of the metals in study (Yang *et al*., 2014). It can additionally provide information about the reaction behaviour of individual solid phases as their presence may be inferred from their dissolved products in leachates extracted in speciation studies (Rodgers *et al*., 2015).

Factors such as extraction fluid pH, temperature and duration of extraction have a direct correlation to the concentration of metals extracted from the sample (Mossop and Davidson, 2003; Oyeyiola *et al*., 2011). The commonly used sequential extraction reagents are typically chosen for the leaching of metals in each fraction. Acetic acid is chosen to extract all exchangeable, acid and water-soluble metals.

Hydroxyl ammonium chloride is used to extract all reducible metals, while ammonium acetate with hydrogen peroxide is chosen to extract all oxidizable metals. Finally, acid combinations such as aqua regia, are introduced to extract all remaining, non-silica bound metals (Castillo *et al*., 2011). These reagents are chosen based on their extraction strengths to leach out metals from the different fractions of the sediments.

In each of the extraction steps, reagents and buffer solutions are added and the sample is shaken using mechanical shaker followed by sonication. This is done to leach out the trace metals from the residue of the previous step, simplified as washing to minimise carry-over effects. After each extraction step, the supernatant liquid is separated from the solid phase by centrifugation.

Trace elements in sediments are available in different physicochemical forms, which in turn influence availability and the identity of the possible source of pollutant (Benson *et al*., 2013). Although these procedures have been extensively applied to aquatic sediments and soils, they are still the subject of controversy.

Sequential extraction is limited by the fractionation step which dislocates chemical elements from compounds. This often results in chemical alterations of the sample matrix and inaccurately indicates the presence of particular chemical phases in the sample (Bacon and Davidson, 2008).

Furthermore, selectivity of the extraction reagents, sample matrix, pre-treatment, extraction time and sample to reagent ratio may result in uncertainties in the metal fractionation or inconsistencies among the different extraction methods.

Controversy around the sequential extraction protocol declares it not to be considered a fully quantitative method due to re-distribution of analytes among solid phases during each extraction step. This occurs because extraction occurs within the aqueous leachate. Additionally, the limited number of certified reference materials to account for the different types of extraction methods limit reliability of the method. Although the extraction stages require ample time to leach out the target metals from samples, the extraction method is commonly incomplete, thus, requiring the residues to be digested with aqua regia. In addition, new solid phases may be precipitated from the leachate. Particle loss during sample handling by centrifugation, decantation and washing, is also another key limitation to the multiple extraction stages (Bacon and Davidson, 2008).

2.5.2.2 The BCR Sequential Extraction

In 1987, the BCR proposed a programme of harmonising the methodology of sequential extraction schemes, used for determination of metals in different environmental samples (Ure *et al.,* 1993). The BCR protocol combined stages 1 and 2 of classical work. Most of the modified procedures provide ambiguity over the type of procedure to choose for sample preparation due to the differences in sample matrices and sampling sources. Determination of potentially toxic elements in sediments can be strenuous due to the lengthy extraction times and countless extraction steps which in most cases provide inconsistent results.

In this study, the Rauret *et al.* (1999) BCR protocol was selected together with the modified microwave-assisted sequential extraction procedure by Castillo *et al*. (2011). They were chosen to examine and clarify the benefits and limitations of each procedure addressed by the sediments study. Rauret *et al*. (1999) named the fractionation steps exchangeable, reducible, oxidizable and residual. These are also often referred in the literature as exchangeable, weakly absorbed, hydrous-oxide bound, organic bound, and lattice material components, respectively (Maiz *et al*., 2000).

Typically, metals of anthropogenic inputs tend to reside in the first three fractions and metals found in the residual fraction are of natural occurrence in the parent rocks and sediments (Ratuzny *et al*., 2009; Zimmerman and Weindorf, 2010).

The exchangeable fraction is removed by changing the ionic composition of water allowing metals bound to the exposed surfaces of sediments to be removed easily. A salt solution is commonly used to remove the exchangeable fraction (Zimmerman and Weindorf, 2010). The carbonate-bound fraction is responsive and sensitive to changes in pH. The carbonate complexes increase with pH (Elder, 1989). Metals bound to Fe-Mn oxides are particularly susceptible to reducing conditions (Salomons, 1995). To remove metals bound in the organic phase, an organic material must be oxidised with an oxidising reagent. The residual fraction consists of metals incorporated into the crystal structures of primary and secondary minerals. This fraction is the hardest to remove and requires the use of strong acids to break down silicate structures after the completed extraction stages (Tessier *et al.*, 1979).

In the choice of reagents for the sequential extractions, particular emphasis was placed on the potential selectivity of each leaching solution. Procedures currently used in sediments chemical analysis were considered, together with those developed in recent studies of (Quevauviller, 1998; Bird *et al*., 2005; Larios *et al*., 2012). Acetic acid is commonly chosen to extract all exchangeable acid and water soluble metals.

Hydroxyl ammonium chloride is used to extract all reducible metals while hydrogen peroxide is chosen to extract all oxidizable metals and finally, aqua regia with HF is used to extract all remaining non-silica bound metals (Nemati *et al*., 2011).

This multi-step procedure assures that all the metals of concern are completely extracted from the sample. The results from all the different steps are calculated and used to determine the accurate concentrations under different conditions.

Factors such as pH of the acid used for adjustment, temperature and duration of extraction are the critical factors that control the concentration of metal extracted from the sample (Ahnstrom and Parker, 1999).

Better understanding of the environmental risks of potentially toxic elements is provided through the study of the geochemical fraction of elements.

This is done by calculating the contamination factor of each element to have a better understanding of the degree of risks posed by potentially toxic elements. Estimation of the relative retention time of elements in sediments associates higher individual contamination factor (ICF) value with lower retention time. A higher ICF value indicates the risk of metal toxicity to the environment. The ICF of potentially toxic elements in this study was calculated following the formula by (Nemati *et al*., 2011, Fathollahzadeh *et al*., 2014).

The ICF values were obtained by dividing the concentrations of metals in the mobile phase from the three fractions (F1+F2+F3), by the concentrations in the residual stage (F4). The summarised equation by Nemati *et al*. (2011) and Fathollahzadeh *et al*. (2014) is shown below as:

$$\text{ICF} = \frac{[F1+F2+F3]}{F4} \qquad \text{Equation…..1}$$

2.5.2.3 Microwave-assisted sequential extraction

Microwave-assisted sequential extraction is defined as a technique that applies the strength of microwave energy to heat reagents in contact with a solid sample in order to partition analytes from the sample matrix into the solvent (Sanchez-Prado *et al*., 2015). The amount of the sample and the extraction time required solely depend on the microwave power to be applied during the extraction stages.

In most cases, sequential extraction reagents do not completely dissolute the sample matrix, hence residues are visible and should be accounted for during analysis of sequential extraction stages.

Hu *et al*. (2008) stipulated that increasing the microwave power improves the extraction yield, thus resulting in shorter extraction time. However, there are limitations on setting the microwave power to the maximum.

At high microwave power, poor extraction yield occurs due to the degradation of metals which are sensitive to high temperature. Microwave-assisted extraction is a modified technique that minimises the extraction conditions employed by the BCR sequential extraction stages by replacing the mechanical shaker used in the BCR protocol, with the microwave heating.

2.6 DETECTION TECHNIQUES FOR POTENTIALLY TOXIC ELEMENTS IN WATER AND SEDIMENTS

The type of analytical technique to be chosen for determination of potentially toxic elements in sequential extracts should be able to account for the complexity and the occurrence of selected elements. This may be influenced by element concentrations ranging from main components in mg/kg to trace elements in µg/kg. There are many instrumental techniques that can be used to determine different elements in water and sediments samples (APHA, 1992).

Atomic spectrometric techniques are commonly used for the quantification of the levels of analytes in samples of interest. The atomic spectrometric technique is chosen based on detection limits expected prior to analysis. These include F-AAS, GF-AAS, ICP-AES and ICP-MS. A brief description of principles, instrumentation and analytical features of these widely used techniques are given below.

2.6.1 Flame-Atomic Absorption Spectrometry (F-AAS)

Flame-atomic absorption spectrometry is an analytical technique commonly used for the determination of trace elements due to its high specificity, low investment and running costs. The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relationship between the measured absorbance and the analyte concentration, thus relies on the Beer-Lambert Law (García and Báez, 2012; Punukollu and Chappidi, 2012)

The basic F-AAS instrument includes a light source, nebuliser, spray chamber, fuel and oxidant pipe system, drain, burner, detector and a read output device. A block diagram of such typical system is shown in figure 2.1.

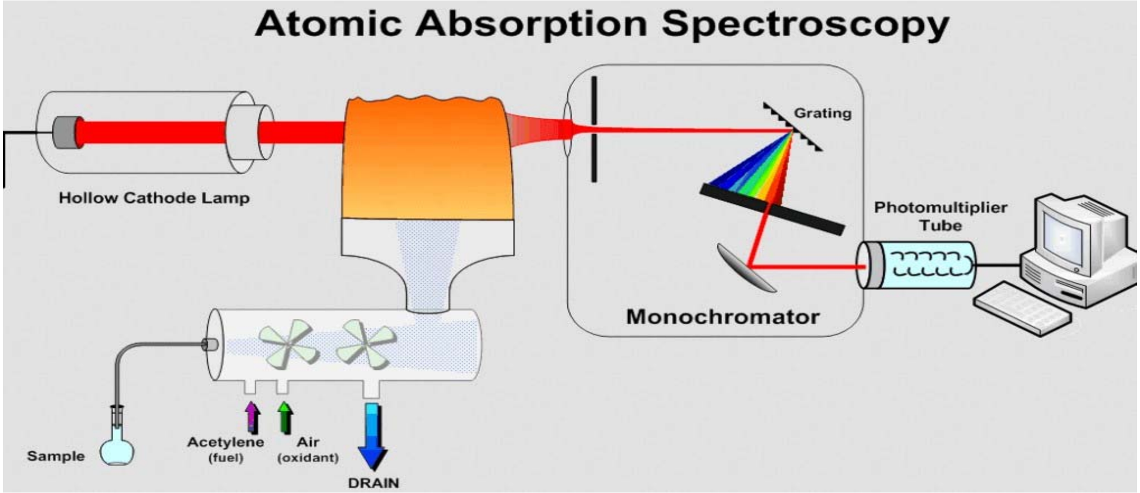

Figure 2.1: A system block diagram of the F-AAS

2.6.2 Graphite Furnace-Atomic Absorption Spectrometry (GF-AAS)

The technique is similar to F-AAS except that the flame is replaced by an electrically heated closed graphite tube having transparent end windows. The graphite tube generates a cloud of atoms which is exposed to the light from the hollow cathode lamp. A higher atom density and longer residence time in the furnace tube provide a lower LOD. The LODs are improved by a factor of up to 1000 times compared to F-AAS, thus detecting as low as ppb range (Johnson *et al*., 1997). The GF-AAS has limited dynamic ranges which can also detect concentrations in the ppt range of 100-1000. Therefore, solutions must be held in a narrow range of concentrations.

2.6.3 Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)

An ICP-AES is a type of technique used for determination of potentially toxic elements in different sample matrices. The plasma source discharge atomic emissions in a radial or axial configuration. The emitted atoms are collected with a lens and imaged onto an entrance slit of a wavelength selection device. A monochromator is used as a wavelength selection device. The use of ICP-AES is competitive with most other spectroscopic analysis of elements with regards to sample throughput, sensitivity and detection limits for analytes (Bernazzani and Paquin, 2001).

Figure 2.2 shows the simplified diagram of the process of introducing a sample till the results output for analysis in an ICP-AES. In this figure, the sample in a solution form is introduced into the nebuliser chamber via a peristaltic pump attached to an automatic sampler.

A peristaltic pump operates by sequentially compressing flexible tubing with evenly spaced and rotating rollers that pull the liquid through the system. The flow of the sample and argon gas through the small aperture of the nebuliser creates small droplets that form a mist of µm-sized particles in the spray chamber (Hou and Jones, 2000). The small droplets from the sample travel with the argon flow and enter the torch region while the larger droplets are carried to the drain system.

In the plasma region, evaporation, atomisation, and ionisation occur at maximum temperatures reaching 10 000K. Visible and UV radiation emitted from the sample constituents enter the monochromator through a small slit where the wavelengths are separated by a grating before being captured and measured by the detector. From the detector, intensity of the emitted lines is measured converting those readings to the actual sample concentration which can be processed from the computerised data output (Hou and Jones, 2000).

Since ICP-AES is used for multi-element quantification, two types of the instrument are commonly known for the elemental analysis.

The sequential spectrometer is one of the instruments selected whereby it uses a monochromator to scan different emission lines in sequence. The wavelength of choice during analysis is controlled and chosen by an analyst on the instrument software, manually.

The second common instrument is the direct reading spectrometer which uses a polychromator with as many as 64 detectors located at exit slits in the focal point (Hou and Jones, 2000).

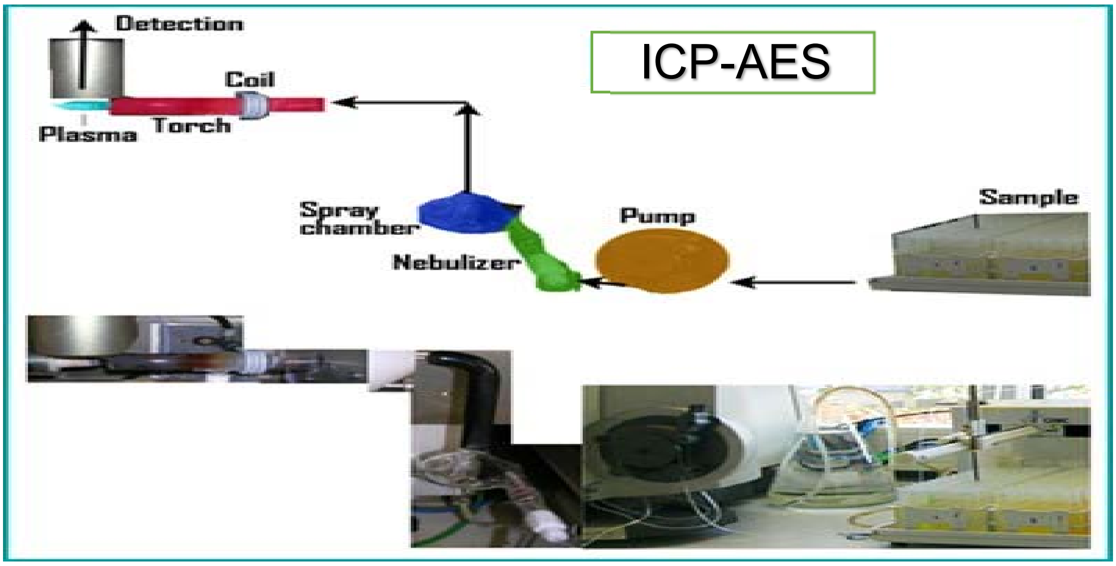

Figure 2.2: Schematic diagram of a typical ICP-AES

2.6.4 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

An ICP-MS is a hyphenated method that combines a high-temperature inductively coupled plasma (ICP) source with a mass spectrometer (MS). Figure 2.3 shows the schematic diagram of the ICP-MS from sample introduction till the processed results. A liquid sample is introduced into the ICP plasma as an aerosol by aspirating the liquid into the nebuliser. The hot region of the ICP torch converts the elements in the aerosol to gaseous atoms where ionisation takes place in the plasma region. The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated by mass spectrometer and detected (Jones, 1992).

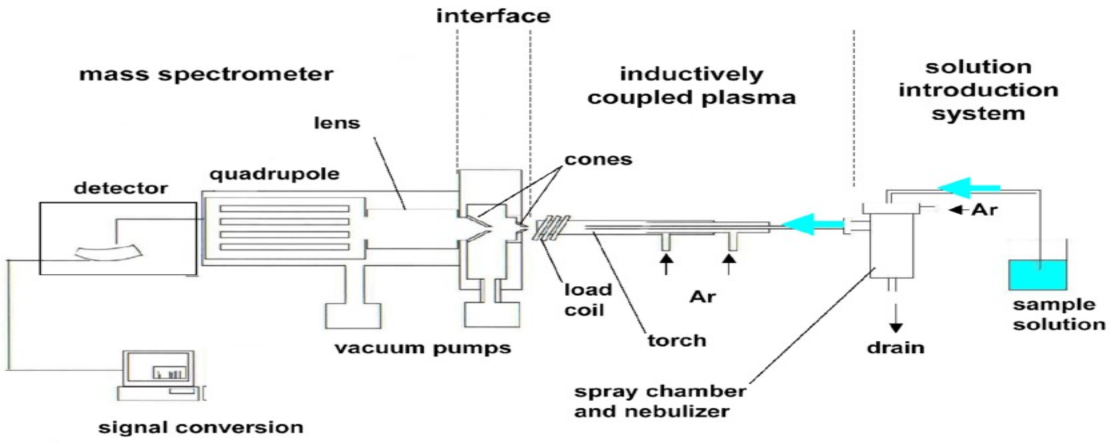

Figure 2.3: Schematic diagram of ICP-MS

The type of sample matrix can influence the detection capabilities, which can in turn affect the degree of ionisation that will occur in the plasma or allow the formation of species that may interfere with the analyte determination (Jarvis *et al*., 1992). The speed, accuracy and multi elements that can be determined in a single analysis make the ICP-MS a very cost-effective technique. The advantage using plasma compared to other ionisation methods, such as flame ionisation, are that complete ionisation is guaranteed and as such prevents oxide formation since it occurs in a chemically inert environment.

Additionally, the benefits associated with the use of ICP-MS include the ability to perform speciation studies when coupled with separation techniques such as HPLC. The technique enables measurement of isotopic composition in nutritional studies and to identify sources of environmental exposure (Hill, 2007; Petridis *et al*., 2014). Consequently, the ICP-MS limitations are based on the amount of total dissolved solids in the samples of interest. High total dissolved solids cause instrumental drifts, decreased sensitivity and detection capability due to the blockages that may occur in the apertures of the cones. To account for this, proper dilutions should be carried out prior sample analysis to minimise the high levels of total dissolved solids (McCurdy and Proper, 2014).

2.7. ANALYTICAL PERFORMANCE CHARACTERISTICS FOR DETERMINATION OF POTENTIALLY TOXIC ELEMENTS

2.7.1 Calibration techniques

Proper selection of calibration techniques is fundamental for the accurate determination of the analyte concentrations. Calibration techniques are just a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard solutions of known concentration.

2.7.1.1 Internal standardisation

An internal standard is defined as a chemical substance added in constant amounts to blanks, calibration standards and samples, in a chemical analysis (Cuadros-Rodrigues *et al*., 2007; De Oliveira *et al*., 2010).

Subsequently, an internal standard is used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte standard concentration to correct analyte losses during sample preparation (Zenkevich and Makarov, 2007). The calculated ratio of the analyte signal and the internal standard signal for the samples is then used to determine the concentration of the analyte from a calibration curve.

Furthermore, an internal standard should have a similar trend to the response of an analyte (Skoog *et al*., 1998). An internal standard with mass number close to that of the analyte in study, improves the precision. The choice of an internal standard should be based on the analyte in study.

The use of internal standard methods is based on condition that one internal standard will not be adequate for a wide range of analytes varying in atomic masses (Vanhaecke *et al*., 1992; Bechlin *et al*., 2014). Such use accounts for the correction of the instrument fluctuations and signal drifts such as signal enhancement and reduction by sample matrix mismatch. For ICP-AES, the choice of an internal standard is based on the existence of similar excitation energies for the internal standard and the analyte of interest and the absence of spectral interferences between an analyte and an internal standard (Chiweshe *et al*., 2016).

Atomic weight, ionisation energy closely to the analyte and the absence of isotopes of internal standard in the sample are some of the selection parameters on the choice of an internal standard in ICP-MS (Sartoros and Salin, 1999).

2.7.1.2 External calibration

In an external calibration method, a known data from a calibration standard and unknown data from the sample are combined to generate a quantitative report. The external calibration method is advantageous since it is simple to perform and is applicable to different analysis. From the responses, a plot of concentration versus detector signal is made for each respective analyte. Linearity of the plot allows easier statistical calculations and extrapolation to estimate the concentration levels of analytes in samples. This linearity is verified by performing a linear regression $R^2=1$ on the data set obtained. The closer the R^2 value to 1, the more accurate the data is (Harris, 2007).

2.7.2 Limit of detection (LOD)

The limit of detection (LOD) is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limited generally to 1%. The LOD of an analytical method tells how low a concentration can be said to be measurable (Proctor, 2008). The LOD is estimated from the mean of the blank, and the standard deviation of the blank (MacDougall and Crummett, 1980).

For methods that employ a calibration curve, the LOD is defined as the analyte concentration yielding a response of concentration factor k higher than the standard deviation of the blank (Sb). The LOD can be calculated as three times the value of the standard deviation of the blank concentration values (Ingle and Crouch, 1988). The LOD formula was reported as:

$$LOD = 3Sb_1 \qquad \text{Equation.....2}$$

Where $3Sb_1$ is three times the standard deviation of the blank concentration.

2.7.3 Limit of quantification (LOQ)

Limit of quantification (LOQ) is the lowest concentration at which the analyte in a sample is detectable at good precision and accuracy.

The LOQ is expressed as the analyte concentration which corresponds to the blank sample plus 10 times the standard deviation of the results for a series of replicates which are used to determine the LOD. It describes the lowest detectable concentration of the analyte measured by the employed analytical sample preparation procedure. The distinguished equation used for the calculation of LOQ was reported by Shrivastava and Gupta (2011):

$$LOQ = 10Sb_1 \qquad \text{Equation.....3}$$

Where $10Sb_1$ is ten times the standard deviation of the blank concentration.

CHAPTER 3

EXPERIMENTAL WORK

3.1 INTRODUCTION

The purity of reagents, reference materials and standards is described in this section followed by an outline of the analytical procedures used for sample preparation and analysis. Furthermore, the analytical instruments and operating conditions employed are described backed with some illustrations.

3.2 DESCRIPTION OF THE STUDY AREAS

3.2.1 The Mokolo River study area

This study focuses on Mokolo River shown in Figure 3.1, located at Lephalale town, previously known as Ellisras, Limpopo province, South Africa. The river is situated about 40km from the Botswana border. The Mokolo River is a tributary of the Limpopo River joining it in the north region (IDP, 2009/10). The river's catchment area comprises of 8387 square kilometres (Maswuma *et al*., 2011).

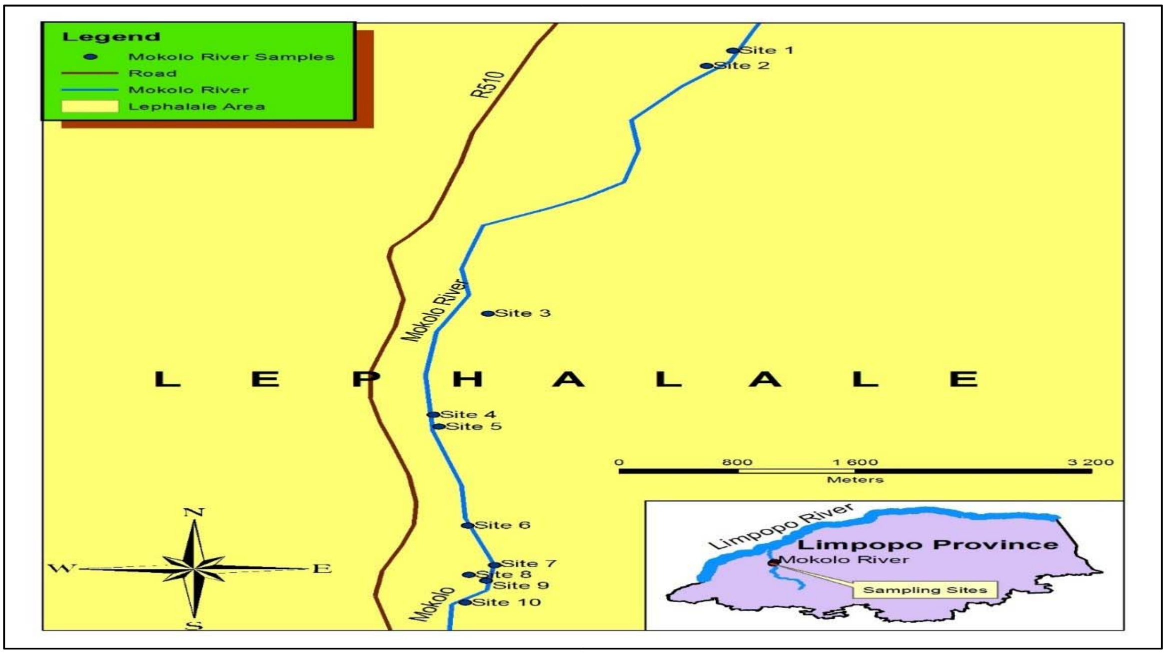

Figure 3.1: Map of Mokolo River showing 10 sampling sites

The name Lephalale is inclusive of the Onverwacht and Maropeng residential areas. The town is situated in a semi-arid climate zone, with an annual average rainfall of less than 400 mm and an annual evaporation of higher than 2000 mm, making water highly vulnerable to droughts; however, the Mokolo River constantly has water (IDP, 2009/10).

The most important tributaries of the Mokolo River are Sand River, Klein Sand River, Sandspruit, Sondagsloop, Loubadspruit, Grootspruit, Sterkstroom, Brakspruit, Malmanies, Bulspruit, Rietspruit, Sandloop, Poer se Loop and the Tambotie R4ivers. The Mokolo Dam is the only large dam in the Lephalale area (River Health Programme, 2006).

The Mokolo River flows through irrigated farmlands but for the most part it passes through privately owned game reserves. The Mokolo Dam confiscates the river in the middle of the gully which lies within the provincial Mokolo Dam Nature Reserve. The dam supplies water to downstream irrigation farms, the town of Lephalale, Matimba power station and Kumba Colliery (Maswuma *et al*., 2011). Sand mining is one of the regular activities due to the fine sand at the banks of the river and as such riverine vegetation is being removed with less monitoring (River Health Programme, 2006).

Lephalale is a major role-player in economic development within Limpopo. Local coal deposits are leading to industrial and mining development and this continual economic development requires development of sustainable water resources and therefore the need to investigate every possible water resource option (IDP, 2012/13). Lephalale local municipality falls within a water-scarce region in which the demand for water is increasingly high and cannot be met by the available water resources in the area (Schachtschneider *et al*., 2010; Oosthuizen, 2013). The majority of people in Lephalale live in scattered rural settlements, which puts a strain on the supply of potable water (IDP, 2009/10). Figure 3.2 shows the images taken during sample collection whereby (a) oily sludge was visible on the river banks and also (b) generator pumps used for irrigation purposes were evident in the vicinity of the river.

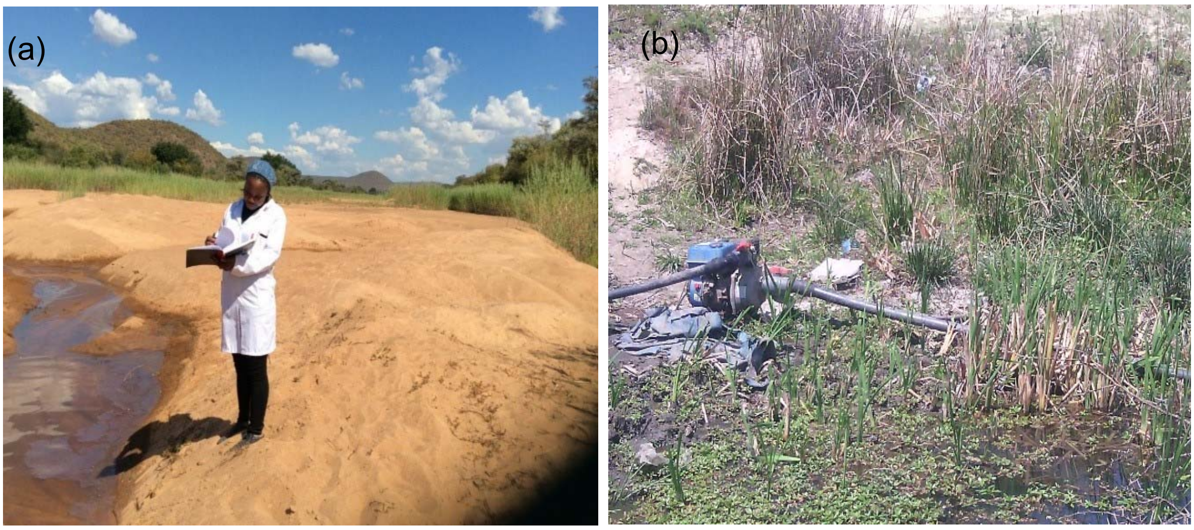

Figure 3.2: Images illustrating the conditions in and around Mokolo River

Irrigation in S1, S4 and S6, and commercial farming in S1, S5 and S6 were the common activities carried around the Mokolo River. Sand mining and supply to local contractors is carried out by trucks as observed on sites 9 and 10. Fishing and swimming carried out by local residents could be seen in site 8. Summary description of the sampling sites at Mokolo River is given in table 3.1.

Table 3.1: Description of Mokolo River sampling sites

Sampling site	Code	Site description
Site 1	S1	Irrigation pumps and sand mining
Site 2	S2	Under a bridge close a golf course and a farm
Site 3	S3	Distant from the bridge
Site 4	S4	Irrigation pumping system and cattle grazing
Site 5	S5	Agricultural activity close to an industrial complex
Site 6	S6	Direct irrigation pump and sand mining
Site 7	S7	Under a bridge
Site 8	S8	Fishing and swimming activities
Site 9	S9	Sand mining
Site 10	S10	Sand mining

3.2.2 The Blood River study area

The Blood River originates in the west of the Polokwane municipal area (IDP, 2012-2013/15), and is located at Seshego area about 14.5 km away from Polokwane. According to the Polokwane municipality report (IDP, 2012-2013/15), municipal and domestic wastes contribute to en7vironmental pollution. This occurs due to lack of waste removal services, illegal dumping sites close to the river banks and lack of storm water drainage among others (IDP, 2012/13). Figure 3.3 shows the map of the study area and the selected sampling sites.

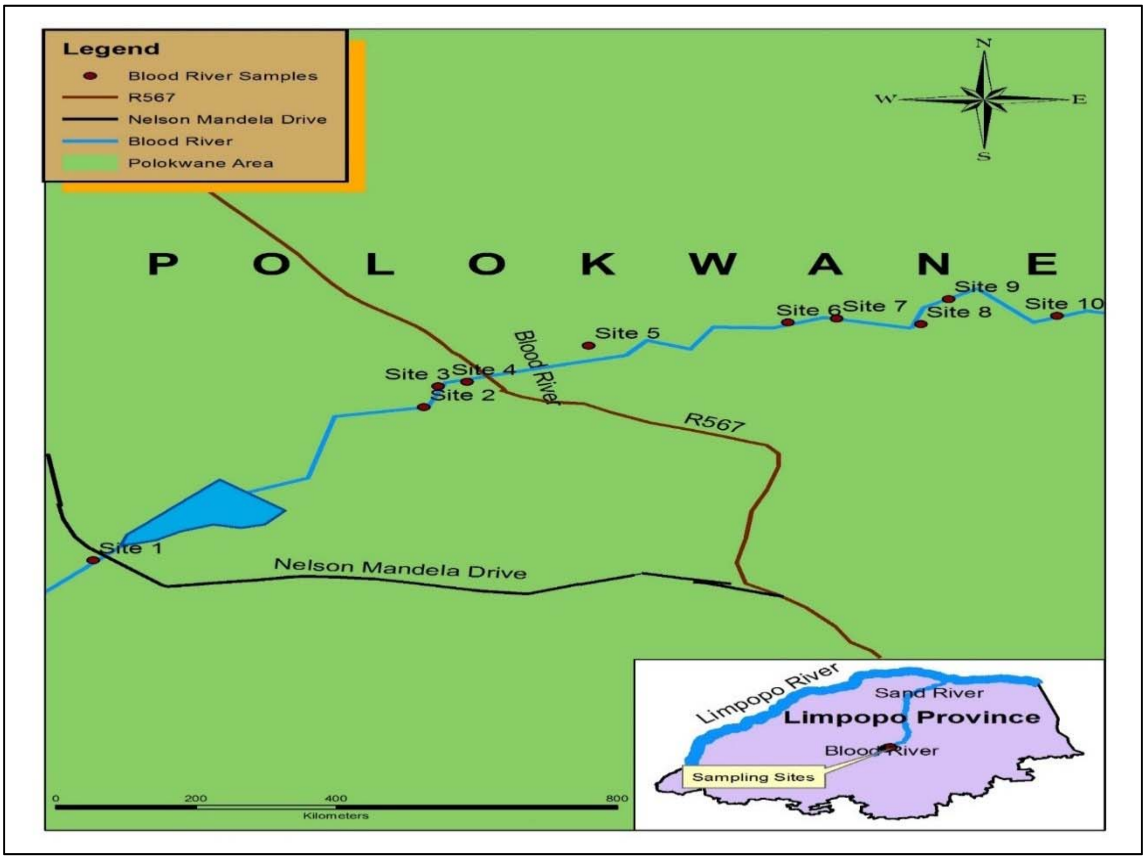

Figure 3.3: Map of Blood River showing 10 sampling sites

Seshego area has been reported to have approximately 340 water leaks per month, 180 sewage leaks flowing directly in to the river and is surrounded by illegal dumping sites due to increase in the number of informal settlements. (IDP, 2012/13). Dumped materials such as broken construction materials and computer parts were visibly scattered around the Blood River bed while dead remains of animals, were found lodged under bridges and against dam walls after being swept downstream (IDP, 2012/13).

The Blood River flows alongside the Seshego Waste Water Treatment Plant and an abandoned fishery. Raw untreated sewage flowing from damaged pipes to the river is a common sight. Residents surrounding the Blood River used to catch barbell, carp and tilapia fish in the river and currently there is no fish, birdlife, insects or other signs of life from the middle to downstream of the river except for small fish and tadpoles visible in the upstream of the river closer to the Seshego Dam.

The conditions in the Blood River are shown in figure 3.4 (a-d). Most rivers in South Africa are heavily polluted with faecal matter and when these rivers are used for irrigation of food products they will pose a potential threat to human health (Seanego and Moyo, 2013). Just like the condition in the Blood River, farmers use the water as their direct source of irrigation system for crops. This is expected to pose a threat to humans and animals *via* food chain.

Illegally dumped domestic wastes could be a possible source of contaminants in the river and are found next to sampling points 4 and 5. Conditions of sampling sites 6 and 7 in Blood River are presented in Figures 3.4 (a – d).

Figure 3.4: Images illustrating the condition and possible sources of pollution in Blood River from picture (a-d), respectively.

Description of the sampling sites is given in table 3.2. Untreated sewage leaking was found flowing opposite sampling sites 7 and 8 that flow directly into the river and this might be a possible source of contamination. The sampling sites 6 and 7 could also be acting as a drain, receiving waste water from residents residing in the squatter camps close of the proximity of the river. Farming and recreational activities such as swimming and fishing were carried out in the lower basin of the river, sites 1, 2 and 3 adjacent the Seshego Dam.

Table 3.2: Description of Blood River sampling sites

Sampling site	**Code**	**Site description**
Site 1	S1	Next to a road adjacent to a crop farm, a close distance from Nelson Mandela drive
Site 2	S2	Adjacent to Seshego Dam
Site 3	S3	Adjacent to Seshego Dam
Site 4	S4	Adjacent to an illegal dumping site and close to an open cattle grazing land, close to a squatter camp, afar from R567 road.
Site 5	S5	Adjacent to an abandoned fishery and close to a squatter camp close to R567 road
Site 6	S6	Adjacent to a wastewater treatment works and close to an open cattle grazing land
Site 7	S7	Leaking untreated sewage, illegal industrial dumping site
Site 8	S8	Adjacent to the leaking sewage, illegal industrial dumping site
Site 9	S9	Direct sand mining
Site 10	S10	Adjacent to a Sand mine

3.3 SAMPLE COLLECTION

The chemical and physical analysis of sediments and water can be used as a tool for monitoring pollutant discharges in river system. Sediments were sampled to help locate non-point, historical, or irregular discharges that may not be readily evident using samples collected from the water column.

Water and sediments samples were also used to identify the locations of these sources by upstream incremental collection of samples from possibly contaminated sites flowing downstream (USEPA, 2001; Ohio EPA, 2015).

3.3.1 Collection and preservation of water samples

Water samples were collected from Blood River and Mokolo River, Limpopo Province, South Africa, with 250 mL low density polyethylene bottles. The water samples were collected during high flow season (September 2015) and low flow season (March 2016) from 10 different sampling sites of each river.

The polyethylene bottles were soaked in 10% HNO_3 for 24 hrs prior to sampling day and then rinsed with ultrapure water and finally air dried at room temperature. Water sampling was performed following the method published by Miller-Ihli and Baker (2001). The water samples were collected from a depth of ≈25 cm below water level. The collected water samples were carried to the lab in a cooler box and upon arrival were immediately filtered with 0.25 µm syringe filters and acidified with 1% (v/v) of the 65% ultrapure HNO_3 for preservation and to stabilise the analytes of interest. The samples were stored in a refrigerator at 4 ^{o}C until removed on the day of analysis.

3.3.2 Sediments samples collection and preservation

Sediments samples were collected during the high flow season (September 2015) and low flow season (March 2016) from 10 different sampling sites of each river. They were sampled at a depth of ≈ 20 cm by a polyethylene sampling spatula. Sediments consisting of silt-sized and clay-sized particles trap potentially toxic elements to protect water quality, but it also presents the potential for further water contamination. Special focus was given to these types of sediments during sample collection. The samples were labelled and stored in 500 mL polyethylene bottles and transported to the laboratory in a cooler box. Large particles such as stones, leaves and pieces of contaminants were removed from the collected samples. The sediments from each sampling site were air dried at room temperature. An agate mortar and pestle were used to grind the dried sediments samples. They were sieved through a 100 µm sieve mesh. The powdered sediments samples were preserved in polyethylene bottles prior to extraction and digestion for analysis.

3.4 REAGENTS, STANDARD REFERENCE MATERIALS AND STANDARDS

High purity reagents were employed in all experiments. Ultrapure water with a resistivity of 18.2 MΩ.cm, obtained from Milli-Q water system (Ultrapure water system, He3al force) produced by Shanghai Carrex Analytic Instrument Co., Ltd, (Shanghai, China) was used for cleaning of glassware and making dilutions.

High purity 48% HF, 65% HNO_3, 35% H_2O_2 and 37% HCl reagents purchased from (Merck, Darmstadt, Germany) were used for mineralisation of sediments samples.

Standards of 1000 mg/L (Sigma-Aldrich® Chemie GmbH, Buchs, Switzerland) were used for preparation of calibration standard solutions. Reference materials SRM 8704 Buffalo River sediments and CRM 280R Lake sediments (NIST, Gaithersburg, USA) were used to evaluate the accuracy of the method employed for quantification of pseudo-total concentrations of potentially toxic elements in sediments samples.

Glacial acetic acid (ACS reagent ≥ 99.7%) purchased from (Sigma-Aldrich® Chemie GmbH, Steinheim, Germany) was used in the first fraction. Hydroxylamine hydrochloride (99.999% trace metal basis) from (Sigma-Aldrich® Chemie GmbH, Steinheim, Germany) was used in the second fractionation. In the third stage, a combination of hydrogen peroxide (Pedrogen™ 30% w/w) and ammonium acetate (Fluka TraceSELECT® ≥ 99.9995 metals basis) from (Sigma-Aldrich® Chemie GmbH, Steinheim, Germany) was used. A certified reference material BCR-701 obtained from NIST (Gaithersburg, USA) was used for the validation of the sequential extraction procedure employed. Nitric acid (ISO, for determinations with dithizone ≥ 65%) purchased from Sigma-Aldrich® Chemie GmbH (Steinheim, Germany) was used for preservation of the water samples after collection, prior to analysis. A standard reference material (SRM 1643f) purchased from NIST (Gaithersburg, USA) was used to evaluate the accuracy of the levels of trace elements in water samples.

3.5 EXPERIMENTAL PROCEDURE FOR PREPARATION OF REAGENTS AND STANDARD SOLUTIONS

Saturated boric acid solution was prepared by adding 7 g of powdered boric acid (ACE reagent, Johannesburg, RSA) to 100 mL of ultrapure water in a clean Teflon bottle.

