SPECIATION OF CHROMIUM IN WATER AND SEDIMENTS FROM MOKOLO AND BLOOD RIVERS, LIMPOPO PROVINCE

by

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DISSERTATION

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DECLARATION

I Mokgohloa Conny Putsane hereby declare that this research project titled '**Speciation of chromium in water and sediments from Mokolo and Blood Rivers, Limpopo Province'** submitted for the degree Master of Science in Chemistry at University of Limpopo, is my own work and that all the sources I have used have been indicated and acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other institution.

DEDICATION

To my daughter Mokgohloa Ntopa Lesedi and my sister Mamabolo Ntlametlwana Irene, I would not have gone this far without their understanding, patience and support.

ACKNOWLEDGEMENTS

ABSTRACT

Rivers provide the main water sources for domestic, industrial, and irrigational purposes; however, they could be polluted by receiving wastes from municipal and industrial sources as well as runoff from agricultural land. This could leave rivers contaminated with chromium (Cr) and other potentially toxic elements. Chromium can be either essential or carcinogenic depending on the chemical form.

The aim of this study was to assess and quantify trivalent Cr [Cr(III)] and hexavalent Cr [Cr(VI)] in water and sediment samples, collected from Mokolo and Blood Rivers in the Limpopo province. Water and sediment samples were collected from ten sampling sites from each river. Microwave assisted acid digestion and microwave assisted extraction methods were used to obtain a simple, rapid and safe method for the determination of total Cr and Cr(VI) in sediments. Water samples were acidified with ultra-pure HNO_3 and analysed directly to quantify total Cr. The accuracy of the method was evaluated by employing a sediment standard reference material (SRM 8704) and trace elements in water reference material (SRM 1643f). Total Cr and Cr(VI) in water and sediment samples were quantified using flame atomic absorption spectrometry (F-AAS) and graphite furnace-atomic absorption spectrometry (GF-AAS). The Cr concentrations in water and sediment samples collected from Blood River were found in the range 1.56 to 6.11 µg/L and 129.2 to 252.9 µg/g, respectively. The concentrations of Cr in water and sediment samples obtained from Mokolo River ranged from 1.34 to 3.53 µg/L and 25.7 to 156.4 µg/g, respectively.

A new solid phase extraction method was developed using chromabond-NH_2 column to determine Cr(VI) in water. In order to achieve separation of Cr(VI), the sample was pre-concentrated and passed through a conditioned chromabond-NH_2 column at a flow rate of 3 mL/min. Hexavalent Cr was selectively adsorbed onto the column and the contents were dried under vacuum. The retained Cr(VI) was subsequently eluted with two column volumes of 2 M HNO_3 and diluted to a final volume of 10.0 mL. The limit of detection was 0.105 µg/L and the relative standard deviations were less than 2%. The validation of the procedure was performed by spiking standard solutions containing Cr(III) and Cr(VI) and the percentage recoveries were higher than 88%. The concentrations of Cr(VI) in Blood and Mokolo Rivers ranged from 0.13 to 1.99 µg/L and 0.13 to 0.55 µg/L, respectively.

Hexavalent Cr in sediment samples was determined by employing leaching reagents of 0.1 M Na_2CO_3 and 0.01 M Na_3PO_4. Determination of Cr(VI) by using 0.1 M Na_2CO_3 as leaching reagent followed by atomic spectrometric measurements provided satisfactory results with percentage recoveries of 94.9 to 105%. This was achieved by extracting the sediment–reagent solution mixture for a period of 15 min at maximum pressure and temperature of 700 psi and 120 ºC, respectively. The leached Cr(VI) was then quantified by GF-AAS after filtration of the sample solutions through a hydrophilic Millipore PVDF 0.45 µm filter. The quantified levels of Cr(VI) leached by 0.1 M Na_2CO_3 in sediment samples of Blood and Mokolo Rivers were in the range 0.41 to 1.32 µg/g and 0.17 to 0.82 µg/g, respectively. The concentrations of Cr(VI) obtained employing the leaching reagent of 0.01 M Na_3PO_4 were found to be below LOD value of 0.06 µg/g in Mokolo River in all the sites and were in the range of 0.07 to 0.09 µg/g in Blood River.

The low values of Cr(VI) in water and sediments show that Cr(III) did not oxidise to Cr(VI) during sample preparation. In Blood and Mokolo Rivers, although the concentrations of Cr(VI) are low, their values suggest a link with the industrial and agricultural activities in the area.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic absorption spectrometry
ATSDR	Agency for Toxic Substances and Disease Registry
DLLME	Dispersive liquid liquid microwave extraction
DMSPE	Dispersive micro solid phase extraction
EDTA	Ethylenediaminetetraacetic acid
ERL	Effect range low
ERM	Effect range medium
FAAS	Flame atomic absorption spectrometry
FAO	Food and Agriculture Organisation
FIA	Flow injection analysis
GC-ICPMS	Gas chromatography-inductively coupled plasma mass spectrometry
GF-AAS	Graphite furnace-atomic absorption spectrometry
HPLC	High performance liquid chromatography
ICP-AES	Inductively coupled plasma-atomic emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
IDP	Intergrated development plan
LLE	Liquid liquid extraction
LOD	Limit of detection
LOQ	Limit of quantification
LPME	Liquid phase micro extraction
MAE	Microwave assisted extraction
MPLs	Maximum permissible levels

NIOSH   National Institute for Occupational Safety and Health

OECP   Organisation for Economic Co-operation and Development

OSHA   Occupational Safety and Health Administration

PEL   Portable effect level

SANS   South African National Standards

SPE   Solid phase extraction

SPME   Solid phase micro extraction

SQGs   Sediment quality guidelines

SRM   Standard reference material

SWWTW   Seshego wastewater treatment works

TBAB   Tetra-n-butylammonium bromide

TEL   Threshold effect level

USEPA   United States Environmental Protection Agency

WHO   World Health Organisation

CHAPTER 1: INTRODUCTION

1.1 PROBLEM STATEMENT

Contamination of water and sediments is a global threat to biodiversity and human beings. It comes as a result of the unnoticed accumulation of heavy metals in the environment (Nguyen *et al.*, 2005). One such heavy metal is chromium (Cr), which naturally occurs in the earth's crust. It exists primarily in two forms: trivalent and hexavalent. Trivalent Cr is an essential nutrient (Flegal *et al.*, 2001). Trivalent Cr-based supplements are generally used to build muscles or trigger weight loss (Levina and Lay, 2008). However, hexavalent Cr is known to be toxic and carcinogenic, causing health problems such as liver damage, pulmonary congestions, vomiting, and severe diarrhoea (Das and Mishra, 2008). Hexavalent Cr can easily go through the cell wall and transfer its noxious influence in the cell itself, causing the onset of cancer (Chandar and Kulshreshtha, 2004).

Chromium is utilised as a part of numerous mechanical procedures, for example, plating, alloying, tanning of skins, and as a water erosion inhibitor (Avudainayagam *et al.*, 2003). These uses of Cr from industrial processes have resulted in the release of large amounts of Cr emissions into the air, water and terrestrial environments. Humans are exposed when inhaling air, drinking water and eating food that may contain Cr.

The Limpopo province is under threat of environmental pollution from mining activities and discharge of wastewater. One river of particular interest is Mokolo River, which passes in the vicinity of Medupi and Matimba power stations as well as Grootegeluk coal mine situated in Lephalale area (Department of Water Affairs, 2008). The Seshego wastewater treatment works (WWTW) discharges its effluent into the Blood River and people residing in the vicinity of Blood River dump domestic wastes at the river causing the river to be more polluted. Chromium from welding activities in Seshego township, domestic and municipal wastes and runoff during the rainy season could be the source of contamination of this river. To the best of our knowledge, no Cr speciation studies have been conducted in water and sediment samples from Mokolo and Blood Rivers.

1.2 BACKROUND AND MOTIVATION

1.2.1 Chemical speciation

Speciation is the distribution of an element amongst defined chemical species in a system (Clough *et al.*, 2012). It is essential for understanding manufactured destructiveness, bioavailability, and environmental fate and transport of elements. It also allows the division between oxidation states, coordinated and uncoordinated particles, cationic, anionic and impartial structures. It is useful to identify the various classes of species of an element to determine their summed concentration in each class (Palmisano *et al.*, 1997). Metals can exist in a scope of physiochemical frames in ecological examples, including hydrated metal particles, inorganic and organic complexes, and adsorbed on natural and inorganic colloidal particles (Lokothwayo, 2008). Another case is the point at which the oxidation state of a component significantly influences its toxicity. For instance, chemical speciation helps in understanding fates of considerable parts of elements in coal amid burning, weathering, change, sifting, and cleaning (Finkelman, 2004). Speciation is separated into two classes; physical and chemical. Physical speciation is imperative for the comprehension of sorption and relocation wonder of overwhelming metals in an earthly framework. Chemical speciation can be divided into four subclasses as presented in Figure 1.1.
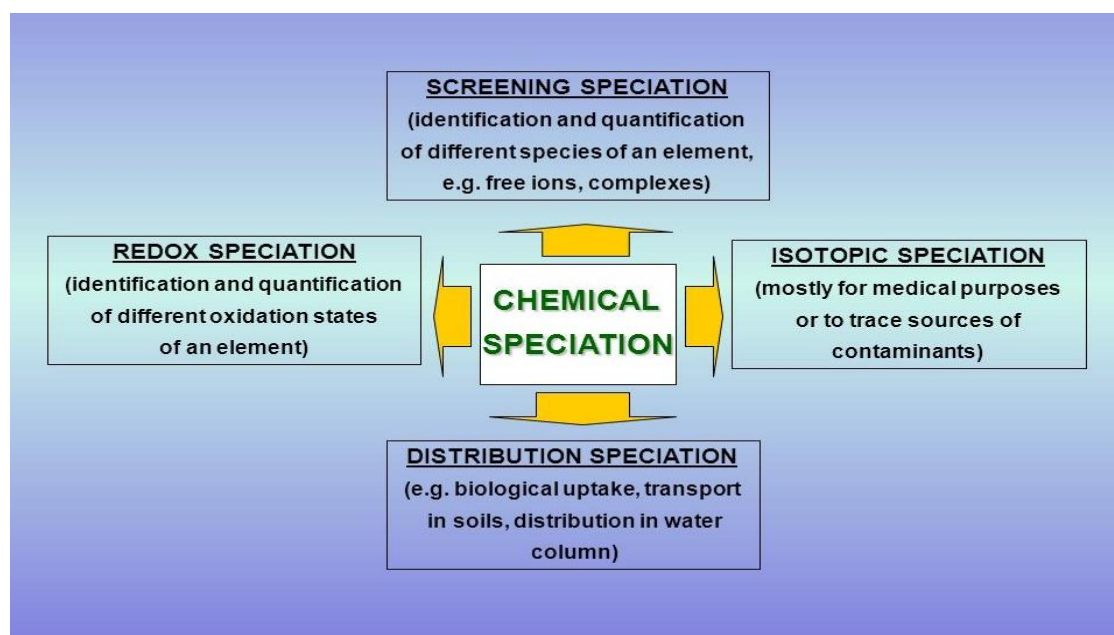
Figure 1.1: Classes of chemical speciation (Templeton *et al.*, 2000).