Afterwards, the boric acid in the sealed bottle was allowed to dissolve with heat on a hot plate in a fume hood. Once dissolved, crystals formed and the acid was filtered and ready for use.

Reagents used during the sequential extraction technique were prepared following the BCR protocol (Rauret *et al*., 1999) as briefly described below:

Solution A (Acetic acid 0.11 mol/L), was prepared by adding 25 mL of glacial acetic acid (≥99.7%) (Sigma-Aldrich® Chemie GmbH, Steinheim, Germany) to a 500 mL vial and made up to 1000 mL with ultrapure water. A 250 mL of this solution (acetic acid 0.44 mol/L) was diluted to 1000 mL to obtain a 0.11 mol/L acetic acid solution.

An exact mass of 34.75 g of hydroxylamine hydrochloride (ACS reagent ≥ 99.999% trace metal basis) purchased from Sigma-Aldrich® Chemie GmbH (Steinheim, Germany) was dissolved in ultrapure water. This solution was acidified to pH≈1.5 with 2 mol/L nitric acid solution (prepared by adequate dilution of concentrated nitric acid) and made up to 1000 mL with ultrapure water. This solution was prepared on the same day of extraction to obtain solution B (hydroxylamine hydrochloride, 0.5 mol/L). Solution C (hydrogen peroxide, 8.8 mol/L, Perdrogen ™ H_2O_2 (w/w) ≥ 30%) was used as supplied by the manufacturer with no dilution.

Solution D (ammonium acetate, 1.0 mol/L) was prepared by dissolving 77.08g of ammonium acetate (Fluka ammonium acetate TraceSELECT ≥99.9995% metals basis) in water and this solution was acidified with concentrated nitric acid to pH≈1.5 and made up to 1000 mL with de-ionized water.

The calibration solutions were prepared prior to analysis from the stock solutions using ultrapure water. The calibration curve was constructed from five readings of the serial standards. Standard solutions were prepared daily, by appropriate dilutions from a stock standard solution of 1000 mg/L to 100 mg/L intermediate solution for each analyte.

3.6 APPARATUS AND INSTRUMENTATION

A portable multi-parameter meter 900P (Mettler Toledo, Mississauga, Canada) was used to measure pH readings of the water samples during sample collection.

A mechanical shaker (Lenton furnaces & Scientific Co, Johannesburg, South Africa) was used to agitate the sediments samples in the centrifuge tubes for extraction of potentially toxic elements. A centrifuge (Lenton furnaces & Scientific Co, Johannesburg, South Africa) was used for centrifugation of the sediments samples in which the centripetal forces were applied to separate the heterogeneous mixture of the sediments samples with the reagents added for extraction. An ultrasonic bath from (Lenton furnaces & Scientific Co, Johannesburg, South Africa) was used for agitating the sediments samples and the supernatant liquid before transferring the liquid to a vial for storage and analysis purposes.

Microwave-assisted extraction and digestion system (CEM Corporation MARS 5, North Carolina, USA) was used for extraction and digestion of the potentially toxic elements in the sediments samples. The determination of potentially toxic elements in both water and sediments samples was done by ICP-MS purchased from PerkinElmer, Elan 6100 (USA) for ultra-trace elements, ICP-AES from ICPE-9000 Shimadzu (Columbia, USA) and F-AAS purchased from PerkinElmer, Syngistix (Singapore) for selected elements.

3.7 SAMPLE PREPARATION

3.7.1 Microwave-assisted acid digestion (Method A)

The microwave-assisted acid digestion method was developed and performed following the protocol by Castillo *et al*. (2011). The operating conditions such as reaction time and selection of reagents were based on the outcome of complete sample digestion. Figure 3.5 depicts the microwave assisted acid digestion system used for digestion of the sediments samples.

Sediments samples were air dried, ground, homogenised, sieved and mineralised using a microwave-assisted acid digestion system for total elemental determination by ICP-MS. A mixture of reagents (HNO_3, H_2O_2 and HCl) yielded complete digestion for sediments samples from Blood River, whereas a mixture of HCl, HF, HNO_3 and H_3BO_3 was employed for the mineralisation of Mokolo River sediments samples.

The microwave-assisted acid digestion procedure was validated using standard reference materials of sediments (SRM 8704 and BCR 280R) for pseudo-total elemental analysis Preparation of the samples for analysis was done in triplicates for each site.

Figure 3.5: Photo depicting the microwave-assisted acid digestion system

The test portion of up to 0.25g of sediments sample from Mokolo River, was digested in a stepwise acid combination of 4 mL concentrated HCl, 2 mL of HNO_3, 2 mL of HF and 2 mL H_3BO_3 by means of microwave heating in a Teflon vessel. Sediments from Blood River were digested with 2 mL of HNO_3, 2 mL of H_2O_2 and 2 mL HCl. The vessel was sealed with a torque wrench and subjected to heating in the microwave unit. After cooling, the vessel contents were allowed to settle and then diluted to volume and analysed by the appropriate quantitative methods of FAAS and ICP-MS. From the percentage recoveries obtained on the test analysis, further sample preparation was done as the method proved to be reliable.

The parameters set and used for digestion of sediments with the use of microwave digestion system are summarised in the table 3.3. Blood River sediments were digested following the Stage 1 instrumental parameters and Mokolo River sediments were mineralised following the two stages. This was on the basis of the different matrices of the sediments.

Table 3.3: Instrumental conditions for microwave-assisted acid digestion

Parameters	**Stage 1 conditions**	**Stage 2 conditions**
Temperature (°C)	150	200
Ramp time (min)	20	20
Hold time (min)	20	15
Cooling time (min)	10	10
Pressure (psi)	800	800
Power (W)	1600	1600
Volume of reagent (mL)	4:2:2	2
Reagents	HCl, HNO_3 and HF	H_3BO_3

3.7.2 BCR sequential extraction procedure (Method B)

Test analysis for the sequential extraction procedure was performed on a sample as to check reliability of the method to be employed. A change in the pH of solutions from 2 to 1.5 was implemented after low percent recoveries were reported in the second and third stages.

In the first step (exchangeable fraction), acetic acid was used to extract all the exchangeable, acid and water-soluble metals. Second step was the reducible fraction in which hydroxyl ammonium chloride was used to extract all the reducible metals and hydrogen peroxide was used to extract all oxidizable metals.

Lastly, acid combination of HCL, HNO_3, and HF was employed to extract all remaining non-silica bound metals (Rauret *et al*., 1999).

During stage 1 of the BCR protocol, 1g of each powdered and sieved sediments sample was weighed into a pre-cleaned 50 mL centrifuge tube. An exact amount of 40 mL of solution A was transferred into the centrifuge tube then shaken for 16 hrs on the mechanical shaker.

The extraction solution was separated from the solid residue by centrifugation at 3000 rpm for 15 min and decantation of the supernatant liquid was done. The residue was washed by adding 4 mL of solution A and shaking for 15 min at 3000 rpm in the centrifuge. Further washing was performed, and the latter was then removed with a pipette and combined with the extracted fraction.

The mixture was finally transferred into a 50 mL centrifuge tube topped to the mark with ultrapure water and stored at 4 ºC in a refrigerator. The residue was used to proceed with stage 2 of the procedure.

In stage 2, the extraction was performed as described in stage 1 in which the residue from stage 1 was mixed with 40 mL of solution B and shaken for 16 h at room temperature. Washing was carried out similarly to stage 1 and the extractant was stored while the residue was continually used in stage 3 of the fractionation procedure.

In stage 3, the residue stored from stage 2 was mixed with 10 mL of solution C and the centrifuge tube was covered and left to react at room temperature for 1 h. The extraction was continued for a further 1h at 80 ºC in an ultra-sonic bath and the volume was reduced by further heating of the uncovered vial. Another 10 mL aliquot of solution C was added, and the vial was covered and heated again to 80 ºC for 1 h. The cover was removed, and the volume reduced to a further ≈2-3 mL. About 40 mL of solution D was added to the cool residue and the extraction performed as in stage 1.

An additional fourth stage was added where the residue was mineralised following the procedure carried out in method A. The solution was diluted to 50 mL with ultrapure water and subjected to analysis with ICP-MS.

Table 3.4 summarises the BCR sequential extraction method employed to extract potentially toxic elements in sediments samples following the procedure applied by Castillo *et al*. (2011).

Table 3.4: Instrumental conditions for the BCR sequential extraction procedure

Fractionation	**Fraction**	**Reagent**	**Volume (mL)**	**Time**
F1	Exchangeable	Acetic acid	40	16 h
F2	Reducible	Hydroxyl ammonium chloride	40	16 h
F3	Oxidizable	Hydrogen peroxide & Ammonium acetate	10:40	1 h/16 h
F4	Residual	HCl, HNO3, HF & H_3BO_3	4:2:2:2	Ramp: 15 min Hold: 15 min

3.7.3 Microwave-assisted sequential extraction procedure (Method C)

Microwave-assisted sequential extraction procedure was employed on the samples collected from the Blood River during the low flow season, modifying the method reported by Castillo *et al*. (2011) as summarised in table 3.5. A method that enables safe and fast extraction of potentially toxic elements in sediments using smaller volume of reagents was computed (Ciceri *et al*., 2008).

About 0.25 g of the powdered, sieved and grain sized fraction of the sediments sample was weighed directly into each pre-cleaned microwave Teflon vessel. The reagents from the BCR sequential extraction protocol were added as described with respect to the different fractions. The vessels were placed inside a rotor of the microwave system, sealed, tightened using a torque wrench and finally submitted to the microwave extraction program. After cooling, the supernatant liquid was separated from the solid phase by centrifugation at 3000 rpm for 30 min.

Acetic acid was used for the first leach, hydroxylamine hydrochloride was used to extract elements in the second stage and a combination of hydrogen peroxide and ammonium acetate was used in the third extraction stage. The residue from the third extraction stage was subjected to digestion with H_2O_2, HCl and HNO_3.

After complete digestion, the solutions were then transferred into 25 mL flasks and filled to the calibrated mark with ultrapure water. The different fractions were analysed by ICP-AES. Summary of the instrumental conditions and parameters set on the microwave system for extraction protocol is given in table 3.5.

Table 3.5: Instrumental conditions for microwave-assisted sequential extraction procedure

Parameters	**Stage 1**	**Stage 2**	**Stage 3**	**Residual**
Reagents	Acetic acid (0.11 mol/L)	Hydroxylamine hydrochloride (0.5 mol/L)	H_2O_2 (32%) and ammonium acetate (1.0 mol/L)	HCl, HNO_3, HF & H_3BO_3
Volume of reagent (mL)	4	4	1:4	4:2:2:2
Temperature (ºC)	200	150	200	200
Ramp time (min)	20	15 min	20 min	15 min
Hold time (min)	20 min	15 min	20 min	15 min
Cooling time (min)	10 min	10 min	10 min	10 min
Pressure (psi)	800	800 psi	800 psi	800 psi
Power (W)	1600	1600 W	1600 W	1600 W

3.8 SAMPLE ANALYSIS

Analysis of water and sediments samples was done using ICP-MS at Department of Chemistry, University of Johannesburg. A F-AAS from UL Department of Chemistry and ICP-AES from Limpopo Agro-Food Technology Station were also employed for sample analysis.

Concentrations of potentially toxic elements in water and sediments samples were determined using an external calibration curve. Blanks, standard solutions and all sample solutions were aspirated to the ICP-MS, ICP-AES and F-AAS nebulisers. Each solution of standard and sample was followed by purging with ultrapure water for at least 30 seconds for ICP-MS and ICP-AES, and 10 seconds for F-AAS analysis, respectively, in order to avoid cross contamination of solutions.

The levels of elements were obtained from the average of the measured values as replicates by the ICP-MS, ICP-AES and F-AAS. The blank solutions were also analysed and the intensity or absorbance of each analyte in the blank sample was subtracted from that of the sample.

Tables 3.6, 3.7 and 3.8, tabulate the parameters and instrumental settings applied during the ICP-MS, ICP-AES and F-AAS analysis, respectively.

Table 3.6: Instrumental conditions of ICP-MS

Parameters	**Instrumental settings**
Model	PerkinElmer Elan 6100
Lens voltage (V)	10
RF power (W)	1150
Main water temperature (ºC)	18
Interface water temperature (ºC)	32.6
Analog stage voltage (V)	-2250
Pulse stage voltage (V)	1050
Torch box temperature (ºC)	31.6
Auxiliary gas flow (L/min)	1.2
Nebuliser gas flow (L/min)	0.95
Peristaltic pump speed (rpm)	24
Nebuliser	Concentric
Spray chamber	Cyclonic

Table 3.7: Instrumental conditions of ICP-AES

Parameters	Operating conditions
Instrument	ICPE-9000 Shimadzu
Ignition mode	Normal (water)
RF generator power (Kw)	1.20
Plasma gas flow rate (L/min)	10
Nebulizer gas flow rate (L/min)	0.7
Auxiliary gas flow rate (L/min)	0.60
Nebulizer type	Cross flow
Plasma torch	Mini torch
View direction	Axial
View position	Low
Gas type	Argon

Table 3.8: Instrumental conditions of F-AAS

Parameters	Instrumental settings
model	PerkinElmer PinAAcle 500
Software programme	Syngistix Touch
Gas Flow rate (L/min): Air : Acetylene	3.30 10.0
Nebulizer type	Stainless steel
Spray chamber type	Teflon

3.9 DETERMINATION OF LIMITS OF DETECTION AND LIMITS OF QUANTIFICATION

To determine the LOD values, reagent blanks were prepared as described in the experimental procedure employed for quantification of elements in the sediments and water samples. The LOD value for each analyte was calculated based on three times the standard deviation of the average of six prepared blank concentrations. The LOQ for each analyte was calculated based on ten times the standard deviation of the average of six prepared blank concentrations.

3.10 ANALYTICAL DATA QUALITY ASSURANCE/QUALITY CONTROL

Standard reference materials (SRM 8704 Buffalo River sediments and BCR 280R Lake sediments) were used for the assessment and validation of the accuracy of the analysed pseudo-total concentrations of elements in the river sediments. The reference material of BCR-701 was used for validation of the method employed during determination of elements in sequentially extracted sediments. The quality control of trace elements in the water samples was evaluated by the use of the water standard reference material (NIST SRM 1643f).

3.11 STATISTICAL ANALYSIS

The data was analysed using one-way analysis of variance (ANOVA) to examine statistical significance of differences in the mean concentration of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Se, V, and Zn, determined in sediments and water samples. Statistical analysis for variability of sediments and water was done using a probability level of P=0.05, considered as statistically significant. The data was discussed based on the seasonal variability of the elements in the sediments and water. Microsoft Excel was used for calculations of average, standard deviation and drawing of graphs.

CHAPTER 4

LEVELS AND TRENDS OF POTENTIALLY TOXIC ELEMENTS IN WATER AND SEDIMENTS SAMPLES FROM MOKOLO AND BLOOD RIVERS: RESULTS AND DISCUSSION

4.1 INTRODUCTION

In this chapter, analytical figures of merit along with the results obtained following the methods employed for analysis of water samples and digestion of sediments from Mokolo and Blood Rivers are given in tables and the trends in figures. Seasonal changes affect the rate of weathering of rocks both physically and chemically. This is due to fluctuations in temperature, rainfall and wind pressure. Furthermore, high wind pressure, river flow rate and water levels influence transport of metals from the point source to other localised areas within the river (Sultan *et al*., 2011). As such, discussion was done on the effects of seasonal variability based on concentrations of elements.

Detailed discussions of the results are given with the concentrations of potentially toxic elements in water compared with MPLs of elements in drinking water set by different organisations. Furthermore, the maximum concentrations of elements in sediments were compared with the sediments quality guidelines of different organisations. Analytical data quality and reliability were discussed.

4.2 SELECTION OF ISOTOPES OF POTENTIALLY TOXIC ELEMENTS

The possible interferences caused by sediments and water matrix on the signal of each considered mass, were investigated during ICP-MS analysis. When an element had two or more isotope masses, at least two isotopic masses per element were monitored. The isotope that showed less interference from the matrix was selected taking into consideration the abundance of the isotope of each element. For ICP-MS analysis, the following isotopes were measured based on their high abundance and absence of isobaric interferences, where possible: ^{51}V, ^{53}Cr, ^{55}Mn, ^{57}Fe, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, Se^{82}, ^{114}Cd and ^{208}Pb (Mekonnen *et al*., 2012).

4.3 CONSTRUCTION OF CALIBRATION CURVES

An external calibration curve was obtained for each element for determination of the element concentrations in the water and sediments samples. Figure 4.1 shows an example of a calibration curve obtained during detection of Pb in water using ICP-MS. Similar calibration curves were obtained for As, Cd, Cu, Cr, Fe, Mn, Ni, Se, V and Zn, and the calibration parameters are summarised in tables 4.1.

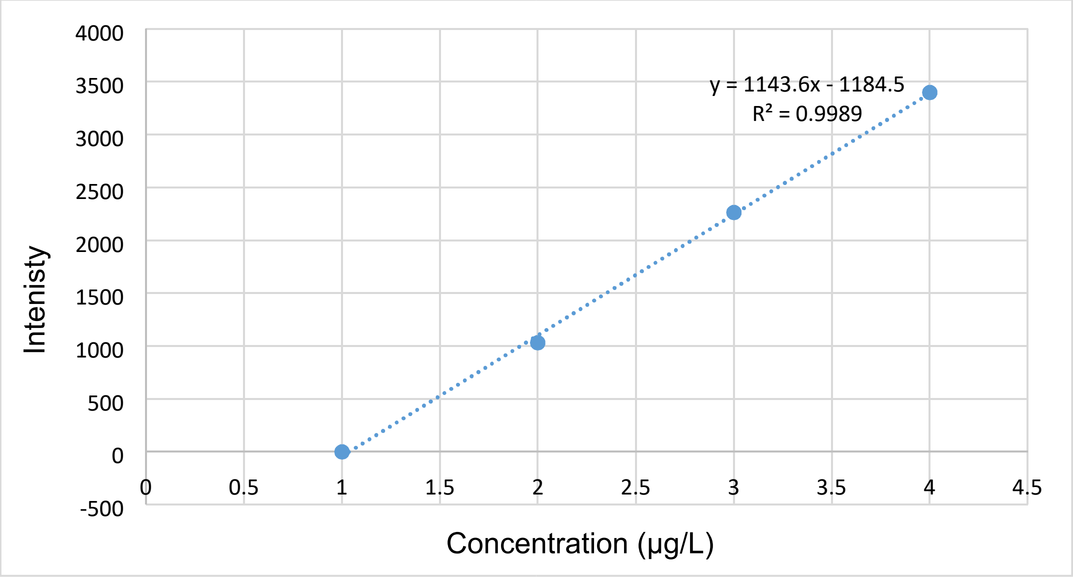

Figure 4.1: Calibration curve obtained for determination of Cu by ICP-MS in water samples

The regression coefficients for the calibration curves ranged from 0.9842 (Se) to 0.9998 (Zn) for the elements determined. The linearity of the calibration curves was very good for majority of the elements (>99%) except for Se, Cr and Fe although acceptable (>98%), as can be seen in figure 4.1.

Table 4.1: Calibration parameters obtained for determination of the selected elements in water and sediments samples by ICP-MS

Element	**Linear Regression R^2 value**	**Linear equation y = mx + c**
Mn	0.9997	y = 10482x – 7667
As	0.9953	y = 731.5x – 489.33
Se	0.9842	y = 73x – 45.667
Pb	0.9994	y = 1184.5x + 136.33
Cd	0.9963	y = 1940.5x – 1383.7
Zn	0.9998	y = 424x – 570.33
Cu	0.9989	y = 1143.6x – 1184.5
Cr	0.9882	y = 134.5x – 215
V	0.9996	y = 1012x – 617
Ni	0.9984	y = 80.5x – 25.333
Fe	0.9876	y = 739.5x + 332.33

4.4 LIMIT OF DETECTION (LOD)

Reagent blanks were prepared following the same procedure for digestion of sediments samples. A blank solution of 1% (v/v) ultrapure HNO_3 solution was prepared for the water analysis method. The intensity of six blanks was measured and divided by the slope of the calibration curve to give blank concentrations. Standard deviations were calculated from the concentrations of these blanks. The LOD for each element under study was calculated based on three times the standard deviation of the average of six individually prepared blank solutions and are given in table 4.2. The LOQs were calculated as 10 times the SD of the blanks.

Determination of Fe in sediments samples was done using F-AAS based on the high concentrations of the element during the test analysis. For determination of potentially toxic elements in water samples by ICPMS, the LODs ranged from 0.009 to 2.48 µg/L and the LOQs ranged from 0.030 to 8.27 µg/L. The LODs and LOQs found in the water and sediments methods are given in Table 4.2.

Table 4.2: Limits of detection obtained for the analysis of potentially toxic elements in digested sediments and water samples by ICP-MS

	Sediments analysis		**Water analysis**	
Element	LOD ng/g	LOQ ng/g	LOD µg/L	LOQ µg/L
Mn	0.28	0.94	0.049	0.16
Zn	1.77	5.90	0.99	3.30
Ni	0.62	2.08	0.05	0.17
Cu	0.48	1.60	0.010	0.033
Cr	0.11	0.37	0.011	0.037
V	0.53	1.77	0.19	0.63
As	0.010	0.033	0.14	0.47
Se	0.12	0.40	2.48	8.27
Cd	0.030	0.10	0.030	0.10
Pb	0.17	0.57	0.009	0.030
Fe	0.049*	0.16*	0.017	0.057

*LOD and LOQ values obtained by F-AAS as mg/kg

The study conducted by Mekonnen *et al*. (2012) reported LOD values of 0.015, 0.416, 0.017, 0.045, 0.035, 0.186, 0.005, 0.937, 0.012 and 0.136 for V, Cr, Mn, Fe, Ni, Cu, Zn, Se, Cd, and Pb in river water employing ICP-MS. The LODs for all the other elements were measured in µg/L except for Fe and Zn, which were detectable in mg/L. The LODs for selected elements obtained, in this study, were slightly comparable, whereby Cr, Fe, Cu and Pb, were found to be lower than those reported by Mekonnen *et al*. (2012). Although the same method was employed, whereby the water samples were acidified with 1% HNO_3 prior to analysis, Mekonnen *et al*. (2012) reported higher detection limits as compared to this study, which could probably be associated with a difference in reagent purity.

In sediments samples of this study, the LODs ranged from 0.010 to 1.77 ng/g. The LOQs ranged from 0.033 to 5.90 ng/g by ICP-MS. Iron in sediments samples was analysed with F-AAS and its LOD and LOQ values were 0.049 mg/kg and 0.16 mg/kg, respectively.

The LOD values in this study were found to be 0.28, 1.77, 0.62, 0.48, 0.11, 0.53, 0.010, 0.12, 0.030, 0.17 ng/g and 0.049 mg/kg for Mn, Zn, Ni, Cu, Cr, V, As, Se, Cd, Pb and Fe, respectively.

Following the same procedure, with slight variation in the volume of the reagents, Melaku *et al*. (2005) reported LODs values of 0.40, 0.83, 0.34, 0.59, 0.08, 0.67, 0.64, 0.16, 0.95, 0.03 and 0.15 for V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Cd and Pb, respectively. These LODs were comparable with the LODs in this study for Mn, Cu, As, Cd and Pb.

4.5 ACCURACY AND PRECISION

4.5.1 Analysis of water certified reference material

Validation of the method used for quantification of trace elements in water samples was done by analysing the standard reference material of water (SRM 1643f) by ICPMS with the aid of an external calibration method. The results were generally precise (%RSD = 0.0085% to 7.7%) and in good agreement (>97%) with the certified values as shown in Table 4.3, indicating validity of our methods for analysis of the water samples except for over recovery observed for Cr (148%) and Pb (123%).

Based on the respective reproducibility values of 1.5% and 0.66% for both elements, the percent recoveries can be accounted for by the element distribution in the samples based on the matrix effect.

Table 4.3: Comparison of the measured concentrations of elements in SRM 1634f obtained by ICP-MS with the certified concentrations ((µg/L)

	Measured value	**Certified value**	**Percent Recovery**
Element	Mean ± SD	Mean ± SD	(%)
Mn	37.6±0.75	37.14±0.60	101
Zn	82.4±7.7	74.4±1.7	111
Ni	62.1±0.16	59.8±1.4	104
Cu	24.8±0.011	21.66±0.71	114
Cr	27.4±0.42	18.50±0.10	148
Pb	22.8±0.15	18.49±0.0084	123
V	38.4±1.2	36.07±0.28	106
Cd	6.44±0.010	5.89±0.13	109
As	57.8±1.2	57.42±0.13	101
Se	13.1±0.0085	11.70±0.081	112
Fe	90.2±1.8	93.4±0.78	97

*n=3 (triplicates)

4.5.2 Analysis of sediments certified reference materials

Two certified reference materials of sediments (SRM 8704 and BCR 280R) were used for the assessment and validation of the accuracy and precision of the method employed for determination of pseudo-total concentrations in the river sediments. The use of two different certified reference materials for the same method of digestion was based on the non-availability of the certified values for some elements of interest in the certified reference material. In this study, the certified concentrations available on the SRM 8704 certificate were for As, Cd, Cr, Fe, Mn, Ni, Pb, V and Zn, while the available certified values from the BCR 280R certificate are for As, Cd, Cu, Cr, Ni, Se and Zn.

The measured concentrations obtained for the certified reference materials by ICP-MS and F-AAS are shown in tables 4.4 in comparison with the certified values. The results were generally in good agreement with the certified values, indicating validity of the method for analysis of sediments samples.

Table 4.4: Analysis of certified reference materials of sediments (BCR 280R and SRM 8704)

Element	**BCR-280R**			**SRM 8704**		
	Measured	Certified	Percent Recovery	Measured	Certified	Percent Recovery
	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	(%)	Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	(%)
Zn	208±0.91	224±25	93	402±2.0	408±15	98
Ni	63.3±2.6	69.0±5.0	92	38.6±10	42.9±3.7	90
Cu	61.4±0.63	53.0±6.0	116		N/A	
As	38.1±0.65	33.4±2.9	114		N/A	
Se	0.251±0.044	0.46±0.09	55	a		
Cd	0.912±0.002	0.85±0.10	107	3.33±0.023	2.94±0.29	113
Cr	111±14	126±7.0	88	a		
Mn		N/A		577±1.9	544±21	106
V		N/A		97.6±0.059	94.6±4.0	103
Pb		N/A		210±3.8	150±17	140
Fe		N/A		3.69[b]	3.97±0.10	93

N/A: not available on the SRM certificate; ([a]): not analysed in this study; ([b]): analysed with F-AAS in % w/w. n: triplicates

Validation of Fe in the sediments samples was done with the use of reference material (8704 Buffalo River sediments) and measured by F-AAS. Table 4.4 summarizes the results obtained for the analysis of the certified reference materials (BCR 280R and SRM 8704) by ICP-MS and F-AAS.

The percent recovery for most of the elements was 90–120%, which showed reliability of the proposed method. Good percent recovery of Zn, Ni and Cd was observed in both BCR 280R and SRM 8704.

Zinc was recovered at 93% and 98%, Ni at 92% and 90%, while Cd was recovered at 107% and 113% from BCR 280R and SRM 8704, respectively.

Comparing the measured values with the certified values in the two reference materials, good percent recoveries were obtained for Mn (106%), Cu (116%), As (114%), V (103%) and Fe (93%). Although lower percent recovery was observed for Cr at 88%, it was still within the acceptable limit of 85%-115% as set by USEPA, (2001) except for Se at 55%. A higher percent recovery of Pb 140% was obtained. According to (FDA, 2015), when an element recovery is >120%, while irreproducibility is less than 20%, issues with matrix effect and method specificity can be investigated to account for the recovery range. In this study, the irreproducibility value for Pb was well within the acceptable range since it was calculated as 1.8%.

For clarity, coding was used to label different samples based on the sample type, location where the sample was taken, method employed on the sample and the season during which the sample was taken. In this study different coding used were code wM1H means w is water sample; M is Mokolo River; 1 is the site number and H is the high flow season. Furthermore, “d” is digested sediments samples; “M” is Mokolo River; “1” is the site number and “H” is the high flow season, thus “dM1H”. Alternatively, for the coding “wM1L” and “dM1L”, “L” is the low flow season. The same coding was followed for Blood River analysis were “B” is Blood River in the coding “wB1H”, “dB1H”, “wB1L” and “dB1L”.

4.6 DETERMINATION OF POTENTIALLY TOXIC ELEMENTS IN WATER AND SEDIMENTS COLLECTED FROM MOKOLO RIVER

Three different sample preparation techniques were employed in this study prior to the determination of potentially toxic elements in the sediments samples.

For the determination of pseudo-total concentrations of potentially toxic elements in sediments, a digestion protocol by Melaku *et al.* (2005) was carried out and labelled as (Method A).

4.6.1 Concentrations of potentially toxic elements in water collected from Mokolo River during high flow season

The concentrations of potentially toxic elements quantified in water during the high flow sampling season are shown in table 4.7 appendix A. The concentrations obtained from water analysis are simplified with the plot in figure 4.2.

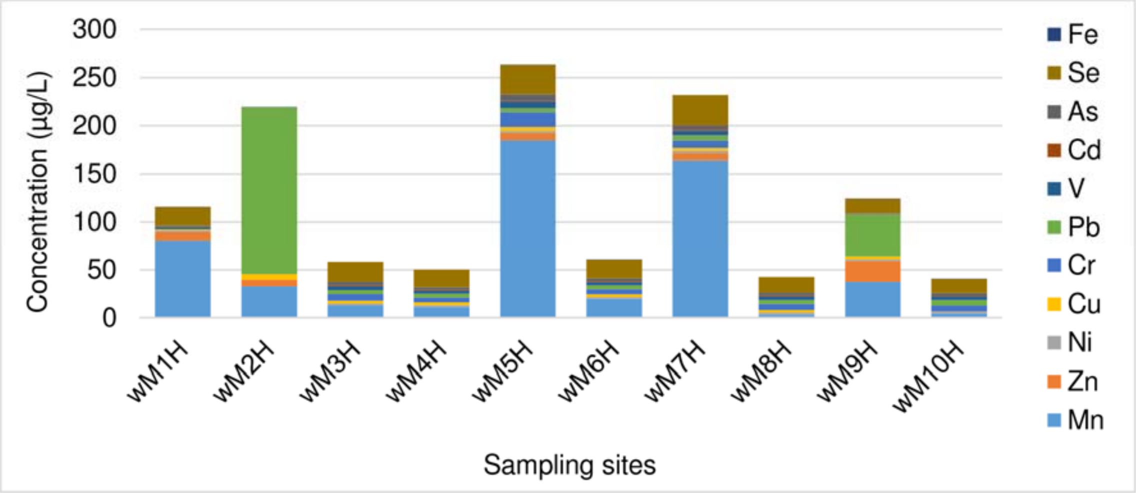

Figure 4.2: Concentrations of potentially toxic elements in water samples collected from Mokolo River during the high flow season

In this study, the concentrations of potentially toxic elements in water of Mokolo River during the high flow season were found to be variable from site to site. The levels of the elements ranged from 3.36 to 185 µg/L, <1.77 to 21.6 µg/L, <0.62 to 2.28 µg/L, <0.48 to 5.74 µg/L, <0.11 to 7.47 µg/L, 0.514 to 174 µg/L, <0.53 to 6.80 µg/L, <0.030 to 0.986 µg/L, 0.010 to 6.19 µg/L, <0.12 to 31.8 µg/L and <0.044 to 0.321 µg/L for Mn, Zn, Ni, Cu, Cr, Pb, V, Cd, As, Se and Fe, respectively.

In this study, Mn was the only element in the water that showed notable spatial variation in all the sites except in S2. Mokolo River had high water levels during the high flow sampling season due to the rainy season at the time. The elevated water level may have caused an increment in the Mn concentration as the element settles at the surface of the river water (Teuchies, 2011). High levels of Mn was detected at S5 (185 µg/L), S7 (164 µg/L) and S1 (80.9 µg/L). Relative to these high levels of Mn, S2 and S9 had 32.8 and 37.5 µg/L, respectively.

Major parts of the Mokolo River flow through agricultural areas where the application of fertilizers is widespread. Consequently, the elevated levels of Mn could be associated with the production of slow-release multi-micronutrient fertilizers of Zn, Fe, Mn, and Cu (Bandyopadhyay *et al*., 2014).

Potentially this accounts for the elevated levels at S1 and S5 due to the crop farming and irrigation that were evident near the river bank. The low levels were recorded at S8 (3.36 µg/L) and S10 (4.72 µg/L). The trend in Mn levels at each site followed the order of S5>S7>S1>S9>S2>S6>S3>S4>S10>S8.

High levels of Pb in water can be attributed to heavily travelled roads that run along the river. Higher levels of Pb often occur in water bodies near highways and large cities due to high gasoline combustion (Saeed and Shaker, 2008). This statement is in good agreement with the trend in this current study since the maximum Pb level detected in S2 (174 µg/L) was close to a bridge where residents cross on a busy tar road. The level of Pb at S9 was at 43.8 µg/L close to a sand mining area. The findings of this study agree with the statement by Saeed and Shaker (2008) in relation to Pb levels and gasoline since trucks are used to collect the sand and sell to local residents for construction purposes. As the trucks and vans move around the sand pits, they emit high gasoline combustion and oil spills were evident from leaking diesel tanks. The levels of Pb in water during the high flow season, followed the order of S2>S9>S10>S7>S5>S3>S8>S6>S4>S1.

Zinc was not detected in S3, S4, S6, S8 and S10. The highest concentration of Zn was measured at site 9 (21.6 µg/L) followed by 9.03 µg/L at S1 and 7.87, 7.38 and 6.90 µg/L at sites S7, S5 and S2, respectively.

Levels of Zn in water bodies may be influenced by the existence of industrial activities due to the release of toxic effluents into the river systems (Wali *et al*., 2014). Mokolo River is surrounded by mining industries and power stations, thus potentially accounting for the reported elevated levels of Zn in the water.

The level of Ni in S2 was below the LOD value of 0.62 µg/L. The levels of Ni were low in all the sites. Concentrations of Ni in the sites followed the order 2.22, 2.28, 2.16, 2.12, 2.05, 1.95, 1.81, 1.67 and 1.35 µg/L in S7, S10, S8, S5, S4, S6, S3, S9 and S1, respectively.

Low concentrations were determined varying from site to site for elements such as Cd, V, As and Cr. Availability of Cd, Cr and As in rivers is commonly from agricultural runoffs which may contain pesticides with As, Cd or Cr (Fu *et al*., 2014). As such, these elements were at maximum concentrations at S5 could be associated to the agricultural activity close to the site.

High concentrations of Se were determined at S5 (30.6 µg/L) and S7 (31.8 µg/L). These elevated levels may be related to the agricultural activities partaking at S5 and S7, which was adjacent to a farm. The concentration at S2 was below the LOD value of 0.12 µg/L. Substantial levels of Se were quantified at sites 1 (19.4 µg/L), S3 (20.7 µg/L), S4 (18.2 µg/L), S6 (19.3 µg/L), S8 (16.1 µg/L), S9 (15.3 µg/L) and S10 (14.7 µg/L).

Low levels of Fe were quantified in the following decreasing order from different sites: S6 (0.321 µg/L), S10 (0.295 µg/L), S1 (0.202 µg/L), S5 (0.161 µg/L), S9 (0.148 µg/L) and S2 (0.052 µg/L). The concentration of Fe in sites 2, 3, 7 and 8 was below the LOD value of 0.049 µg/L. A study by Daiana *et al*. (2013) reported that Mn was often found in combination with Fe, and in many minerals. However, the levels of Fe detected in the Mokolo River doesn't reveal this correlation.

Average concentrations of potentially toxic element in water were found in the following order of Mn > Se > Zn > Cr > Pb > V > Cu > As > Ni > Cd > Fe (Figure 4.2). The concentrations varied from site to site with S5 showing the overall high concentrations for most of the detected elements.

The pH varied between 7.18 (site 4) and 9.35 (site 7) as shown in table 4.7, appendix A. The measured pH was found to be in acceptable pH range of 6.0 to 9.0 (APHA, 1998), except for site 7 (9.35) which was slightly above the recommended limit for survival of aquatic species. The pH range during the high flow season appears to be good for the normal functioning of the aquatic species such as fish and the bottom dwelling invertebrates like corals and snails. Abida *et al*. (2009) reported that the pH values from Madivala Lake varied between 5.6 and 7.5 and this low pH range of water may be attributed to discharge of acidic water by agricultural and domestic activities. Although this was the case, the pH of Mokolo River indicated that it was still within tolerable limits even with the existence of agricultural activities partaking near the river.

4.6.2 Concentrations of potentially toxic elements in water collected from Mokolo River during low flow season

Table 4.8 in Appendix A, lists the concentrations of potentially toxic elements in water quantified by ICP-MS during the low flow season. The trend in the concentrations of the elements is simplified by figure 4.3.

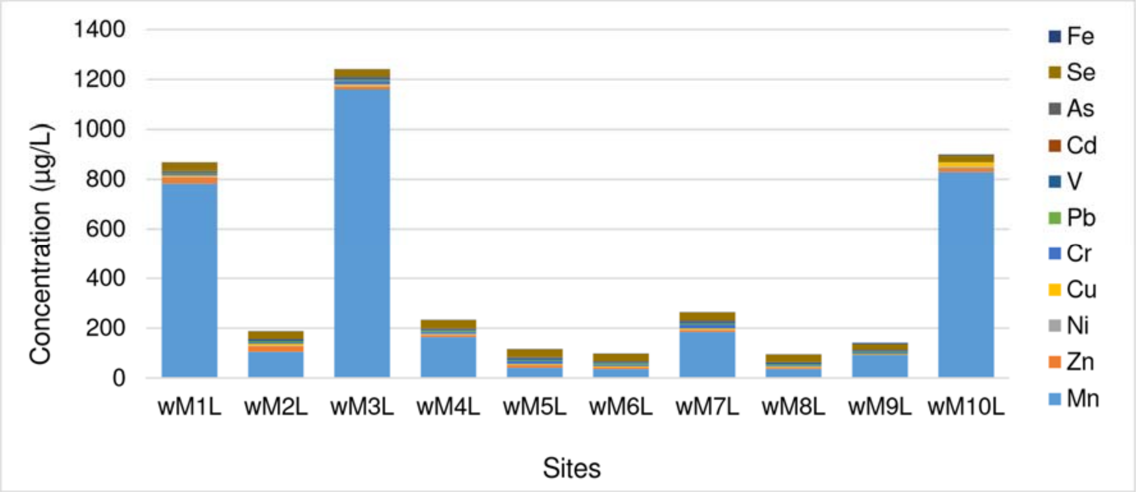

Figure 4.3: Concentrations of potentially toxic elements quantified in water from Mokolo River during the low flow season

The levels of potentially toxic elements in water quantified during the low flow season ranged from 35.1 to 1160 µg/L, <1.77 to 25.6 µg/L, 1.98 to 6.18 µg/L, 2.87 to 17.5 µg/L, <0.11 to 15.4 µg/L, 1.79 to 5.41 µg/L, <0.53 to 6.80 µg/L, <0.030 to 0.990 µg/L, <0.010 to 6.19 µg/L, 22.6 to 33.0 µg/L and 0.23 to 5.02 µg/L for Mn, Zn, Ni, Cu, Cr, Pb, V, Cd, As, Se and Fe, respectively.

The most probable cause of high Mn concentration in the Mokolo River water during the low flow season may be from the type of rock found in the Sand River catchment area since it serves as a tributary to the Mokolo River. Granite is one of the major basement rocks found in the Sand River catchment area (Polokwane Municipality SDF, 2011). On weathering, this rock produces Mn. This might be related to the windy weather during the low flow season were weathering is maximum.

Maximum level of Mn was quantified in S3 at 1160 µg/L, which was 33 times higher than the lower concentration quantified in S6 at 35.1 µg/L.

The concentrations varied from site to site indicating substantial difference in the levels of the element at each site.

The USEPA (2012) guide has established maximum contamination limit for Se in water at 50 µg/L. In their report, they stated that Se levels in water systems often occur from agricultural runoff in normal, dry undeveloped lands. Contamination in ground water is associated with industrial pollution. This statement is in agreement with the trend and level of Se in this study where the maximum level of Se during low flow season is associated with the dry season and the existence of agricultural and industrial effluents in the Mokolo River. The concentrations ranged between 22.6 µg/L in S9 to 33.0 µg/L in S1.