The use of traditional analytical methods for direct speciation measurement requires significant complexity and hyphenated techniques (Yan and Ni, 2001; Moldovan *et al.*, 2004).

1.2.2 Chromium speciation

Chromium speciation has been a critical undertaking in history because of broad usage of this metal in different businesses, for example, metallurgical (steel, ferro-and nonferrous compounds), refractories (chrome and chrome-magnesite) and substance (shades, electroplating, tanning and other). Chromium speciation does not only impact its toxicity, but the mobility of Cr relies upon its chemical form, in this manner detailed knowledge of each Cr species as opposed to the total Cr level is required to appropriately assess physiological and toxicological impacts of Cr, its chemical change in water, soil and transport in the environment (Bobrowski *et al.*, 2004; El-Shahawi *et al.*, 2005).

Speciation of Cr has been broadly considered, mainly in drinking water. One of the primary purposes behind this is the low maximum allowable level of Cr in drinking water (50 µg/L) prescribed by the World Health Organisation (WHO), notwithstanding its known health impacts (WHO, 2017). However, the most extreme permissible concentrations of the Cr species in wastewater have been set up at 1 mg/L for Cr(III) and 0.1 mg/L for Cr(VI) (WHO, 2003). It is important to conduct a study of Cr speciation in water and sediments collected from Blood and Mokolo Rivers because they serve as a source of water for domestic and agricultural use by nearby residents. It is also important to reveal the levels of Cr species in water and sediments to meet regulatory compliance. Hexavalent Cr concentration limits for drinking water now being considered by the California Environmental Protection Agency (EPA) give experts the requirement for quantification limits in the sub-mg/L range (US EPA, 2018). The total Cr concentration in groundwater is low (<1 µg/L), whereas, in surface water it is observed to be in the range of 0.5 to 2 µg/L while dissolved Cr content is 0.02 to 0.3 µg/L (Miguel *et al.*, 2015).

Chromium is non-biodegradable in the environment. Upon entry to the environment, it undergoes a range of changes in its physicochemical form. Chromium is a trace component of which toxicity relies upon its chemical form. The two types of Cr have distinctive chemical, physicochemical and biochemical reactivity and additionally

fundamentally extraordinary environmental (Kotas and Stasicka, 2000). Total Cr measurements alone cannot determine the real environmental effect and potential toxicity risks. In this way, there is a need to carry out Cr speciation which will determine the concentrations of each Cr species in a sample. Speciation analysis of Cr is, however, a challenging task. Conditions during sample collection and treatment can easily affect the stability of different Cr species (Kutsher *et al.*, 2012). Errors can easily arise from contamination. On the other hand, considering the low concentrations of Cr found, for example, in natural water samples, it is important to follow a pre-concentration procedure before the detection process (Porto *et al.*, 2017).

Existing methods for accurately determining Cr(VI) in natural waters have one or more of the following weaknesses: poor sensitivity, time-instability of the Cr redox species, or necessity for expensive instrumentation that is not commonly available (Ball and McCleskey, 2003). Since both the mobility and toxicity of Cr depend on its oxidation state, speciation of Cr is important in determining its fate in the environment and its risk to human health. Main selective techniques known for speciation of Cr species are: co-precipitation, separation with ion exchange and/or chelating resin, solid phase extraction (SPE) and solvent extraction. Solid phase extraction procedures have been widely used due to their simplicity, selectivity, high enrichment factors, safety and ease of automation (Cimen *et al.*, 2013). In this work, chromabond-NH_2 columns were employed for selective pre-concentration of Cr(VI) in water. Ambushe *et al.* (2009) used the chromabond-NH_2 column for the speciation of Cr in milk samples prior to ICP-MS detection. To the best of our knowledge, the use of chromabond-NH_2 column for the pre-concentration and separation of Cr in water is yet very limited.

The most frequently used techniques that have been employed to determine Cr(VI) and Cr(III) are atomic spectrometric techniques in the form of either flame atomic absorption spectrometry (FAAS) or graphite furnace-atomic absorption spectrometry (GF-AAS), inductively coupled plasma-atomic emission spectrometry (ICP-AES) or inductively coupled plasma-mass spectrometry (ICP-MS) (Xiao *et al.*, 2013). Inductively coupled plasma-atomic emission spectrometry is one of the well-established techniques with good sensitivity, low detection limits and rapid sample analysis. Inductively coupled plasma mass spectrometry is used when more sensitive determinations are required.

Graphite furnace-atomic absorption spectrometry is employed in the current study for the detection of Cr(VI) in water and sediments and total concentrations of Cr in water.

This is due to its low running cost, excellent detection limits, low sample utilisation, high flexibility from interferences, simplicity of handling with full automation and proven reliability in a huge number of research facilities around the world (Perkin Elmer, 2012). Moreover, total Cr in sediments were determined by FAAS in the current study. Typical detection limits of FAAS are of the order of 1 – 100 µg/L (Garcia and Baez, 2012), making it a perfect tool for the determination of Cr in contaminated sediment samples.

1.3 AIM

The aim of this study is to assess and quantify different species of Cr in water and sediment samples, collected from Mokolo and Blood Rivers in Limpopo province.

1.4 OBJECTIVES

The specific objectives are to:

i)   determine the total Cr concentrations in sediments using FAAS after microwave assisted digestion;

ii) determine the total Cr concentrations in water using GF-AAS;

iii) separate Cr(VI) in water by SPE technique using Chromabond-NH_2 column and detection of separated species by GF-AAS

iv) extract Cr(VI) in sediments using Na_3PO_4 and detection by GF-AAS;

v)   extract Cr(VI) in sediments using Na_2CO_3 and detection by GF-AAS and

vi) validate the methods using Standard Reference Materials (SRMs) of water and sediments.

CHAPTER 2: LITERATURE REVIEW

2. INTRODUCTION

This chapter presents a summarised literature survey of Cr in water and sediments. Importance and scarcity of water, contamination and environmental distribution of Cr in water and sediments, toxicity and chemistry of Cr, speciation of Cr in water and sediments, sample preparation and methods used for Cr speciation are reviewed. An overview of the FAAS and GF-AAS are given, along with their principles.

2.1 Importance of water and its scarcity

Water, the key wellspring of life and Earth's important key resource, is accepting a fundamental part in human progress. Expanding populace, urbanisation and industrialisation has provoked the lessened availability of water. It is imperative to improve the use of water and limit the run off surface water by employing methods for water harvesting, groundwater preservation and water re-use. Ground water from springs and wells is regularly the fundamental sources of water supply especially in towns and residential communities (Hay *et al*., 2012). Only 2.5% of the water is fresh water and just less than 0.3% of all freshwater is in streams, lakes, and the climate (Gleick, 1996). The distribution of Earth's water is shown in Figure 2.1.
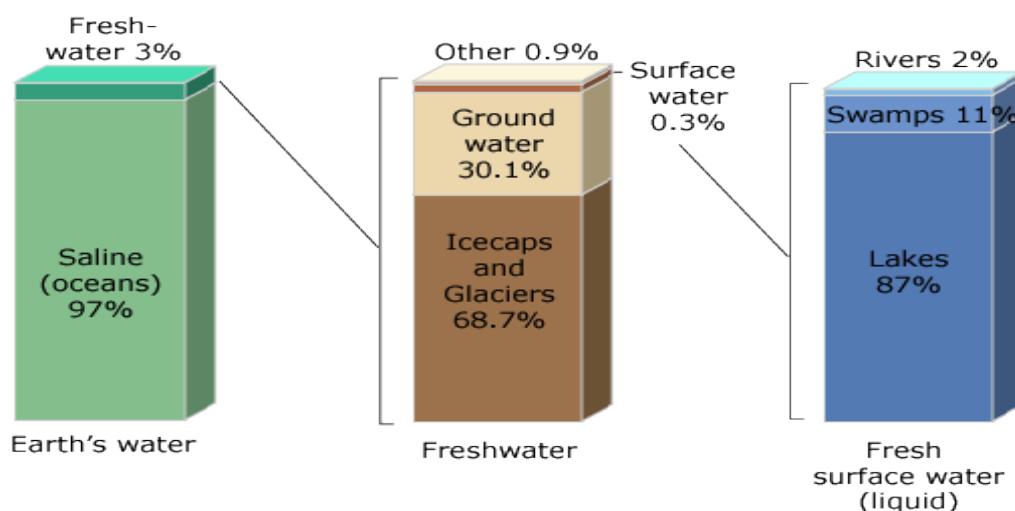
Figure 2.1: Distribution of Earth's water (Gleick, 1996)

Water fills in as a basic part on the planet economy. This is the major wellspring of sustenance for root makers (plants) in all support networks on earth. In that capacity, the stream of life inside living species starts from water. Water is helpful and critical for

aquaculture, household, industrial, water system, animals, mining, public supply, thermoelectric power and hydroelectric power use (Hutson *et al.*, 2004). It hovers through the land also as it does through the human body, transporting, dissolving, and reviving supplements and regular issue, while occupying waste material. Bearing in mind again that the body is about 75 % water, it is easy to understand that water must be the body's most essential daily ingredient (Montain *et al.*, 2006). Water scarcity problems are universal to developed or developing countries, and they are bound to increase in future due to rapid population growth which is forecasted to reach over 10 billion people in 2050 (Hanna *et al.*, 2007).

Safe and clean water is scarce. South Africa is one of water scarce countries. Climate change, such as weather patterns including droughts or floods, an increase in freshwater use, destruction of river catchments caused by urbanisation, deforestation and an increase in pollution attribute to the growing water crisis in South Africa (Muller *et al.*, 2009). More than 60% of South Africa's rivers are currently being overexploited. According to the Organisation for Economic Co-operation and Development (OECD), only one-third of the country's main rivers are in good condition and one-quarter of its river ecosystems are critically endangered (OECD, 2015). About 71% of the global population (4.3 billion people) lives under conditions of moderate to severe water scarcity at least 1 month of the year (Mekonnen and Hoekstra, 2016). Approximately 1.2 billion people live in areas of scarcity (FAO, 2007). According to Global Water Institute, almost 700 million people suffer from water scarcity (Global Water Institute, 2013). Figure 2.2 shows the physical water scarcity in 2010 and projected change in water scarcity by 2050 based on the middle-of-the-road scenario.
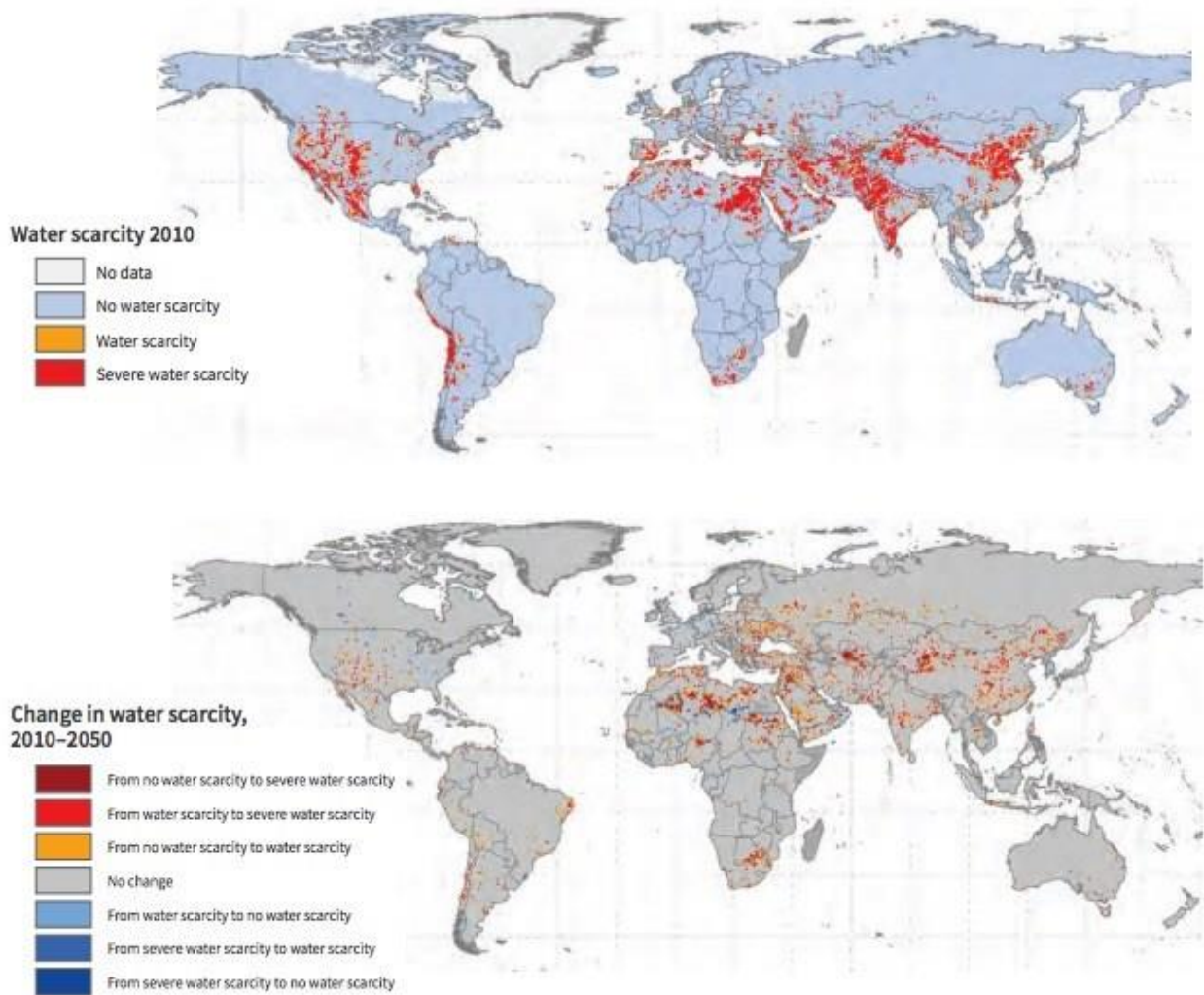
Figure 2.2: Physical water scarcity in 2010 (upper figure) and projected change in water scarcity by 2050 (lower figure) based on the middle-of-the-road scenario (Burek et al., 2016).

Burek *et al.* (2016) stated that nearly half the global population are already living in potential water scarce areas at least one month per year and this could increase to some 4.8 – 5.7 billion in 2050. About 73% of the affected people live in Asia (69% by 2050) (Burek *et al.*, 2016). In 2010, South Africa was facing mild to severe water scarcity (Rodda *et al.*, 2016). According to the Department of Water and Environmental Affairs, the demand for water will outstrip supply in Gauteng by 2013, and in the whole South Africa by 2025 (DWA, 2013). Western Cape is considered the most seriously affected area. In February 2018, the Breede-Gouritz catchment and Berg-Olifants catchment were low at average dam levels of 20 and 30%, respectively (van der

Merwe, 2018). During that period, Western Cape province was going through a severe drought affecting the highly populated urban area of Cape Town and its water supply. As shown in Figure 2.2, Western Cape area will be under threat of water shortage between 2010 – 2050.

2.2 Contamination of water and sediments

Organic and inorganic pollution result from organic and inorganic substances which are released into the environment because of domestic, agricultural and industrial water activities (Lim *et al.*, 2000). Increasing population, urbanisation, industrial growth and agricultural activities have a significant impact on the demand of scarce water resources. In addition, sources of contamination along the water catchment area and the pipe water supply system pose challenges to the supply of fresh water. The transfer of contaminants from one water body to another is a major challenge (Li *et al.*, 2017). Sources of water and sediment pollution are illustrated in Figure 2.3
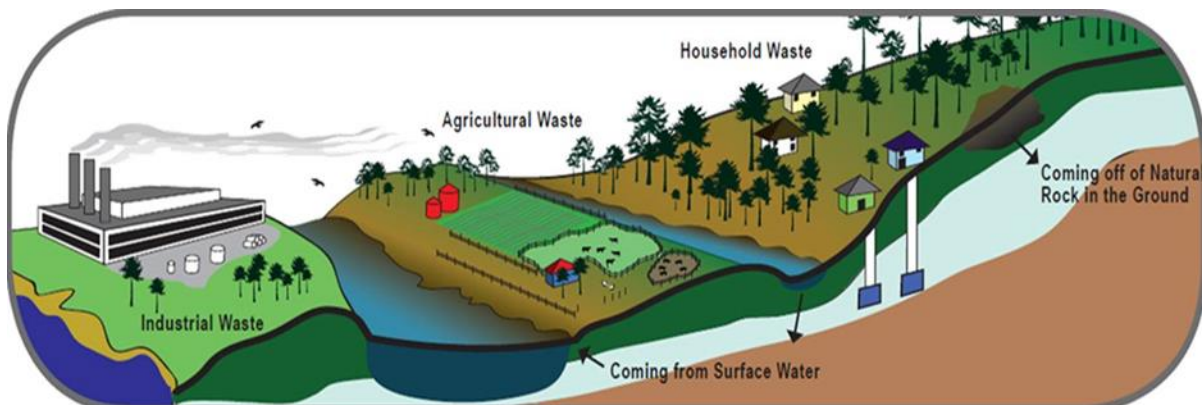
Figure 2.3: Sources of water and sediment pollution (NC Department of Health and Human Services, 2011)

Chromium in water originates from natural sources, such as weathering of rock constituents, wet precipitation and dry fallout from the atmosphere, and run-off from the terrestrial environments. Chromium can also reach drinking water supply systems from the corrosion inhibitors used in water pipes and containers (Sarin and Pant, 2006). Polluted areas receiving wastewater contain Cr concentration higher that can reach several μM (Perrin *et al.*, 2014). Transport and fate of Cr in surface water can be explained by using three subsystems namely rivers, lakes and oceans. The transport

pathways of Cr are controlled by conditions such as temperature, depth, degree of mixing, oxidation conditions and the amount of organic matter (Stanin and Piernie, 2004). These conditions can change Cr from one form to another in water and sediments.