The level of Zn in the sites showed high variation. The concentrations ranged from below LOD value of <1.77 µg/L to 25.6 µg/L. Common sources of Zn in the water systems include industrial wastes, fertilisers and insecticides among others (DWAF, 1996e). The existence of such activities may have had an influence on the level of Zn in the downstream region of the river from S1 to S6. Greenfield *et al*. (2012) reported Zn level up to 130.00 µg/L in water sample from the Nyl River in South Africa. The level was about 5 times higher than the maximum concentration in this study.

Iron has been quantified in many South African river systems such as the Dzindi River, Umtata River, Plankenburg River, Diep River and Mvudi River, among others (Fatoki *et al*., 2002; Edokpayi *et al*., 2014). The levels were associated with industrial wastes, sewage effluents and agricultural runoffs.

Concentration of Fe in the water of this study ranged from 0.23 to 5.02 µg/L. These levels were lower than those reported from other rivers in South Africa.

The study conducted by Abida *et al*. (2009) quantified Pb, Cd, Cr and Ni concentrations in water, sediments and fish from Madivala Lake. The potentially toxic elements concentrations in the water samples followed the order Pb > Cr > Cd > Ni. In their study, Cr ranged between 0.22-2.5 µg/L in water. The maximum level of Cr in Madivala Lake was 6.16 times lower than the maximum level of Cr found in the Mokolo River water during the low flow season of 15.4 µg/L.

The highest concentrations of Ni, Cd and Pb observed in water samples were 6.4, 4.9 and 7.2 µg/L, respectively. The maximum concentration of Ni (6.18 µg/L) in water sample of Mokolo River, in our study, was comparable to the level reported by Abida *et al*. (2009).

The concentrations of Cd and Pb, in current study, were found to be 4.91 and 1.30 times lower than the levels reported by Abida *et al*. (2009), respectively.

The levels of V and As ranged from <0.53 to 6.80 µg/L and <0.01 to 6.19 µg/L, respectively. The lowest concentrations were quantified at S10 and the highest levels at S7 for both elements. This indicates that the elements may be associated with the same source of pollutant. The average concentrations of the elements in the water during the low flow season followed the order of Mn>Se>Zn>Cr>Cu>V>As>Pb>Ni>Cd>Fe (Figure 4.3). The concentrations were significantly different from site to site ($p<0.05$).

The mean pH of the water during low flow season in the Mokolo River was 6.91 with site 10 having the lowest pH value of 6.50 and the highest pH value of 7.07 reported at site 8 as shown in table 4.7. The average pH value was within the acceptable pH range (APHA, 1998).

4.6.3 Comparison of potentially toxic elements in water collected from Mokolo River during the high and low flow seasons

Following the high flow season analysis of water collected from Mokolo River, the sampling and analysis of water during low flow season was done for comparison of the seasonal variation for the levels of elements in water. The seasonal variation is shown in figure 4.4. Except at site 5, the measured concentrations of potentially toxic elements were higher in the low flow season compared to the high flow season.

The low levels of potentially toxic elements detected in water at high flow season could be attributed to the dilution effect during the rainy season.

The seasonal climate has a major influence on the concentration and distribution of potentially toxic elements, in the case of Fe, Cu and Mn during dry seasons (low flow) which suggests significant evaporation phenomena in the area as well as low mobility of sediments (Montalvo *et al*., 2014).

Furthermore, Mekonnen *et al*. (2015) stated that slow currents of water in dry seasons give room for heavy metals to settle down and accumulate in water without turbulence. Dilution of the water during the rainy season is another factor, which might have reduced the elemental concentration in most sites during high flow season.

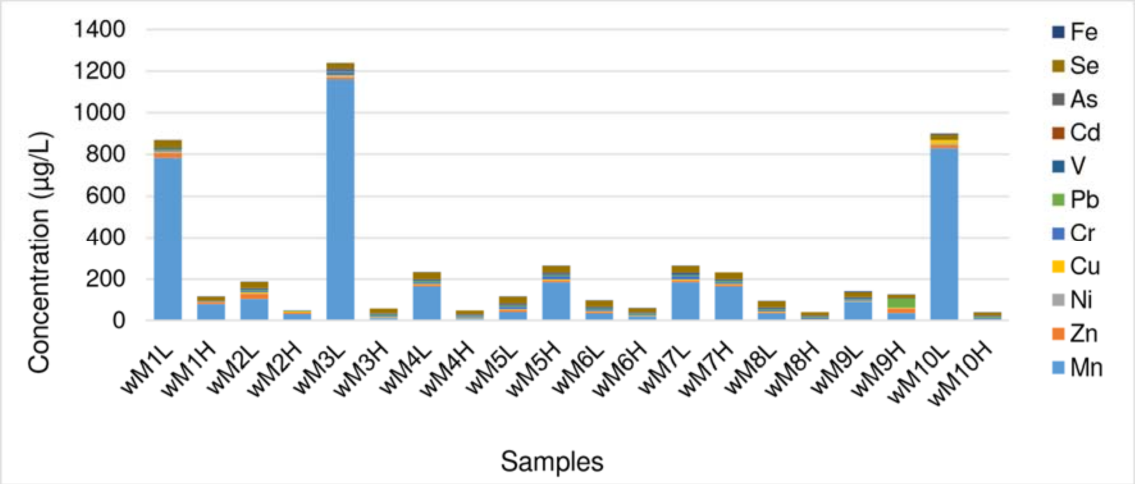

Figure 4.4: Seasonal comparison of potentially toxic elements in water from Mokolo River

The concentrations of Mn, Zn, Ni, Cu and Fe in water were found to be 6.27, 1.19, 2.71, 3.05 and 15.6 times higher during low flow season than in high flow season, respectively.

The ANOVA analysis revealed that there were significant ($p<0.05$) variations in the levels of the potentially toxic elements detected in water samples during the high and low flow seasons.

The results obtained indicated that the pH of the water ranged from 7.18 to 9.35 (mean = 7.96) and 6.50 to 7.07 (mean = 6.91) in the high and low flow seasons, respectively. The pH of the water from the Mokolo River did not differ significantly ($P > 0.05$). Previous studies of the Mokolo River catchment, reported pH values of 6.6 before the year 1988 and 7.6 in 2011 (Burne, 2015). These proved to be an increasing trend from then till to date, particularly for the high flow season in our study.

4.6.4 Pseudo-total concentrations of potentially toxic elements in sediments collected from Mokolo River during high flow season

The concentrations of potentially toxic elements quantified in sediments during the high flow sampling season are shown in table 4.9 of Appendix A. The pseudo-total concentrations in sediments obtained employing method A, are summarized in figure 4.5.

Concentrations of potentially toxic elements varied from site to site and element to element, from 16.3 to 85.0 mg/kg, 15.4 to 35.2 mg/kg, 5.70 to 40.6 mg/kg, 7.45 to 11.6 mg/kg, 55.4 to 171 mg/kg, 17.3 to 44.0 mg/kg, 14.5 to 111 mg/kg, <0.12 ng/g, 0.385 to 0.954 mg/kg, 2.14 to 35.4 mg/kg and 2180 to 3760 mg/kg for Mn, Zn, Ni, Cu, Cr, V, As, Se, Cd, Pb and Fe, respectively.

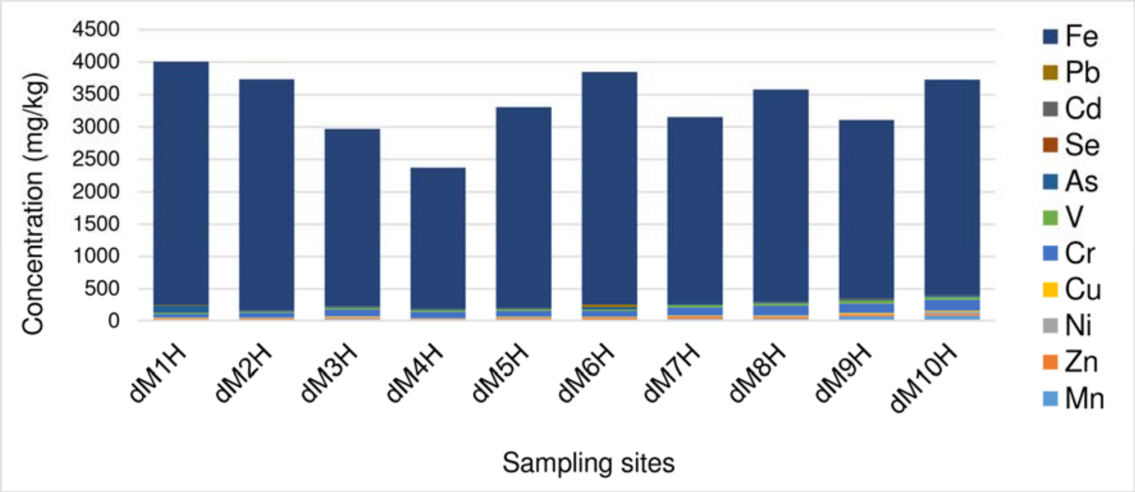

Figure 4.5: Pseudo-total concentrations of potentially toxic elements in sediments samples collected from Mokolo River during the high flow season

Li *et al.* (2009) emphasised that Mn oxides control the adsorption of metals in superficial sediments. High levels of Mn were recorded at S10 (85 mg/kg) followed by S9 (74.5 mg/kg) and both S8 and S7 at 38.0 mg/kg. Sand mining was observed at these sites.

Manganese levels of 405 mg/kg were reported in the sediments of Mvudi River during the wet season (Edokpayi *et al*., 2016). These values exceeded the Mn levels determined in this study. Similar to the Mokolo River, Mvudi River is occupied with domestic, recreational and agricultural activities.

The average concentrations of Cu and Zn recorded in the sediments of Mvudi River during the wet season were 33.68 and 29.11 mg/kg, respectively (Edokpayi *et al.*, 2016). In this study, the highest level of Cu (11.6 mg/kg) was lower than the value in the Mvudi River sediments. Whereas, the highest level of Zn (35.2 mg/kg), in this study, was slightly above the level recorded in the Mvudi River sediments.

Distribution of V and Ni in sediments showed a similar trend. The highest concentration of V was recorded at S9 (44.0 mg/kg) and the lowest at S1 (17.3 mg/kg), while Ni ranged from 5.70 mg/kg in (S1) to 40.6 mg/kg in (S10). The report by Department of Water Affairs and Forestry (DWAF, 1996c) showed the similarity in chemical behaviour of the two elements. Thus, the observed similar trend in distribution of V and Ni could be explained by this similar chemical behaviour.

The DWAF (1996c) reported that As is strongly adsorbed by soil and as such, accumulation is expected to be maximum is sediments. Elevated concentrations of As can occur in agricultural soils, due to previous use of organo-arsenic pesticides that persist as long-lasting residues in the soil (DWAF, 1996c). In this study, the highest level of As was recorded at S1 (111 mg/kg) and the lowest at S2 (14.5 mg/kg). The maximum levels recorded at S1 may be attributed with the presence of agricultural activities partaking in the area. Furthermore, substantial level of the element in the sediments was observed in S4 (21.0 mg/kg), S7 (21.2 mg/kg), S8 (22.0 mg/kg), S9 (22.8 mg/kg) and S10 (25.7 mg/kg).

Chromium is also strongly adsorbed by soil. Its occurrence in soil and sediments is associated with industrial pollutions. This is in agreement with the trend shown in this study since consistently high Cr levels were recorded in areas where industrial activities are closer to the river. Higher Cr levels were recorded in the downstream region as compared to the upstream. This might be from the flushed industrial effluents, which flow from upstream and settle in the downstream sediments. Edokpayi *et al*. (2016) reported Cr levels ranging from 44.23 to 149.52 mg/kg in the sediments of the Mvudi River. The levels were associated with industrial pollutants.

The levels of Cd ranged from 0.385 to 0.954 mg/kg from S4 and S9, respectively. Site 4 was close to an irrigation system were livestock farming was taking place, while sand mining was carried out at S9.

The DWAF (1996e) report associated the presence of Cd in water bodies with anthropogenic activities. The anthropogenic activities could be the source of Cd in this study. The level of Cd in the Mvudi River during the wet season was found to be below the LOD (Edokpayi *et al*., 2016). Low levels of Cd were determined in all the sites in this study. This agrees to the study by Olujimi *et al*. (2015), which states that Cd concentration has not been commonly reported in many river systems in South Africa.

The concentrations of Fe ranged from 2180 mg/kg (S4) to 3760 mg/kg (S1). These levels were lower than the level recorded in the sediments (4371 mg/kg) of Mvudi River during the wet season (Edokpayi *et al*., 2016). DWAF (1996b) associated the presence of Fe with coal formations.

The Mokolo River is situated in the vicinity of coal-fired power stations and coal mine. These industrial activities could be some of the possible sources of the metal in the river although other sources are not ruled out.

Selenium concentration was below the LOD value of 0.12 ng/g in all the sites. This consistent distribution is probably due to the lithology of the area as there is no clear identifiable source of Se in the Lephalale Municipality.

The levels of Pb in the sediments followed the order S6 > S1 > S9 > S3 >S5 > S10 > S8 > S4 > S2 >S7. Lead levels up to 3.87 mg/kg were recorded in the sediments of Mvudi River during the wet seasons (Edokpayi *et al*., 2016) and these levels were comparable to the levels in sediments of Mokolo River. The river flows alongside a tar road while sand mining was the common activity and as such, oil spills from the trucks loading the sand and cars travelling on the roads were possible contamination sources of the river system. Such activities might have contributed to the elevated levels of Pb in the sediments.

The average concentrations of potentially toxic elements in sediments of Mokolo River during the high flow season were in the following order: Fe > Cr > Mn > V > Zn > As Ni > Cu > Pb > Cd > Se (Figure 4.5).

During the wet season, the two important weather-related factors that can lead to variations in trace metal levels in rivers are surface runoffs from various land use activities in the catchment.

This includes settlements, dumpsites, agriculture urban runoff, and dilution due to high precipitation (Edokpayi *et al*., 2016). These conditions are the likely factors that affected the levels of potentially toxic elements in the Mokolo River during the high flow season.

4.6.5 Pseudo-total concentrations of potentially toxic elements in sediments of Mokolo River collected during low flow season

Table 4.10 of Appendix A shows the pseudo-total concentrations of potentially toxic elements in sediments quantified by ICP-MS and F-AAS collected during the low flow season. The concentrations are simplified by the plot in figures 4.6.

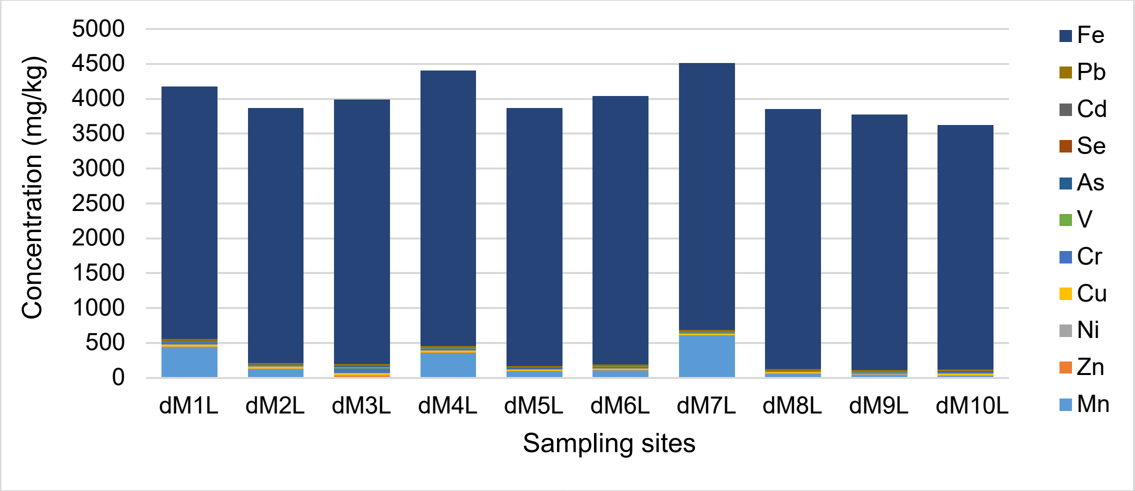

Figure 4.6: Pseudo-total concentrations of potentially toxic elements in sediments of Mokolo River during the low flow season

The concentrations of potentially toxic elements determined during the low flow season ranged from <0.28 ng/g to 624 mg/kg, 4.29 to 27.3 mg/kg, 5.21 to 29.0 mg/kg, 2.49 to 15.1 mg/kg, 17.3 to 63.8 mg/kg, 12.5 to 45.2 mg/kg, 0.928 to 3.05 mg/kg, <0.030 ng/g to 0.477 mg/kg, 4.01 to 13.5 mg/kg and 3500 to 3950 mg/kg for Mn, Zn, Ni, Cu, Cr, V, As, Cd, Pb and Fe, respectively. Selenium was below the LOD value of <0.12 ng/g in all the sites. Cadmium was also below the LOD value of <0.03 ng/g in most sites except at sites 3 and 10.

The levels of potentially toxic elements determined in the sediments of Mokolo River during the low flow season were consistently high at most of the sites. Barker (2006) reported that metals which are bound in sediments have no direct danger to the aquatic ecosystem if they remain bio-unavailable. This is because they not easily leachable during normal environmental changes. Harsh and rapid changes to the water column such as pH, water hardness, salinity or temperature may leach out heavy metals from the sediments to the water (Uzairu *et al*., 2009). Furthermore, during the low flow season, evaporation from water bodies may lead to an increase in the concentrations of contaminants as the dilution factor is removed.

The levels of Mn recorded in the sediments during the low flow season of this study ranged between <0.28 ng/g (S3) and 624 mg/kg (S7). The levels of Mn recorded in the sites followed an increasing order of <0.28 ng/g (S3), 48.8 mg/kg (S10), 56.7 mg/kg (S9), 79.8 mg/kg (S8), 114 mg/kg (S5), 127 mg/kg (S6), 136 mg/kg (S2), 371 mg/kg (S4), 443 mg/kg (S1) and 624 mg/kg (S7).

Botes and Van Staden (2004) detected Mn in the sediments of Blesbokspruit River (31.12 µg/g), Crocodile River (1406 µg/g) and the Olifants River (411.3 µg/g) in South Africa. These levels were comparable to the levels of Mn detected in this study, except the levels from the Crocodile River. The measured concentration in sediments of Crocodile River was 2.25 times higher than the maximum level detected in this study.

The concentrations of Cu determined in the sediments during the low flow season were in the range of 2.49 (S7) to 15.1 mg/kg (S3). These levels were very low compared to the mean levels of Cu determined in the Mvudi River during the dry season. In their study, the recorded average concentration of Cu was 33.68 mg/kg (Edokpayi *et al*., 2016).

The levels of Cu recorded in the sampling sites followed an increasing order of 2.49 mg/kg (S7), 2.60 mg/kg (S8), 2.67 mg/kg (S5), 2.73 mg/kg (S6), 2.96 mg/kg (S9), 4.29 mg/kg (S10), 4.65 mg/kg (S4), 7.00 mg/kg (S1), 10.2 mg/kg (S2) and 15.1 mg/kg (S3).

Iron concentrations in the sediments also varied among the sampling sites. As reported by many researchers, the existence of Fe in the river system is due to anthropogenic activities (Dlamini *et al*., 2013; Moyo *et al*., 2015).

The acid mine drainage, untreated sewage, landfill leachates and the corrosion of iron and steel are some of the listed activities contributing to Fe levels in water systems (DWAF, 1996c).

Concentrations of Fe in the sediments were relatively high throughput the sampling locations. Site 4 had highest accumulation of the element recorded at 3950 mg/kg while site 10 had lowest concentration of 3500 mg/kg. The presence of dumpsites where Fe chips were dumped might have contributed to the elevated levels at S4. High level of Fe (31666.7 mg/kg) has been reported in the sediments from Quarry Dam, Swaziland (Dlamini *et al*., 2013). The levels of Fe in their study, were associated with acid mine drainage.

These levels were much higher than the levels reported in this study. Akan *et al*. (2012) reported lower concentrations compared to this study. The maximum level in their studied sites was 32.86 µg/g and were associated with agricultural runoffs.

Arsenic levels were relatively low, ranging from 0.928 to 3.05 mg/kg in S9 and S3, respectively. Site 2 (1.97 mg/kg), S4 (1.17 mg/kg), S5 (1.12 mg/kg), S6 (1.50 mg/kg) S7 (1.49 mg/kg), S8 (1.30 mg/kg) and S10 (1.47 mg/kg) showed comparable levels. These levels indicate the presence of As in the sediments may be from a similar point source. Arsenic levels have been reported in the sediments of Flag Boshielo Dam at average concentration of 1.8 mg/kg (Kekana, 2013). These levels were in the same range when compared to the measured concentrations in this study.

Zinc concentrations recorded in the sediments of Mokolo River ranged from 4.29 mg/kg (S8) to 27.3 mg/kg (S3). Distribution of the element in the river system indicates that it was influenced by the activities that partake in the upper section of the sampling sites. In comparison with other studies, maximum Zn concentrations exceeding the levels in this study have been reported.

In the Quarry Dam, Zn level as high as 65.29 mg/kg was reported (Dlamini *et al*., 2013). Zinc in the sediments of Lake Chad, Nigeria was found to be 165.34 mg/kg.

Accumulation of Cr in the sediments during low flow season was maximum in S3 at 63.8 mg/kg. Site 1 (49.0 mg/kg), S2 (25.8 mg/kg), S3 (63.8 mg/kg), S4 (30.3 mg/kg) and S10 mg/kg) showed higher Cr levels as compared to the other sites. Due to calm and relatively low water flows, precipitation of the water might have caused an increment in the Cr levels in the upstream as compared to the downstream. This was also observed in the low water levels observed in the sites during sampling.

Chromium level as high as 220.76 mg/kg was reported in the Quarry Dam, exceeding the levels in this study (Dlamini *et al*., 2013). It was reported that acid mine drainage from industrial effluents influenced the content of the metal in the Quarry Dam.

In this study, mine industries surround the Mokolo River. However, the level of Cr reported by Akan *et al*. (2012), had maximum level of 43.23 µg/g. This level was in the same range as the levels detected in this study. In this study, minimum and maximum levels of Ni, V and Pb in the sediments showed similar trend.

The maximum levels of these elements were detected at S3 and the minimum levels at S8 with an exception for Pb, which was minimum level at S9. The levels of Ni, V and Pb ranged between 5.21 and 29.0 mg/kg, 12.5 and 45.2 mg/kg and 4.01 and 13.5, respectively. Dlamini *et al*. (2013) reported maximum levels of Ni and Pb at 217.86 and 81.32 mg/kg. These levels were attributed to acid mine drainage and extremely higher than the Ni and Pb determined in this study.

Levels of potentially toxic elements in the sediments of Mokolo River during the low flow season followed the order of Fe > Mn > Cr > V > Zn > Ni > Pb > Cu > As > Cd > Se (Figure 4.2).

4.6.6 Comparison of potentially toxic elements in sediments samples collected from Mokolo River during the low and high flow seasons

Zahra *et al*. (2014) reported low levels of selected elements in sediments in a season of low rainfall. They further elaborated the correlation of concentrations of elements during low and high flow seasons to the differences in pollution sources seeing that the main impacts at the points in the stream were agricultural in nature.

Element concentrations would be increased during rainfall seasons as fertilisers and other agricultural chemicals containing metals are washed into the river, increasing the metal accumulation in the sediments and water.

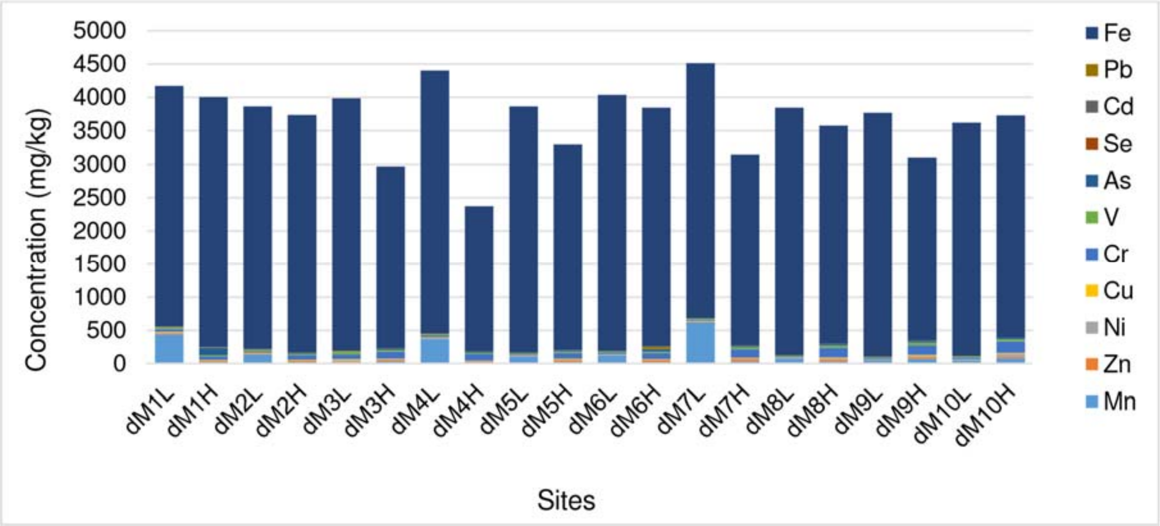

Figure 4.7: Seasonal comparison of potentially toxic elements in sediments from Mokolo River

Since the sediments represent a reservoir of potentially toxic elements, the high correlation levels between the metals Fe and Mn may suggest that these elements have a common source of generation, being the most abundant as determined in this study (Montalvo *et al*., 2014). In the sediments, accumulation during the low flow season was notably higher than the high flow season. The elements Mn, Cu and Fe were found to be 7.34, 1.09 and 1.05 times higher in sediments during low flow season than in high flow season. Additionally, the maximum level of V during low flow season exceeded the level recorded during the high flow season.

The elements, Zn, Ni, Cr, Pb, Cd and As were 1.29, 1.40, 2.68, 2.62, 2.00 and 36.4 times higher during the low flow season than the high flow season, respectively. This may be attributed to the water dilution that occurred during the high flow rainy season. Changes in the environmental conditions may have caused the mobilisation of these elements from water to sediments (Yuan *et al*., 2004). The maximum level of Se was below the LOD for both seasons.

Weathering of rocks, fluctuations in water temperature, and velocity of running water as an influence from water levels during low and high flow seasons, changes in eutrophication among others are some of the environmental conditions favouring the mobilisation of these elements.

In the dry season, the major factor is evaporation from water bodies, which can lead to an increase in the concentrations of contaminants as the dilution factor is removed. There was a statistical variation (p>0.05) in the levels of potentially toxic elements in the sediments seasonally.

4.6.7 Comparison of potentially toxic elements in water and sediments samples collected from Mokolo River during the high flow season

The plot shown by figure 4.8 illustrates the comparison between the levels of potentially toxic elements determined in sediments and water. From Mokolo River, comparison of the levels of potentially toxic elements in water and sediments is done to show relative distribution of the elements in the water and sediments. The concentrations of potentially toxic elements in sediments were higher than the levels determined in water.

This is because the sediments act as a reservoir for all contaminants and dead organic matter descending from the possible sources of pollution (Saeed and Shaker, 2008).

Concentrations of Mn in water samples were found to be substantially higher than all elements levels. A study by De Jonge and Bervoets (2012) reported that Mn ions precipitate and accumulate in the sediments. This is due to Mn oxides under oxidising conditions in the aerated cores.

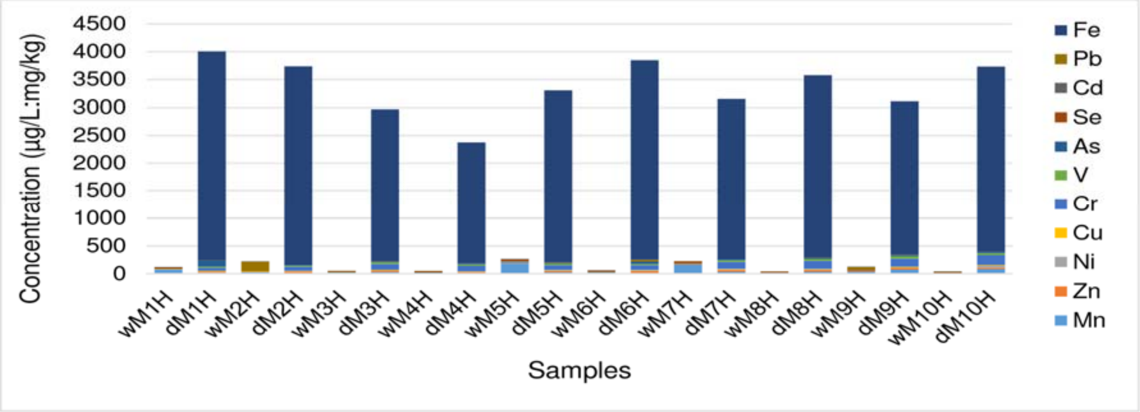

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Figure 4.8: Comparison of the levels of potentially toxic elements in water ((µg/L) and sediments (mg/kg) collected from Mokolo River during the high flow season

Considering the levels of potentially toxic elements from the high flow season, Mn, Fe, V and Pb were accumulated in sediments at high concentrations amounting to thousands of times their levels quantified in water. Manganese, Zn, Se and Pb were detected in high concentration in water but not exceeding the levels in sediments. Therefore, the potentially toxic elements are highly accumulated in sediments than in water. A significant variation ($p<0.05$) was observed between the levels of potentially toxic elements determined in water and sediments analysed during the high flow season.

4.6.8 Comparison of potentially toxic elements in water and sediments samples collected from Mokolo River during the low flow season

Concentrations of potentially toxic elements in sediments and water were measured to check the distribution of the elements between water and sediments.

A graph was constructed as illustrated by figure 4.9, to compare the concentrations of potentially toxic elements in water and sediments collected from Mokolo River during the low flow season.

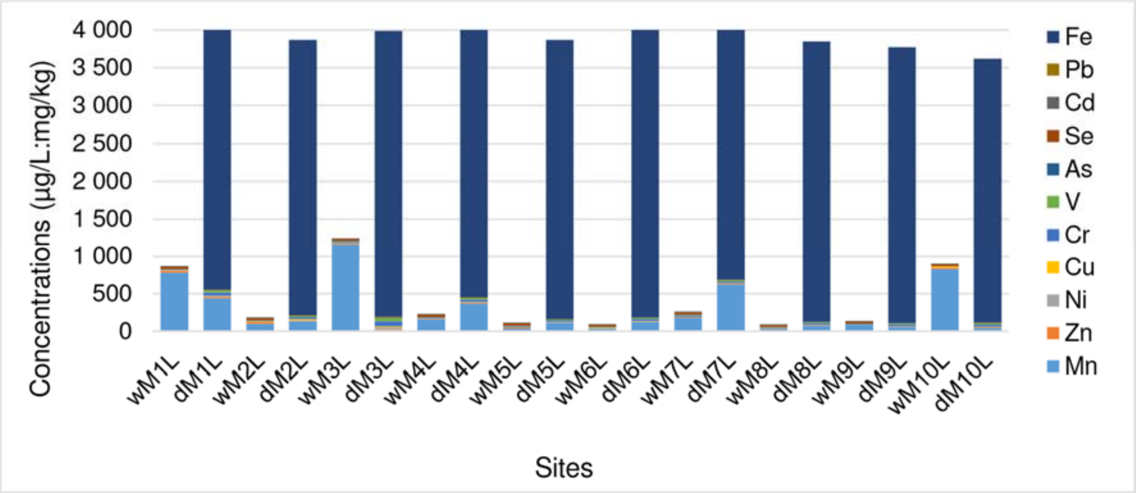

Figure 4.9: Comparison of the levels of potentially toxic elements in water (µg/L) and sediments (mg/kg) collected from Mokolo River during the low flow season

Manganese concentrations were found to be significantly higher compared to all the elements in water. The concentrations of potentially toxic elements in water followed the order Mn > Se > Zn > Cr > Cu > V > As > Pb > Ni > Cd > Fe.

In sediments samples, Fe was detected in high concentrations in all the sites than all the other elements. The concentrations of potentially toxic elements in sediments followed the trend Fe > Mn > Cr > V > Zn > Ni > Pb > Cu > As > Cd > Se.

The levels of potentially toxic elements quantified in water were lower than their levels in sediments samples (Figure 4.9). Based on the maximum concentrations of each element quantified in each site, site 3 showed maximum accumulation in sediments for most of the elements while site 7 and 10 showed high concentrations for most of the elements in water. These metals may be from the fertilisers used for agricultural activities.

Considering the levels of potentially toxic elements from the low flow season, Mn, Fe, V, Cr Zn, Ni and Pb were accumulated in sediments at high concentrations amounting to thousand times those quantified in water.

Manganese, Zn, Se, Cr, Cu and Pb were detected in high concentrations in water. There was a significant variation ($p<0.05$) in the levels of potentially toxic elements in the water with the sediments analysed during the low flow season.

4.7 DETERMINATION OF POTENTIALLY TOXIC ELEMENTS IN WATER AND SEDIMENTS COLLECTED FROM BLOOD RIVER

4.7.1 Concentrations of potentially toxic elements in water collected from Blood River during high flow season

Figure 4.10 is a plot used to simplify the levels of potentially toxic elements in water during the high flow season. The detailed concentrations are shown in table 4.11, appendix B.

A study conducted by Shanbehzadeh *et al*. (2014) examined potentially toxic elements levels in water and sediments of upstream and downstream of the entry of the sewage to the Tembi River in Iran. The Blood River, in this study, also receives sewage effluents. Seanego and Moyo (2013) studied and reported about the water quality of the Sand River which is joined by the Blood River on the left North side of Polokwane. Although they didn't conduct determination of heavy metals, their study focused on the water quality parameters (P, TDS, BOD, N, O and salinity).

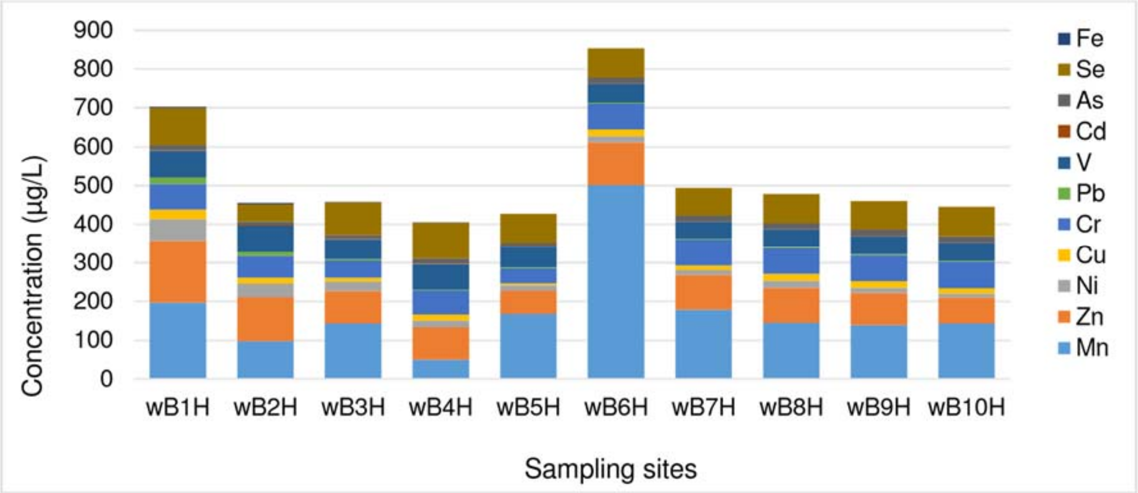

Figure 4.10: Concentrations of potentially toxic elements in water samples collected from Blood River during the high flow season

From the study by Shanbehzadeh *et al*. (2014), Mn concentration was reported at 1400 µg/L in water from Tembi River, Iran during the high flow season. This concentration value was higher than the maximum level of Mn obtained in this study at S6 (table 4.11, appendix B).

The concentration was found to be 2.79 times higher than that reported in this study. The high concentration of Mn at S6 was associated with the sewage effluents that come from the leaking untreated sewage into the river. The release of Mn into river systems has been associated with industrial discharges, fertilisers as well as acid mine drainage (Howe *et al.,* 2005). The presence of such activities contributed to the elevated levels in Tembi River (Shanbehzadeh *et al*., 2014) and was comparable with the water system of Blood River. Low concentrations**,** ranging from 13 to 46 µg/L, were reported in the water from Flag Boshielo Dam (Kekana, 2013).

Cadmium was recorded at low concentrations ranging from 0.799 to 1.80 µg/L. These concentrations were much lower than the level (300 µg/L) reported by Shanbehzadeh *et al*. (2014). Cadmium is mainly found in bottom sediments and suspended particles (Friberg *et al.*, 1986). Municipal sewage, runoff and leachates of solid wastes might have contributed to the levels of Cd in the water.

Arsenic concentrations ranged between 9.21 µg/L and 16.3 µg/L (Appendix B: table 4.11). There was a significant difference of As concentrations between sites ($p<0.05$).

DWAF (1996b) reported that As might occur at high concentrations in water bodies subjected to industrial wastes, or agricultural activities that used As-containing compounds such as fertilisers and arsenic-containing pesticides. Given the fact that there are agricultural activities and municipal wastes in the vicinity of the Blood River, it can be assumed that the As from these sources could have contributed to the current levels of As.

Chromium was recorded at relatively high concentrations at all the sites. The recorded concentrations during high flow season ranged between 40.4 µg/L (S5) and 69.2 µg/L (S10). Chromium has been reported as a scarce metal and that the occurrence and amounts of it in aquatic ecosystems are usually very low (DWAF, 1996e).

High concentrations have been reported in studies conducted on African rivers and traced to anthropogenic activities (Greenfield *et al*., 2012; Kekana, 2013; Shanbehzadeh *et al*., 2014; Mekonnen *et al*., 2015). The Blood River receives municipal wastes, runoffs from industries and leachates from solid wastes. These sources could have contributed to the levels of Cr in Blood River.

The concentrations of Cu ranged from 4.42 µg/L (S5) to 24.1µg/L (S1). The study by Shanbehzadeh *et al*. (2014) reported Cu as high as 630 µg/L and this level exceeded the concentration of Cu at all sites of this study. Availability of Cu in the aquatic environment is due to weathering processes and anthropogenic activities such as sewage treatment plants and runoff from the use of Cu-containing fungicides and pesticides among others (DWAF, 1996e). These sources might have contributed to the levels of Cu in the Blood River.

Anthropogenic sources such as emissions from leaded petrol, industrial and municipal wastewater discharges are some of the main sources of Pb in water (DWAF, 1996e). Although the leaded gasoline was banned in South Africa, the residues of Pb from past use of leaded gasoline could have contributed for the current level. Municipal wastewater discharges and domestic wastes also contribute to the elevated Pb levels in the river. During the high flow season of this study, Pb was detectable in all the sites with maximum detected concentration of 17.1 µg/L. The concentrations varied from site to site with a decreasing order from S1 to S5. Furthermore, relatively low levels of Pb were recorded from S6 to S10, ranging from 2.02 to 3.07 µg/L.

The concentration of Pb as high as 1020 µg/L, was reported in the Tembi River in Iran and this high level was attributed to municipal wastes (Shanbehzadeh *et al*., 2014). This level was about 60 times higher than the maximum level of Pb detected in the Blood River.

In this study, the maximum and minimum concentrations of V were recorded at S1 (68.6 µg/L) and S7 (43.7 µg/L), respectively. The V compounds are useful in industrial applications for catalysis (APHA, 1989). Reduction of V in water has been easily achieved by precipitation and filtration (DWAF, 1996d).

The common source of Ni in the environment is through the release of burning of fossil fuels (WHO, 2007). The concentrations of Ni ranged between 9.51 µg/L and 56.8 µg/L during the high flow season. The concentrations increased from S1 to S5 and fluctuated from S6 to S10 with the lowest concentration of Ni at S10. The Ni in the water of Blood River could be from both natural sources and anthropogenic such as burning of the fossil fuels. The concentrations of Ni up to 650 µg/L were reported by Shanbehzadeh *et al*. (2014). This level was about 11.4 times higher than the highest level measured in this study.

The aforementioned trend was similar for Zn with the highest concentration reported at S1 (160 µg/L). The concentrations decreased from S1 to S5 and then increased at S6 with an increasing and decreasing trend from S7 to S10.

The lowest concentration of Zn was recorded at S5 (59.6 µg/L). The levels of Zn in the water were different from site to site at $p>0.05$. Zinc level of 1350 µg/L have been reported in the Nyl River during similar weather conditions of this study (Greenfield *et al.,* 2012).

Although not associated with specific source of pollutant, these levels were reported to be influenced by anthropogenic activities. Possibly, domestic wastes might have been swept into the river during the rainy season and contributed to the Zn levels of this study, although lower than other studies.

The report by DWAF (1996d) stated Se as an element, which occurs in association with sulphide ores of heavy metals such as Cu, Fe and Zn. It was also stated that elevated concentrations of Se occur in ground waters in seleniferous areas.

Selenium occurs in water through industrial discharges during manufacturing of paint pigments, ceramics, and plastic among others (DWAF, 1996d). Illegal dumpsites were evident in the Blood River area. These dumpsites contain broken glasses, dumped pieces of ceramic tiles and plastic bags. Such materials end up in the river system during rainfalls and contribute to the levels of Se in the water body. Selenium levels in this study ranged from 45.3 µg/L (S2) to 95.4 µg/L (S1). These levels decreased from S3 to S7.

The flow of water may have resulted in decreasing concentration of Se as the Se-containing waste materials are leached into the water from upstream to downstream. Lower Se levels compared to this study were reported in the water of Nyl River at maximum concentrations of 10 µg/L. No direct source of Se was identified in the Nyl River (Greenfield *et al*., 2012).

Concentrations of Fe in the sediments collected from S5, S6, S7, S8, S9, and S10 were all below the LOD value of 0.049 mg/kg. Site 1, S2, S3 and S4 showed detectable concentrations of the element and this could be contributed from the dumped domestic wastes from the residents in the squatter camp. Dumped metals could be seen near the riverbanks close to the upper region of the sampling sites. Such materials might have contributed to the detectable levels of Fe in the Blood River. Shanbehzadeh *et al*. (2014) reported maximum concentration of Fe (1100 µg/L) during similar season as this study. The reported levels exceeded the concentrations of this current study.

The average pH of the Blood River during the high flow season was found to be 8.26, with the lowest pH recorded at site 1 (7.81) and the highest recorded at site 4 (8.93).

The pH values recorded for Blood River were within the recommended range (DWAF, 1996d; CCME, 1999). The pH of water varies because of industrial discharges, acid mine drainage, sewage effluents, runoffs and acidic rainfalls (Dallas and Day, 2004). The effluents change the water chemistry by modifying the pH levels.

4.7.2 Concentrations of potentially toxic elements in water collected from Blood River during low flow season

A graphical representation of the total concentrations of selected elements in water is given by figure 4.11. Furthermore, the concentrations of potentially toxic elements in water during low flow season are given in table 4.12, appendix B.

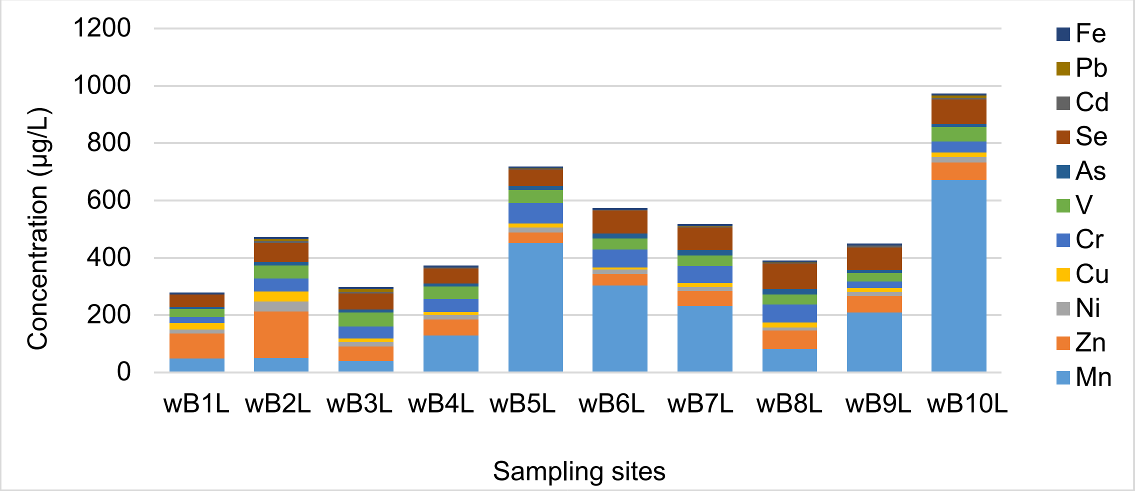

Figure 4.11: Concentrations of potentially toxic elements in water samples collected from Blood River during the low flow season

Possible sources of Zn along the Blood River include plating, galvanising, steel and iron workshops. Friberg *et al*. (1992) reported that Zn and Cd are used in conjunction in electroplating, hence it may be assumed that they would follow similar trends of industrial input into the river. However, this was not the case in this study as the distribution of the elements in water is not uniform across the river because of changes in the environmental conditions.

There are variations in the flow of the water, temperature, pH and possible source of the elements in the river. The concentration of Cd ranged between 0.767 µg/L (S7) and 1.88 µg/L (S4) while Zn ranged from 37.1 µg/L (S5) to 162 µg/L (S2).

The increase in the concentrations of the elements in the upstream indicates that it may likely be from the domestic waste and workshops from the informal settlements in the area.

The concentration of Zn measured in this study was lower than those reported from other South African rivers. Zinc levels of 230 µg/L, 1100 µg/L and 4400 µg/L were reported in South African rivers (Edokpayi *et al*., 2016).

The spatial distribution shows the sites having higher levels of Mn in the lower region as compared to the upper region of the river (Figure 4.11), which could indicate the anthropogenic source.

The concentrations of Mn ranged between 40.7 µg/L (S3) and 672 µg/L (S10). These levels were much lower than the levels reported from Umtata River (2040 µg/L) and Diep River (1300 µg/L) (Edokpayi *et al*., 2016).

The concentrations of Pb during the low flow season were found to be between 1.64 µg/L (S6) and 14.4 µg/L (S3). These levels were lower than the levels reported in the study of Umtata River (1110 µg/L) and Dzindzi River (50 µg/L) but higher than Diep River which was below LOD (Edokpayi *et al*., 2016). The spatial distribution of the element in the water did not show consistency in the levels from S1-S10. Weathering of bedrock and corrosion of metal pipes are some of the sources of element (Linder and Lundéhn, 2002).

Car brake linings release Cu and the corrosion from corrugated roofs and drain pipes also contribute to the concentration of Cu from urban runoffs (Linder and Lundéhn, 2002). The informal settlement surrounding the Blood River generates domestic wastes dumped to the vicinity of the river. High concentrations of Cu in S1, S2, S8, S9 and S10 could be associated with these activities. The concentrations of Cu during the low flow season ranged between 8.00 µg/L (S6) and 34.9 µg/L (S2). Compared to the reported concentrations from other rivers, the levels of Cu in water from the Blood River were low (Fatoki *et al*., 2002; Jackson *et al*., 2009; Edokpayi *et al*., 2014; Edokpayi *et al*., 2016).

Iron has been reported to be readily available into the environment, as such, high concentrations are always expected in the river systems. It has been reported to be highly abundant in freshwater environment than other elements due to its abundance in the earth (DWAF, 1996c).

Anthropogenic sources of Fe include mining of coal and steel productions (Moyo *et al*., 2015). In this study, the levels of Fe ranged from 0.119 to 1.82 µg/L.

These low levels of Fe in the water of Blood River, indicate that the source might be from natural conditions such as weathering, and not from anthropogenic sources. The concentration of Fe in water from Mvudi River was 2 786 times higher than the highest level of Fe detected in this study (Edokpayi *et al*., 2016).

Selenium ranged between 44.6 µg/L (S1) and 93.6 µg/L (S8) during the low flow season. Similarly, V concentrations ranged between 27.3 µg/L (S1) and 48.5 µg/L (S10). The levels of these elements increased further downstream. This indicates that during the flow of the water, the elements are carried from the entrance point in the upper region and accumulate in the lower region of the river.

The concentrations of As also increased from upstream to downstream. The high concentrations at downstream were adjacent to the leaking sewage, illegal dumping site and close to the sand mining areas. The highest level of measured As was 19.3 µg/L and the lowest was 6.30 µg/L. WHO (2011) attributed As levels with the existence of anthropogenic activities. The anthropogenic activities might have contributed to the levels of As in the river.

The levels of Ni during the low flow season ranged between 11.6 µg/L (S2) and 34.4 µg/L (S8) (Table 4.12). Nickel is commonly found in the production of stainless steel. Squatter camps in informal settlement surrounding the Blood River were dominant with steel houses. Corrosion of the housing materials might have caused the release of Ni into the water system.

Chromium concentrations have been reported in water from rivers in South Africa. The levels reported from the Dzindi River ranged between 30 and 100 µg/L (Edokpayi *et al*., 2014). Chromium levels as high as 593 µg/L were reported from the Mvudi River (Edokpayi *et al*., 2016). The levels in these studies were attributed to wastewater treatment plants, agricultural runoffs, emissions and poor waste disposal. These activities are also common in the proximity of the Blood River. In this study, Cr levels ranged between 21.8 to 72.1 µg/L. These levels were lower than the levels reported from Dzindi and Mvudi Rivers (Edokpayi *et al*., 2014; Edokpayi *et al*., 2016).

The average concentrations of potentially toxic elements in the water from Blood River during the low flow season were recorded in the following order: Mn > Zn > Se > Cr > V > Ni > Cu > As > Pb > Cd > Fe (Figure 4.11).

4.7.3 Comparison of the concentrations of potentially toxic elements in water collected from Blood River during the low and high flow seasons

The levels of potentially toxic elements in the water from Blood River during low and high flow seasons are compared in Figure 4.12.

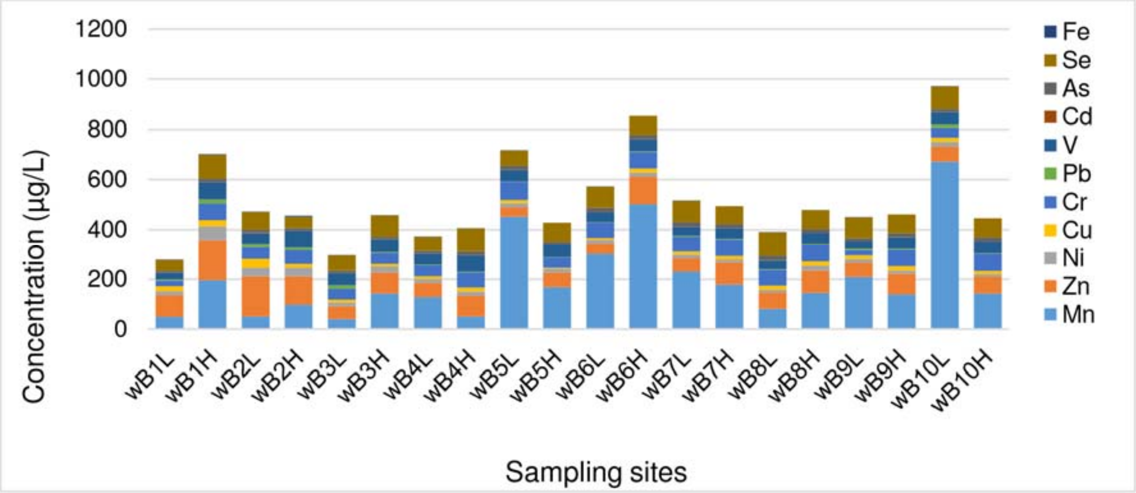

Figure 4.12: Seasonal comparison of the levels of potentially toxic elements in water samples collected from Blood River

The findings, in this study, revealed the seasonal variations of the concentrations of potentially toxic elements in water. The levels of Zn, Ni, Cr, V, Se, As and Fe in water during low flow season were higher than the levels measured in high flow season. Whereas, the levels of Mn, Cu, Cd and Pb were higher in samples from high flow season than in low flow seasons. These differences could be accounted by identifying the possible sources varying seasonally. Different sources such as illegal dumpsites, untreated sewage, and informal settlements may have contributed to the concentrations of the elevated levels in the water samples. A study by Olafisoye *et al*. (2013) reported that heavy metal concentrations in the water were higher in dry season compared to wet season. This is because of slow currents of water in dry seasons, which allows heavy metals to settle down and accumulate in water without turbulence (Okoro *et al*., 2015).

The average concentrations of potentially toxic elements in the water during the low flow season followed the order of Mn > Zn > Se > Cr > V > Ni > Cu > As > Pb > Cd > Fe. The concentrations of potentially toxic elements in the water during high flow season followed the order of Mn > Se > Zn > Cr > V > Ni > Cu > As > Pb > Cd > Fe.

4.7.4 Pseudo-total concentrations of potentially toxic elements in sediments collected from Blood River during high flow season

Determination of the total concentrations of potentially toxic elements in sediments was achieved after microwave-assisted acid digestion. The levels detected in sediments are simplified with the plot in figure 4.13 and the detailed concentrations are given in tables 4.13.

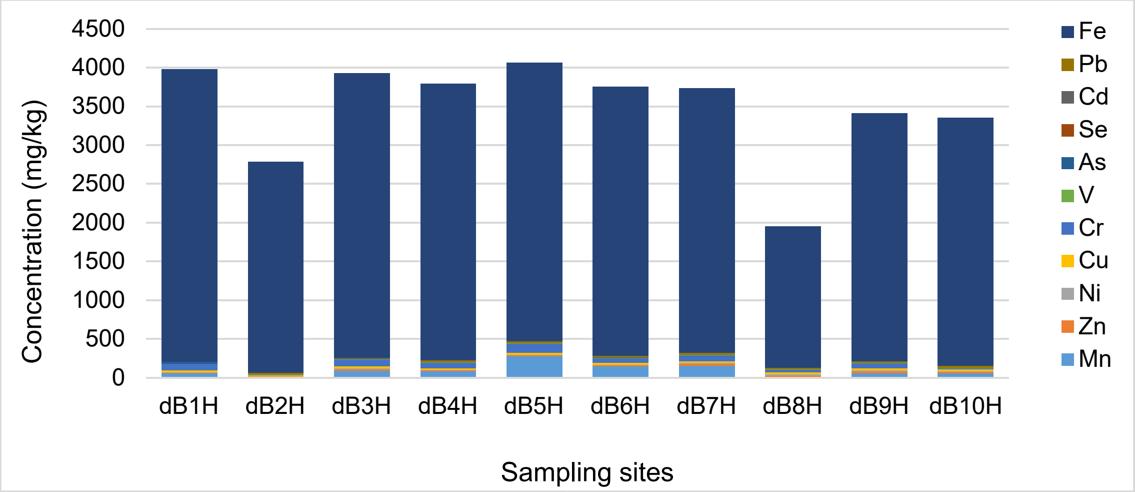

Figure 4.13: Pseudo-total concentrations of potentially toxic elements in sediments collected from Blood River during the high flow season

In sediments, the concentrations measured during the high flow season ranged between 18.9 and 284 mg/kg, 6.35 and 29.7 mg/kg, 3.84 and 42.4 mg/kg, 2.01 and 7.72 mg/kg, 12.3 and 56.2 mg/kg, 5.12 and 21.8 mg/kg, 0.290 and 1.85 mg/kg, 0.030 and 0.0588 mg/kg, 2.26 and 6.48 mg/kg and 1820 and 3770 mg/kg for Mn, Zn, Ni, Cu, Cr, Pb, V, Cd, As and Fe, respectively. Selenium was below the LOD value of 0.12 ng/g for all the sites. Concentrations of the potentially toxic elements varied from site to site and element to element. The concentrations were varied in the following order: Fe > Mn > Cr > Ni > V > Zn > Cu > Pb > As > Cd > Se.

Manganese is commonly used in the industries in the manufacturing of pigments and also in cathode material in standard and alkaline disposable dry cells and batteries. It is also used in metal alloys, particularly in stainless steels.

Other uses of it is in treatments for rust and corrosion prevention on steel (Zhang and Cheng, 2007). The high levels of Mn were recorded in the middle section of the river at S5 (284 mg/kg), S6 (158 mg/kg) and S7 (160 mg/kg).

The concentration of Mn decreased from upstream to middle stream and increased downstream. The higher levels reported downstream may be attributed to the rainfall followed by high river discharge from upstream environment, industrial effluents and municipal wastes. Shanbehzadeh *et al*. (2014) reported Mn levels ranging between 278 and 310 mg/kg in sediments of Tembi River, Iran, similar to the sampling season of this study. These levels were higher than the Mn levels recorded in this study. Difference in sources of Mn and environmental conditions in the Blood River compared to Tembi River contributed to the different concentrations.

The concentrations of Fe ranged from 1820 mg/kg at S8 to 3770 mg/kg at S1. These levels were lower than the mean concentration of 4371 mg/kg reported in the sediments of Mvudi River during the season similar to this study (Edokpayi *et al*., 2016). Availability of this element in high concentrations was attributed to the existence of wastewater effluents, runoff from agricultural soil and landfill sites close to the river.

Insoluble oxidized iron (rust) can give water a red tint, manganese oxide causes a black discoloration, and a combination of the two can yield a yellow-brown hue (DWAF 1996a). This might have been the cause of the yellowish red colour of the Blood River water illustrated by figure 3.4 (c). These is also consistent with the high levels of Fe and Mn in the water samples.

The smelting of nonferrous metals, burning of fossil fuels and municipal wastes are the major sources contributing to the presence of Zn in the environment. Zinc levels increased from upstream to further middle section of the stream. Maximum level of Zn was determined in S7 (29.7 mg/kg). This area was associated with direct leakage of untreated sewage and illegal dumpsites contributed by residential areas surrounding the middle to upper section of the river.

Sites 2 and 3 showed low concentrations, which may result from the flow of the river being carried the element further down the stream, thus reducing the levels upstream. Zinc concentrations between 21 and 24 mg/kg have been recorded in sediments from Tembi River during the similar sampling season to this study (Shanbehzadeh *et al*., 2014). These levels were attributed to effluents from residential and industrial area as well as abattoir wastewater.

Low concentrations of Cu were recorded at S2 (2.01 mg/kg) and S8 (2.13 mg/kg). This implies that despite the presence of sewage effluents in the area, the levels of Cu in the Blood River may be of less concern.

Nickel toxicity is generally low, but elevated levels have been reported to cause sublethal effects (Nussey *et al*., 2000). The levels of Ni ranged between 3.84 mg/kg and 42.4 mg/kg. There was random distribution in the levels of this element in the Blood River.

Anthropogenic sources of V include oil and coal combustion, steel alloy production and run offs from traffic (WHO, 1988). Site 5 was close to busy R567 roads were oil and diesel from vehicles might have been washed off to the river. As such, the highest concentration of V was recorded in S5. The concentrations of V in this study ranged from 5.12 mg/kg (S2) to 21.8 mg/kg (S5). Distribution of the element in the sediments varied from site to site.

Most of the lead entering aquatic ecosystems besides natural weathering of sulphide ores can be associated with suspended sediments (DWAF, 1996e). High levels of Pb were recorded at S7 (5.90 mg/kg) and S5 (6.48 mg/kg). Mohiuddin *et al*. (2012) associated Pb levels with effects from point and non-point sources such as leaded petrol, municipal and domestic runoffs, as well as atmospheric deposition. In this study, S5 and S7 were close to squatter camps, illegal dumpsites and leaking raw sewage. These sources could have contributed to the elevated levels of Pb.

The presence of Cd in soil or sediments is mainly from agricultural sources such as insecticides, fungicides and commercial fertilisers. Sludge may also contribute to the levels in sediments. Levels of Cd in sediments have been reported from Mvudi River during the wet season (Edokpayi *et al*., 2016).

The maximum concentration from their study was 0.233 mg/kg which was higher than the maximum level in this study (0.062 mg/kg). The levels of Cd, in this study were less than the LOD value of 0.030 ng/g in most of the sites and detected in S3, S4, S6 and S7.

Concentrations of As have been reported in sediments of Flag Boshielo Dam (Kekana, 2013). The maximum concentration was 1.8 mg/kg. This level was comparable to the maximum level of As in this study, which was 1.85 mg/kg (S6).

Chromium compounds are found in the environment, due to erosion of Cr-containing rocks, decayed animals and plants (DWAF, 1996c). High concentrations of Cr were recorded at S3 (51.8 mg/kg), S4 (41.9 mg/kg), S5 (56.2 mg/kg) and S7 (55.2 mg/kg). These sites are close to the densely populated informal settlement. Domestic wastes from the informal settlement and raw sewerage directly discharged into the river could be the sources of the metal.

4.7.5 Pseudo-total concentrations of potentially toxic elements in sediments samples collected from Blood River during low flow season

Accumulation of potentially toxic elements during low flow season in the sediments followed the order of Fe > Mn > Cr > Zn > V > Ni > Cu > Pb > As > Cd > Se. This trend is shown graphically by figure 4.14.

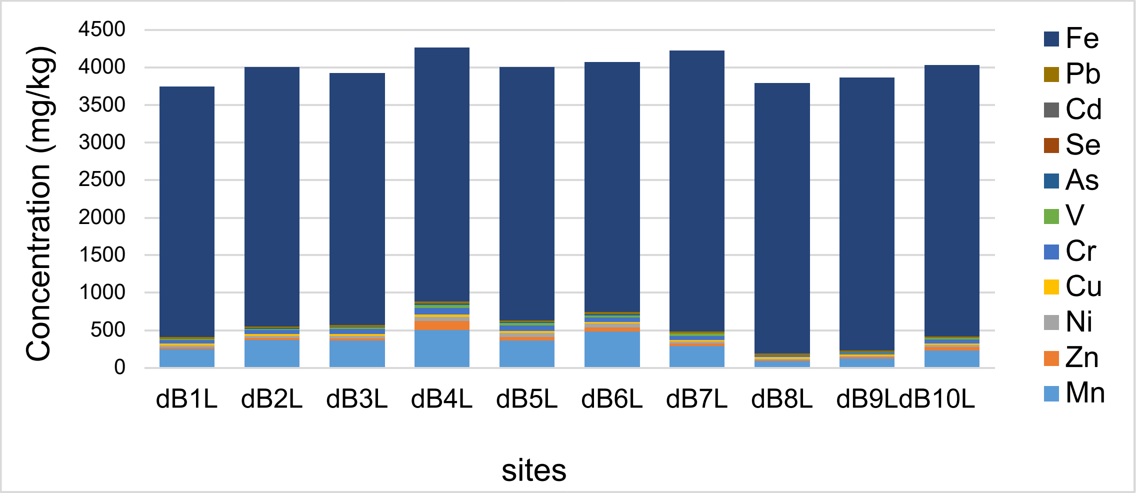

Figure 4.14: Pseudo-total concentrations of potentially toxic elements in sediments collected from Blood River during the low flow season

The concentrations of potentially toxic elements were between 99.3 and 509 mg/kg, 15.4 and 119 mg/kg, 14.4 and 51.6 mg/kg, 10.0 and 33.7 mg/kg, 23.2 and 89.3 mg/kg, 17.6 and 51.9 mg/kg, 1.20 and 5.52 mg/kg, <0.030 ng/g and 0.115 mg/kg, 3.26 and 23.2 mg/kg and 3330 and 3740 mg/kg for Mn, Zn, Ni, Cu, Cr, V, As, Cd, Pb, and Fe, respectively.

Selenium was below the LOD value of 0.12 ng/g. At current levels, Se and Cd won’t pose threat to the ecosystem. However, the availability of Cd in the river system is not desirable. It may accumulate in sediments and pose threat in future.

Iron levels are of concern due to its elevated levels in all the sites ranging from 3330 mg/kg to 3740 mg/kg. These levels were much lower than those reported from sediments of Olifant Rivers (Olifants River, Klein Olifants River, and Olifants River tributary) in Mpumalanga (Moyo *et al*., 2015). The level of Fe in their studied sites, ranged from 52300 to 67700 µg/g.

The source of Zn in water is through anthropogenic activities, although available naturally by weathering processes (Adriano, 2001). Occurrence of Zn in rivers is through industrial effluents, pesticides and insecticides, domestic wastes and metal plating (Adriano, 2001). The element has been reported to be toxic when in high concentrations in sediments (Olaniran *et al*., 2014). Mitileni *et al*. (2011) reported Zn levels ranging from 12.5 to 36.2 mg/kg in Mandzoro River, while Olafisoye *et al*. (2013) reported levels of Zn between 31.54 and 66.90 mg/kg from dumpsites. In this study, Zn levels were high in the middle parts of the sampling sites in the Blood River (S4, S5 and S7). The levels in the upper part (S1, S2 and S3) and lower areas (S8, S9 and S10) were within the range of reported Zn levels by Mitileni *et al*. (2011) and Olafisoye *et al*. (2013).

The major pathway of Cr is through landfill leachates or burning of Cr-containing dumps. Chromium levels between 26.98 and 46.58 mg/kg were reported in soil from dumpsites of Lagos state during dry season. These levels were in the same range with the detected levels, in this study, for most sites (23.2 to 89.3 mg/kg). Moyo *et al*. (2015) reported levels in the range of 186 to 333 mg/kg. These levels were much higher than the levels detected in sediments of Blood River.

Lead is toxic even at low levels. The concentration of Pb was high in the middle stream of the sampling sites (S4 (23.2 mg/kg), S5 (12.4 mg/kg), S6 (14.9 mg/kg) and S7 (18.5 mg/kg)). Lead as high as 630. 07 mg/kg was reported in dumpsites during the dry season (Olafisoye *et al*., 2013). This level was about 27 times higher than the level in this study.

In the low flow season, Ni concentration in sediments ranged from 14.4 to 51.6 mg/kg. These levels increased from S1 (33.7 mg/kg) to S4 (51.6 mg/kg). From S4 the concentrations decreased further down the river. The concentrations were lower than the levels reported by Olafisoye *et al*. (2013). The authors reported Ni level as high as 85.43 mg/kg.

The mean concentration of Mn in sediments from Olifants River was 1660 µg/g, with minimum and maximum concentrations of 1310 and 2310 µg/g, respectively (Moyo *et al*., 2015). These levels were much higher than the levels in this study, which ranged between 99.3 and 509 µg/g.

High levels of Cu were mainly distributed in the middle part of the sampling sites of the river (S4 (33.7 mg/kg), S5 (28.5 mg/kg) and S6 (28.1 mg/kg)). Relatively low concentrations of Cu were recorded in downstream (S7, S8 and S9). A similar trend was observed for the levels of V in the sediments. High concentrations of V were recorded at the middle sampling area of the river (S4 (51.9 mg/kg), S5 (49.8 mg/kg) and S6 (47.8 mg/kg)). The concentrations increased from S1 until S5 indicating that the elements have a tendency to accumulate in the sediments moving into depositional areas downstream (Zhang *et al*., 2012).

High concentrations of As may be attributed to anthropogenic activities such as Ascontaining fertilisers (Fu *et al*., 2014). In this study, As concentrations ranged from 1.20 to 5.52 mg/kg in sediments samples. Islam *et al*. (2015b) determined As levels in sediments during dry season. The average As level was 27 mg/kg during dry season of their study (Islam *et al*., 2015b). This level was 4.89 times higher than the level recorded during the low flow season of this study.

Overall, S4 showed the highest levels for all the elements quantified. Site 4 was found to be close to a dumping site for household wastes such as broken bottles, plastic bags and other wastes.

Thus, domestic wastes from the informal settlement could be the main sources of the elevated levels of potentially toxic elements at S4 and the middle part of the sampling sites in the Blood River. There was a significant difference in the levels of potentially toxic elements in the sediments from site to site ($p<0.05$).

The average pH of the water during low flow season in the Blood River was found to be 8.37 with the maximum pH recorded at site 4 (9.32) and the lowest recorded at site 5 (7.77). Table 4.11 in appendix shows the pH values in each site.

The average pH value was within the acceptable range (APHA, 1998). The pH values at sites 4 and 10 were slightly above the recommended range by DWAF (1996d) and APHA (1998).

4.7.6 Comparison of potentially toxic elements in sediments samples collected from Blood River during the high and low flow seasons

The average concentrations of potentially toxic elements in the sediments during high flow season followed the order: Fe > Mn > Cr > Ni > V > Zn > Cu > Pb > As > Cd >Se. The levels of potentially toxic elements in the sediments during low flow season followed the following order: Fe > Mn > Cr > Zn > V > Ni > Cu > Pb > As > Cd > Se. This trend is shown in Figure 4.15

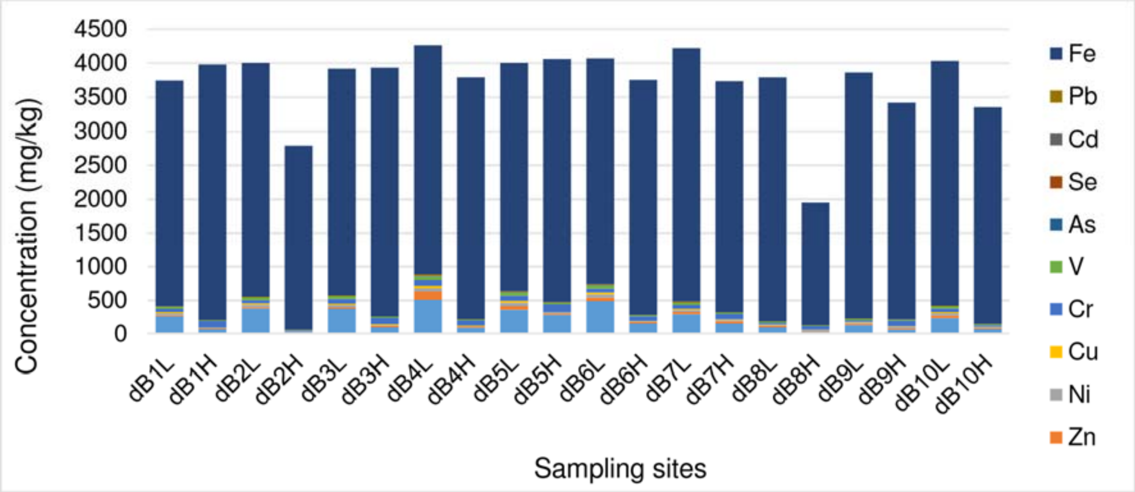

Figure 4.15: Seasonal comparison of potentially toxic elements in sediments collected from Blood River

The results, in this study, showed the levels of potentially toxic elements in the low flow season were higher than the levels recorded in high flow season for both sediments and water. Dilution factor accounts for the low levels of potentially toxic elements in the water body during high flow season (Islam *et al*., 2014).

Other studies also reported that low flow season levels exceeded high flow season levels (Mohiuddin *et al*., 2012; Edokpayi *et al*., 2016). Concentrations of all the elements in sediments during the low flow season were consistently higher than the concentrations quantified during high flow season. The maximum concentrations of Mn, Zn, Ni, Cu, Cr, V, As, Cd and Pb in the sediments during low flow season were found to be 1.79, 4.01, 1.21, 4.37, 3.00, 2.38, 2.98, 2.00, and 3.58 times higher than the levels in high flow season.

The concentrations of Fe quantified during high flow season were slightly higher than the levels during low flow season. The levels of potentially toxic elements in the sediments during low flow season were significantly higher than the levels measured during high flow season ($p<0.05$). This could be attributed to the variation in water capacity of the river where low flow resulted in precipitation of the elements, hence increasing its concentration (Islam *et al*., 2014; Islam *et al*., 2015b).

4.7.7 Comparison of potentially toxic elements in water and sediments samples collected from Blood River during the high flow season

A study by Mohammad-Salah *et al*. (2012) reported the correlation of potentially toxic elements in sediments with water quality standards. They identified sediments as the primary source of toxic elements in water by chemically leaching potentially toxic elements from water drainage basins and runoffs. The concentrations of the potentially toxic elements in water and sediments are compared in figure 4.16.

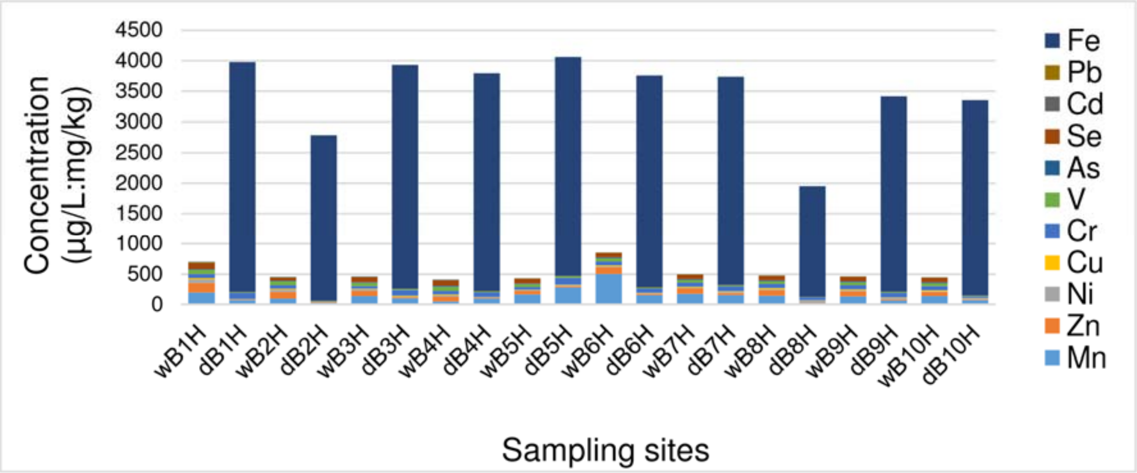

Figure 4.16: Comparison of the levels of potentially toxic elements in water (µg/L) and sediments (mg/kg) from Blood River during the high flow season

Concentrations of potentially toxic elements in the sediments were higher than the levels in the water. This is commonly caused by constant mixing and flow of the river (Mutia *et al*., 2012). Selenium was detected in water and sediments samples.

Iron was present in considerable levels in the sediments, although not detected in some of the sites in the water. The high levels of Fe in sediments justifies the sediments as a sink for metal contaminants. Re-adsorption from the water to the bottom sediments might have caused an increase in the concentration of the element in the sediments.

Appendix B, table 4.11 gives the minimum and maximum concentrations of each element. The average concentrations of potentially toxic elements in the water during high flow season followed the order of Mn > Se > Zn > Cr > V > Ni > Cu > As > Pb > Cd > Fe.

The concentrations of potentially toxic elements in the sediments during high flow season followed the order of Fe > Mn > Cr > Ni > V > Zn > Cu > Pb > As > Cd >Se. The downstream area indicated high concentrations for most of the elements. The levels of potentially toxic elements in the sediments were significantly higher than in water.

4.7.8 Comparison of potentially toxic elements in water and sediments samples collected from Blood River during the low flow season

The relationship between the concentrations of the elements in sediments and water is shown by figure 4.17. These concentrations are used to evaluate the trend of potentially toxic elements in water and sediments.

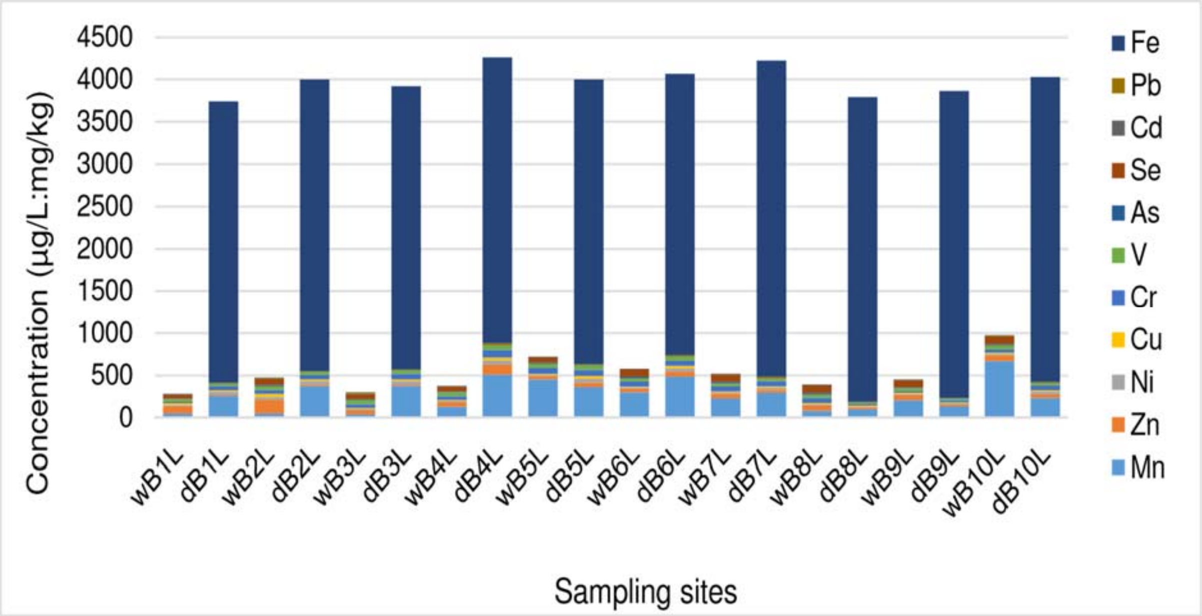

Figure 4.17: Comparison of the levels of potentially toxic elements in water (µg/L) and sediments (mg/kg) from Blood River during the low flow season

The concentrations of each element in water ranged from 40.7 to 672 µg/L, 37.1 to 162 µg/L, 11.6 to 34.4 µg/L, 8.00 to 34.9 µg/L, 21.4 to 72.1 µg/L, 1.64 to 14.4 µg/L, 27.3 to 48.5 µg/L, 0.767 to 1.88 µg/L, 6.30 to 19.3 µg/L, 44.6 to 93.6 µg/L and 0.119 to 1.82 µg/L for Mn, Zn, Ni, Cu, Cr, Pb, V, Cd, As, Se and Fe, respectively.

The concentrations of potentially toxic elements in the water during the low flow season followed the order: Mn > Zn > Se > Cr > V > Ni > Cu > As > Pb > Cd > Fe.

The concentrations of potentially toxic elements in the sediments during low flow season followed the order of Fe > Mn > Cr > Zn > V > Ni > Cu > Pb > As > Cd > Se. The levels in downstream part of the sampling sites found to be high for most of the elements.

Due to the turbulence created by increase of water flow, sediments are displaced and carried away from the riverbed. As summer starts, the rise in temperature causes evaporation during the end of the rain season which results in the rise in heavy metals concentration in water and finally in sediments because metal ions are transferred from water to sediments (Shanbehzadeh *et al*., 2014). This trend was similar in the water and sediments of this study. Potentially toxic elements determined in water were lower than in sediments.

Manganese concentrations were found to be significantly higher in water samples in comparison to all the elements and significantly elevated in the sediments.

Iron was found in maximum concentrations in sediments in all the sites monitored. The levels of potentially toxic elements quantified in water were lower than the levels in sediments.

The maximum concentrations of Mn, Pb, Se and Cd in sediments and water samples from Mokolo River during the low flow season had significant differences from most of the sites. There were no significant differences observed in the Zn, Cu, Cr, V and As mean concentrations among the sediments at the 95% confidence level for both high flow and low flow seasons. The level of Fe in water samples was significantly higher during low flow season than the level in high flow season at a 5% level of significance.

A study by Seanego (2014) reported maximum levels of Fe in fish of the Sand River, which is a tributary of Blood River. Although WHO (2011), have classified the element as a non-toxic element, its high levels can lead to hemochromatosis in people, which is a vague illness, identified by flu-like symptoms, aching of joints and fatigue. Seanego (2014) recommended that people be banned from catching fish in the Sand River as a food source. Furthermore, it was recommended that a study need to be undertaken to compare heavy metal concentrations during the dry and the wet season (Seanego, 2014), concluding that Fe was bioavailable.

Consequently, in this study selected potentially toxic elements were studied during the proposed seasons in Blood River. There was a significant variation ($p<0.05$) in the levels of potentially toxic elements in the water and sediments analysed seasonally. Hence, the levels of potentially toxic elements in sediments exceeds those detected in water.

4.8 COMPARISON OF CONCENTRATIONS OF POTENTIALLY TOXIC ELEMENTS IN SEDIMENTS AND WATER WITH MAXIMUM PERMISSIBLE LEVELS

The water quality standards and the sediments quality guidelines (SQGs) have been developed and used to identify potentially toxic elements of concern in aquatic ecosystems. This was done by providing the maximum permissible levels (MPLs) of the potentially toxic elements in the sediments and water systems based on applications of choice (MacDonald *et al*., 2000; Mohammad-Salah *et al*., 2012). Comparison with guideline values was based on maximum concentration levels of each element during the high and low flow seasons in water and sediments.