The sediment comes from the settling of suspended matter, either from surface run-off or riverine input, as well as precipitation reactions in the water body itself, and settling organic detritus. Some contributions emanate directly from the soil fauna, like shells or corals. Since sediments play an important role in physicochemical and ecological dynamics, any change in toxic concentrations of heavy metal residues on the sediments will affect the natural aquatic life support systems (Jumbe and Nandini, 2009). Toxic substances, heavy metals such as Cr may accumulate in sediments (Vries *et al.*, 2007) and enter food chains through plants or animals (Dosumu, 2003). Hexavalent Cr in sediments can be leached into surface water or ground water, and taken up by plants (Wang *et al.*, 2011) hence Cr (VI) incurs a significant risk to human health when released into the environment (Huang, 2009). Further, it has the potential to produce leachates that pollute surface and groundwater resources (Mandina and Mugadza, 2013). High levels of turbidity caused by sediment pollution limit penetration of sunlight into the river water, thereby limiting growth of algae and rooted aquatic plants. Azmat *et al.* (2016) conducted a study based on the contamination of Cr in water, sediments and its bioaccumulation in India major carps in Chenab River, Pakistan. Water and sediments from Mouttas River in Algeria were reported to be contaminated with Cr produced from the discharge of wastewater from the tannery of Jijel (Leghouchi *et al.*, 2009). Shil *et al.* (2017) investigated the heavy metal (Cr, Cu, Ni, Pb and Fe) contamination in water, sediment and fish from the Passur River near the Sundarbans Mangrove of Bangladesh. Their results indicated that the concentrations of elements found were mainly attributed to geological sources.

2.2.1 Mining and industrial activities

Mining displaces whole communities, forced off their land by expanding mines. This poses major risks to societal sustainability, affects and disturbs the balance of the ecosystem (Resane, 2015). Despite all these negative impacts, South Africa coal

mines have generated significant benefits to the economy and society, from increased output, revenues, investment, exports and foreign exchange, to employment, local economic development, training opportunities and new technologies (Chamber of Mines, 2013; Montmasson-Chair, 2015). Rural and urban areas in South Africa use over 70 % of surface water drawn from rivers, streams, lakes, ponds and springs (DWAF, 2004). This water gets polluted by acid mine drainage, toxic waste and abandoned mines, which continue to threaten the health of South Africa's communities and its environment (Boularbah *et al.*, 2006).

According to several researchers, the decline of water quality in rivers, lakes and groundwater has progressively become a global issue of concern (Khan *et al.*, 2013; Behmel *et al.*, 2016). Local communities near mining operations can be negatively affected by mining activities due to the scale of mining operations, extracted waste rock, overburden and tailings volumes, mining and processing of lower grade ores, the intensity of resource use (e.g. energy and water) per unit of production, and implementation of low-cost bulk mining techniques (Mudd, 2010). According to Mhlongo *et al.* (2006) the quality of water in mining and water stressed regions is negatively impacted. The coal mining industry is facing complex water resource management challenges such as avoiding non-compliant discharge of mine affected water (Gao *et al.*, 2017).

Coal provides a major source of energy for both rural and urban industries. During coal combustion, Cr portions in ash products, such as bottom ash and fly ash can be released into the atmosphere as fine particulates that are not captured by particulate capture device (Goodarzi, 2009). Figure 2.4 shows the estimation of Cr(VI) in coal-derived fly ash.
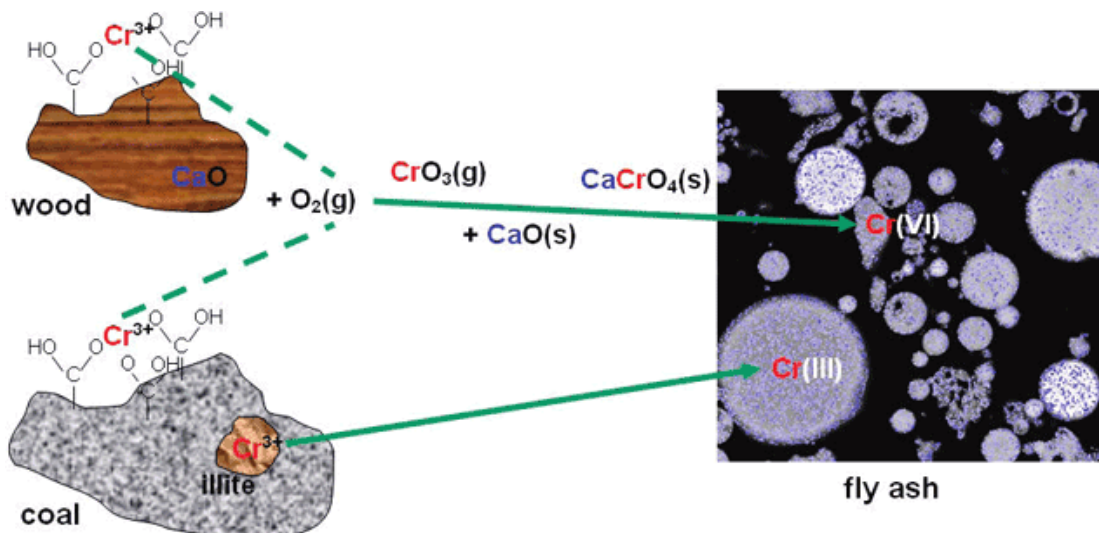
Figure 2.4: Estimation of Cr(VI) in coal-derived fly-ash (Stam *et al.*, 2011)

Fly ash has been successfully used for many years in a wide range of applications including material applications, asphalt, concrete pavements, soil stabilisation, road base, structural fill, embankments, mine reclamation, mineral fillers or fertilisers as well as small scale applications such as production of zeolites and geo-polymers (Izquierdo and Querol, 2012). During pulverised coal combustion, Cr-bearing minerals change and react with other mineral-derived components to form Cr-bearing crystalline phases and glass. In the process, Cr(III) oxidizes to Cr(VI) (Stam *et al.*, 2011). The coal fly ash contains many trace metals including Cr, which are harmful in nature and due to this; serious environmental concerns have been raised by wet disposal of coal fly ash (Tiwari *et al.*, 2015). The impact of coal ash leachates on receiving waters apart from increased elemental concentrations causes change in water pH with implications for trace element mobility (Carlson *et al.*, 1993). Finkelman *et al.* (2004) investigated coal leaching and found that 40% of its Cr was leached. In coal mine water, minerals may be transformed and release Cr under suitable conditions of pH, redox potential and temperature. Coal ash leaches Cr in amounts that can greatly exceed the United States Environmental Protection Agency's (US EPA) threshold for hazardous waste at 5000 parts per billion (ppb) (US EPA, 2009) and the Cr that leaches from coal ash is "nearly 100 % Cr(VI) (Electric Power Research Institute, 2000).

Most bituminious coals contain Cr in the form of Cr(III) in organic association and primarily as Cr(VI) in illite (Huggins *et al.*, 2000; Huggins and Huffman, 2004; Goodarzi *et al.*, 2009), whereas the evidence of Cr(VI) is rarely found (Huggins et al. 1999). Total Cr (82%) can be leached from a polish fly ash in environmental conditions (Jeon *et al.*,

2016). Chromium (between 5 and 25 ppm) is found entirely in coal in the trivalent oxidation state. Hexavalent Cr produced during coal burning affect air quality which eventually lead to water and soil contamination. Moreover, approximately one-third of atmospheric emissions of Cr calculated as 3,400-22,000 Mt/yr (Hope, 2008) are thought to be in the most toxic Cr(VI) form (Kieber *et al.*, 2002).

Other mining activities that release Cr in the environment include chromite ore mining and ferrochrome production. Waste rock, dust and tailings water are produced during chromite ore mining (Amponsah-Dacosta, 2017). These waste materials are contaminated with Cr and other heavy metals of concern (Godfrey *et al.*, 2007). During the production of ferrochrome, air pollutants such as nitrogen oxides, carbon oxides, sulfur oxides and particulate dust are emitted. The particulate dust contains heavy metals such as Zn, Pb, Ni, Cd and Cr in the hexavalent state (Strobos *et al.*, 2004).

Hexavalent Cr compounds are used as pigments in dyes, paints, inks and plastics, as anticorrosive agents added to paints, primers, and other surface coatings. The Cr(VI) compound, chromic acid, is used to electroplate Cr onto metal parts to provide a decorative or protective coating. The Cr(VI) particles released during smelting of ferrochromium ore as well as emissions from welding fumes during stainless steel and nonferrous chromium alloys welding have become a serious concern (OSHA, 2006; NIOSH, 2013; Pesch *et al.*, 2015; Leese *et al.*, 2017). Welding or cutting materials containing Cr is likely to give Cr compounds in any particulate fume that is generated during the process. Chromium can be present in the fumes in several different valence states, including Cr(III) and Cr(VI) (Leese, 2017).

2.2.2 Agricultural activities

Rivers give the primary water sources to residential, industrial and irrigational purposes; however, they are effortlessly contaminated due to their critical roles in transporting municipal and industrial contamination and runoff from horticultural land (Singh *et al.*, 2005). Agriculture is by far the largest consumer of the Earth's available freshwater: 70% of water that is withdrawn from waterways and groundwater is used for agriculture (Loucks *et al.*, 2005).

Water pollution from agriculture and its consequences are a source of increasing concern (Vorosmarty *et al.*, 2010). It has direct negative impacts on human health. Aquatic ecosystems are also affected by agricultural pollution; for example, eutrophication caused by the accumulation of nutrients in lakes and coastal waters has impacts on biodiversity and fisheries (Mateo-Sagasta *et al.*, 2017). Large quantities of agrochemicals, organic matter, drug residues, sediments and saline drainage are discharged by farms into water bodies (Abdel-Raouf *et al.*, 2012). In South Africa, chromated copper arsenate (CCA) is still commonly used for the treatment of wood. A study by Naidoo *et al.* (2013) revealed that some local food vendors in Cape Town are cooking food over fires fuelled with CCA-treated wood.

Agricultural activities found in the proximity of Blood and Mokolo Rivers could be sources of potentially toxic elements in the rivers. The activities found near the Mokolo River include livestock (cattle, sheep, piggery and poultry) farming, and also agricultural practices that produces crops such as sorghum, wheat, maize and sunflowers (IDP, 2014). According to Gambus and Wieczorek (2012), phosphorus and multielement fertilisers used to produce maize contain Cr in small fractions, which may impact the quality of the Mokolo River due to the proximity of the farm to the river. In addition, inefficient farming practices around the Seshego area results in soil erosion and build up in the Blood River. Runoff of herbicides and pesticides containing Cr(VI) would increase the water pH to very alkaline conditions, which could kill or harm fish by damaging their outer surfaces, preventing them from excreting metabolic wastes and increasing the uptake of salts due to osmoregulation (Evans *et al.*, 2005). The contaminated water of Blood and Mokolo Rivers used for irrigation is one further source of pollution. Irrigation can mobilise salts accumulated in soils, which are then transported by drainage water to receiving water bodies and cause salinization. A horse-stable found near Mokolo River produces manure which are rich in nitrogen and phosphorus (Moss, 2008). Manure has serious implications for water quality (FAO, 2006).

2.3 Toxicity of Cr species

Contamination of the environment by Cr, especially Cr(VI), has become a major area of concern. Leaching and seepage of Cr(VI) from the soils into the groundwater poses a considerable health hazard (Zayed and Terry, 2003). The toxicity of Cr(III) and Cr(VI)

differs considerably. Trivalent Cr, is an essential nutrient, required in trace amounts for sugar and lipid metabolism (Anderson, 2000). It is also essential for the maintenance of protein metabolism, whereas Cr(VI) is extremely toxic to human beings, plants, aquatic animals and bacteria (Mohanty and Kumar, 2013). Trivalent Cr based nutritional supplements are widely used as human mineral supplements, particularly marketed for weight loss and performance enhancement (Eastmond *et al.*, 2008). Recently, the toxicity of Cr(III) has also attracted increasing attention. Trivalent Cr revealed genotoxicity to *Eisenia fetida* earthworm in natural spiked soils after 4 days of exposure (Bigorgne *et al.*, 2010). The toxicity of Cr(III) to freshwater algae is even more severe than Cr(VI) depending on the certain specific conditions (Vignati *et al.*, 2010). For example, overdose intake of Cr(III) could result in considerable genotoxic risks to both human and animals (Eastmond *et al.*, 2008).

Hexavalent Cr has been confirmed as a human carcinogen and a powerful epithelial irritant (Das and Mishra, 2008). By inhaling Cr(VI) containing materials, one can experience nasal septum, asthma, bronchitis, pneumonitis, inflammation of the larynx and liver and increased incidence of bronchogenic carcinoma. Skin contact of Cr(VI) compounds can induce skin allergies, dermatitis, dermal necrosis and dermal corrosion (Das and Mishra, 2008). Other toxic effects of Cr(VI) include: renal tubular necrosis, mild liver abnormalities and occupational exposure has been associated with increased lung cancer mortality (ATSDR, 2000). The toxic effects of Cr(VI) are dependent on the duration of exposure and the dose. A knowledge of reactions that will influence its fate must be obtained due to the hazard imposed by Cr(VI). Hexavalent Cr can be reduced to Cr(III). This alleviates the hazard imposed by Cr(VI), but one should always consider the possibility of Cr(III) oxidation back to Cr(VI) (Pedersen *et al.*, 2015). Reports by Lind *et al.* (2001) and Pillay *et al.* (2003) indicated that there is gradual oxidation of trivalent to hexavalent Cr when slag is in the presence of atmospheric oxygen. This transmits more mobile and toxic Cr(VI) into the environment. Some crops are not affected by low Cr(VI) concentration; Cr(VI) is toxic to most plants at 100 μM/kg dry weight (Davies *et al.*, 2002). Changes in environmental conditions (natural and anthropogenic) can strongly influence the behaviour of both essential and toxic elements by affecting the forms in which they occur.

2.4 Chemistry of Cr

Chromium was found in 1797, by Louis Vauquelin, in France. It is ordinarily found in rocks, creatures, plants, soil, waterways and seawater and volcanic exhaust, and also in Earth's hull at a grouping of around 100 mg/kg (Scancar and Milacic, 2014). It exists in all oxidation states from 0 to VI, however Cr(III) and Cr(VI) are generally predominant. The pervasive types of Cr may experience, a progression of changes, changing from one into another affected by different physicochemical processes. Hexavalent Cr exists as an anion, either as chromate (CrO_4^{-2}) or dichromate ($Cr_2O_7^{-2}$) and Cr(III) is available in the cationic form as Cr^{3+} (Bhateria and Dhaka, 2017). Trivalent Cr is not very soluble and Cr(VI) is dissolvable. Essential Cr(0) isn't found in the earth crust and is naturally dormant. Divalent chromium Cr(II) is a solid reductant which oxidises to the trivalent state. This explains why Cr(II) isn't accessible in natural systems (Pechova and Pavlata, 2007). Hexavalent Cr is the second most stable form and a solid oxidising specialist, particularly in acidic media (Mishra and Bharagava, 2015). Trivalent Cr has 3d electrons that are in a high turn state in an octahedral complex. The isotopes of Cr are ^{50}Cr, ^{52}Cr, ^{53}Cr and ^{54}Cr with relative plenitude of 4.31, 83.76, 9.55 and 2.38 %, respectively (de La Gaurdia and Morales-Rubio, 2003).

The pH of the media plays a crucial role in the inter-conversion of both stable states of Cr. Because of the low dissolvability of Cr(III) (≤5 μg/L) inside the regular pH range of common waters (6.5 - 8.5) (Saputro *et al.*, 2014), the hexavalent form is required to dominate at whatever point expanded levels of naturally leached Cr happen. The circulation of Cr(VI) species relies upon pH, redox potential and aggregate Cr(III) concentration. By and large, disturbance of the chemical balance between specific species firmly relies upon pH value. At neutral pH, the Cr(III) - Cr(VI) extent is identified with the oxygenation of solution.

Trivalent Cr is the most stable state in water with a standard potential of - 1.74V (de La Guardia and Morales-Rubio, 2003), in this manner a lot of energy would be expected to change it to lower or higher oxidation states. Trivalent Cr can be converted to Cr(VI) in the presence of manganese oxides. Although Cr(VI) reduction is less privileged thermodynamically, this process can be carried out because the concentrations of reductants are high enough (Stanin *et al.*, 2004). Hexavalent Cr in acidic arrangement has a high positive redox potential (~ 1.38V) (de La Guardia and

Morales-Rubio, 2003) which means that it is oxidising and insecure within the sight of electron benefactors.

2.5 Chromium speciation in water and sediments

Elemental speciation in water and sediments has become an essential part of environmental studies due to the varied toxicity of different chemical forms of many elements (Komorowicz and Barałkiewicz, 2011). To obtain sufficient information on the toxicity and biotransformation of Cr, it is important to speciate the different oxidation states of Cr (Yousefi and Shemirani, 2013). Due to leakage, poor storage and improper disposal, Cr(VI) has become one of the most frequently detected contaminant in water and sediments (Thacker *et al.*, 2006). Because of the dangerous effects of hexavalent Cr and its high mobility in the environment, several Cr speciation studies have been conducted in water and sediments to assess water quality thereby protecting communities exposed to Cr.

Markiewicz *et al.* (2016) accurately quantified total Cr and its speciation form Cr(VI) in water by inductively coupled plasma dynamic reaction cell isotope dilution mass spectrometry (ICP-DRC-IDMS) and high performance liquid chromatography inductively coupled plasma dynamic reaction cell isotope dilution mass spectrometry (HPLC/ICP-DRC-IDMS). Dynamic reaction cell (DRC) completely removed the spectral interferences, predominantly occurring in Cr determination. Isotopically enriched ^{53}Cr(VI) was prepared adequately to avoid artefacts in Cr speciation. Isiaka *et al.* (2016) focused on speciation of Cr and vanadium (V) in medicinal plants from Zizameleni farm, Gauteng Province. The concentration of Cr(VI) in medicinal plants varied between 3.1 µg/g and 9.4 µg/g and that of V ranged from 1.1 µg/g to 17 µg/g. It was established that elements species taken up by plants do not depend on metal content in soil but is a function of plant properties. Loock *et al.* (2014) conducted a survey of Cr(VI) contamination of surface water in the proximity of ferrochromium smelters in South Africa. Hexavalent Cr levels present in surface water within the vicinity of ferrochrome smelters located in the Bushveld Igneous Complex were monitored for a period of one year. The surface water in the proximity of ferrochrome smelters was mostly unaffected by Cr(VI) pollution according to their results. Leśniewska *et al.* (2017) focused on separation of Cr species in soils. The studies showed that the physical state of waste, initial form and oxidation state of Cr and soil

properties influenced the final Cr species and their mobility in soil, which have an impact on contamination of environment.