4.8.1 Comparison of concentrations of potentially toxic elements in water with water quality guidelines

The National Water Act 36 of 1998 guides the management of water resources in South Africa (DWAF, 1997). This act was implemented to regulate and emphasise the protection of water resources such as water quality to ensure sustainability of water systems (Gordon and Muller, 2010). The Department of Water and Sanitation is the custodian of South Africa's water resources. The department initiated the development of the South African Water Quality Guidelines, of which this is the second edition.

The South African Water Quality Guidelines serve as the primary source of information for determining the water quality requirements of different water uses and for the protection and maintenance of the health of aquatic ecosystems (DWAF, 1996c).

Different countries and organisations have established standards and regulations to prevent toxicity and risks associated with polluted water serving as the primary source of link to the food chain. The Canadian Council of Ministers of the Environment (CCME) is the primary intergovernmental forum for collective action on environmental issues of national and international concern. The council developed water and sediments quality guidelines for maximum permissible limits of potentially toxic elements in water and sediments to allow protection of aquatic ecosystems (CCME, 1999).

The World Health Organisation (WHO) is additionally one of the major bodies with the water safety plans to ensure human and aquatic safety. The WHO body regularly revises its standards for drinking water, aquatic survival and sediments quality (WHO, 2011). United States Environmental Protection Agency (US EPA) is an agency of the United States, which was created because of serving protection to human health and the environment. The agency works closely with industries to prevent and monitor pollution in the environment (US EPA, 1999).

In this study, comparison with MPLs for irrigation water was done to evaluate the suitability of water for irrigation purpose and to conduct risk assessment through food chain since water from both rivers is used for irrigation through agricultural activities. Based on ongoing research and review of local and international information, the water quality guidelines exist but are continuously updated and modified on a regular basis.

Comparison of potentially toxic elements in this study with MPLs set by different organisations was done based on maximum levels of each element during the high and low flow seasons in water. Table 4.5 shows comparison of the potentially toxic element concentrations in water obtained during this study with national and international guidelines.

Table 4.5: Comparison of maximum concentrations of potentially toxic elements in water (µg/L) with MPLs for drinking and irrigation water

Element	**Mokolo River**		**Blood River**		**DWAF Irrigation guidelines**	**SANS drinking water standards**	**WHO drinking water standards**	**CCME irrigation guidelines**
	HF	LF	HF	LF				
Mn	185	1160	502	672	20	100	500	200
Zn	21.6	25.6	160	162	1000	5000	123	200
Ni	2.28	6.18	56.8	34.4	200	150	70	200
Cu	5.74	17.5	24.1	34.9	200	1000	2000	-
Cr	15.4	15.4	69.2	72.1	100	100	25	-
Pb	174	5.41	17.1	14.4	200	20	10	200
V	6.80	6.80	68.6	48.5	100	200	-	100
Cd	0.986	0.990	1.80	1.88	10	5	3	5.1
As	6.19	6.19	16.3	19.3	100	10	10	100
Se	31.8	33.0	95.9	93.6	20	20	40	-
Fe	0.321	5.02	3.93	1.82	5000	200	-	5000

* (DWAF, 1996c); (SANS, 2005); (WHO, 2011); (CCME, 1999); (-): data not available

The comparison study was done to evaluate the levels of toxicity risks that may be encountered by people using the water from Mokolo and Blood Rivers. The seasonal distribution of elements in water from Mokolo River differed from Blood River. The highest concentrations in the water were recorded during the low flow season as compared to the high flow except for few elements such as Pb in water from Mokolo River and Ni, Pb, V, Se and Fe in water from Blood River.

Manganese has been reported to be naturally found in food sources such as leafy vegetables, nuts, grains and animal products (IOM, 2002). Furthermore, Mn intake from drinking water is normally lower than intake from food (WHO, 2011). The study by Howe *et al*. (2005) associated the levels with fertilizers and acid mine drainage which was comparable in this study with the existence of agricultural activities and industries surrounding the Mokolo River.

A maximum permissible level of Mn in water is not available in the WHO (2011) and CCME (1999) drinking water quality guidelines. The maximum Mn concentration in the water of Mokolo River was found to be 1160 µg/L during low flow and 185 µg/L during high flow seasons. The maximum Mn concentration recorded during the low flow season exceeded MPL of 20 µg/L (DWAF, 1996c), 100 µg/L (SANS, 2005), 500 µg/L (WHO, 2011) and 200 µg/L (CCME, 1999). In high flow season, the concentration of 185 µg/L exceeded the MPL set by DWAF (1996c) and SANS (2005), but lower than the limits set by WHO (2011) and CCME (1999). The maximum concentration of Mn in the water of Mokolo River indicates that using the water for irrigation of crops pose a serious threat to humans via food chain.

The maximum level of Mn in the water of Blood River during the low flow season was found to be 672 µg/L, which exceeded the maximum concentration of 502 µg/L recorded during the high flow season. The levels during both seasons were above the MPL of Mn in water, which set by DWAF (1996c), SANS (2005), WHO (2011) and CCME (1999). Comparison with these guidelines indicate that the water from Blood River pose a threat to humans through exposure by consumption of crops that irrigated with the water from the river.

Arsenic contamination is usually through anthropogenic and natural sources such as domestic wastewater, mining and smelting, fertilizers, chemical production and paint pigments (Cooksey, 2012). Some of these sources are found in the vicinity of Mokolo River. The maximum concentrations recorded for both high and low flow seasons, were 6.19 µg/L. This level was below the MPL of As in water set by DWAF (1996c) and CCME (1999) for irrigation purposes and SANS (2005) as well as WHO (2011) guidelines for drinking water.

The level of As in the water of Blood River during low flow season exceeded the high flow season concentration. The levels in both seasons were below the guidelines set by DWAF (1996c) and CCME (1999) for irrigation water but were higher than the standards set by SANS (2005) and WHO (2011) for drinking water. Exposure to humans via food chain through consumption of crops irrigated with the water is minimal. However, lack of control on disposal of potential contaminants may pose as a threat to humans in future.

In Mokolo River, the maximum level of Pb recorded during high flow season of 174 µg/L was extremely higher than that measured during the low flow season (5.41 µg/L). The level of Pb measured during the high flow season was lower than the guideline value set by DWAF (200 µg/L) and CCME (200 µg/L) for irrigation water but higher than the guideline values set by SANS (2005) and WHO (2011) for drinking water.

During the low flow season, the Pb level was below the SANS (2005), DWAF (1996c), WHO (2011) and CCME (1999) guideline values. Additionally, DWAF (1996b) reported association of Pb with industrial and municipal wastewater discharges as well as combustion of fuel. Oil spills from vehicles might have been washed off into the river system causing the increment in the Pb concentration of Mokolo River. Continuous monitoring is required to control the levels although exposure is minimal through consumption of food irrigated with the water at current level. A similar trend of Pb levels in the water of Mokolo River was observed in the water of Blood River. The maximum concentration during the high flow season of 17.1 µg/L exceeded the maximum concentration recorded during low flow season of 14.4 µg/L. The levels during both seasons were below the guidelines set by DWAF and CCME for irrigation water but higher than the level set by WHO (2011) for drinking water. Exposure to humans through consumption of crops irrigated with the water is of less concern. However, it is of threat through drinking of the contaminated water.

Iron concentrations were much lower than the MPLs set by DWAF, SANS and CCME in water from both rivers. This trend was observed in both high and low flow seasons. There is no standard guideline value for Fe in water set by (WHO, 2011). The levels of Fe are of less concern in the water from Mokolo and Blood Rivers.

The source of Cr in water systems has been reported to be industrial, domestic runoff and sewage sludge (Redfern, 2006). Blood River was mainly associated with domestic runoff and sewage sludge. In this study, high levels of Cr were detected in water from Blood River during the high and low flow seasons. The levels (69.2 µg/L in high flow season and 72.1 µg/L in low flow season) were below the MPL set by DWAF and SANS of 100 µg/L but exceeded the limit by WHO of 25 µg/L.

The maximum level of Cr in the water samples from Mokolo River for both seasons was 15.4 µg/L. This concentration was below the MPL of 100 µg/L set by SANS and DWAF, and 25 µg/L set by WHO. The measured level of Cr doesn’t show any risk.

A report by Kekana (2013) associated Ni release into the environment with burning of fossil fuels and further emphasized that Ni as insoluble in water and other common solvents. Thus, sediments and soil tend to tie up relatively large quantities of it. The levels of Ni in the water of both rivers for both high flow and low flow seasons did not exceed the limits of 200 µg/L by DWAF, 150 µg/L by SANS, 70 µg/L by WHO and 200 µg/L by CCME.

The report by DWAF (1996c) stated that the chemical behaviour of Cu, Pb, Zn and Cd was similar to the chemical behaviour of Ni. Sources of Zn into the aquatic environment are processes such as weathering and erosion from rocks and ores and industrial wastes, fertilizers, insecticides and pharmaceuticals. Although these sources are familiar around Mokolo River, the levels of Zn, Cu and Cd during the low and high flow seasons did not exceed the irrigation and drinking guidelines set by DWAF (1996), CCME (1999), SANS (2005) and WHO (2011). No MPL value is set for Cu by CCME. Exposure to humans of these elements through food chain and direct use of the water is of less concern. The same trend was observed in the water from Blood River where the maximum Cu and Cd levels were recorded.

However, the maximum concentrations of Zn in water samples from Blood River during high flow season of 162 µg/L and low flow season of 160 µg/L exceeded the WHO guideline value of 123 µg/L but were below the guideline values set by DWAF of 1000 µg/L, SANS of 5000 µg/L and CCME of 200 µg/L.

Vanadium compounds have various industrial applications and are used as catalysts in the chemical industry, in certain glassware and ceramic products (APHA, 1989). Vanadium can be expected to be retained in the soil surface layers due to sorption by the soil exchange complex, and in cultivated land it is expected to accumulate in the plough layer (DWAF, 1996c).

The maximum level of V in water samples from Blood River during high flow season was found to be 68.5 µg/L, which exceeded the low flow season maximum level of 48.5 µg/L. The maximum level of V in the water of Mokolo River for both high and low flow seasons were found to be 6.80 µg/L. This level was below the MPL set by DWAF (1996c), SANS (2005) and CCME (1999). No guideline value is set for V by WHO. Exposure to this element through the water from both rivers is of less concern.

The maximum levels of Se in the water of Mokolo River during the high flow and low flow seasons were 31.8 and 33.0 µg/L, respectively. These levels were above the DWAF and SANS guideline value of 20 µg/L but lower than the WHO guideline value of 40 µg/L. In the water from Blood River, the maximum levels during high flow season of 93.6 µg/L exceeded the low flow season level of 95.9 µg/L. These levels were higher than the MPL set by DWAF (20 µg/L), SANS (20 µg/L) and WHO (40 µg/L). No MPL value was set by CCME for Se in water.

The elevated levels of Se indicate risk to humans that this element can cause through drinking water or via food chain by consumption of crops irrigated with the water from the rivers. potentially toxic elements in the water of Blood River were at tolerable levels in comparison to the MPLs set by DWAF (1996c), SANS (2005), WHO (2011) and CCME (1999). Most of the elements were below the irrigation guideline values set by DWAF and CCME. Thus, proving to be of less danger to humans through consumption of crops irrigated with the water, except for Mn and Se which were slightly above the MPLs. Municipal and domestic wastes surrounding Blood River need to be controlled and monitored as they are potential sources of potentially toxic elements in the water system. The potentially toxic elements levels in the water from Mokolo River were within the recommended guideline values except for Mn and Se.

Even though some of the elements were recorded at below allowable limits, the fact that they are present in the water is a cause for concern since they can bio-accumulate in aquatic organism overtime causing health hazards though consumption of fish and irrigated crops.

4.8.2 Comparison of concentrations of potentially toxic elements in sediments with sediments quality guidelines

South Africa has no sediments quality guidelines. However, organisations such as WHO, US EPA and CCME have the sediments quality guidelines to regulate and protect aquatic environment.

The maximum concentrations of potentially toxic elements in sediments are compared with the MPLs of the elements in sediments as shown in table 4.6.

Table 4.6: Comparison of maximum concentrations (mg/kg) of potentially toxic elements in sediments with sediments quality guideline values

Mn	85.0	624	284	509		30	-
Zn	35.2	27.3	29.7	119	123	110	200
Ni	40.6	29.0	42.4	51.6	20	16	45
Cu	13.8	15.1	14.4	28.5	25	16	63
Cr	171	63.8	56.2	89.3	25	25	64
Pb	35.4	13.5	6.48	23.2	-	40	70
V	44.0	45.2	21.8	51.9	-	-	130
Cd	0.954	0.477	0.062	0.115	6	0.6	1.4
As	111	3.05	1.85	5.52	-	-	12
Se	<2.48	<2.48	<2.48	<2.48	-	-	1
Fe	3760	3950	3770	3740	-	30	-

*(WHO, 2011); (USEPA, 1999); (CCME, 2001); (-): data not available

Kekana (2013) reported the levels of elements in sediments and muscle tissue of two types of fish (*S. intermedius and L. rosae*) found at Flag Boshielo Dam, Olifants River System. In that study, Cd level was 0.3 mg/kg in the muscle tissue of *L. rosae* and was not detected in *S. intermedius* muscle tissue while the level was 0.8 mg/kg in sediments.

These levels were comparable with the levels in the sediments and muscle tissue of fish from Mooi River (Van Aardt and Erdmann, 2004). The maximum level of Cd, in this study, was found to be 0.954 mg/kg during high flow season and 0.477 mg/kg in the low flow season in sediments samples from Mokolo River.

The maximum concentrations of Cd recorded during high and low flow seasons were lower than the SQG set by WHO (2011) of 6 mg/kg and CCME (2001) of 1.4 mg/kg.

However, the concentration recorded during the high flow season (0.954 mg/kg) exceeded the SQG of 0.6 mg/kg set by US EPA (1999). Whereas, in the sediments of Blood River, Cd proved to be an element of less concern to the river system. The concentrations determined during the low flow season of 0.115 mg/kg and high flow season of 0.062 mg/kg were below the SQGs of 6 mg/kg (WHO, 2011), 0.6 mg/kg (US EPA, 1999) and 1.4 mg/kg (CCME, 2001).

High levels of Mn were reported in the sediments of Mokolo River during the high and low flow seasons. Manganese level in sediments that measured during high flow season was found to be 2.8 times higher than the limit set by USEPA (1999) of 30 mg/kg although it is lower than the concentration recorded during the low flow season of 624 mg/kg. Manganese can bio-accumulate in lower organisms but not in higher organisms, and bio magnification in food chains is not expected to be significant (ATSDR, 2008). No SQGs by WHO (2011) and CCME (2001) are available for Mn. In the Blood River, Mn in the sediments samples found to be 284 mg/kg during the high flow season and 509 mg/kg during low flow season. These concentrations exceeded the level set by US EPA (30 mg/kg).

Zinc in sediments of Mokolo River had maximum concentrations of 35.2 and 27.3 mg/kg during high and low flow seasons, respectively. These levels were higher than the levels recorded in sediments from Hout River Dam which was below the detection limit of 0.004 mg/kg for both low and high flow seasons (Sara *et al*., 2013). However, there are studies in the Fumani Mine Tailings Dam (96.4 mg/kg) which recorded levels higher than our recent study (Ogola, 2010).

Zinc levels recorded during both seasons were below the SGLs of 123 mg/kg, 110 mg/kg and 200 mg/kg, set by WHO (2011) US EPA (1999) and CCME (2001), respectively.

Zinc levels in the sediments of Blood River for both seasons were below WHO (123 mg/kg) and CCME (200 mg/kg) sediments quality guideline values.

The maximum level was 119 mg/kg during low flow season, which was slightly above the US EPA guideline of 110 mg/kg. The domestic wastes, could have caused the elevated levels of Zn in the sediments of Blood River.

Nickel is normally released into the environment through the burning of fossil fuels (WHO, 2007). Average Ni concentrations up to 47 mg/kg have been reported in the sediments of Flag Boshielo Dam. Nickel has been reported to be more mobile in sediments under acidic conditions hence indicating its ability to seep into groundwater.

A report by ATSDR (2005) stated that plants can take up Ni and thus exposure to humans through food chain is possible. In this study, high levels of Ni were reported in the sediments from Mokolo and Blood Rivers during high and low flow seasons. The levels were 40.6 and 29.0 mg/kg during high and low flow seasons in sediments samples collected from Mokolo River. These levels were above the SQGs of 20 mg/kg set by WHO (2011) and 16 mg/kg set by US EPA (1999), but lower than 45 mg/kg set by CCME (2001).

The Ni concentration reported in the sediments samples from Blood River during the low flow season exceeded the high flow season level. The concentration during the low flow season of 51.6 mg/kg exceeded the SQGs set by WHO, USEPA and CCME. During the high flow season, the maximum measured concentration was 42.4 mg/kg, which exceeded the guideline values set by WHO, US EPA but lower than the guideline value set by CCME. The high levels of Ni in sediments from both rivers could pose risk to the aquatic organisms.

The concentrations of Fe in sediments from Mokolo River were 3950 mg/kg and 3760 mg/kg in low and high flow seasons, respectively. Similarly, high levels of Fe were measured in sediments from Blood River in both seasons. The levels were 3740 mg/kg in low flow season and 3770 mg/kg in high flow season. The level of Fe as high as 42834 mg/kg was reported in the sediments of Flag Boshielo Dam, Olifants River System (Kekana, 2013).

The levels of Fe in sediments, in current study, from both rivers were slightly lower than those reported from Flag Boshielo Dam, Olifants River System. Iron exists as an element that is naturally released into the environment from weathering of sulphide ores and igneous, sedimentary and metamorphic rocks (DWAF 1996c).

Anthropogenic activities may have contributed to the elevated levels of Fe in the river systems. Iron can be introduced into the aquatic environment by anthropogenic activities such as burning of coal, acid mine drainage, mineral processing, sewage, landfill leachates and the corrosion of iron, among others (Xing *et al*., 2006; Xing and Liu, 2011; Moyo *et al*., 2015).

These sources contribute to the maximum levels of Fe in the Mokolo and Blood Rivers, which are surrounded by coal-fired power stations and mines as well as sewage and landfill leachates, respectively. The maximum levels of Fe in the sediments of both rivers exceeded the SQGs set by USEPA (30 mg/kg). No SQGs are set by WHO (2011) and CCME (2001) for Fe.

Acid mine drainage and sewage leakage into the Olifants River catchment area were some of the anthropogenic activities that contributed to the elevated levels of Fe in the river system (Claassen *et al*., 2005). The maximum levels of Fe in the Blood River may have been caused by seepage of untreated sewage into the river system. Maximum levels of Fe up to 3770 mg/kg and 3740 mg/kg were recorded during the high and low flow seasons, respectively. These levels were extremely higher than the SQG set by US EPA (30 mg/kg).

Chromium levels in the sediments samples collected from Mokolo River had maximum levels of 171 mg/kg and 63.8 mg/kg during high and low flow seasons, respectively. The measured levels of Cr in the sediments were much higher than the maximum level reported in sediments from Swartkop River, South Africa that found to be 30 mg/kg (Nel, 2014). The levels of Cr in both seasons exceeded the SQG value of 25 mg/kg set by WHO and US EPA. For high flow season the level of Cr in sediments exceeded the SQG value set by CCME, whereas during the low flow season, the recorded level of 63.8 mg/kg, was approximately equal to the CCME SQG value of 64 mg/kg. The high levels of Cr in sediments could pose the risk to the ecosystem.

In the sediments of Blood River, the recorded Cr level during the low flow season of 89.3 mg/kg was higher than the guideline values set by WHO (2011), US EPA (1999) and CCME (2001). The maximum level recorded during the high flow season of 56.2 mg/kg exceeded the guideline values set by US EPA and WHO but the level was below the SQG value set by CCME.

These levels in the sediments are likely to increase considering the current trend of uncontrolled disposal of domestic waste near the river.

Copper is moderately soluble in water, binds easily to sediments and organic matter, and can bio-accumulate (DWAF, 1996c). Blood River receives untreated sewage effluents and the Cu from this source may bind to the organic matter that could contribute to the accumulation of Cu in the sediments. The level of Cu during the low flow season (28.5 mg/kg) exceeded the level during the high flow season (14.4 mg/kg) in Blood River sediments. The concentration during the low flow season exceeded the SQGs set by WHO of 25 mg/kg and US EPA of 15 mg/kg but was below the guideline value set by CCME of 63 mg/kg. The maximum level of Cu in sediments in high flow season was below the guideline values by WHO (2011), US EPA (1999) and CCME (2001).

The level of Cu in the sediments of Mokolo River was approximately comparable in both seasons although the level in sediments during the high flow season (15.1 mg/kg) was higher than the level during the low flow season (13.8 mg/kg). Despite the levels being lower than the SQG values set by WHO (25 mg/kg), USEPA (16 mg/kg) and CCME (63 mg/kg), the element may accumulate in the sediments depending on future contamination.

The levels of V and Pb in sediments for both high and low flow seasons in both Rivers were all below the SQG values set by US EPA and CCME. Continuous monitoring of the levels of these metals is required since the levels of Pb and V could elevate due to the ongoing activities in and around the rivers.

The existence of As in the river system may be associated with the release of As from arsenic-containing pesticides. The maximum level recorded during the high flow season of 111 mg/kg was in the sediments sample from Mokolo River, which could be associated with agricultural activities.

This level exceeded the SQG value set by CCME of 12 mg/kg but the low flow season level of 3.05 mg/kg was below the CCME SQG value. In the sediments of Blood River, the levels of As recorded during both seasons, were below the CCME SQG value of 12 mg/kg. No SQGs set by WHO (2011) and US EPA (1999) for As.

CHAPTER 5

SEQUENTIAL EXTRACTION OF POTENTIALLY TOXIC ELEMENTS IN SEDIMENTS:

RESULTS AND DISCUSSION

5.1 INTRODUCTION

In this study, sequential extraction was employed to study the abundance and distribution of potentially toxic elements in sediments by subjection to different reagents under certain operating conditions of sequential extraction procedure. This was done to determine the distribution of potentially toxic elements in the exchangeable, reducible, oxidizable and residual fractions.

Although this method is more time consuming than pseudo-total metal analysis, it provides information on the physical and chemical factors affecting the mobilization, bioavailability and transportation of the potentially toxic elements in sediments (Ure *et al*., 1993; Hanay *et al*., 2008). Collectively, these fractions constitute the pseudo-total concentrations of potentially toxic elements in sediments samples, thus suitable for comparison studies. The sequential extraction method employed following the protocol summarised in table 3.4 will be referred to as Method B in this section.

5.2 SEQUENTIAL EXTRACTION OF POTENTIALLY TOXIC ELEMENTS IN SEDIMENTS APPLYING MODIFIED BCR PROCEDURE

5.2.1 Determination of limit of detection for modified BCR sequential extraction procedure

The reagent blanks were prepared following the method B employed for sequential extraction. From standard deviations of the blank concentrations, the LODs were calculated as three times the standard deviation from each fractionation stage. Table 5.1 shows the LODs for the four fractions following method B by ICP-AES.

Table 5.1: Limit of detection in mg/kg obtained for the sequential extraction procedure by ICP-AES

Element	Stage 1	Stage 2	Stage 3	Residual
Cd	0.008	0.038	0.0064	0.125
Cr	0.218	0.040	0.0064	0.041
Cu	0.008	0.013	0.0742	0.022
Fe	0.087	0.008	0.0573	0.005
Ni	0.017	0.004	0.0658	0.003
Pb	0.276	0.169	0.503	0.026
Zn	0.023	0.004	0.0806	0.041

The LODs for the exchangeable (stage 1) ranged from 0.008 to 0.276 mg/kg. The LODs for the reducible (stage 2), oxidizable (stage 3) and residual (stage 4) ranged from 0.004 to 0.169 mg/kg, 0.0064 to 0.503 mg/kg and 0.003 to 0.125 mg/kg, respectively. The LODs reported by Ciceri *et al*. (2008) were 0.066, 0.23, 0.018, 0.41, 0.026 and 0.0059 mg/kg in the first stage, 0.16, 0.18, 0.016, 0.11, 0.0069 and 0.0010 mg/kg in the second stage and lastly 0.50, 0.14, 0.023, 0.76, 0.0076 and 0.043 mg/kg in the third stage for Cr, Ni, Cu, Zn, Cd and Pb, respectively. These values were found to be higher than the LODs obtained in this study.

5.3 VALIDATION OF THE SEQUENTIAL EXTRACTION PROCEDURE

Validation and evaluation of the method was carried out by comparing the measured and certified values for BCR 701 certified reference material. The percent recoveries for most analytes were within the acceptable ranges for all three stages. Table 5.2 shows the percent recoveries obtained for the analysis of the sediments certified reference material (BCR 701) by ICP-AES following method B.

Table 5.2: Concentrations and percent recoveries of potentially toxic elements in the sequentially extracted sediments certified reference material (BCR 701) by ICP-AES

Fraction	**Element**	**Measured value**	**Certified value**	**Percent recovery**
		Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	(%)
F1	Cd	7.13±0.25	7.30±0.40	98
	Cr	2.69±0.050	2.26±0.16	119
	Cu	52.7±3.0	49.3±1.7	107
	Ni	13.2±0.28	15.4±0.9	86
	Pb	2.87±0.092	3.18±0.21	90
	Zn	223±3.5	205±6.0	109
F2	Cd	3.05±0.36	3.77±0.28	81
	Cr	50.3±1.0	45.7±2.0	110
	Cu	126±4.0	124±3.0	101
	Ni	25.8±2.3	26.6±1.3	97
	Pb	107±18	126±3.0	85
	Zn	126±9.3	114±5.0	111
F3	Cd	0.218±0.010	0.27±0.06	81
	Cr	110±4.0	143±7.0	77
	Cu	63.1±2.1	55.0±4.0	115
	Ni	18.2±0.78	15.3±0.9	119
	Pb	9.25±0.49	9.30±2.0	99
	Zn	46.5±2.7	46.0±4.0	101

The percent recovery of the elements in extraction stages ranged from 81-119% which shows the reliability of the applied method, with an exception of Cr (77%) in the third fraction. The low percent recovery observed for Cr could be attributed to low extraction efficiency of the reagent employed in oxidizable fraction.

The sequential extraction method was further validated by comparing the sum of concentrations of potentially toxic elements extracted by four stages in sequential extraction procedure with the results of the pseudo-total metal concentrations (Nemati *et al*., 2011).

The percent recovery of the sequential extraction method was calculated as follows, and the results are summarised in tables 5.10-table 5.19, in Appendix C where F1, F2, F3 stand for the first, second and third fractions, respectively, and R stands for residual fraction.

$$\text{Recovery (\%)} = \frac{[F1+F2+F3+R]}{Pseudo-total\ digestion} \times 100$$

The results obtained using both methods showed good agreement.

5.4 FRACTIONATION OF POTENTIALLY TOXIC ELEMENTS IN SEDIMENTS SAMPLES EMPLOYING BCR PROCEDURE

The concentrations of potentially toxic elements in different fractions of sediments samples collected from Blood River in low flow season were determined using ICPAES. The detailed concentrations obtained during determination of the elements in the fractionation stages are given in the tables 5.10 to table 5.19 in Appendix C. The results are presented in figures in this section.

Figure 5.1 shows the levels of Cd in different fractions of sequentially extracted sediments following method B. The sum of the concentrations which are obtained by adding the concentrations of Cd in the four stages of sequential extraction procedure (Method B) are compared with pseudo-total concentrations determined after microwave-assisted acid digestion (Method A). These results are shown in figure 5.2.

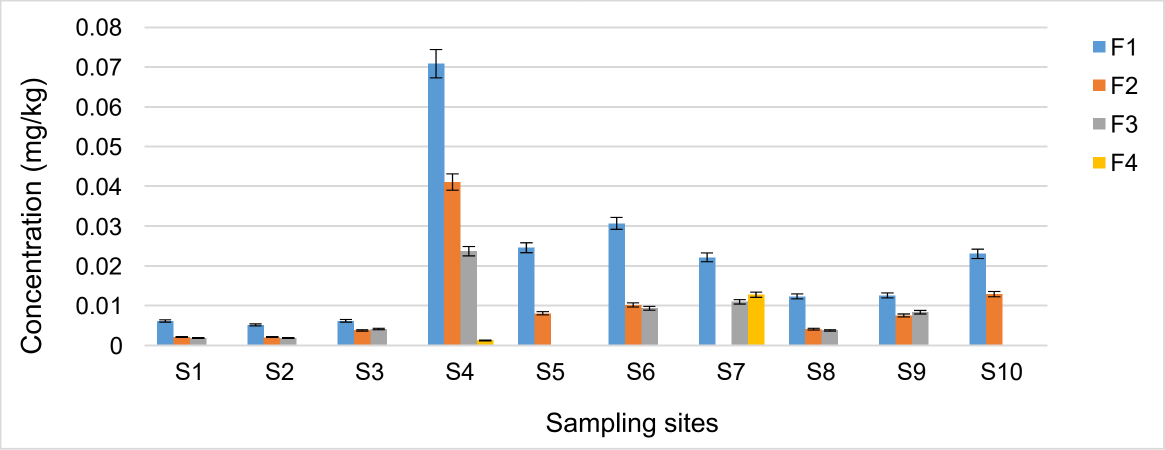

Figure 5.1: Levels of Cd in different fractions of sediments samples obtained employing method B

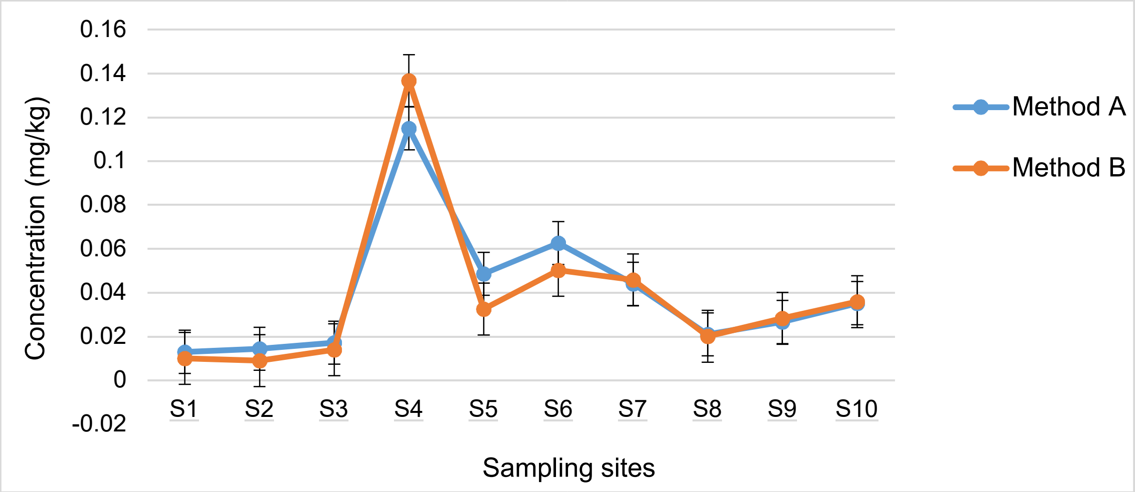

Figure 5.2: Comparison of the sum of concentrations of Cd in different fractions (method B) and pseudo-total concentrations for digested sediments samples (Method A)

The level of Cd in the sequentially extracted sediments need to be monitored due to its high toxicity since it has been reported as one of the most labile elements (Marmolejo-Rodrıguez *et al*., 2007). The distribution order of Cd in the sediments of Blood River was found to be mainly in the exchangeable fraction followed by reducible, oxidizable and then the residual fraction. In this study, Cd was found to be the mobile element as its highest portion (44-75%) was in exchangeable fraction at all sites. The results were in agreement with the study by Marmolejo-Rodrıguez *et al*. (2007) and Díaz-de Alba *et al.* (2011), which showed Cd as among the most labile elements in their studies. Additionally, Delgado *et al*. (2011) and Kong *et al*. (2015) also reported high percentages of Cd in the first fractionation.

A noticeable portion of Cd was found in reducible fraction (20-36%) except at S7, and the oxidizable fraction (17-29%) at sites S1-S9 excluding S5 and S10. Relatively low to no Cd measured in the residual fraction except site 7 (Figure 5.1). A similar trend was reported by Li *et al*. (2007) where Cd was contributed by untreated domestic waste, roadside run offs, agricultural and industrial activities in the study area. Such activities may have contributed to the presence of the element in the sediments of the Blood River. Other studies reported Cd to be effectively bound to organic matter (Sundaray *et al*., 2011; Zhang *et al*., 2015).

A study conducted by Shozi (2015) emphasised Cd as a mineral that is very rare in the Earth's crust and associated its presence in the rivers with anthropogenic activities. This statement is in good agreement with the highest concentration of Cd obtained in this study at site 4. Site four (S4) of the Blood River was found to be adjacent to an illegal dumping site and close to an open cattle grazing land. The wastes associated with such activities may have contributed to the elevated levels of Cd in the sediments. For most of the sampling sites, Cd in the residual stage was below the LOD value of 0.125 mg/kg. A more sensitive analytical instrument such as the ICP-MS may be used to detect the concentration at ppb levels to account for the existence of the element at low levels. Fractionation of Cd followed the pattern F1>F3>F2>F4.

An internal check was performed on the results of the sequential extraction by comparing the sum of the extracted concentrations from the four stages with the pseudo-total concentrations obtained after digestion. A good agreement was observed between the two methods (Figure 5.2).

Figure 5.3 shows the levels of Cr in different fractions of sequentially extracted sediments measured by method B. Moreover, the sum of the concentrations which are obtained by adding the concentrations of Cr in the four stages of sequential extraction procedure (Method B) are compared with pseudo-total concentrations determined after microwave-assisted acid digestion (Method A).

The correlation is shown in figure 5.4. The concentrations of Cr from each extraction step are shown in tables 5.10 - table 5.19, appendix C.

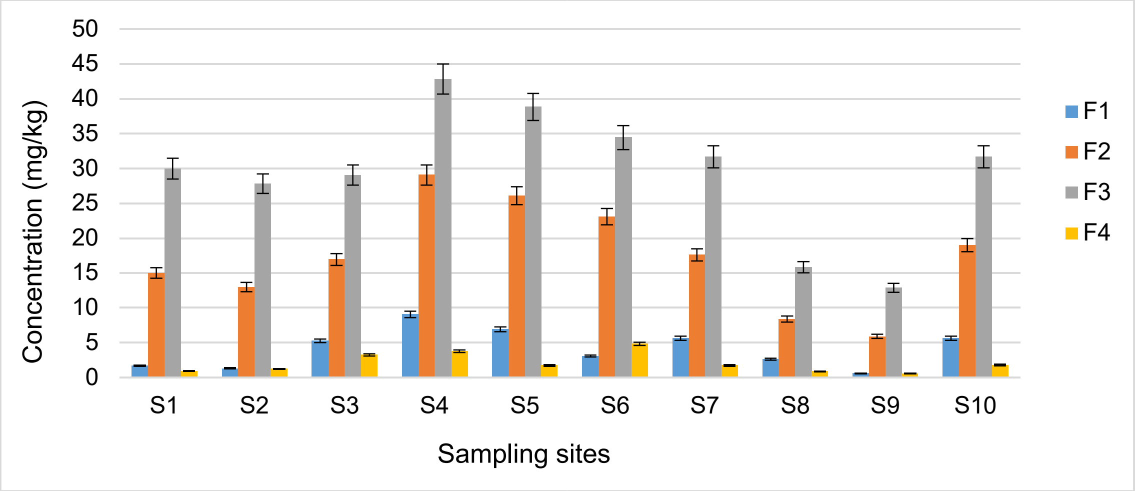

Figure 5.3: Levels of Cr in different fractions of sediments samples obtained employing method B

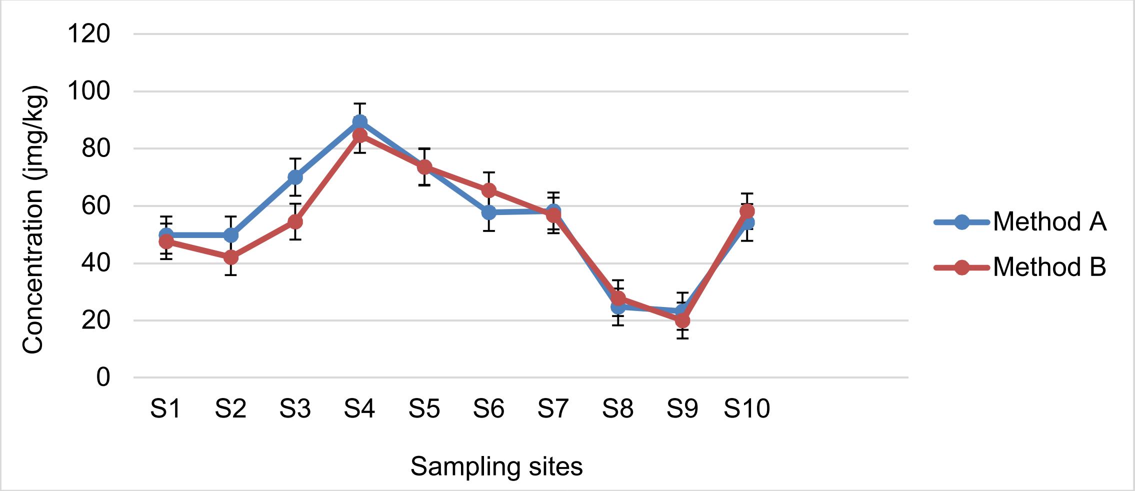

Figure 5.4: Comparison of the sum of concentrations of Cr in different fractions (method B) and pseudo-total concentrations for digested sediments samples (Method A)

The partitioning pattern of Cr was similar in all the sites except S6. The distribution of Cr in this study indicated that Cr was mostly dominant in the oxidizable fraction (5165%) similar to the results reported by Passos *et al*. (2010) and Borgese et al. (2013).

A noticeable fraction of Cr was found in F2 (30-35%), F1 (3-11%) and F4 (2-7%) fractions, respectively. However, the amount of Cr in fractions 1, 2 and 4 was less than 37% at all sites indicating the soil development source of Cr (Borgese *et al*., 2013).

The study carried out reported that Cr levels in the exchangeable fraction were favourable with acidic pH levels than alkaline levels (Chaudhary and Banerjee, 2004).

However, this is not the case in this study since the exchangeable Cr was higher in site 4 and 10 than in all the sites for the basic pH levels recorded at S4 of 9.32 and S10 of 9.29. These concentrations are contributed from the metal bound to organic matter in sediments, which may elevate the levels of Cr in the water. The analysis of BCR 701 sediments certified reference material showed the fractionation pattern of Cr in the order: F3>F2>F1. This pattern was consistent with the fractionation pattern of Cr in this study in sediments samples (F3>F2>F1>F4).

Reliability of the results obtained in the sequential extraction method B was checked by comparison with the pseudo-total digestion concentrations (Method A). A good agreement between the two methods was shown by the correlation in figure 5.4.

The partitioning pattern of Cu in the reducible fraction was highly favourable in all the sites except site 4. Overall, the reducible fraction (37-53%), was the primary association for Cu, followed by the F1 (21-33%), F3 (20-24%) and lastly the F4 (211%). The fractionation pattern followed the trend F2>F1>F3>F4. This trend was shown graphically in figure 5.5.