A study by Edokpayi *et al.* (2014) assessed the variation pattern in trace metals contamination in Mvudi River water and sediments for the period of January – June 2014. Gilbert and Avenant-Oldewage (2006) studied the accumulation of trace elements in the water, sediment and tissues of *Labeobarbus kimberleyensis* from the Vaal Dam, South Africa. The high sediment element levels indicated that there was a low bioavailability of elements for accumulation by biota in the Vaal Dam.

2.6 Sample preparation methods for speciation analysis

Several methods including co-precipitation (Duran *et al.*, 2009; Sacmaci, 2017), liquid–liquid extraction (LLE) (Beni *et al.*, 2007), dispersive liquid–liquid microextraction (DLLME) (Bahadir *et al.*, 2016), cloud point extraction (Yildiz *et al.*, 2011; Nafti *et al.*, 2017; Lopez-Garcia *et al.*, 2017), dispersive micro solid phase extraction (DMSPE) (Kazemi *et al.*, 2017), microwave assisted extraction (MAE) (Arain *et al.*, 2008; Araujo-Barbosa *et al.*, 2017) and solid phase extraction (SPE) (Hazer and Demir, 2013; Leśniewska *et al.*, 2016; Li *et al.*, 2017) have been reported for the extraction of different species in different matrices. Rezaee *et al.* (2006) reported DLLME as one of the liquid phase micro-extraction (LPME) greatly used as a pre-concentration method. The method is based on the ternary component solvent system. Moradi *et al.* (2010) later reported a surfactant-assisted DLLME which utilises a surfactant, as disperser agent. In this method, a mixture of solution containing surfactant and extraction solvent is quickly injected into the aqueous sample to produce an emulsified solution. However, recent research trends include the use of more environmental friendly analytical pre-concentration procedures such as LPME. Several reviews about the basic principles and main applications of LPME procedures are at present available in the scientific literature (Dadfarnia and Shabani, 2010 and Pena-Pereira *et al.*, 2010). For example, LPME procedures have been applied for the pre-concentration of inorganic species, including Cr species, in environmental samples (Hemmatkhah *et al.*, 2009; Majidi and Shemirani, 2012; Wen *et al.*, 2013).

In the present study, the speciation of Cr in sediment and water samples were performed using MAE and SPE technique, respectively. Microwave assisted extraction

has been widely used for different analytical purposes such as environmental detection, food inspection, and agricultural sample analysis (Yi *et al.*, 2017). This is due to its low solvent consumption, short extraction time, low cost, and high extraction rate (Delazar *et al.*, 2012). Microwave assisted extraction is based on heating the solvent through absorption of microwave energy by polar molecules, thus increasing the solvent penetration into the sample matrix (Duarte *et al.*, 2014.) Mamatha *et al.* (2014) used MAE combined with ion exchange separation and ICP-OES for the determination of Cr(VI) and Cr(III). The species of interest were extracted from samples using a solution containing 0.1 M EDTA, 1% tetrabutyl ammonium bromide (TBAB) and little HF in a domestic microwave oven. Microwave assisted extraction was used for determination of Cr(VI) in river sediments by speciated isotope dilution ICP-MS (Drincic *et al.*, 2018). The addition of 0.4 M $MgCl_2$ prevented oxidation of Cr(III) during the extraction step. Jamali *et al.* (2009) conducted a study based on speciation of heavy metals in untreated sewage sludge using microwave assisted sequential extraction procedure. They reported that the Cr(VI) levels in untreated waste water sewage sludge were associated with organic matter/sulphide fractions. Solid phase extraction is designed for fast, selective sample preparation and purification prior to chromatographic analysis. Solid phase extraction has become more popular for analyte pre-concentration and matrix removal, due to its simplicity and economy in terms of time and solvent (Pico *et al.*, 2007; Pawliszyn *et al.*, 2010).

The principle of SPE involves partitioning of solutes between two phases (sample matrix) and a solid (sorbent) phase. Solid phase extraction allows concentration and purification of analytes from solution by sorption on a solid sorbent. The general approach is to load a solution onto the SPE solid phase, wash away undesired components, and then wash off the desired analytes with another appropriate solvent into a collection tube (Camel, 2003). Solid phase extraction steps are presented in Figure 2.5.
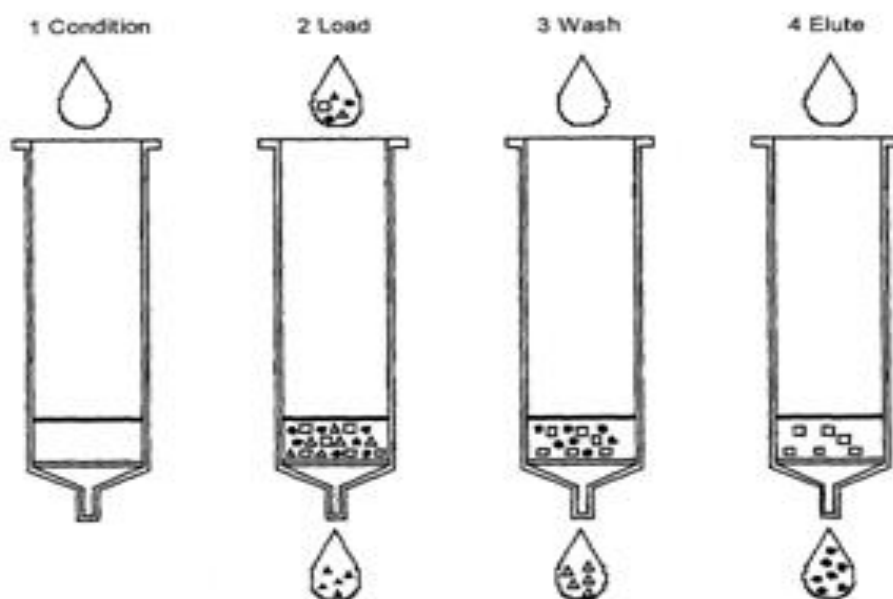
Figure 2.5: Solid phase extraction steps (Zwir-Ference and Bizuik, 2006)

The SPE technique has been widely used for Cr speciation in environmental samples (Narin *et al.*, 2006; Tuzen and Soylak, 2006) and pharmaceutical samples (Soylak *et al.,* 2008). Jayasinghe *et al.* (2003) used SPE for quantification of total Cr and Cr(VI) in drinking water followed by electro-thermal atomic absorption spectrometry. They concluded that the method can be successfully employed as an alternative to the commonly used pre-concentration and speciation analytical techniques.

Chromabond-NH$_2$ columns were applied to determine Cr(VI) levels in infant milk products by Soares *et al.* (2000). Ambushe *et al.* (2009) used the chromabond-NH$_2$ column for the speciation of Cr in raw milk samples prior to ICP-MS detection. The literature report on application of these columns for speciation of Cr in water samples is, however, limited.

2.7 Microwave-assisted digestion for total Cr determination in sediments

Microwave-assisted sample preparation techniques are becoming widely used in analytical laboratories all over the world. Microwave digestion is used to dissolve heavy metals in the presence of organic molecules. This is an important advancement in analytical chemistry tools for routine analysis because the fast evolution of instrumentation must be parallel to the development of straightforward and simple strategies for sample preparation or this initial step will remain as a bottleneck in the whole analytical procedure (Nobrega *et al*., 2012).

These techniques are susceptible to loss of volatile analytes and cross-contamination. Other problems often associated with conventional sample preparation techniques include danger due to the use of strong oxidisers, incomplete digestion and the need for large amounts of reagents, constant supervision and special fume hoods. Temperature is the most critical parameter that ensures a complete decomposition of organic matter and thus provides a clear solution (Schindlbacher *et al*., 2010). Real time temperature specification mostly around 200 ^{o}C is enabled to increase the boiling temperature of the acid which ensures the complete digestion of the sample by microwave digestion system (Ghanthimathi *et al*., 2012). The high pressure generated using closed vessels (special vessels that can operate at up to 11 040 kPa) raises the boiling point of the acid(s) used and substantially increases the dissolution rates of these organic matrices. For example, HNO_3 boils at 176 ^{o}C when heated by microwaves at 5 atm in a closed container but under normal circumstances it boils at 120 ^{o}C (Smith and Arsenault, 1996). This is 56 ^{o}C above its normal boiling point and results in an increase in the oxidation potential, thereby speeding up reactions.

Microwave-assisted sample preparation with cavity oven and closed vessels are increasingly being used due to its compatibility with most modern instrumental methods, promotion of better analytical blank control, use of smaller volume of acids, improved safety conditions, and avoid losses by volatilisation or contaminations commonly present in open systems (Araujo *et al*., 2002; Bizzi *et al*., 2014). Microwave-assisted digestion can be performed using dilute acid solutions without any significant negative influence on the digestion efficiency, which improves analytical blanks, and reduces costs and residues generation (Trevizan *et al*., 2007). Closed-vessel microwave-assisted digestion with diluted acid solutions is an efficient alternative for sample preparation of organic samples for inorganic analysis using spectrochemical

techniques (Dugenest *et al.*, 1998). Based on green chemistry principles, the use of diluted acids for digestions offers advantages, such as cost reduction, minimization of residues, reduction of blank values and prevention of damages to components of the equipment (Araujo *et al.*, 2002).

2.8 Leaching

Leaching is the procedure by which constituents of a solid material are discharged into a leaching water phase. Although a few species might be a greater amount of an environmental concern than others, the leaching procedure is in-discriminant with respect to all constituents that are discharged under a typical set chemical which may incorporate mineral disintegration, desorption and complexation, and mass transport forms (Trevisan, 2007). Thus, these phenomena are influenced by specific factors that can modify the rate or degree of leaching. Among these variables are:

- internal chemical and physical reactions
- external stresses from the surrounding environment
- physical degradation of the solid matrix due to erosion or cracking, and
- loss of matrix constituent due to the leaching process itself.

Hexavalent Cr leached from a variety of man-made processes and disposal of Cr(VI)-rich waste materials may lead to contamination of soil, water and sediments, especially during big flooding events (Milacic *et al.*, 2017; Vidmar *et al.*, 2017). Mandiwana (2008) conducted a rapid leaching of Cr(VI) in soil using Na_3PO_4 in the determination of Cr(VI) by electro-thermal atomic absorption spectrometry. Leaching of Cr(VI) from the ferrochrome slag causes sustainable contamination to the environment (Mandina and Mugadza, 2013). Jean-Soro *et al.* (2012) used EDTA and citric acid to leach out Cr and Ni in contaminated soil. In their findings, citric acid leached more Cr than EDTA, certainly because of a substitution reaction with Cr(VI). An evaluation of the leaching of Cr from 100% cement waste forms was carried using a refined biofilm formation method (Idachaba *et al.*, 2004).

2.9 Methods used for Cr speciation

The Cr speciation methods include offline and online (Kumbasar, 2008). In online methods, the separation system is coupled with the detection system whereby separation, identification and quantification of Cr are carried out in a one-step analytical

process. In offline methods, separation and pre-concentration of Cr species are carried out before the sample insertion into the detection instrument. Offline methods are somewhat complicated, time-consuming, resulting often in losses of the analyte and/or contamination appearance (Kambusar, 2008).

Speciation techniques that involve separation include flow-injection analysis (FIA) and high performance liquid chromatography (HPLC) which includes ion chromatography (IC), ion-pair chromatography (IPC) and reversed-phase (RP) chromatography (Kotas and Stasicka, 2000). The most frequent pre-treatments used are complex formation (about 38%) and pre-concentration by column/ionic exchange (about 23%). About 14% of the pre-treatments are based on oxidation-reduction reactions that can convert Cr(III) to Cr(VI) and vice versa to determine the total Cr in one of these forms. Only 3% of the pre-treatments are determined by digestion and separation processes, because in liquid samples the species are usually solubilised (Marqués *et al.*, 2000). Sequential leaching methods (Koschinky and Hein, 2003), hyphenated techniques such as gas chromatography-inductively coupled plasma mass spectrometry (GC-ICP-MS) (Hirata *et al.*, 2006) and X-ray spectroscopic techniques (Ohnuki *et al.*, 2005) have been developed for chemical speciation of elements in the environmental samples. Significant information on the chemical forms of elements in the environment is provided by these methods analysis (Adamu, 2006).

The analysis techniques must be coupled with extraction and pre-concentration techniques such as LLE to achieve higher sensitivity (Dadfarnia *et al.*, 2010). Other examples are SPE (Marguí *et al.*, 2013) and co-precipitation (Manzoori *et al.*, 2009) but these methods use large volumes of organic solvents which pose problems to health and the environment. In addition, these techniques use large volumes of sample and time-consuming extraction steps.

To overcome these, techniques such as solid phase micro-extraction (SPME) and liquid phase micro-extraction (LPME) (Rutkowska *et al.*, 2014) must be used. Few attempts have been made to sample Cr with diffusion gradients in thin films (DGT). Ernstberger *et al.* (2002) showed that a conventional DGT device equipped with a chelex binding phase quantified Cr(III) only. Leese and co-workers (2016) used micro-liquid chromatography- inductively coupled plasma-mass spectrometry (LC-ICP-MS) for the simultaneous detection of Cr(III) and Cr(VI) in exhaled breath condensate samples and it was found that the limits of quantification were 0.040 µg/L and 0.013

µg/L for Cr(III) and Cr(VI), respectively. The fact that Cr(III) compounds are typically cationic in solution and Cr(VI) compounds are typically anionic in solution, speciation using ion exchange chromatography methodologies is an analytical challenge. Trivalent Cr and Cr(VI) compounds are cationic and anionic in solution, respectively, have resulted in analysis either only determining Cr(VI) using colorimetric analysis with diphenylcarbohydrazide (Scindia *et al.*, 2004) or by subtraction of Cr(III) from determined Cr(VI) and total Cr (Chen *et al.*, 2010). Simultaneous determination of Cr(III) and Cr(VI) has been conducted using ICP-MS coupled to reverse phase chromatography combined with ion-pairing reagents (Wolf *et al.*, 2007).

Fabric phase sorptive extraction established itself as a viable alternative to the other conventional pre-concentration techniques (Kumar *et al.*, 2014). It is used for the pre-concentration of Cr metal ions followed by HPLC-UV detection. Target analytes are pre-concentrated fast, while at the same time minimizes the number of laborious and cumbersome sample preparation steps. Hexavalent Cr can be determined after reaction with 1,5-diphenyl carbohydrazide using colorimetry (Zhang and Koropchak, 2000).

2.10 Techniques used for Cr speciation in the current study

Despite the significant advances that have been made in metal speciation analysis techniques over the past 25 years, much remains to be done. There is an urgent need for routine Cr species determination. The standardisation and simplification of the speciation schemes, development of controlled, reliable and reproducible sampling, storage and pre-treatment procedures as well as study of certified reference materials (CRMs) are essential to achieve this goal. Published papers on Cr speciation for the most part show high selectivity and sensitivity, yet the requirement for metal species preservation at the detection stage has gotten little consideration (Bahadir *et al.*, 2016; Markiewics *et al.*, 2016). An evident handicap in speciation analysis is that Cr in environmental compartments is commonly at trace or ultra-trace levels, and consequently, its different chemical species are even more elusive. Consequently, Cr speciation must rely on superior separation and detection capabilities, using methods of guaranteed accuracy and precision (Kotas and Stasicka, 2000).

One way in which speciation is determined: laboratory analysis provides a direct measure of different forms of a target chemical. The most frequently used techniques that have been used to determine Cr(VI) and Cr(III) after separation of the species are atomic spectrometric techniques in the form of either FAAS or GF-AAS.

2.10.1 Flame atomic absorption spectrometry

The FAAS is an analytical technique for the quantitative determination of chemical elements using the absorption radiation (light) by free atoms in the gaseous state. In this case, the technique is used for determining the concentration of Cr in sediments. Flame atomic absorption spectrometry is used mostly for trace element detection due to its low cost, low detection limit, moderate interferences that can be easily corrected, very good precision and easy automation of the measurement (Garcia and Baez, 2012). However, one of the disadvantages of using FAAS is that it is a single-element analysis technique which is time consuming (Bader, 2011).

With FAAS, the light beam emitted by the source, which must be at the wavelength required for measurement, passes through the flame in which the element is in its atomic state. The beam is then focused on the entrance slit of the monochromator. A monochromator is used to select the specific wavelength of the light that is absorbed by the sample and to exclude other wavelengths. The selection of the specific wavelength of light allows for the determination of the specific element of interest when it is in the presence of other elements. The light selected by the monochromator is directed onto a detector. The detector used is typically a photomultiplier tube which convert the light signal into an electrical signal proportional to the light intensity (Martinenghi *et al.*, 2016; Garcia and Baez, 2012).

When the level of Cr species is very low, they may undergo changes during sample handling and analysis, especially in pre-concentration procedures. Atomic spectrometric detection, both alone or coupled with other techniques to pre-concentrate and separate the two main Cr species, Cr(III) and Cr(VI), is the most widely employed methodology reported in the literature (de La Guardia and Morales-Rubio, 2003). Figure in 2.6 shows the basic components of any type or brand of a FAAS.
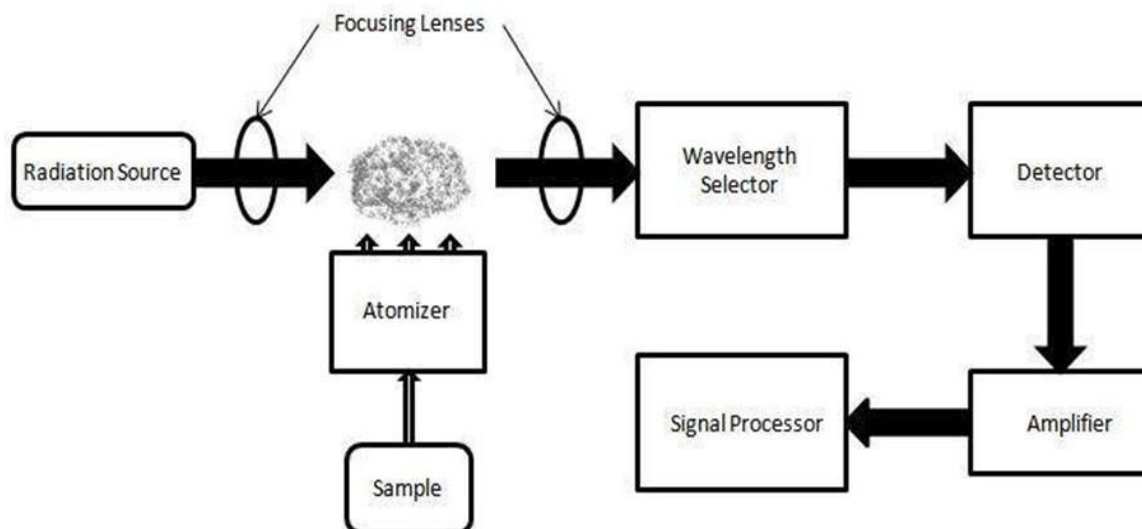
Figure 2.6: Schematic diagram of FAAS

2.10.2 Graphite furnace-atomic absorption spectrometry

A GF-AAS is equipped for distinguishing metals at levels three orders of extent lower than the regular FAAS. The instrument uses lower amounts of samples (ordinarily 5-60 µL) and the atomisation procedure is more proficient contrasting with AAS (Sardans *et al.*, 2010). Despite these advantages, GF-AAS suffers from a couple of limitations. A GF-AAS is ordinarily a single component strategy unless a special optical arrangement or multi-component source is utilised and requires in the order of 1 – 2 min for each atomisation cycle. Since AAS is generally a traditionally single element detection technique, the quantification of numerous metals in a single sample is a time-consuming procedure (Helaluddin, 2016).