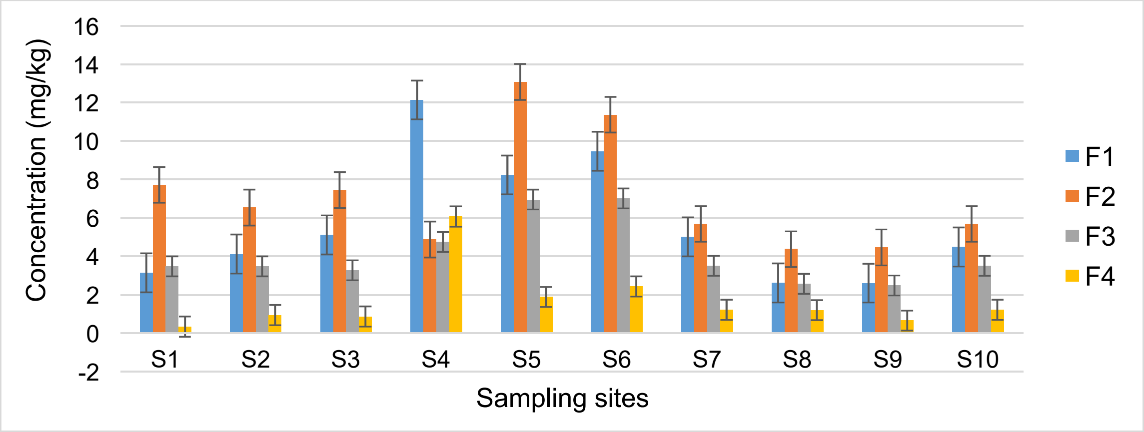

Figure 5.5: Levels of Cu in different fractions of sediments samples obtained employing method B

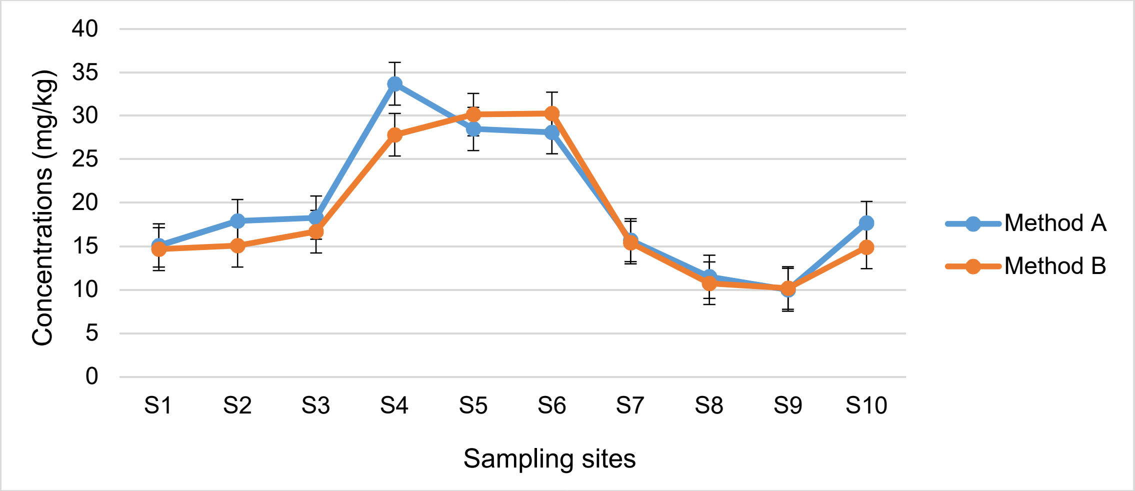

Figure 5.6: Comparison of the sum of concentrations of Cu in different fractions (Method B) and pseudo-total concentrations for digested sediments samples (Method A)

A different fractionation pattern was observed in site 4 where Cu was dominant in the exchangeable fraction (44%) followed by the residual fraction (22%), reducible fraction (18%) and finally the oxidisable fraction (17%). Wali *et al*. (2014) and Davutluoglu *et al*. (2011), reported the dominance of Cu in the exchangeable and residual fractions in their studies. Wali *et al*. (2014) reported Cu fraction of 39.70% in the exchangeable fraction and 60.30% in the residual fraction.

Although the sum of concentrations from the extracted Cu in some of the sites was lower than the concentration from pseudo-total concentration obtained after digestion, reliability and efficiency of method B was shown by the good recoveries obtained as indicated by the tables in Appendix C. The recoveries ranged between 83% in site 4 (Table 5.12) and 108% in site 6 (Table 5.15). Reliability of the results obtained, in this study, was shown by the partitioning trend of Cu in the BCR 701 sediments certified reference material. The fractionation pattern was similar to the findings of this study, following the order: F2>F3>F1 with an additional stage F4 of this study showed lowest fraction.

Figure 5.7 shows the partitioning trend of Ni in the sediments from S1 to S10. Comparison of methods A and B is shown in figure 5.8.

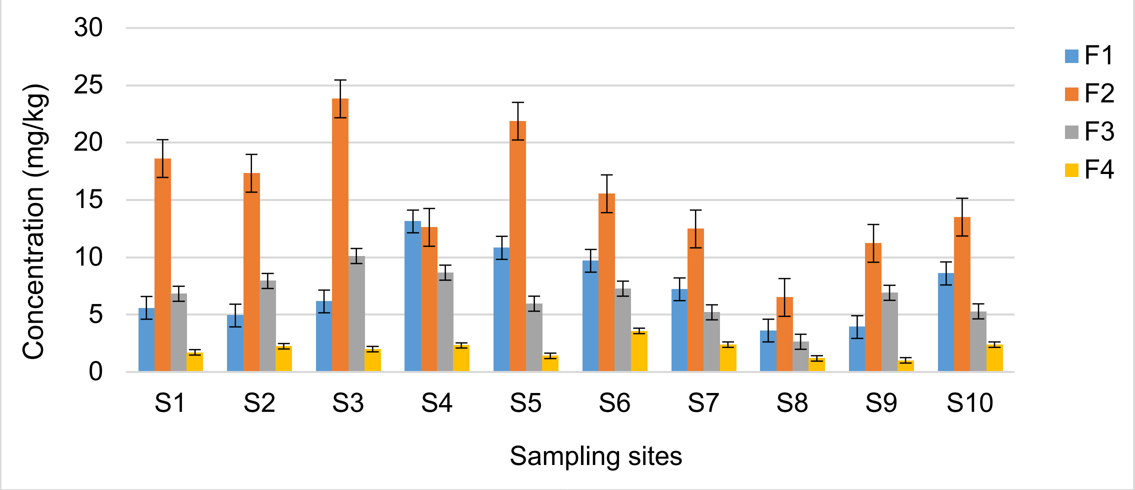

Figure 5.7: Levels of Ni in different fractions of sediments samples obtained employing method B

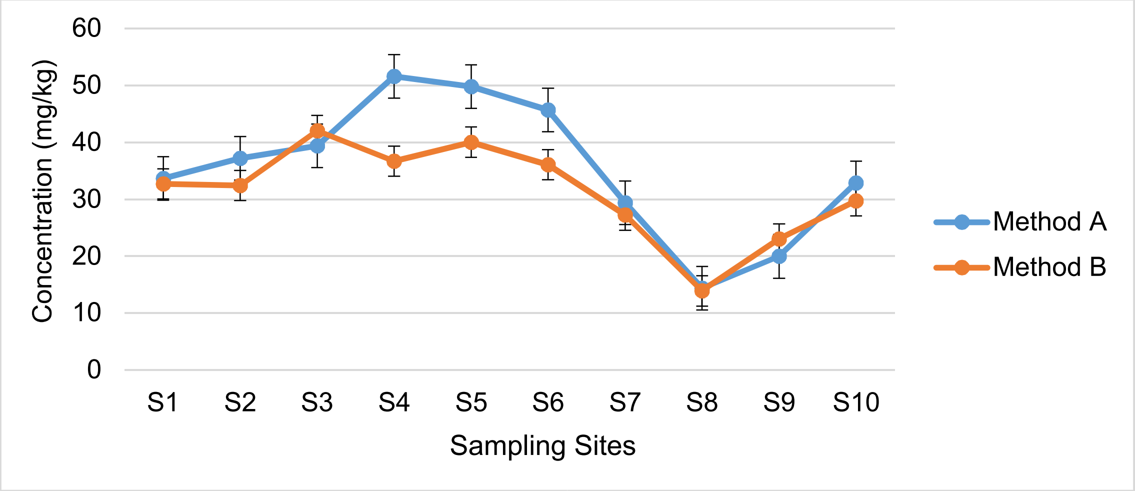

Figure 5.8: Comparison of the sum of concentrations of Ni in different fractions (method B) and pseudo-total concentrations for digested sediments samples (Method A)

The proportion of Ni concentration (43-57%) was measured in the reducible fraction. A noticeable amount (17-29%) was found in the exchangeable fraction followed by oxidizable fraction (19-24%) and residual fraction, which contained the lowest portion (4-10%). The elements associated with the residual fraction are likely to be incorporated in alumina-silicate minerals and so are unlikely to be released to porewaters through dissociation (Tüzen, 2003).

Davutluoglu *et al*. (2011) and Favas *et al*. (2011) reported the dominant presence of Ni in the residual fraction compared to the other fractions.

The concentrations obtained from addition of the extracted fractions were lower than the pseudo-total concentration from the digestion method employed. The pattern was observed in figure 5.8. The comparison of the two methods showed good agreement in majority of the sites except for the low percent recoveries at site 4 (71%) table 5.12 and site 6 (79%) table 5.15, appendix C. The fractionation pattern of Ni in this study followed the order F2>F1>F3>F4. Furthermore, the reliability of the method B was shown by similarity in the partitioning trend of this method with the trend observed for BCR 701 following the order: F2>F1>F3.

Figure 5.9 shows the partition of Pb in the sediments from S1 to S10. Reliability of the method B is checked by comparison with method A (figure 5.8).

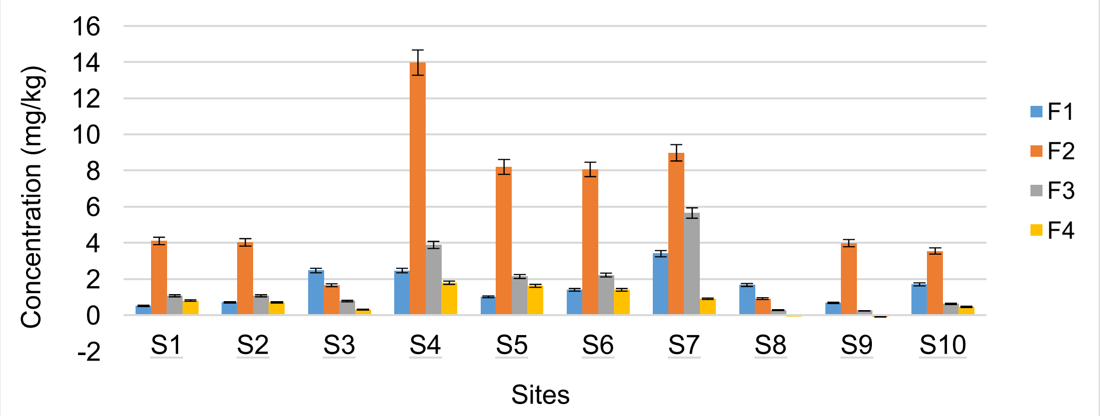

Figure 5.9: Levels of Pb in different fractions of sediments samples obtained employing method B

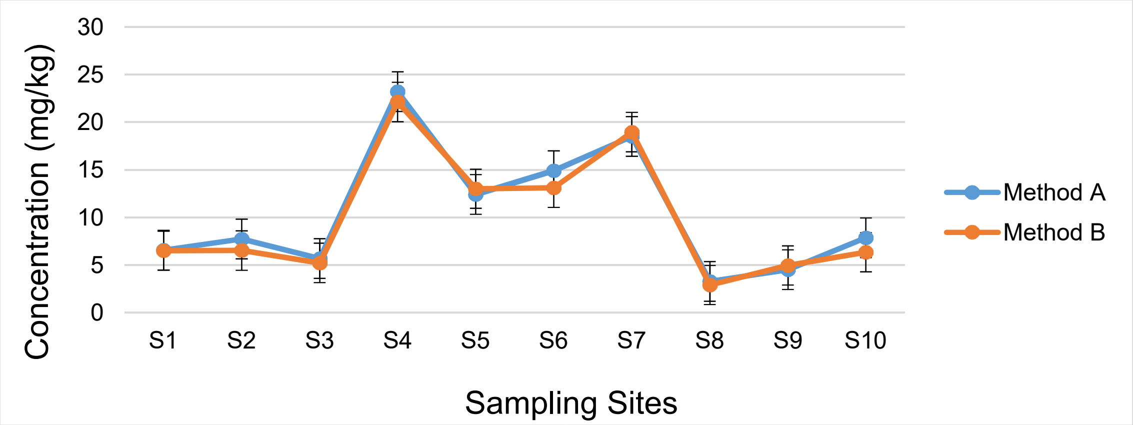

Figure 5.10: Comparison of the sum of concentrations of Pb in different fractions (method B) and pseudo- total concentrations for digested sediments samples (Method A)

Naturally occurring Pb minerals are not very mobile under normal environmental conditions, thus the sources of Pb are likely anthropogenic (Jain, 2004). Elevated Pb concentrations were expected in the Fe/Mn (reducible) fraction. The Fe-Mn oxyhydroxides are important scavengers of Pb in sediments and Pb is able to form stable complexes with Fe-Mn oxyhydroxides (Li *et al.*, 2001; Wong *et al.*, 2007).

The fractionation pattern of Pb followed the order F2>F3>F1>F4. Sites 3 and 8, showed a different trend in the fractionation stages whereby the exchangeable fraction was the most favourable. The high concentration of Pb in exchangeable fraction at S3 and S8 show great mobility, bioavailability and toxicity in the sediments of Blood River.

Zhao *et al*. (2012) reported the maximum portion of Pb in the exchangeable and easily reducible fractions. In their study, they traced the existence of Pb in the exchangeable fraction with anthropogenic activities. In this study, similar findings observed at S3 and S8 showing dominance of Pb in F1. These sites are adjacent to a residential area and elevated concentrations may come from the leaking sewage or illegally dumped wastes. The higher percentage of Pb was obtained in F2 (47-82%) while less than 30%, 27% and 12% in F3, F1 and F4, respectively. The Blood River is strongly influenced by intensive human activities and Pb is mainly derived from municipal wastes, atmospheric inputs and automobile exhaust (Li *et al*., 2007). These activities in the area might be the major source of Pb in the sediments.

The reliability of the method B for fractionation of Pb was evaluated by comparing the sum of the concentrations obtained in four stages with the pseudo-total concentrations obtained by method A. The percent recoveries obtained for the method B show the reliability of the method with quantitative percent recovery of 81 to 110%, as presented by tables 5.18 and 5.19 of appendix C.

Figure 5.11 shows the partition of Zn in sediments. The comparison of the sum of concentrations of Zn in different fractions (method B) and pseudo-total concentrations for digested sediments samples (Method A) is displayed in figure 5.12.

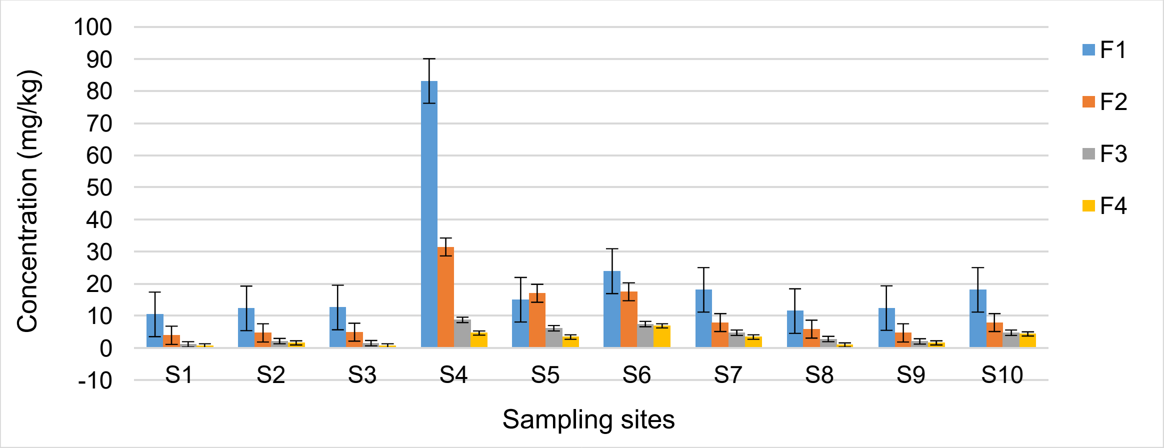

Figure 5.11: Levels of Zn in different fractions of sediments samples obtained employing method B

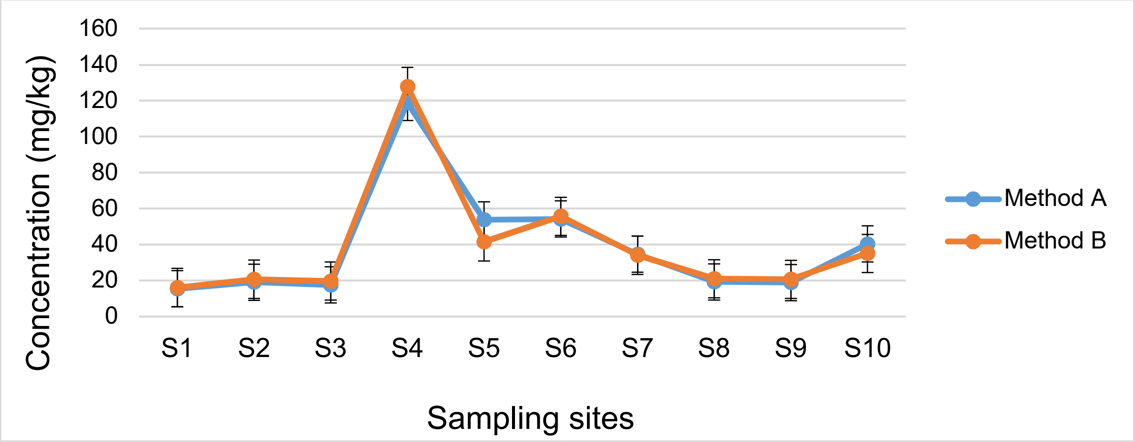

Figure 5.12: Comparison of the sum of concentrations of Zn in different fractions (Method B) and pseudo-total concentrations for digested sediments samples (Method A)

The solubility of Zn is primarily determined by pH. Zinc may precipitate at pH values greater than 8.0 and it may also form stable organic complexes (CCME, 2016). Zinc showed to be easily leachable by soluble salts and ions (WHO, 2001).

Thus, the high concentrations were measured in the exchangeable fraction. The maximum level in the exchangeable fraction shows that Zn is very mobile.

The high level of Zn at site 4 may have resulted from the release of exchangeable Zn from illegal dumping sites. Zinc partitioning followed the order F1>F2>F3>F4 (Figure 5.11) except in S5. Complexation of Zn with dissolved organic matter has been reported to increase its solubility and mobility (Houben and Sonnet, 2012).

Distribution of Zn in the sediments of Blood River was maximum in the F1 (43–65%), followed by F2 (22-31%), F3 (7-15%) and lastly F4 (3-12%).

Comparison of the sum of concentrations of Zn in different fractions (method B) and pseudo-total concentrations for digested sediments samples (Method A) showed good agreement between the methods (Figure 5.12).

In this study, the highest level of Fe was measured in the reducible fraction. Higher percentage of Fe was obtained in the reducible fraction (46-67%) followed by oxidizable fraction (18-27%), whereas less than 15% recorded in exchangeable and residual fractions at all the sites (Figure 5.13). Fractionation of Fe in the sediments followed the order F2>F3>F4>F1. The Fe–Mn oxy-hydroxide is the main scavenger for all metals. The lower percentage of Fe in the organic fraction (oxidizable fraction) may be attributed to the competition with Mn organic complex (Passos *et al*., 2010; Rath *et al*., 2009).

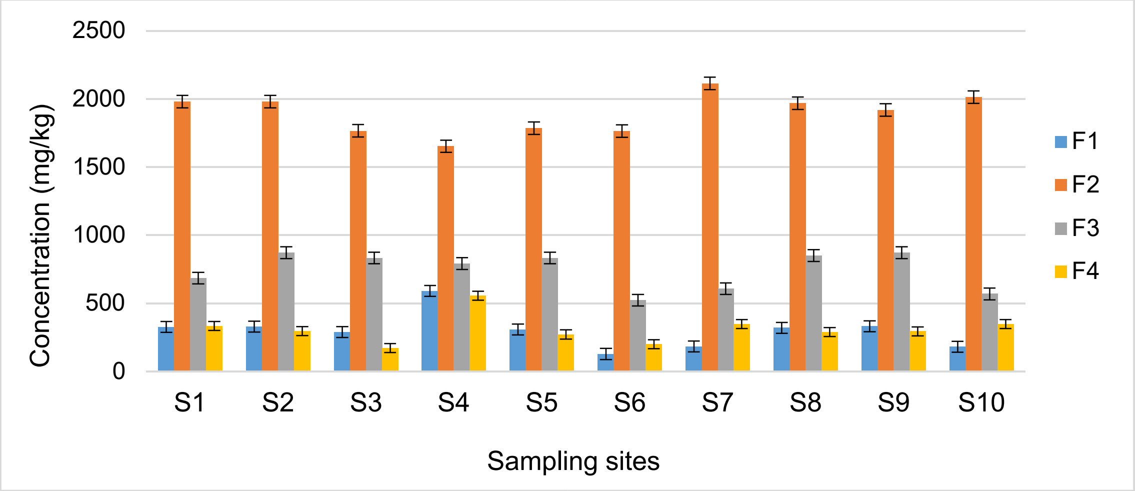

Figure 5.13: Levels of Fe in different fractions of sediments samples employing method B

Additionally, Figure 5.14 shows comparison of the sum of concentrations of Zn in different fractions (method B) and pseudo-total concentrations for digested sediments samples (Method A).

Due to lack of certified value for Fe in the BCR-701 reference material, validation of the analytical results was done by comparing the sum of the extracted concentrations of Fe (method B) with the pseudo-total concentrations (Method A).

The percent recoveries obtained for the internal check of the methods showed the good agreement of the methods at 86 to 106% as shown in tables 5.19 and 5.20, appendix C.

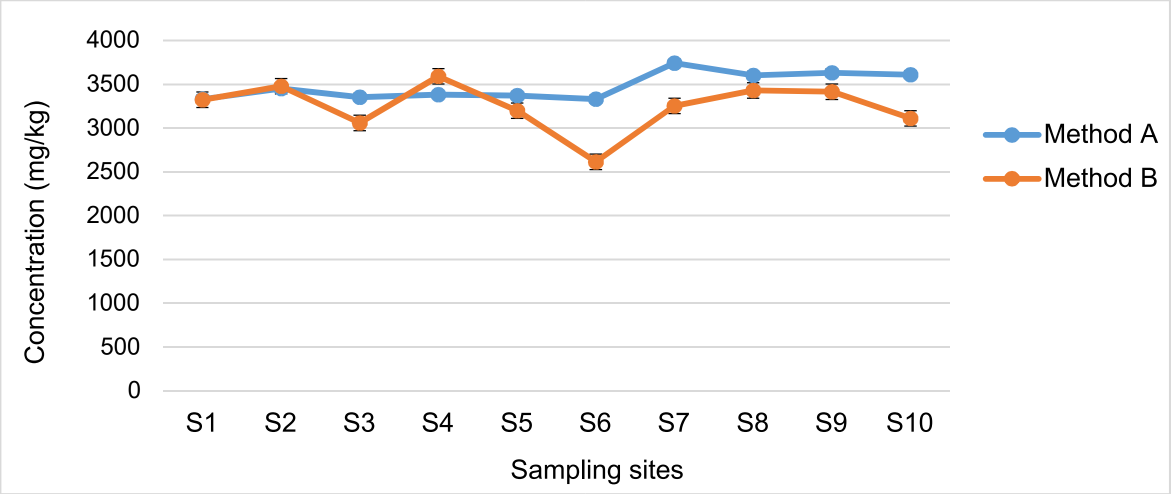

Figure 5.14: Comparison of the sum of concentrations of Fe in different fractions (method B) and pseudo- total concentrations for digested sediments samples (Method A)

5.5. ENVIRONMENTAL IMPLICATIONS

The individual contamination factor (ICF) and the global contamination factor (GCF) have been used to assess the environmental risks and estimate possible damage to aquatic organisms and environment caused by contaminated sediments and water (Nemati *et al*., 2011; Nasr *et al*., 2015). This is based on the potentially toxic elements being able to bind to different sediments fractions leading to bioavailability of the elements from the sediments to the water and the environment (Jain, 2004).

5.5.1. Individual Contamination Factor and Global Contamination Factor

The ICF and GCF values were calculated following the method by Nemati *et al*. (2011). The ICFs for the different sediments were obtained by dividing the sum of the non-residual fractions (F1 + F2 + F3) by the residual fraction (F4) of each sample.

$$\text{ICF} = \frac{[F1+F2+F3]}{F4}$$

The GCF for each site was calculated by summing the ICF of all the metals obtained for the sediments samples (Ikem *et al*., 2003).

GCF = Σ ICF

The ICF and GCF classifications were discussed following the interpretation by Zhao *et al*. (2012) where, ICF < 0 and GCF < 6 indicates low contamination, 1 < ICF < 3 and 6 < GCF < 12 indicate moderate contamination.

Additionally, when 3 < ICF < 6 and 12 < GCF < 24 then this indicates considerable contamination and lastly ICF > 6 and GCF>24 is classified as high contamination. The ICF and GCF values for the ten studied sites are calculated and given in table 5.3.

Table 5.3: Individual contamination and global contamination factors of Blood River during the low flow season obtained employing method B

Element	**ICF**										
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Average
Cd	0.0802	0.0724	0.112	108	0.260	0.402	2.600	0.161	0.227	0.287	11.4
Cr	48.9	33.7	15.7	21.4	40.4	12.5	31.4	29.6	34.0	31.1	29.9
Cu	42.4	15.0	18.2	3.6	15.0	11.5	11.7	7.97	14.6	11.2	15.1
Fe	8.98	10.8	16.9	5.5	10.8	12.1	8.3	10.9	10.7	7.9	10.3
Ni	18.1	13.5	20.0	14.8	27.2	9.10	10.4	10.6	21.8	11.4	15.7
Pb	7.05	8.27	16.5	11.3	7.02	8.35	19.9	188	190	13	46.9
Zn	26.6	1.06	30.4	26.5	11.3	7.08	9.05	21.9	12.2	7.0	15.3
GCF	152	82	118	191	112	61	93	269	283	82	144

Based on the calculated values of ICF, low contamination by Cd in S1, S2, S3, S5, S6, S8, S9 and S10, and S7 showed a moderate contamination by Cd, whereas site 4 had an ICF value of 108, indicating a high contamination risk of Cd to the environment. This was based on the anthropogenic activities that were dominant at S4. The values of ICF for Cr and Cu were above the maximum threshold for high contamination, which shows the risk associated with these elements.

The ICF values of Fe in all the sites were in the high contamination level, except for S3, which was classified as moderate contaminant. However, with continuous pollution occurring in the area, the Fe level in S3 will likely increase.

The ICF values of Ni in all sites were in the high contamination level. Monitoring of the element should be carried out to control possible threat to the aquatic system.

The Pb was classified under high contamination level in all the sites since the ICF values exceeding the ICF of 6 from S1-S10. Similarly, Zn was also classified as high contamination risk to the river system based on the ICF values, with an exception in S2, which was under the moderate contamination level.

Overall, the risk assessment results showed the highest ability of Cr, Cu, Fe, Ni, Pb, and Zn to be released from the sediments to overlying water with the average ICF values of 29.9, 15.1, 10.3, 15.7, 46.9 and 15.3, respectively. The average ICF values followed the order: Pb, Cr, Ni, Zn, Cu, Cd and Fe. Although Cd was under the low classification in most of the sites, it was overall classified under high-risk contamination level based on the average ICF value of 11.2 (Table 5.3).

The residual concentration of any element is considered a non-mobile fraction and is an important partition influencing the mobility nature of the elements, while the non-residual fractions are considered mobile. The results showed Cr, Cu, Fe, Ni, Pb, and Zn have high potential mobility. The mobility of the elements shows the increased possible risk of these elements to the surrounding environment and aquatic organisms.

The GCF values of the analytes were greater than 24 in all the sites, indicating high contamination in the sediments. Thus, the analysed elements in sediments pose high risks to the ecosystem.

The GCF values were 152, 82, 118, 191, 112, 61, 93, 269, 283 and 82 from S1 to S10, orderly. The average GCF value was 114, thus classified as high contamination level.

5.5.2. Risk Assessment Code

The risk assessment code (RAC) was first introduced by Perin *et al*. (1985), and was applied to assess the mobility and bioavailability of potentially toxic elements in surface sediments (Mortazavi *et al*., 2016). The RAC value of each analyte is calculated by the following equation:

$$\mathrm{RAC} = \frac{F1}{\Sigma F1 - F4} \times 100$$

The RAC classifications were discussed following the interpretation by Perin *et al*. (1985), where RAC value of <1 indicates No risk, between 1 and 10 indicates low risk, 11 to 30 is medium risk, 31 to 50 is high risk and finally, >50 is classified very high risk. Table 5.4 shows the calculated percent RAC values of the analytes in the sediments of Blood River.

Table 5.4: Risk assessment code of potentially toxic elements in sediments from Blood River during low flow season

Element	**RAC (%)**										
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	Average
Cd	4.54	3.81	4.42	51.7	89.1	17.5	48.4	8.48	8.15	14.3	25.0
Cr	3.55	3.02	9.70	10.7	9.41	4.72	9.95	9.45	2.98	9.70	7.32
Cu	21.4	27.3	30.6	43.5	27.3	31.2	32.5	24.3	25.6	30.1	29.4
Fe	9.78	9.46	9.44	16.5	9.62	4.90	5.63	9.33	9.66	5.79	9.01
Ni	17.1	15.2	14.6	35.7	27.0	26.9	26.4	25.9	17.1	28.8	23.5
Pb	7.85	10.8	47.5	11.1	7.85	10.8	18.0	58.0	13.9	26.9	21.3
Zn	65.4	33.2	64.2	65.0	36.2	43.0	53.0	54.8	60.1	51.5	52.6

Following the contamination assessment evaluated by ICF and GCF values, the RAC is applied to assess the risk based on the percentage of metal in exchangeable fraction and bound to carbonate (Saeedi *et al*., 2013). Based on the calculated RAC values in table 5.3, an average of 52.6% of total Zn of the study sites was either adsorbed to exchangeable, or carbonate bound.

Thus, it is in the high to very high-risk category to local aquatic and non-aquatic environment and can easily enter into the food chain. The RAC values ranged between 33.2 and 65.45 in the sites, indicating high risk in S2, S5 and S6 and very high risk in S1, S3, S4, S7, S8, S9 and S10.

Chromium and Fe were classified under the low risk category indicating lower availability from which they cannot be easily leached out to the aquatic environment. The RAC values of Cr ranged between 2.98 and 10.7% with an average value of 7.32%. On average, Fe was 9.01%, ranging from 4.90% in S6 to 16.5% in S4, which falls under the moderate risk category.

The concentration of Cd was significantly low in the sediments of Blood River, however the RAC value indicated it as a potential risk to the aquatic and surrounding environment of the river. The average RAC value in the sediments was 25.0% indicating medium risk, while the risk assessment ranged between 3.81% (low risk) and 89.1% (very high risk). Cadmium in S1, S2, S3, S8 and S9 was classified as high risk and in S6 and S10 as moderate risk.

The average RAC values of Cu (29.4%), Ni (23.4%) and Pb (21.3%) classified the elements as medium risk to the environment. The RAC values of Cu ranged from 21.4% in S1 to 43.5% in S4. Although Cu was classified under the medium risk category, S3, S4, S6, S7 and S10 were classified under the High risk. The existence of Ni in the non-residual fraction categorised its existence in the sediments as medium risk to the environment in the sites with an exception in S4 (35.7%) which was of high risk. Partitioning of Pb in the sediments resulted in the RAC values of Pb ranging from 7.85 % in sites 1 and 5 to 58.0% in S8. The existence of this element in S3 and S8 indicates that it poses high risk to the aquatic organisms in the Blood River. Overall, the analyte was classified under the low (S1, S2, S5 and S6) to medium risk (S4, S7, S9 and S10).

These findings indicate potential risks to the human health of residents surrounding the Blood River. These may be caused by direct exposure to the water and indirect exposure via food chain. The risk assessment of Blood River followed the decreasing order of Zn > Cu > Cd > Ni > Pb > Fe > Cr.

5.6 MICROWAVE-ASSISTED SEQUENTIAL EXTRACTION OF POTENTIALLY TOXIC ELEMENTS IN SEDIMENTS (METHOD C)

5.6.1 Introduction

In this study, the fractionation of Cd, Cr, Cu, Fe, Ni, Pb, and Zn was carried out in sediments collected during low flow sampling season from Blood River using microwave-assisted sequential extraction method (method C). Comparison was done between the two sequential extraction methods and the digestion method for pseudototal concentration determination. Analysis was carried out by ICP-AES.

5.6.2 Determination of limit of detection for microwave-assisted sequential extraction procedure

Reagent blanks were prepared following the Method C procedure. The blank solutions were prepared and obtained from the reagents of each fractionation stage sown in table 3.5 (Chapter 3). The LODs for each element were determined based on three times the standard deviation of the average of 6 individually prepared blank solutions, are given in table 5.5.

Table 5.5: Limit of detection obtained for the determination of potentially toxic elements in extracted sediments by ICP-AES in (mg/kg) obtained employing method C

Element	**F1**	**F2**	**F3**	**F4**
Cd	0.0070	0.0038	0.0006	0.0019
Cr	0.0034	0.0204	0.0043	0.0002
Cu	0.0021	0.0025	0.0250	0.0011
Fe	0.0019	0.0156	0.0021	0.0008
Ni	0.0212	0.0015	0.0658	0.0007
Pb	0.0106	0.0006	0.0010	0.0008
Zn	0.0021	0.0036	0.0008	0.0011

The LODs obtained by employing the microwave-assisted sequential extraction ranged from 0.0019 to 0.0212 mg/kg in the exchangeable fraction (F1). For the reducible fraction, the LODs ranged between 0.0006 and 0.0204 mg/kg.

In the oxidizable and residual fractions, the LODs ranged from 0.0006 to 0.0658 mg/kg and 0.0002 to 0.0019 mg/kg, respectively.

In comparison to the LODs reported, in this study, obtained by applying method B, the LODs determined using method C were very low. The use of the microwave for extraction proves to be more reliable based on complete extractions in each stage. This may be based on the closed system where loss of volatiles and contamination are minimal.

5.6.3 Validation of microwave-assisted sequential extraction procedure

The accuracy of the microwave-assisted sequential extraction protocol was evaluated. The certified reference material BCR 701 was used to evaluate the percent recoveries applying the procedure. The percent recoveries of the elements in the BCR 701 material were in the range of 68-117% (Table 5.6).

Table 5.6: Concentrations and percent recoveries determined by method C for potentially toxic elements in certified reference material of sediments (BCR 701)

Fraction	**Element**	**Experimental value**	**Certified value**	**Percent Recovery**
		Mean ± SD (mg/kg)	Mean ± SD (mg/kg)	
F1	Cd	5.81±0.036	7.30±0.40	80
	Cr	1.86±0.032	2.26±0.16	82
	Cu	40.5±2.1	49.3±1.7	82
	Ni	10.6±0.063	15.4±0.9	69
	Pb	2.39±0.054	3.18±0.21	75
	Zn	213±3.5	205±6.0	104
F2	Cd	2.55±0.015	3.77±0.28	68
	Cr	48.6±0.70	45.7±2.0	106
	Cu	114±0.89	124±3.0	92
	Ni	26.3±0.45	26.6±1.3	99
	Pb	103±1.3	126±3.0	81
	Zn	122±1.4	114±5.0	107
F3	Cd	0.212±0.0011	0.27±0.06	79
	Cr	110±0.71	143±7.0	77
	Cu	63.1±0.71	55.0±4.0	115
	Ni	17.9±0.028	15.3±0.9	117
	Pb	9.22±0.025	9.30±2.0	99
	Zn	45.0±0.67	46.0±4.0	98

The low percent recoveries obtained in F1 for Ni (69%) and Pb (75%), F2 for Cd (68%) and F3 for Cd (79%) and Cr (77%) may have resulted from the loss of analytes. This may have occurred when transferring reagents and sample residue between fractionation stages. Additionally, uncertainties in measurements during analyses may have resulted in either the low or the higher recoveries in the case of Cu (115%) and Ni (117%) in F3.

Furthermore, validation of the modified protocol (method C) was done by evaluating the percent recoveries obtained by the ratio of the sum of fractional metal concentration to that of pseudo-total metal concentration of samples multiplied by 100. The percent recoveries are given in appendix D from S1 (Table 5.20) to S10 (Table 5.24), which showed good agreement.

5.6.4 Determination of potentially toxic elements in sediments applying microwave-assisted sequential extraction procedure

In this study, sediments samples from Blood River collected during the low flow season were analysed using the proposed method C discussed in table 3.5 in chapter 3. Two extraction methods were applied and compared with pseudo-total concentrations from digestion of the sediments. The extraction method was developed by varying the extraction time, sample size, reagent volume and applied instrumental conditions.

For the BCR sequential extraction (method B), the method was developed by monitoring the extraction time, reagents volume, sample size and the strength of the mechanical shaker in addition to the ultrasonicator. For the microwave-assisted sequential extraction method, the extraction was done to obtain the shorter extraction time compared to the mechanical shaker, varying time, temperature and pressure of the microwave system.

Figure 5.15 shows the fractionation trend of Cd employing method C. In figure 5.16, the concentrations obtained in method C is compared with those obtained by applying methods A and B. Coding was applied to simplify the illustration given by the figures.

For example, in the coding S1B, S1 represents site 1 while B represents method B. In S1C, the C represents method C. Furthermore, S2B and S2C represent site 2 of method B and C, respectively. The numerical indicates the site from which the sample was taken.

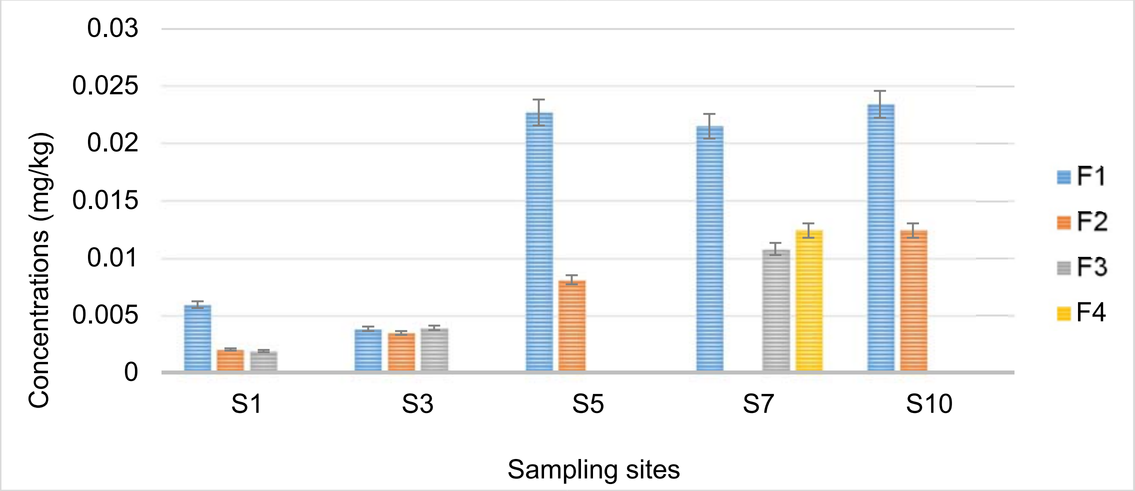

Figure 5.15: Fractionation of Cd employing method C

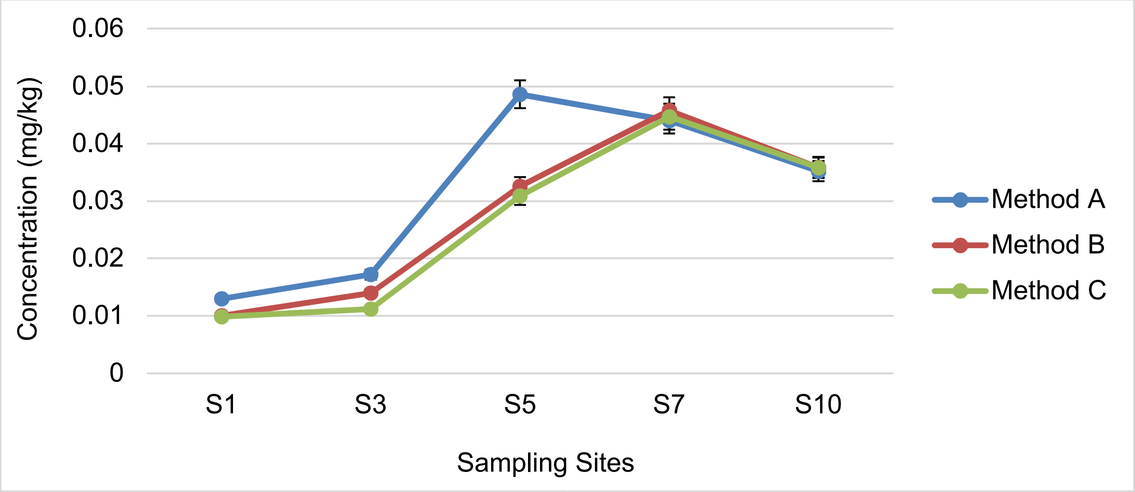

Figure 5.16: Comparison of pseudo-total concentrations for digested sediments samples (Method A) with the sum of concentrations of Cd in different fractions obtained by methods B and C

The Cd released by employing method C, is given by the fractionation pattern as shown in figure 5.15. The distribution of Cd in the four fractions varies from site to site. Long *et al*. (2009) associated the exchangeable fraction as the stage at which the amount of potentially toxic elements is easily released into the environment under acidic conditions.

The higher the concentration of the element in this fraction the more the element causes the adverse impact to the environment. This is based on the mobility of the element in the fraction. In this study, Cd was found at low to below detectable levels at some of the fractions but was dominant in the exchangeable fraction at all the sites.

In the study by Nemati *et al*. (2011), about 50% of total of Cd was recorded in this fraction. Even though the element was available at low concentrations, its existence in the sediments course may pose threat to the surrounding environment.

Considerable levels of Cd were also found in the reducible and oxidizable fractions. The element was not detected in the residual fraction for most of the sites, except at S7. This was consistent with the amount of Cd detected at this fraction (F4) when method B applied. Cadmium was not detectable in the F3 and F4 at S5. The portions of Cd available in the four fractions were, F1 (34-74%), F2 (0-35%), F3 (0-35%) and F4 (0-28%). The partitioning of Cd in the studied sites obtained by employing method C followed the order: F1>F2>F3>F4 (figure 5.15). This trend is consistent with the pattern observed when method B was applied.

The reliability of the method C was further checked by comparing the sum of the concentrations in the fractions with the pseudo-total concentrations obtained by method A. The percent recoveries are reported in appendix D ranging from 64% (S5) to 102% (S7 and S10). Although low percent recoveries were obtained by method C, good agreement was observed between the methods A, B and C (Figure 5.16). There was no significant difference in the fractionation pattern of Cd when method C was compared with B. The statistical variation ($P<0.05$) indicated that the methods A and B are indeed comparable. Thus, method C can reliably be applied for sequential extraction to minimise the long extraction hours and large volume of reagents.

Distribution of Cr in the different fractions is shown by figure 5.17. The comparison of method C with methods A and B is presented in figure 5.18.

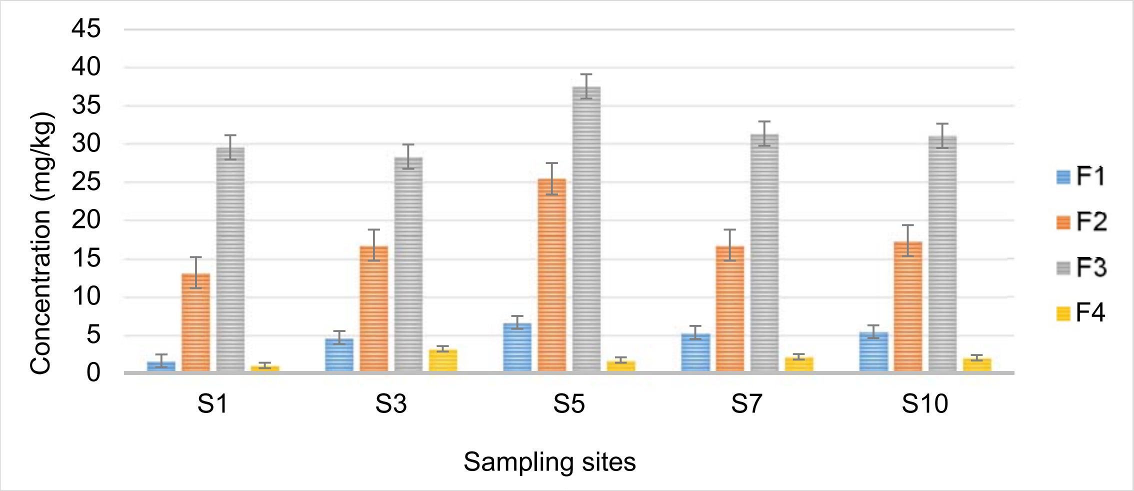

Figure 5.17: Fractionation of Cr employing method C

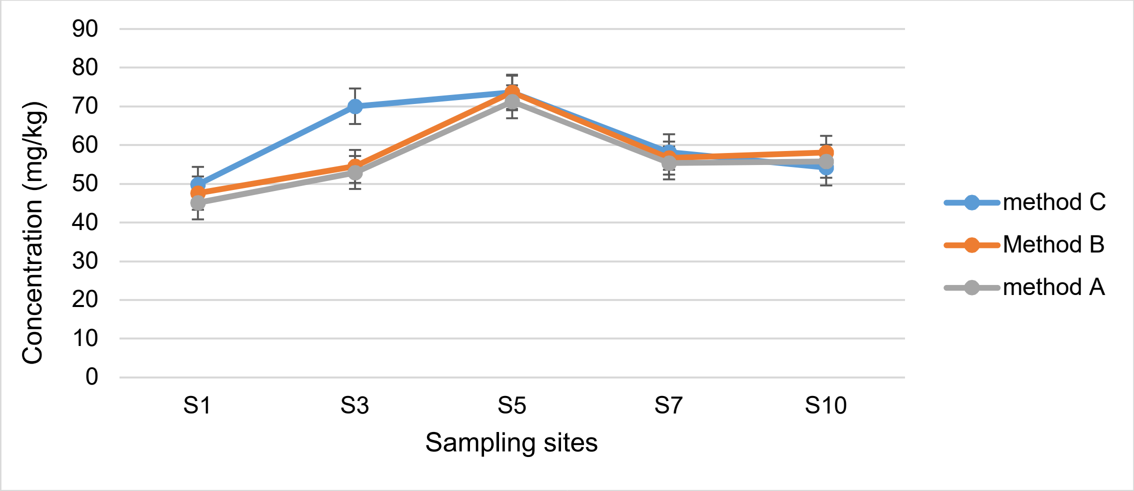

Figure 5.18: Comparison of pseudo-total concentrations for digested sediments samples (Method A) with the sum of concentrations of Cr in different fractions obtained by methods B and C

Chromium was mainly associated in the reducible and oxidizable fractions. The concentration of Cr was dominant in the F3 at levels between 53 and 65% of its total composition. Furthermore, it was also available at F2 between 29 and 36%, F1 at 310% and F4 at 2-6%. Availability of this element in the non-residual fractions (F1, F2 and F3) at higher concentrations than the residual fraction (F4) proves its mobility from the sediments to the water system of Blood River. In agreement with the fraction order obtained by applying method B, the fractionation of Cr in the sediments of Blood River maintained the order: F3>F2>F1>F4.

The sums of the concentrations in the fractions of method C were compared with the pseudo-total concentrations obtained by method A. The percent recoveries obtained ranged from 76% (S3) to 103% (S10). Apart from the low percent recovery of Cr in S1, good agreement was observed between the methods A, B and C (Figure 5.18). The percent recovery of the element in the fractions indicate that the method C can be approved for sequential extraction of the element in sediments to minimise the extraction time, thus increasing turnaround time for analysis. Moreover, the results showed good agreement between the three methods except for site 3 where loss of analyte during sequential extraction could have resulted in the deviation for this sample.

ANOVA was applied to check the consistency and comparability of the method C to the other methods A and B. Comparison of concentrations between the two methods indicated no significant difference (P>0.05).

In all the analysed samples, Cu was dominant in the reducible and exchangeable fractions (Figure 5.17). The distribution was followed by the oxidizable and the residual fractions.

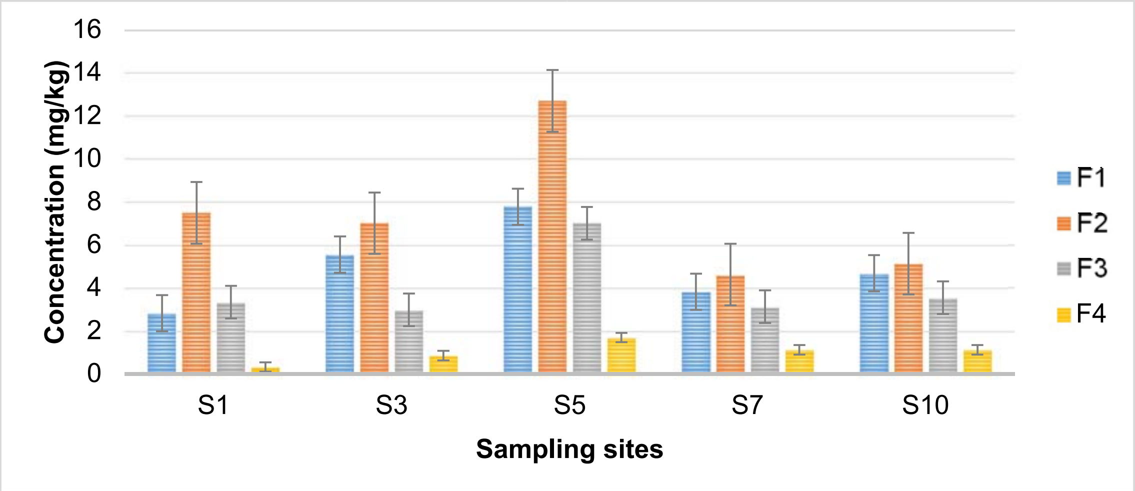

Figure 5.19: Levels of Cu in different fractions of sediments obtained employing method C

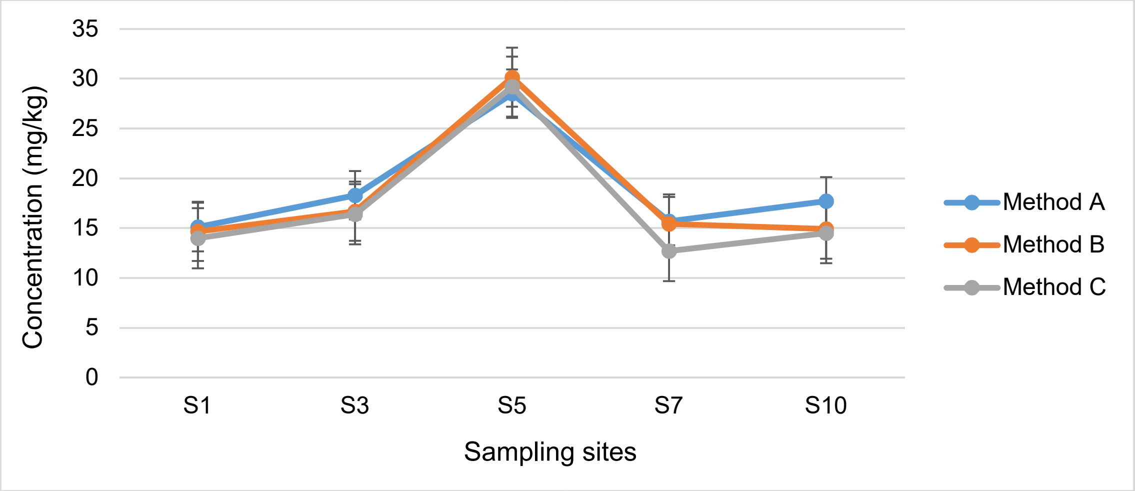

Figure 5.20: Comparison of pseudo-total concentrations for digested sediments samples (Method A) with the sum of concentrations of Cu in different fractions obtained by methods B and C

A previous study has reported Cu as an element that easily forms complexes with FeMn oxides due to the large adsorption surface area (Huang *et al*., 2013). Yang *et al*. (2014) reported the proportions of Cu as 42.2% (F2), 35.9% (F4), 13.5% (F3) and 8.5% (F1). In their study, Cu was dominant in the reducible fraction followed by the residual fraction. In this study, Cu was also dominant in the reducible fraction ranging from 35-54% of the total composition. Copper was partitioned in the four fractions of the sediments sample following the order of F2 (35-54%), F1 (20-34%), F3 (18-25%) and F4 (2-9%). The fractionation pattern was summarised as F2>F1>F3>F4.

The sums of the concentrations in the fractions employing method C were comparable to the pseudo-total concentrations obtained by method A. The method C was reliable since good recoveries were obtained in the range of 81% (S7) to 102% (S5), as indicated in tables 5.20 to 5.24 (Appendix D). The good harmony between three methods was verified by the statistical evaluation, which indicated that the three methods were indeed comparable (P>0.05).

Although fractionation of Fe was not reported on the BCR 701 certificate, its distribution was evaluated in this study. Fractionation of Fe evaluated employing method C is given in Figure 5.21.

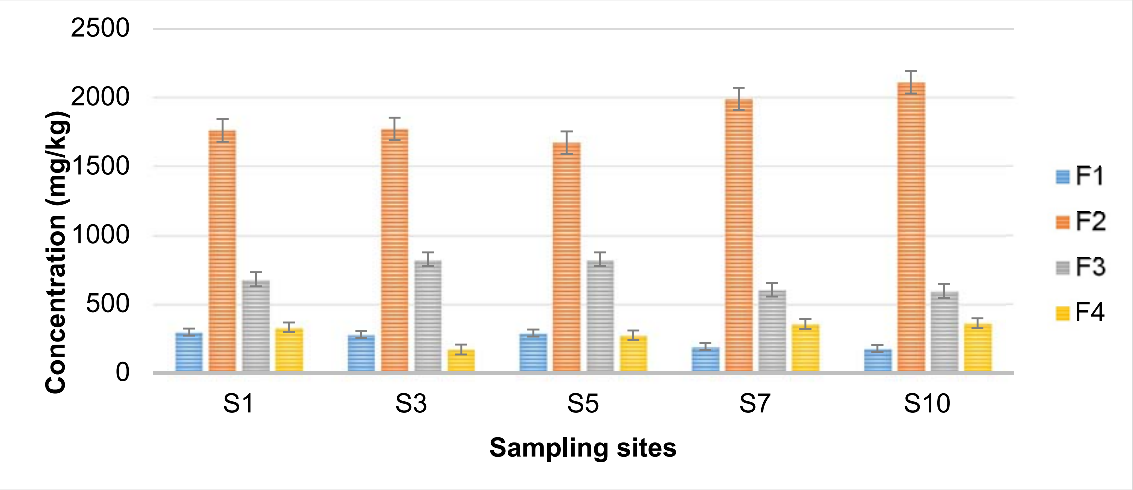

Figure 5.21: Fractionation of Fe obtained by employing method C

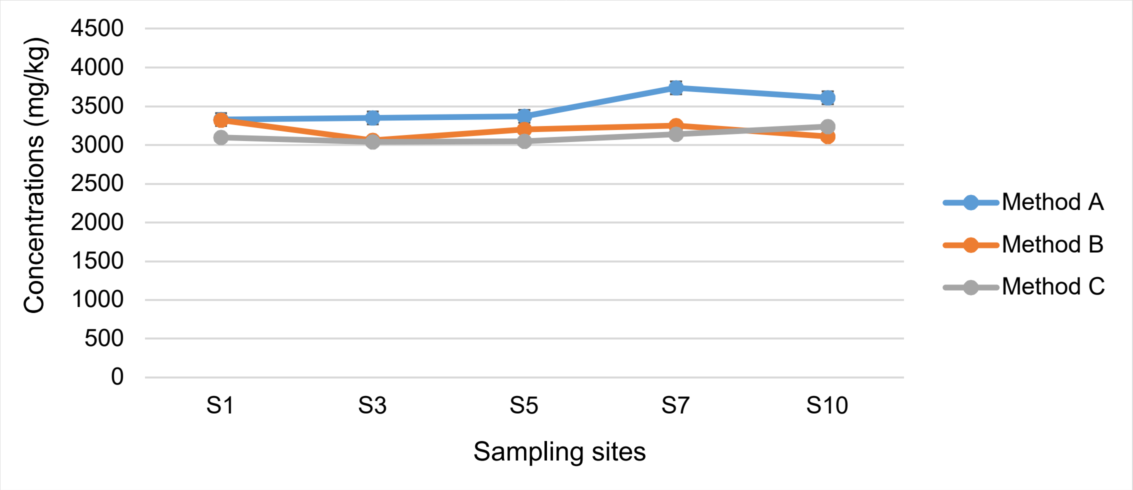

Figure 5.22: Comparison of pseudo-total concentrations for digested sediments samples (Method A) with the sum of concentrations of Fe in different fractions obtained by methods B and C

The fractionation pattern displayed by figure 5.21 indicates that Fe was highly available in the reducible fraction (57-65%) followed by the oxidizable fraction (1827%), residual (6-11%) and lastly the exchangeable fraction at (5-10%). Distribution of the element in the exchangeable and residual fractions was competitive. The trend was consistent with the concentrations obtained by method B.

Availability of the element in the reducible and oxidizable fraction indicates that the resuspension of the sediments due to water movements may leach out the element to the surface water.

This will result in exposure via food chain through consumption of fish or crops irrigated with the water that could pose threat to humans when the element is at high levels.

The percent recoveries obtained by applying method C showed that the method is acceptable for sequential extraction of Fe in sediments. Method C was comparable to method A as confirmed by the percent recoveries in the range of 84-93% and by the good agreement between the methods as shown in figure 5.22. The statistical evaluation showed good agreement between the digestion method for Fe with methods B and C (P>0.05), which proves the efficiency method C for sequential extraction of Fe.

Fractionation of Ni employing method C was dominant in the reducible and exchangeable fractions when compared to the oxidizable and residual fractions (Figure 5.23). Additionally, the reliability and efficiency of the applied microwave-assisted sequential extraction method is further shown by comparison with other methods in figure 5.24.

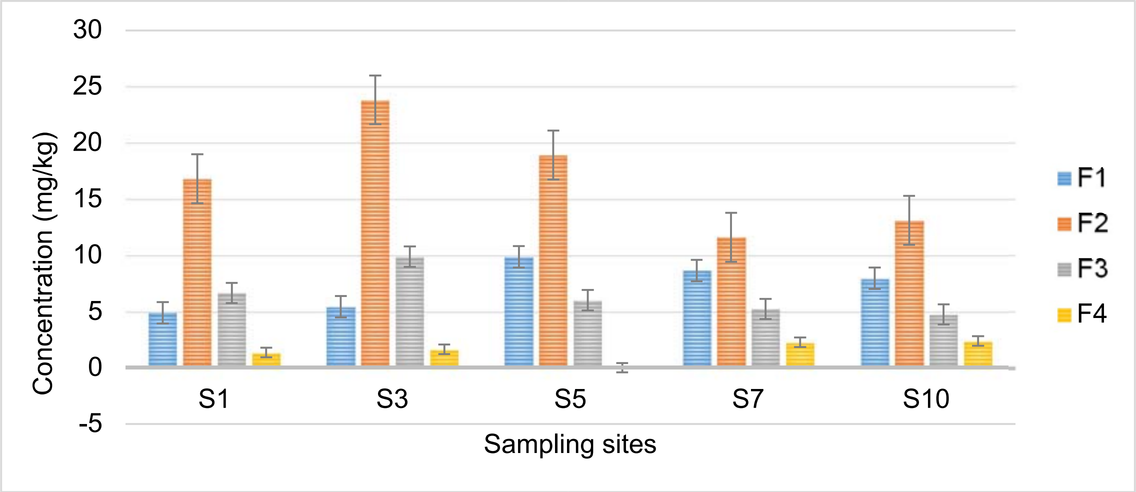

Figure 5.23: Fractionation of Ni obtained by method C

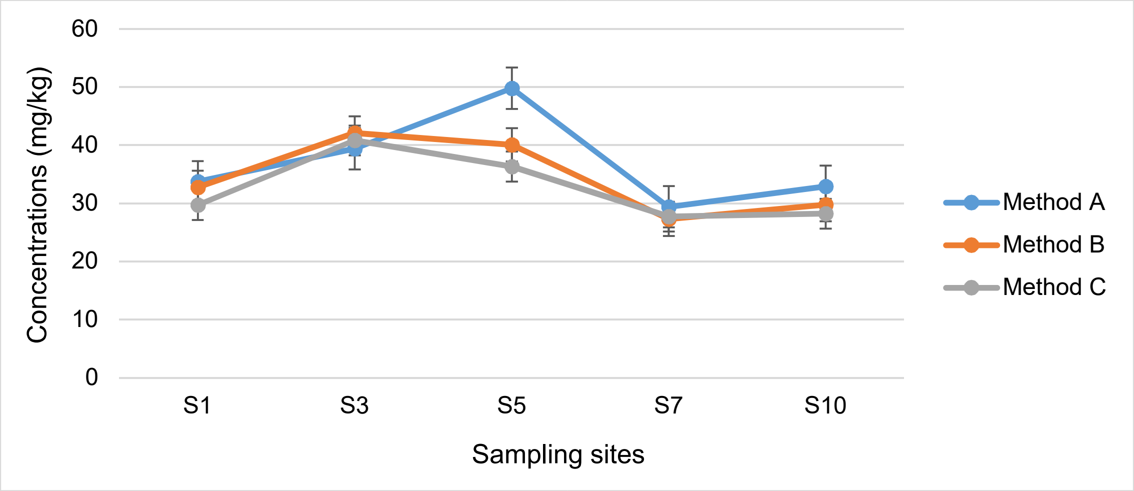

Figure 5.24: Comparison of pseudo-total concentrations for digested sediments samples (Method A) with the sum of Ni concentrations in different fractions obtained by methods B and C

A study by Arain *et al*. (2008) reported three extraction methods, ultrasonic-assisted extraction (USE), microwave-assisted extraction (MAE), and conventional single extraction (CSE). These methods were compared with the modified three-stage BCR sequential extraction procedure (SEP) to determine the levels of selected elements from lake sediments samples. Following the MAE protocol in their study, Ni partitioning followed the order: F2 (38.7%)> F4 (26.9%)> F3 (16.7%)> F1 (12.6%). In their study, Ni was least available in the exchangeable fraction and dominant in the reducible fraction. The fraction of Ni, in this study, indicated that it was available in considerable portions in the exchangeable fraction. The distribution of Ni in different fractions obtained by method C showed 42-58% in F2, 13-31% (F1), 17-24% (F3) and 4-8% in F4. Once again, the maximum availability of Ni in the non-residual fractions indicate its mobility and bioavailability to the water system of Blood River, which may pose risks to ecosystem.

Leaching of Ni in sediments can be achieved by microwave-assisted sequential extraction method to minimise the extraction time and reagents, as shown by good agreement of the methods in figure 5.24. The trend illustrated shows that method B and C are similar, which proves efficiency of the microwave-assisted sequential extraction procedure for Ni fractionation at less extraction time.

Pseudo-total concentrations for digested sediments samples (Method A) were compared with the sum of Ni concentrations in different fractions employed by method C.

This was done to obtain percent recoveries that can be used to validate the efficiency of the modified method. The percent recoveries ranged from 73 to 103% as given in tables 5.22 and 5.21 (Appendix D). The statistical data (p >0.05) indicated that good harmony between methods B and C as there is no significant difference between the methods.

A study by Nemati *et al*. (2011) reported about the fractionation of Pb employing the microwave-assisted method. In their study, they associated dominance of Pb with the reducible fraction at levels between 21 and 24%. In this study, Pb partitioning was dominant in the reducible fraction as illustrated in figure 5.25. This trend was consistent and comparable with the results obtained by method B (Figure 5.26). Efficiency of the method C is also checked against method A and evaluated by the percent recoveries given in appendix D.

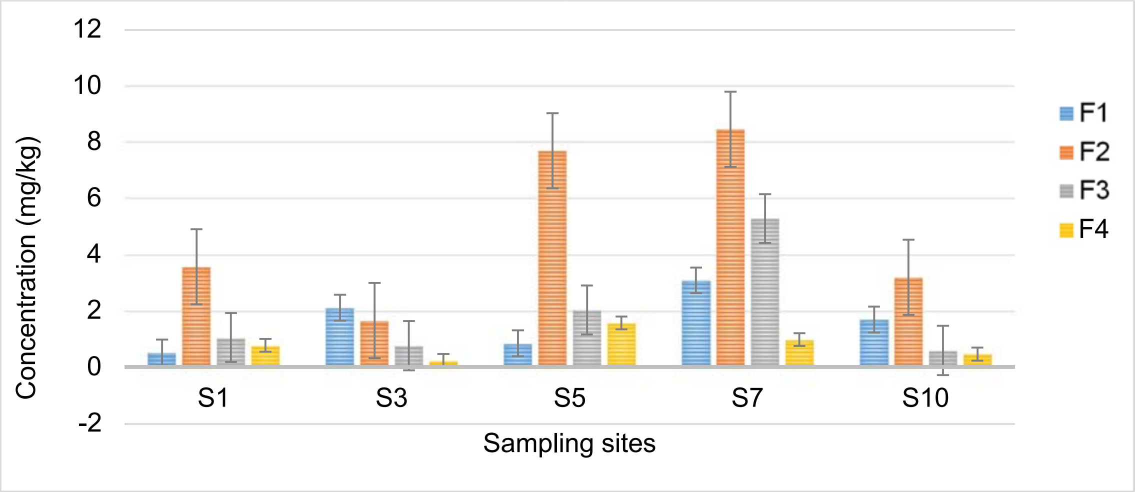

Figure 5.25: Fractionation of Pb obtained by method C

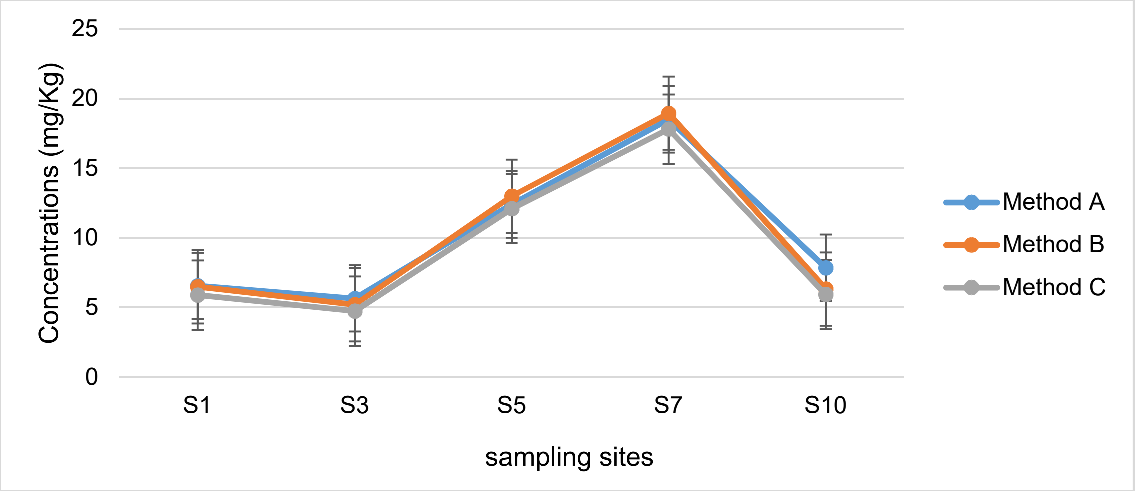

Figure 5.26: Comparison of pseudo-total concentrations for digested sediments samples (Method A) with the sum of concentrations of Pb in different fractions obtained by methods B and C

The BCR sequential extraction method has been applied to explain partitioning of Pb (Yuan *et al*., 2004; Nemati *et al*., 2011; Yang *et al*., 2014). In these studies, Pb was associated mainly the residual fraction. In this study, the portions of Pb available in different fractions were 35-63% in F2, 7-44% in F1, 10-30% in F3 and 5-13% in F4. Diaz-de Alba *et al*. (2011) reported about large portion of Pb being available in the exchangeable fraction and associated its existence with anthropogenic activities. In this study, the substantial levels of Pb recorded in exchangeable fraction could be associated with anthropogenic activities in the Blood River aquatic environment.

The percent recovery check performed on the partitioning of Pb employing the method C, showed the reliability of the method (Appendix D). Good agreement between three methods was achieved as shown in figure 5.26. The value of $P>0.05$ verified that the there is no statistical difference on results obtained by employing methods B and C, thus fractionation of Pb can be achieved at shorter time by microwave-assisted sequential extraction.

The levels of Zn in fractions measured by applying method C for sequential extraction are shown in figure 5.27. The comparison of pseudo-total concentrations for digested sediments samples with the sum of concentrations of Zn in different fractions obtained by methods B and C is given in figure 5.28.

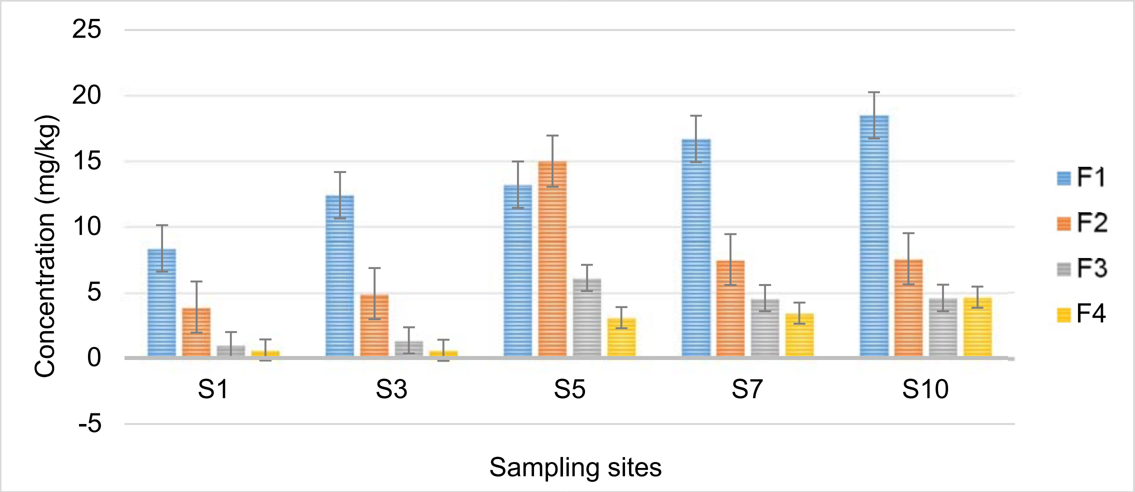

Figure 5.27: Fractionation of Zn obtained by method C

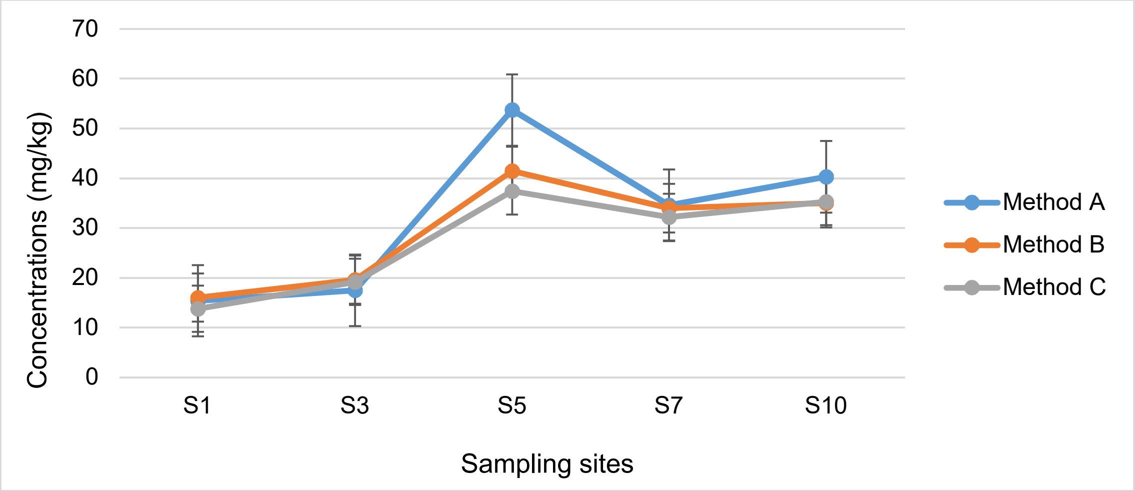

Figure 5.28: Comparison of pseudo-total concentrations for digested sediments samples (Method A) with the sum of concentrations of Zn in different fractions obtained by methods B and C

Zinc was dominant in the exchangeable fraction when compared to the other fractions. This observation was in harmony with our finding by employing method B. The fractionation pattern of Zn in the sediments followed the order: F1 (35-64%)> F2 (21-40%)> F3 (7-16%)> F4 (3-13%). Yang *et al*. (2014) reported mean proportion of the four fractions for Zn as residual (38.0%), exchangeable (29.8%), reducible (26.2%) and oxidizable (6.0%).

Although this fractionation trend was not in agreement with the trend reported in this study, its substantial availability in the non-residual fractions, particularly in exchangeable fraction indicates that Zn could have high degree of leachability from sediments to water. Thus, this could pose risks to the aquatic life and humans via food chain.

Reliability of the proposed method C for sequential extraction of Zn was checked by comparing the sum of concentrations of Zn in the four fractions of method C with pseudo-total concentrations of Zn determined by method A. The percent recoveries obtained ranged from 70 to 110% as given in the tables of appendix D. Although low recovery was obtained in sediments of S5, improvement can be achieved if sample loss is accounted. This is the deviation observed only in a sample analysis for sediments sample from S5. The extraction methods B and C are in good agreement.

Additionally, this was again verified by the statistical evaluation, which indicated that the sequential extraction method C was indeed comparable to method B ($P>0.05$). 5.7 Risk assessment of sediments from Blood River based on fractionation by method C

Individual contamination factors (ICFs) of the elements in the sediments of Blood River during the low flow season were calculated in order to determine the risks associated with the analysed elements. The ICF values were calculated as the sum of the concentrations of elements extracted in the first three fractions of the microwave sequential extraction divided by the concentration in the residual fraction (Nemati *et al*., 2011). The ICF values of each analyte from the five studied sites are given in table 5.7.

Table 5.7: The ICF values of selected elements sediments samples collected from Blood River obtained by method C

Element	**ICF**					
	S1	S3	S5	S7	S10	Average
Cd	0.079	0.090	0.25	2.60	0.286	0.66
Cr	45.6	15.7	41.4	25.1	27.1	31.0
Cu	42,.2	18.2	16.3	10.3	11.9	19.8
Fe	8.30	17.1	10.3	7.86	8.03	10.3
Ni	21.1	23.8	22.3	11.3	10.9	17.9
Pb	6.64	19.5	6.73	17.2	11.8	12.4
Zn	21.4	31.2	11.1	8.4	6.60	15.7
GCF	145	126	108	83	77	108

The ICF results indicated low contamination by Cd at the studied sites except S7 that indicated moderate contamination (Table 5.7). These values were comparable to the ICF values reported in Table 5.3 of this study.

The comparison justified that the availability of the element in the non-residual fraction indicates its possible mobility into the aquatic system of Blood River and may pose as a threat even at the low levels (Tokalioglu *et al*., 2003).

Based on the classification by Zhao *et al*. (2012), the ICF values of the other elements (Cr, Cu, Fe, Ni, Pb and Zn) in this study were all under the high contamination level. The GCF values at S1, S3, S5, S7 and S10 were 145, 126, 109, 83 and 76. These values exceeded the high-risk category (GCF>24) as classified by Zhao *et al*. (2012).

The average ICF values indicated that the studied elements are likely to contaminate the river system of Blood River by transfer from the sediments to the water. Furthermore, this will have a negative impact on the health of the aquatic and nonaquatic organisms of the ecosystem.

Sundaray *et al*. (2011) and Liu *et al*. (2008) determined the availability of sedimentary metals by applying a scale to the proportions of the metals in bioavailable fractions.

Their respective studies associated the concentrations of the elements with anthropogenic activities, which make the elements remain loosely bound to the sediments and dominant in exchangeable fraction.

Furthermore, they emphasised that the elements available in this fraction could easily be released to aquatic environments where they may be transferred to aquatic organisms causing high risk. Thus, risk assessment study was done by evaluating RAC. The RAC levels in sediments of Blood River from the five studied sites are given in Table 5.8.

Table 5.8: Risk assessment code for analysed elements in sediments samples collected from Blood River during low flow season after extraction by method C

Element	**RAC (%)**					
	S1	S3	S5	S7	S10	Average
Cd	4.40	2.81	16.0	48.1	14.6	17.2
Cr	3.50	8.75	9.29	9.55	9.71	8.16
Cu	20.2	33.9	26.7	30.1	32.3	28.6
Fe	9.60	9.15	9.44	6.03	5.46	7.94
Ni	16.5	13.3	27.1	31.2	28.2	23.3
Pb	8.76	44.4	6.97	17.3	28.5	21.2
Zn	60.4	64.4	35.3	51.9	52.4	52.9

The RAC results indicated low risk for Cr and Fe at all studied sites (Table 5.8). Cadmium also manifested low risk at S1 and S3, Pb at S1 and S5, and Ni at S3. Medium risk was indicated by Ni in all studied sites and additionally by Cd at S5 and S10, Cu at S1, S5 and S7 and Pb at S7 and S10.

Moreover, Cd at S7, Cu at S3 and S10, Pb at S3 and Zn at S5 showed a high risk. Zinc manifested very high risk at all sites that could pose toxicity to aquatic organisms and humans.

Overall, the average RAC indicated that although Cd, Cu, Ni and Pb were classified as medium contaminants, they are still mobile and bioavailable in the aquatic environment.

These results were consistent with the RAC results reported in table 5.4 obtained by method B. Therefore, a significant remediation control must be applied since the mobilization of the elements could result in the food chain by irrigating the crops with contaminated water and could be a threat to humans particularly, residing in the studied area.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

INTRODUCTION

In this chapter, the main findings of this study have been summarised, conclusions have been drawn with reference to the objectives of the project, and some recommendations are provided for related future studies and policy actions.

6.1 CONCLUSIONS

6.1.1 Quantification of potentially toxic elements in water samples

Potentially toxic elements in the water bodies are mainly influenced by anthropogenic activities. Apart from the man-made sources of pollution into the river systems, the water chemistry and weather conditions can have an impact on the concentrations of the elements in the water. To assess this impact, the levels of potentially toxic elements in the water samples collected from Blood and Mokolo Rivers during high and low flow seasons were analysed.

During the high flow season, surface runoffs from residential areas surrounding the rivers, dumpsites, industrial and agricultural effluents may result in the chemical composition of the water, hence modifying the water chemistry of the areas. Precipitation during temperature fluctuations may also lead to variations in the levels of potentially toxic element in the rivers. In the dry season, evaporation of the water in the rivers may result in increased concentrations of contaminants.

Validation of the method employed for quantification of potentially toxic elements in water was done by analysing the standard reference material of water (NIST SRM 1643f). Quantitative percent recoveries of greater than 97% were obtained as indicated in table 4.3, proving the reliability of the results obtained in this study, except for Cr. The percent recovery of an element as a percent of total concentration is a function of several factors such as the mode of occurrence in the sample, leach medium, leach time and temperature conditions and pH of the reagent mixture. Such factors might have contributed to the deviation in the percent recovery of Cr.

Concentrations of potentially toxic elements in water from Mokolo River followed the order: Mn > Se > Zn > Cr > Pb > V > Cu > As > Ni > Cd > Fe during high flow season and Mn > Se > Zn > Cr > Cu > V > As > Pb > Ni > Cd >Fe during low flow season. The levels of potentially toxic elements in the water of Blood River were in the order: Mn > Se > Zn > Cr > V > Ni > Cu > As > Pb > Cd > Fe and Mn > Zn > Se > Cr > V > Ni > Cu > As > Pb > Cd > Fe during high and low flow seasons, respectively.

Maximum concentrations of the analysed elements were compared with MPL of elements in drinking and irrigation water set by different organisations. The levels were found to be at considerable levels compared to the guideline values set by DWAF (1996c), SANS (2005), WHO (2011) and CCME (1999). The levels of the elements in the water of Mokolo and Blood Rivers were below the recommended guideline values. There are however, exceptions. Manganese exceeded the MPL set by DWAF (20 µg/L), SANS 2005 (100 µg/L), WHO (500 µg/L) and CCME (200 µg/L). From both rivers, the presence of Se exceeded the MPL of 20 µg/L by DWAF (1996c) and SANS (2005). Additionally, the maximum concentration of As in both high and low flow seasons exceeded the MPL of 10 µg/L (SANS, 2005; WHO, 2011) in water samples collected from Blood River. Cadmium levels from both rivers did not exceed the MPL set by DWAF (10 µg/L), SANS 2005 (5 µg/L), WHO (3 µg/L) and CCME (5.1 µg/L). The maximum levels of Pb from Mokolo River were found below the MPL of the four guidelines. However, in water from Mokolo River they exceeded the levels set by SANS 2005(20 µg/L) and WHO (10 µg/L). Despite the lower levels for some elements in the water compared to the MPLs, the levels of these elements are more likely to elevate in the future if the current activities in and around the rivers are not controlled or minimised.

An ANOVA test on the data indicated that there were significant differences in the levels of potentially toxic elements in water from different sampling sites of Mokolo and Blood Rivers. This could be attributed to the different sources of environmental contaminants in these Rivers at each site that are associated with different point or non-point sources of pollution.

6.1.2 Determination of pseudo-total concentrations of potentially toxic elements in sediments samples

Certified reference materials of sediments (SRM 8704 and BCR 280R) were used to validate the method employed for determination of pseudo-total concentrations of potentially toxic elements in sediments collected from Mokolo and Blood Rivers.

Quantitative percent recoveries were obtained for all the elements using BCR 280R and SRM 8704, except for Se (55%). Thus, the method is reliable for the quantification of the elements in sediments with the exception of Se.

The high temperature and combination of the reagents might have caused Se to lose its volatile materials thus decreasing its concentration.

The pseudo-total levels of potentially toxic elements in sediments of Mokolo River were in the order: Fe > Cr > Mn > V > Zn > As > Ni > Cu > Pb > Cd > Se during high flow season and Fe > Mn > Cr > V > Zn > Ni > Pb > Cu > As > Cd > Se during low flow season.

In the sediments samples from Blood River, the levels of potentially toxic elements followed the pattern Fe > Mn > Cr > Ni > V > Zn > Cu > Pb > As > Cd > Se and Fe > Mn > Cr > Zn > V > Ni > Cu > Pb > As > Cd > Se during the high and low flow seasons, respectively. The results indicated that concentrations of elements determined in sediments during low flow season were significantly higher than high flow season concentrations. This could be attributed to the differences in water levels where low water levels result in precipitation of the elements in sediments.

Maximum concentrations of the selected elements in sediments were compared with SQGs set by WHO (2011), US EPA (1999) and CCME (2001). The levels of Fe, Mn, Ni and Cr in the sediments of Mokolo and Blood Rivers were above the SQGs of WHO, US EPA and CCME. This was observed in the sediments collected both in high and low flow seasons. The pseudo-total arsenic level exceeded the CCME guideline value of 12 mg/kg in the sediments of Mokolo River during the high flow season.
Although the pseudo-total levels of other elements were below the SQG values, their high concentrations raise concern if the current trend of pollution continues in and around both rivers.

6.1.3 Quantification of potentially toxic elements in sediments by sequential extraction methods

Fractionation of Cd, Cr, Cu, Fe, Ni, Pb and Zn in sediments of Blood River was achieved by employing microwave-assisted sequential extraction procedure. The data was validated by analysis of certified reference material of sediments (BCR 701) and quantitative percent recoveries were obtained.

Furthermore, validation of the microwave-assisted sequential extraction method was done by evaluating the percent recoveries obtained by calculating the sum of concentrations from the fractions to that of pseudo-total concentrations determined by digestion method multiplied by 100 to give a percentage. The percent recoveries obtained by this calculation are also quantitative.

Thus, we conclude that the developed microwave-assisted sequential extraction method is reliable and efficient for analysis of the selected elements in sediments. Furthermore, the statistical evaluation indicated that method C can reliably be used for fractionation studies of the analysed potentially toxic elements in sediments. There was no statistical difference ($P>0.05$) observed between methods B and C when the distributions of Cd, Cr, Cu, Fe, Ni, Pb and Zn in different fractions of the sediments of Blood River were assessed.

The ICF and GCF results showed Cr, Cu, Fe, Ni, Pb, and Zn, are likely to be released from the sediments to overlying water. The mobility of these elements from sediments to the water shows the risks to human health through food chain. Based on the average RAC values, it was concluded that although Cd, Cu, Ni and Pb were classified as medium contaminants, they are still mobile and may be bioavailable in the aquatic environment. Therefore, a significant remediation control must be applied to control pollution by these elements.

These findings were justified by consistency in the risk assessment results after calculation of RAC by using the levels of the elements obtained by both microwave-assisted sequential extraction and BCR sequential extraction methods.

6.2 RECOMMENDATIONS

Currently, no regular monitoring of potentially toxic elements is done in the studied rivers. The measured high levels of potentially toxic elements in water bodies of both rivers revealed a clear need for continued monitoring of potentially toxic elements in the water and sediments.

A regular schedule for sampling the main river and tributary streams should be established to evaluate the changing conditions surrounding the river.

Frequency of the sampling should be taken into consideration to identify the types of hazards, seasonal flows, storms and other factors that may change during the year and after the sample have been collected.

There is a need for sediments quality guidelines to be set for South African fresh waters. The process requires interaction and collaboration with scientists, regulators and implementers. Consequently, this document will raise issues that will need further investigation.

This study provided baseline data in the levels of the selected potentially toxic elements in Blood and Mokolo Rivers, which gives a valuable opportunity in understanding the changes brought about by anthropogenic activities in these impacted catchments. Thus, the results of this study may be used to inform the relevant authorities and environmental managers to increase awareness, and potentially lead to environmental pollution reduction interventions.

We recommend risk assessment study to be performed on the Mokolo River system by determining potentially toxic elements in different sediments fractions applying the developed microwave-assisted sequential extraction procedure. The associated risks to human health need to be assessed to determine how the use of contaminated water of Mokolo and Blood Rivers might be affecting the users.

In such study, fish and crops grown by irrigating with the water from both Blood and Mokolo Rivers need to be included to assess the impact via food chain.

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APPENDICES

APPENDIX A: CONCENTRATIONS OF POTENTIALLY TOXIC ELEMENTS IN WATER AND SEDIMENTS OF MOKOLO RIVER

Table 4.7: Concentrations (Mean ± SD, n = 3) (µg/L) of potentially toxic elements in water collected during the high flow season from Mokolo River and analysed by ICP-MS

Site	**M1W**	**M2W**	**M3W**	**M4W**	**M5W**	**M6W**	**M7W**	**M8W**	**M9W**	**M10W**
PH	7.86	7.90	8.06	7.18	7.95	7.75	9.35	7.99	7.86	7.72
Mn	80.9±1.36	32.8±0.12	13.1±0.096	11.3±0.10	185±1.9	19.8±0.33	164±1.0	3.36±0.14	37.5±0.93	4.72±0.17
Zn	9.03±0.24	6.90±0.18	<1.77	<1.77	7.38±0.83	<1.77	7.87±0.97	<1.77	21.6±0.44	<1.77
Ni	1.35±0.091	<0.62	1.81±0.066	2.05±0.031	2.12±0.040	1.95±0.052	2.22±0.059	2.16±0.14	1.67±0.15	2.28±0.10
Cu	1.08±0.18	5.74±0.023	3.10±0.12	2.83±0.037	4.05±0.64	2.89±0.068	3.16±0.001	2.71±0.016	2.94±0.098	<0.48
Cr	<0.11	<0.11	6.91±0.22	5.22±0.049	15.4±2.5	5.31±0.086	7.47±0.33	6.46±0.62	<0.11	6.09±0.003
Pb	0.514±0.042	174±0.36	3.98±0.0057	3.85±0.011	4.53±0.69	3.89±0.010	5.41±0.020	3.95±0.15	43.8±0.55	5.45±0.19
V	1.86±0.13	<0.53	4.01±0.064	3.17±0.11	6.80±0.023	3.40±0.17	4.72±0.10	3.77±0.026	<0.53	3.63±0.017
Cd	0.301±0.047	<0.030	0.962±0.0057	0.884±0.00	0.986±0.025	0.893±0.018	0.947±0.013	0.897±0.0042	0.503±0.011	0.898±0.008
As	1.28±0.11	<0.010	3.70±0.045	2.66±0.082	6.19±0.26	2.93±0.083	4.42±0.051	3.01±0.038	0.599±0.098	2.79±0.040
Se	19.4±0.69	<0.12	20.7±0.24	18.2±2.2	30.6±1.65	19.3±1.6	31.8±1.1	16.1±2.8	15.3±0.19	14.7±2.3
***Fe**	0.202±0.017	0.052±0.0	<0.049	<0.049	0.161±0.0	0.321±0.01	<0.049	<0.049	0.148±0.0	0.295±0.01

Table 4.8: Concentrations (Mean ± SD, n = 3) (µg/L) of potentially toxic elements quantified in water collected during the low flow season from Mokolo River and analysed by ICP-MS

Site	M1W	M2W	M3W	M4W	M5W	M6W	M7W	M8W	M9W	M10W
PH	7.01	7.06	6.89	7.02	7.04	7.01	6.94	7.07	6.60	6.50
Temp(˚C)	31.4	28.8	24.3	25.9	28.2	27.9	30.4	28.3	30.0	28.6
Mn	783±66	105±7.5	1160±110	164±1.0	41.3±2.2	35.1±1.2	185±1.9	36.0±4.86	91.1±2.8	830±19
Zn	25.6±0.030	23.6±2.0	11.0±1.1	7.87±0.97	9.23±0.09	6.46±1.1	7.38±0.83	4.28±0.49	<1.77	14.1±2.7
Ni	2.75±0.090	2.14±0.11	3.36±0.25	2.22±0.06	2.14±0.09	1.98±0.09	2.18±0.04	2.12±0.08	2.08±0.07	6.18±0.21
Cu	3.45±0.17	5.76±0.17	4.85±0.080	3.16±0.0	3.22±0.060	3.11±0.020	4.05±0.64	2.87±0.040	2.97±0.020	17.5±0.48
Cr	5.62±0.080	6.24±0.13	15.3±0.58	7.47±0.33	12.2±0.13	7.49±0.37	15.4±2.5	5.90±0.45	5.25±0.30	<0.11
Pb	4.24±0.10	4.68±0.070	4.13±0.090	5.41±0.020	3.93±0.010	3.99±0.030	4.53±0.69	3.97±0.050	3.94±0.030	1.79±0.045
V	4.61±0.18	4.57±0.020	6.16±0.090	4.72±0.10	5.83±0.14	4.61±0.21	6.80±0.020	4.35±0.11	3.74±0.00	<0.53
Cd	0.970±0.03	0.960±0.02	0.930±0.01	0.950±0.01	0.96±0.030	0.95±0.020	0.990±0.03	0.95±0.020	0.91±0.010	<0.030
As	4.31±0.10	4.28±0.070	5.95±0.21	4.42±0.050	5.43±0.13	4.29±0.20	6.19±0.26	3.98±0.25	3.67±0.16	<0.010
Se	33.0±1.5	29.3±3.6	27.0±0.42	31.8±1.1	30.7±1.0	28.6±2.00	30.6±1.6	28.9±2.1	22.6±1.24	26.5±0.079
Fe	0.41±0.0.021	0.23±0.030	0.44±0.013	0.33±0.011	0.37±0.001	0.51±0.036	0.36±0.029	0.750±0.00	5.02±0.009	4.22±0.061

Table 4.9: Pseudo-total concentrations (mg/kg) of potentially toxic elements in sediments collected during the high flow season from Mokolo River and analysed by ICP-MS

Site	**M1W**	**M2W**	**M3W**	**M4W**	**M5W**	**M6W**	**M7W**	**M8W**	**M9W**	**M10W**
Element	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Mn	23.9 ±4.2	20.2± 0.61	34.7± 0.17	16.3± 0.14	27.0± 0.55	20.0± 0.035	38.0± 1.7	38.0± 0.72	74.5± 0.99	85.0± 3.9
Zn	18.2 ±1.1	22.6± 0.53	23.6± 1.8	15.4± 0.20	21.7± 0.63	29.6± 0.12	35.2± 1.5	27.7± 1.0	29.0± 0.015	26.6± 0.36
Ni	5.70 ±0.41	7.69± 0.24	8.18± 0.54	10.7± 0.15	13.2± 0.37	11.3± 0.0	8.11± 0.35	14.8± 0.19	13.7± 0.13	40.6± 13
Cu	7.45 ±2.0	8.34± 0.26	9.77± 0.67	5.94± 0.056	11.6± 0.31	9.90± 0.055	8.57± 0.61	11.4± 0.68	13.8± 0.33	11.1± 0.016
Cr	55.4 ±3.8	69.1± 0.310	103± 0.054	96.9± 3.2	86.9± 3.0	88.5± 3.8	125± 2.8	145±2.2	141± 0.46	171± 4.7
V	17.3 ±0.21	19.6± 0.020	27.0± 1.1	23.4± 0.64	22.4± 0.75	20.0± 0.75	31.7± 0.79	34.1± 0.46	44.0± 1.3	35.4± 0.85
As	111± 8.5	14.5± 0.025	17.5± 0.54	21.0± 0.45	15.3± 0.24	41.3± 0.24	21.2± 0.55	22.0± 0.48	22.8± 0.27	25.7± 0.46
Se	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48
Cd	0.550±0.14	0.704 ±0.0030	0.931 ±0.065	0.385 ±0.0041	0.864 ±0.017	0.726 ±0.0010	0.828 ±0.045	0.803 ±0.0060	0.954 ±0.043	0.731 ±0.021
Pb	7.43 ±1.5	2.66± 0.035	6.1±1.1	2.82± 0.002	5.58± 0.094	35.4± 0.019	2.14± 0.098	3.35± 0.15	6.46± 0.036	3.54± 1.2
***Fe**	3760 ±98	3570± 26	2740 ±2.0	2180± 590	3100 ±23	3590± 94	2880 ±42	3280± 130	2760 ±110	3330 ±110

Table 4.10: Pseudo-total concentrations (mg/kg) of potentially toxic elements in sediments collected during the low flow season from Mokolo River and analysed by ICP-MS

Site	**M1S**	**M2S**	**M3S**	**M4S**	**M5S**	**M6S**	**M7S**	**M8S**	**M9S**	**M10S**
Element	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Mn	443±9.0	136±1.9	<0.049	371±15	114±3.5	127±0.24	624±15	79.8±4.4	56.7±3.8	48.8±5.4
Zn	15.3±0.30	11.1±1.3	27.3±0.61	8.52±0.33	4.93±0.43	5.80±0.30	7.93±0.13	4.29±0.043	5.43±0.015	6.47±0.077
Ni	12.6±0.067	7.74±0.023	29.0±0.69	10.8±0.20	5.63±0.063	6.47±0.079	7.58±0.13	5.21±0.25	5.80±0.21	5.74±0.063
Cu	7.00±0.94	10.2±2.3	15.1±0.38	4.65±0.010	2.67±0.11	2.73±0.023	2.49±0.061	2.60±0.10	2.96±0.064	4.29±0.27
Cr	40.0±3.4	25.8±1.6	63.8±2.8	30.3±0.79	22.1±2.7	25.3±0.13	22.2±0.73	17.3±1.3	20.4±0.81	28.4±0.99
V	26.0±2.4	17.2±0.42	45.2±1.8	21.3±0.36	13.0±0.30	16.4±0.021	15.9±0.60	12.5±0.97	13.9±0.95	17.9±0.34
As	2.82±0.48	1.97±0.24	3.05±0.18	1.17±0.038	1.12±0.28	1.5±0.018	1.49±0.024	1.30±0.052	0.928±0.0011	1.47±0.086
Se	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48
Cd	<0.030	<0.030	0.035±0.005	<0.030	<0.030	<0.030	<0.030	<0.030	<0.030	0.477±0.013
Pb	8.34±0.15	8.12±0.28	13.5±0.31	9.46±0.45	4.97±0.47	4.37±0.025	5.31±0.13	5.27±0.62	4.01±0.019	5.61±0.15
***Fe**	3620±43	3650±42	3790±10	3950±41	3700±14	3850±77	3830±250	3720±94	3660±43	3500±37

APPENDIX B: CONCENTRATIONS OF POTENTIALLY TOXIC ELEMENTS IN WATER AND SEDIMENTS OF BLOOD RIVER

Table 4.11: Concentrations (µg/L) of potentially toxic elements in water collected during the high flow season from Blood River and analysed by ICP-MS

Site	**B1W**	**B2W**	**B3W**	**B4W**	**B5W**	**B6W**	**B7W**	**B8W**	**B9W**	**B10W**
Ph	7.81	8.56	8.07	8.93	7.94	8.98	8.32	7.97	7.97	8.07
Mn	197±6.1	98.2±1.3	143±0.71	50.2±1.6	168±3.9	502±10	179±3.0	145±1.0	139±0.58	143±2.3
Zn	160±12	113±12	84.0±1.1	83.3±11	59.6±4.5	110±2.8	89.1±14	90.1±15	82.7±7.7	67.6±3.6
Ni	56.8±0.93	34.7±1.3	24.5±0.56	17.3±0.51	14.2±0.25	16.5±0.50	12.0±0.35	18.2±0.74	13.6±0.59	9.51±0.48
Cu	24.1±3.0	15.8±0.33	9.76±0.50	15.6±1.0	4.42±0.60	16.8±1.1	13.0±0.34	18.2±1.8	17.4±3.5	13.7±0.11
Cr	66.4±2.1	56.2±0.29	43.6±1.2	60.8±0.38	40.4±1.9	64.8±0.67	66.7±0.96	67.4±1.4	66.8±0.14	69.2±3.0
Pb	17.1±0.91	10.6±0.19	4.35±0.14	2.84±0.13	2.02±0.05	2.77±0.17	2.72±0.10	3.07±0.055	3.01±0.086	2.17±0.033
V	68.6±3.3	67.6±1.8	51.6±2.3	66.1±1.5	51.8±2.4	48.7±1.2	43.7±1.1	44.4±1.0	46.3±1.7	47.0±2.5
Cd	1.80±0.033	1.24±0.026	1.16±0.076	1.28±0.13	0.902±0.042	0.847±0.027	0.866±0.12	0.820±0.078	0.948±0.10	0.799±0.033
As	12.3±0.094	9.21±0.52	9.81±0.70	13.9±0.91	10.0±0.43	15.5±0.50	15.1±0.55	15.6±0.34	16.3±0.34	15.9±1.0
Se	95.9±1.6	45.3±4.9	85.3±4.3	93.3±11	75.9±9.3	75.7±1.3	72.3±1.3	75.8±4.8	74.8±4.5	77.1±4.3
Fe	2.59±0.013	3.93±0.034	0.382±0.012	0.202±0.026	<0.049	<0.049	<0.049	<0.049	<0.049	<0.049

Table 4.12: Concentrations (µg/L) of potentially toxic elements in water collected during the low flow season from Blood River and analysed by ICP-MS

Site	**B1S**	**B2S**	**B3S**	**B4S**	**B5S**	**B6S**	**B7S**	**B8S**	**B9S**	**B10S**
pH	8.26	8.81	8.11	9.32	7.77	7.90	7.83	7.91	8.53	9.29
Temp(˚C)	19.1	22.5	19.8	23.8	23.0	23.4	24.9	27.8	25.8	24.1
Mn	49.5±0.19	50.3±6.0	40.7±0.24	129±2.3	452±4.6	303±3.9	231±0.67	81.7±0.30	209±5.7	672±2.4
Zn	87.2±0.20	162±11	50.4±2.8	56.4±0.41	37.1±9.2	41.0±0.83	53.5±0.03	64.5±0.50	57.8±4.9	60.7±0.42
Ni	14.3±1.7	34.4±2.5	15.9±1.8	15.0±028	16.9±0.17	14.5±0.06	14.2±2.7	11.6±0.46	14.5±0.73	18.3±0.26
Cu	21.1±0.47	34.9±6.4	11.6±0.20	10.9±1.4	12.9±3.9	8.00±0.061	13.4±0.44	17.0±0.24	14.5±0.47	17.0±2.1
Cr	21.8±3.6	47.3±10	42.9±1.32	45.1±1.9	72.1±1.7	62.5±2.4	59.6±0.42	62.0±0.43	21.4±2.9	39.5±1.3
Pb	5.20±0.81	13.4±2.1	14.4±0.27	2.60±0.010	2.37±0.14	1.64±0.021	3.51±0.13	2.47±0.15	6.38±0.45	11.5±0.20
V	27.3±4.2	43.6±7.0	48.3±0.41	43.5±1.4	45.8±1.6	39.0±0.54	36.0±0.84	35.7±0.14	29.7±1.1	48.5±2.7
Cd	0.990±0.23	1.84±0.31	1.46±0.016	1.88±0.035	1.23±0.22	0.844±0.040	0.767±0.013	0.961±0.055	0.819±0.13	1.05±0.16
As	6.30±0.43	12.5±2.2	9.63±1.2	10.2±0.43	14.6±0.40	17.0±0.87	18.5±0.042	19.3±0.26	11.4±0.29	11.4±0.0
Se	44.6±19	70.7±0.77	60.2±1.8	57.0±5.8	61.3±6.3	85.3±1.3	85.3±1.3	93.6±5.8	83.9±1.8	90.8±4.4
Fe	0.425±0,011	0.526±0.096	0.426±0.01	0.469±0.0	0.119±0.004	0.167±0.004	0.436±0.016	0.301±0.012	0.910±0.037	1.82±0.004

Table 4.13: Pseudo-total concentrations (mg/kg) of potentially toxic elements in sediments collected during the high flow season from Blood River and analysed by ICP-MS

	B1W	**B2W**	**B3W**	**B4W**	**B5W**	**B6W**	**B7W**	**B8W**	**B9W**	**B10W**
Element	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Mn	74.6±1.8	33.7±0.31	103±0.32	97.7±0.043	284±8.5	158±7.2	160±0.37	18.9±0.12	69.7±0.045	71.8±0.11
Zn	6.69±0.26	6.35±0.068	15.3±0.081	10.6±0.11	18.8±2.0	18.6±0.31	29.7±0.17	8.96±0.083	14.4±0.54	11.2±0.50
Ni	10.9±0.20	3.84±0.055	14.1±0.061	13.3±0.017	14.4±0.180	12.7±0.21	20.9±0.12	42.4±0.15	31.4±3.0	23.1±5.3
Cu	3.55±0.12	2.01±0.0042	14.4±0.059	5.37±0.042	7.34±0.059	6.12±0.12	7.72±0.042	2.13±0.010	5.21±0.93	2.84±0.067
Cr	36.4±0.55	12.3±0.21	51.8±0.39	41.9±0.35	56.2±2.1	37.1±0.73	55.2±0.89	17.1±0.21	35.8±1.1	30.6±0.14
V	10.8±0.38	5.12±0.11	17.6±0.086	15.5±0.12	21.8±0.67	14.7±0.34	19.4±0.13	9.49±0.060	15.9±1.0	13.7±0.24
As	0.447±0.009	0.290±0.016	0.637±0.008	0.789±0.021	1.59±0.14	1.85±0.025	1.51±0.056	0.692±0.007	1.28±0.072	1.37±0.008
Se	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48
Cd	<0.030	<0.030	0.0588±0.001	0.0396±0.003	<0.030	0.062±0.0023	0.0467±0.0022	<0.030	<0.030	<0.030
Pb	2.26±0.23	5.32±0.043	2.56±0.086	2.64±0.034	6.48±0.41	3.63±0.087	5.90±0.037	2.60±0.0082	3.440±0.18	2.57±0.16
***Fe**	3770±86	2720±23	3670±16	3570±0.85	3590±43	3470±100	3410±74	1820±28	3200±54	3200±100

Table 4.14: Pseudo-total concentrations (mg/kg) of potentially toxic elements in sediments collected during the low flow season from Blood River and analysed by ICP-MS

	B1S	**B2S**	**B3S**	**B4S**	**B5S**	**B6S**	**B7S**	**B8S**	**B9S**	**B10S**
Element	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	an ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Mn	262±5.9	379±12	376±3.3	509±5.2	361±3.0	487±15	295±10	99.3±1.2	134±3.0	236±1.6
Zn	15.4±0.087	19.0±0.18	17.5±0.88	119±0.10	53.7±0.42	54.2±0.81	34.6±1.2	19.2±1.5	18.8±1.1	40.3±1.0
Ni	33.7±0.43	37.2±1.5	39.4±0.53	51.6±0.093	49.8±0.65	45.7±0.68	29.4±0.12	14.4±0.61	20.0±1.0	32.9±1.2
Cu	15.1±0.50	17.9±1.0	18.3±0.080	33.7±0.10	28.5±0.12	28.1±1.1	15.7±0.88	11.5±0.52	10.0±0.43	17.7±0.64
Cr	49.8±2.4	49.8±1.6	70.0±1.0	89.3±2.1	76.3±4.4	57.7±4.3	58.2±2.9	24.7±0.76	23.2±0.36	54.2±0.84
V	29.3±0.44	40.1±1.7	41.7±0.21	51.9±0.54	49.8±2.3	47.8±0.58	30.4±1.1	17.6±0.0054	21.5±0.15	31.8±0.14
As	2.24±0.013	3.1±0.15	3.49±0.12	5.52±0.002	5.47±0.61	4.64±0.16	2.23±0.041	2.20±0.072	1.20±0.55	2.06±0.69
Se	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48	<2.48
Cd	<0.030	<0.030	<0.030	0.115±0.007	0.0486±0.002	0.0626±0.001	0.0440±0.002	<0.030	<0.030	0.0352±0.002
Pb	6.56±0.15	7.71±0.11	5.66±0.027	23.2±0.048	12.4±0.14	14.9±0.77	18.5±3.3	3.26±0.12	4.49±0.14	7.86±0.15
***Fe**	3330±23	3450±9.6	3350±97	3380±90	3370±77	3330±130	3740±15	3600±42	3630±25	3610±38

APPENDIX C: MODIFIED BCR SEQUENTIAL EXTRACTION FOR BLOOD RIVER SEDIMENTS COLLECTED DURING LOW FLOW SEASON

Table 5.9. Fractionation of potentially toxic elements in sediments sample 1 collected from Blood River by modified BCR sequential extraction method (Method B)

Element	**Site 1**						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ±SD	Σ(F1-F4) Extracted	Pseudo - total digested	Recovery (%)
Cd	0.00613±0.00	0.00204±0.00	0.00186±0.00	-1.70±0.009	0.0100	0.0130	77
Cr	1.69±0.012	15.0±0.075	30.0±0.22	0.954±0.018	47.6	49.8	96
Cu	3.14±0.110	7.71±0.028	3.48±0.007	0.338±0.006	14.7	15.1	97
Fe	325±4.9	1980±120	685±12	333±5.2	3320	3330	100
Ni	5.58±0.10	18.6±0.037	6.82±0.15	1.71±0.010	32.7	33.7	97
Pb	0.509±0.014	4.10±0.16	1.07±0.066	0.806±0.009	6.49	6.56	99
Zn	10.5±0.17	3.93±0.024	1.05±0.060	0.582±0.014	16.0	15.4	104

Table 5.10: Fractionation of potentially toxic elements in sediments sample 2 collected from Blood River by modified BCR sequential extraction method (Method B)

Element	**Site 2**						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudototal digested	Recovery (%)
Cd	0.00511±0.009	0.00204±0.00	0.0019±0.00	-1.70±0.009	0.0116	0.0144	80
Cr	1.31±0.021	13.0±0.16	27.80±0.028	1.25±0.023	42.1	49.8	85
Cu	4.12±0.11	6.54±0.13	3.48±0.0071	0.942±0.006	15.1	17.9	84
Fe	329±2.4	1980±122	872±2.1	295±7.2	3480	3450	101
Ni	4.93±0.047	17.3±0.33	7.95±0.30	2.24±0.028	32.4	37.2	87
Pb	0.704±0.007	4.03±0.071	1.07±0.066	0.702±0.021	6.51	7.71	99
Zn	12.3±0.026	4.66±0.069	2.09±0.024	18±0.014	20.7	19.0	104

Table 5.11: Fractionation of potentially toxic elements in sediments sample 3 collected from Blood River by modified BCR sequential extraction method (Method B)

Element	Site 3						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.00615±0.00	0.00375±0.00	0.00411±0.00	-2.05±0.061	0.0140	0.0172	81
Cr	5.28±0.049	16.9±0.17	29.0±0.36	3.25±0.032	54.5	70.0	78
Cu	5.11±0.020	7.44±0.011	3.27±0.046	0.870±0.015	16.7	18.3	91
Fe	289±1.6	1770±104	833±12	171±2.4	3060	3350	91
Ni	6.15±0.17	23.8±0.21	10.1±0.18	2.00±0.19	42.1	39.4	107
Pb	2.47±0.019	1.65±0.055	0.785±0.009	0.297±0.026	5.21	5.66	92
Zn	12.6±0.071	4.91±0.085	1.49±0.011	0.624±0.008	19.6	17.5	112

Table 5.12: Fractionation of potentially toxic elements in sediments sample 4 collected from Blood River by modified BCR sequential extraction method (Method B)

Element	Site 4						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0708±0.001	0.0411±0.002	0.0237±0.001	0.00126±0.00	0.137	0.115	119
Cr	9.05±0.045	29.0±0.070	42.8±0.0077	3.78±0.022	84.7	89.3	95
Cu	12.1±0.72	4.87±0.039	4.75±0.0002	6.07±0.081	27.8	33.7	83
Fe	591±1.1	1650±7.1	792±8.6	555±7.4	3590	3380	106
Ni	13.1±0.16	12.6±0.095	8.65±0.14	2.32±0.14	36.7	51.6	71
Pb	2.46±0.026	14.0±0.012	3.89±0.079	1.80±0.018	22.1	23.2	95
Zn	83.1±0.74	31.4±0.189	8.68±0.19	4.65±0.11	130	119	107

Table 5.13: Fractionation of potentially toxic elements in sediments sample 5 from Blood River by modified BCR sequential extraction method (Method B)

Element	Site 5						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0245±0.00	0.0080±0.00	-0.929±0.05	-1.70±0.009	0.0326	0.0486	67
Cr	6.93±0.11	26.1±0.066	38.8±0.008	1.78±0.022	73.6	73.6	100
Cu	8.23±0.23	13.1±0.27	6.95±0.014	1.88±0.012	30.1	28.5	106
Fe	308±3.1	1790±1.7	833±12	271±2.4	3200	3370	95
Ni	10.8±0.27	21.9±1.5	5.95±0.25	1.42±0.042	40.1	49.8	80
Pb	1.02±0.029	8.20±0.32	2.15±0.13	1.62±0.017	13.0	12.4	105
Zn	15.0±0.48	17.0±0.10	6.10±0.36	3.38±0.22	41.5	53.7	77

Table 5.14: Fractionation of potentially toxic elements in sediments sample 6 collected from Blood River by modified BCR sequential extraction method (Method B)

Element	Site 6						
	F1 Mean ± SD	Stage 2 Mean ± SD	Stage 3 Mean ± SD	Stage 4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0307±0.0	0.0102±0.0	0.00932±0.0	0.525±0.047	0.0502	0.0626	80
Cr	3.09±0.094	23.1±0.98	34.4±4.3	4.83±0.10	65.4	57.7	113
Cu	9.47±1.5	11.4±0.83	7.02±0.74	2.43±0.16	30.3	28.1	108
Fe	128±3.5	1760±52	524±3.1	199±0.73	3323	3330	100
Ni	9.70±0.26	15.5±0.30	7.28±0.21	3.57±0.23	36.1	45.7	79
Pb	1.41±0.015	8.06±0.14	2.22±0.045	1.40±0.041	13.1	14.9	88
Zn	23.9±0.57	17.4±0.57	7.45±1.2	6.89±0.31	55.7	54.2	103

Table 5.15: Fractionation of potentially toxic elements in sediments sample 7 collected from Blood River by modified BCR sequential extraction method (Method B)

Element	Site 7						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0221±0.00	-0.0609±0.0016	0.0109±0.00	0.0127±0.00	0.0458	0.0440	104
Cr	5.64±0.16	17.6±0.52	31.7±0.85	1.75±0.088	56.7	58.2	97
Cu	5.02±0.35	5.69±0.42	3.51±0.37	1.22±0.082	15.4	15.7	98
Fe	183±3.5	2110±19	608±3.2	348±3.6	3250	3740	87
Ni	7.21±0.33	12.5±0.14	5.20±0.11	2.39±0.034	27.3	29.4	93
Pb	3.41±0.015	8.98±0.004	5.65±0.49	0.907±0.014	18.9	18.5	102
Zn	18.0±0.75	7.86±0.00	4.74±0.027	3.38±0.22	34.0	34.6	98

Table 5.16: Fractionation of potentially toxic elements in sediments sample 8 collected from Blood River by modified BCR sequential extraction method (Method B)

Element	Site 8						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0123±0.00	0.00406±0.00	0.00373±0.00	-0.410±0.019	0.0201	0.021	96
Cr	2.62±0.055	8.39±0.29	15.8±0.42	0.907±0.044	27.7	24.7	112
Cu	2.62±0.080	4.37±0.12	2.57±0.085	1.20±0.12	10.8	11.5	93
Fe	320±1.1	1970±15	851±0.26	289±2.3	3430	3600	95
Ni	3.61±0.17	6.50±0.45	2.64±0.074	1.20±0.017	13.9	14.4	97
Pb	1.68±0.007	0.917±0.066	0.284±0.0067	0.0153±0.00	2.89	3.26	89
Zn	11.5±0.36	5.82±0.14	2.74±0.027	0.915±0.058	21.0	19.2	109

Table 5.17: Fractionation of potentially toxic elements in sediments sample 9 collected from Blood River by modified BCR sequential extraction method (Method B)

Element	**Site 9**						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0125±0.00	0.00751±0.00	0.00831±0.00	-2.04±0.087	0.0283	0.0266	106
Cr	0.595±0.0094	5.90±0.075	12.9±0.38	0.570±0.010	19.9	23.2	86
Cu	2.61±0.13	4.46±0.013	2.48±0.0071	0.656±0.0011	10.2	10.0	102
Fe	330±0.87	1920±84	872±2.1	293±8.5	3410	3630	94
Ni	3.93±0.047	11.2±0.33	6.89±0.73	1.01±0.20	23.1	20.0	115
Pb	0.689±0.025	3.99±0.0035	0.255±0.013	-0.0681±0.003	4.93	4.49	110
Zn	12.4±0.070	4.66±0.069	2.01±0.14	1.56±0.037	20.6	18.8	110

Table 5.18: Fractionation of potentially toxic elements in sediments sample 10 collected from Blood River by modified BCR sequential extraction method (Method B)

Element	**Site 10**						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0230±0.00	0.0129±0.00	-0.348±0.0035	-0.234±0.0043	0.0359	0.0352	102
Cr	5.64±0.16	19.0±1.5	31.7±0.85	1.81±0.088	58.1	54.2	107
Cu	4.49±0.40	5.69±0.42	3.51±0.37	1.22±0.082	14.9	17.7	84
Fe	180±0.12	2010±160	569±58	348±3.6	3110	3610	86
Ni	8.56±0.16	13.5±0.14	5.28±0.15	2.39±0.034	29.8	32.9	90
Pb	1.70±0.0075	3.55±0.086	0.622±0.039	0.454±0.0071	6.33	7.86	81
Zn	18.0±0.75	7.86±0.00	4.74±0.027	4.38±0.22	35.0	40.3	87

APPENDIX D: MICROWAVE-ASSISTED SEQUENTIAL EXTRACTION

Table 5.19: Fractionation of potentially toxic elements in sediments sample 1 collected from Blood River by microwave-assisted sequential extraction method (Method C)

Element	Site 1						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo- total digested	Recovery (%)
Cd	0.00593±0.00	0.00203±0.00	0.00189±0.00	-1.69±0.030	0.00985	0.0130	76
Cr	1.58±0.023	13.1±0.011	29.5±0.56	0.968±0.0074	45.1	49.8	91
Cu	2.82±0.035	7.49±0.065	3.33±.011	0.323±0.001	14.0	15.1	92
Fe	294±7.2	1760±5.5	678±2.0	329±0.41	3100	3330	93
Ni	4.89±0.0098	16.8±0.023	6.65±0.027	1.34±0.014	29.7	33.7	88
Pb	0.517±0.0053	3.56±0.023	1.05±0.016	0.772±0.001	5.90	6.56	90
Zn	8.36±0.10	3.88±0.051	0.981±0.0087	0.618±0.031	13.8	15.4	90

Table 5.20: Fractionation of potentially toxic elements in sediments sample 3 collected from Blood River by microwave-assisted sequential extraction method (Method C)

Element	Site 3						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo- total digested	Recovery (%)
Cd	0.00383±0.00	0.00347±0.00	0.00392±0.00	-1.02±0.006	0.0112	0.0172	65
Cr	4.62±0.045	16.7±0.036	28.3±0.030	3.17±0.013	52.9	70.0	76
Cu	5.55±0.10	7.01±0.0021	2.98±0.011	0.854±0.00	16.4	18.3	9.
Fe	278±1.9	1770±1.0	823±2.1	168±1.9	3040	3350	91
Ni	5.42±0.072	23.8±0.21	9.87±0.049	1.64±0.045	40.8	39.4	103
Pb	2.11±0.070	1.65±0.055	0.762±0.014	0.232±0.005	4.75	5.66	84
Zn	12.4±0.005	4.91±0.085	1.35±0.019	0.599±0.002	19.2	17.5	110

Table 5.21: Fractionation of potentially toxic elements in sediments sample 5 collected from Blood River by microwave-assisted sequential extraction method (Method C)

Element	Site 5						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0227±0.00	0.00812±0.00	0.0138±0.00	-1.56±0.004	0.0309	0.0486	64
Cr	6.61±0.065	25.4±0.69	37.5±0.083	1.68±0.0077	71.2	76.3	93
Cu	7.78±0.12	12.7±0.57	7.01±0.0019	1.69±0.0061	29.2	28.5	102
Fe	288±1.2	1670±3.1	823±2.0	271±2.4	3050	3370	91
Ni	9.85±0.056	18.9±0.77	6.01±0.57	1.56±0.028	36.3	49.8	73
Pb	0.846±7.7	7.69±0.070	2.03±0.0018	1.57±0.016	12.1	12.4	98
Zn	13.2±15.0	15.0±0.20	6.10±0.36	3.09±0.0041	37.4	53.7	70

Table 5.22: Fractionation of potentially toxic elements in sediments sample 7 collected from Blood River by microwave-assisted sequential extraction method (Method C)

Element	Site 7						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0215±0.00	-0.0138±0.0016	0.0108±0.00	0.0124±0.00	0.0447	0.0440	102
Cr	5.29±0.053	16.7±0.042	31.3±0.042	2.12±0.010	55.4	58.2	95
Cu	3.82±0.072	4.62±0.045	3.13±0.009	1.12±0.004	12.7	15.7	81
Fe	189±1.1	1990±7.4	603±1.7	354±2.0	3140	3740	84
Ni	8.65±0.12	11.6±0.019	5.23±0.003	2.25±0.016	27.7	29.4	94
Pb	3.08±0.11	8.45±0.037	5.28±0.071	0.976±0.003	17.8	18.5	96
Zn	16.7±0.20	7.50±0.10	4.56±0.055	3.42±0.62	32.2	34.6	93

Table 5.23: Fractionation of potentially toxic elements in sediments sample 10 collected from Blood River by microwave-assisted sequential extraction method (Method C)

Element	Site 10						
	F1 Mean ± SD	F2 Mean ± SD	F3 Mean ± SD	F4 Mean ± SD	Σ(F1-F4) Extracted	Pseudo-total digested	Recovery (%)
Cd	0.0234±0.00	0.0124±0.00	- 0.288±0.007	- 0.193±0.0081	0.0358	0.0352	102
Cr	5.41±0.066	17.3±0.033	31.0±0.021	1.98±0.008	55.8	54.2	103
Cu	4.68±0.029	5.13±0.001	3.54±0.054	1.12±0.003	14.5	17.7	82
Fe	177±0.93	2110±4.8	594±6.1	359±0.79	3240	3610	90
Ni	7.95±0.047	13.1±0.003	4.74±0.045	2.36±0.010	28.2	32.9	86
Pb	1.69±0.015	3.19±0.010	0.592±0.004	0.463±0.007	5.94	7.86	76
Zn	18.5±0.040	7.56±0.020	4.58±0.024	4.64±0.15	35.3	40.3	88

CONFERENCE PRESENTATION

Lovia Matabane, Abayneh Ambushe and Taddese Godeto, Assessment of potentially toxic elements in river water and sequentially extracted river sediments, 42nd SACI Conference, 29 November to 4 December, 2015, Elangeni Hotel, Durban, South Africa. (Poster Presentation)