With GF-AAS, the sample is introduced directly into a graphite tube, which is then heated in a programmed series of steps to remove the solvent and major matrix components and to atomise the remaining sample. All the analyte is atomised, and the atoms are retained within the tube (and the light path, which passes through the tube) for an extended period of time. As a result, sensitivity and detection limits are significantly improved over FAAS (PerkinElmer, 2012). A schematic diagram of a typical GF_AAS is shown in Figure 2.7.
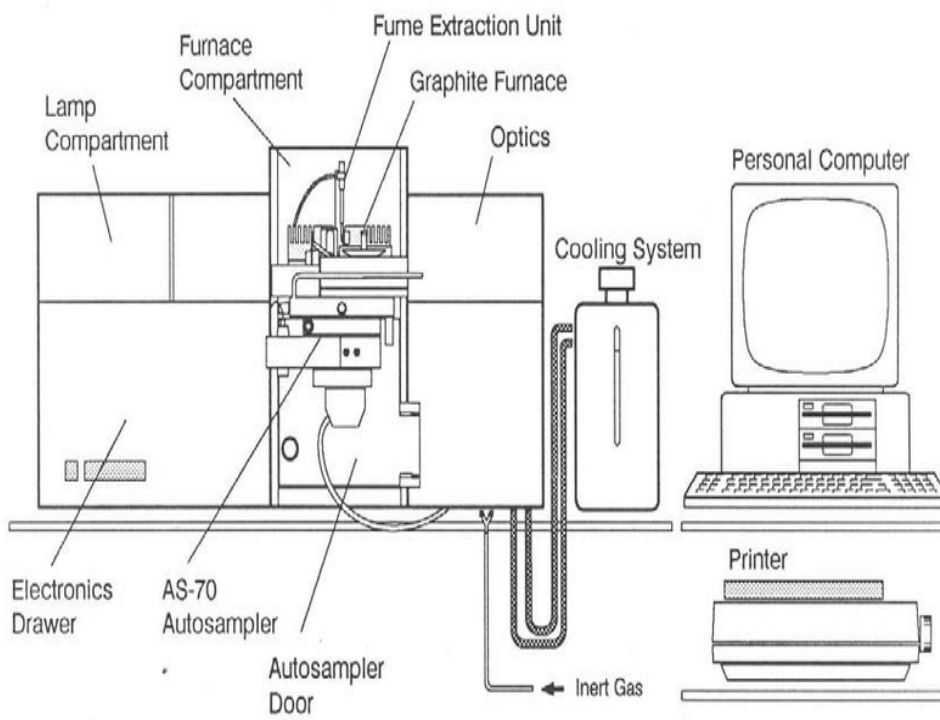
Figure 2.7: Schematic diagram of the cross section of an electrically heated graphite furnace (Melaku, 2005)

CHAPTER 3: EXPERIMENTAL

3. INTRODUCTION

In this chapter, the description of study areas, origin of samples and the analytical procedures used for sample preparation and analysis are described. Equipment and instrumentation as well as instrumental parameters, used during speciation of Cr are included. The purity of reagents, standard reference materials and standards are given.

3.1 Description of the study area and sampling

3.1.1 Blood River

The Blood River, a tributary of Sand River, is a stream running through a township called Seshego outside Polokwane, Limpopo province. It originates in the west of Polokwane municipal area and flows to Seshego Dam. The activities such as fishing, swimming and sand mining take place in the Seshego Dam. All these activities could have a negative impact on the dam and the river as well. Among other activities, animals depend on this river water for drinking. Sampling sites 3 and 4 are located in the Seshego dam. A map showing sampling sites from 1 to 10 in Blood River is given in Figure 3.1.
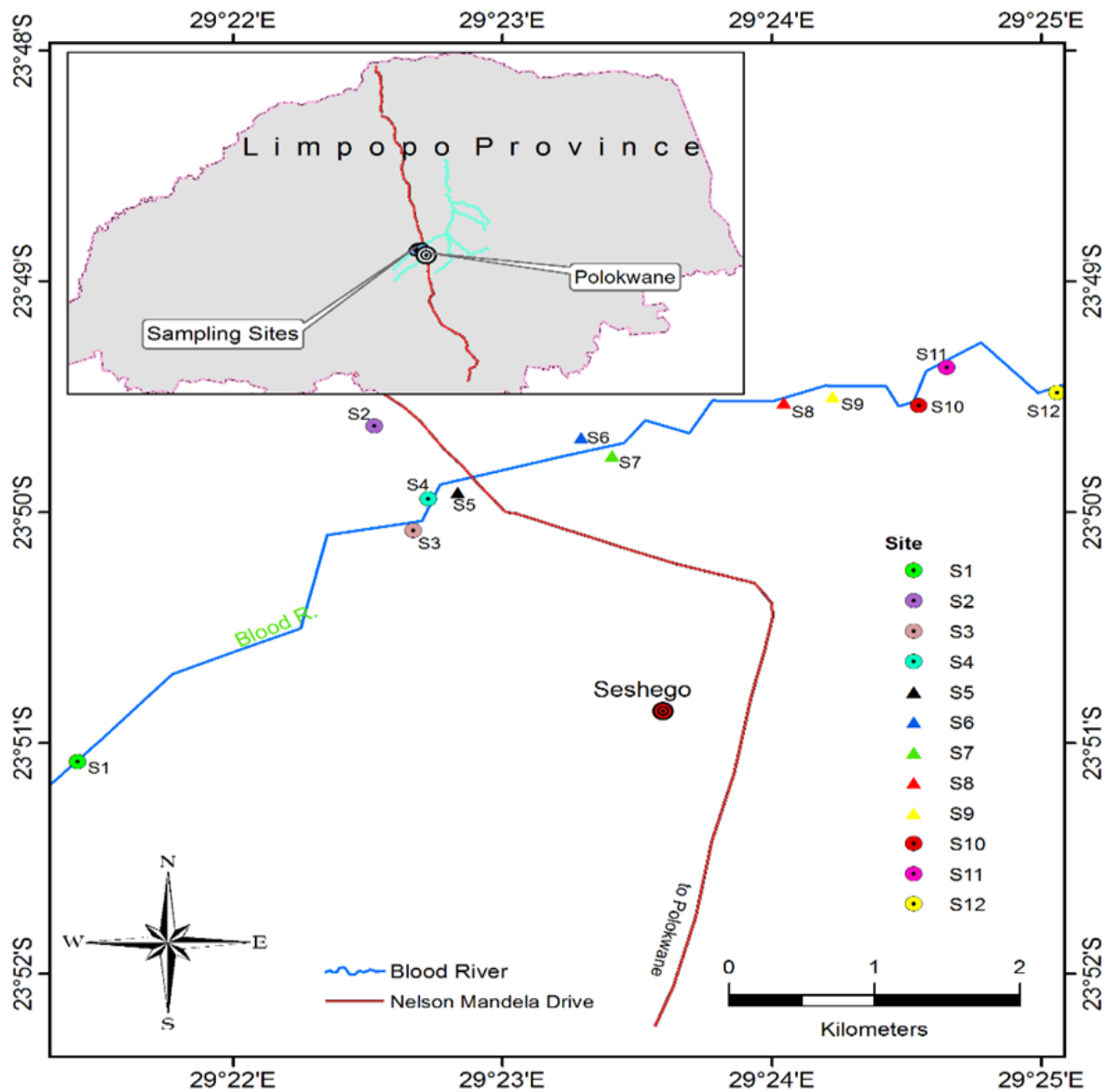
Figure 3.1: Map showing sampling sites at the Blood River, Limpopo Province

Communities residing near the Blood River dump their household waste in and around the river. A photograph showing the dumping site near Blood River is given in Figure 3.2.

Figure 3.2: Dumpsite located near sampling site 2 in Blood River

Blood River is surrounded by several agricultural activities. These agricultural activities use water of Blood River for irrigation purposes. Possible source of pollution and alkalinity in site 4 of Blood River could be due to this irrigation. A picture showing water abstraction pump system for irrigation purpose in site 4 of Blood River is given in Figure 3.3.

Figure 3.3: Water abstraction pump system for irrigation in site 4 of Blood River

The Seshego WWT discharges its effluents directly into the Blood River. A photograph showing wastewater from the sewage is presented in Figure 3.4. This waste water could be a possible source of pollution in site 8 of Blood River.

Figure 3.4: Wastewater discharged from the WWT plant at site 8 of the Blood River

3.1.2 Mokolo River

The Mokolo River catchment is situated in Limpopo province and covers an area of 8387 km² with a mean annual runoff of 312.3 x 10^6 m^3/a (Midgely *et al.*, 1994). It passes in the vicinity of Medupi and Matimba power stations as well as the Grootegeluk coal mine. The catchment extends from the Waterberg mountains through the upper ranges of the Sand River, and incorporates the Mokolo Dam and various small tributaries that join the primary Mokolo River up to its confluence with the Limpopo River (DWAF, 2008). The lower catchment is dominated by game farming, while the upper catchment is dominated by irrigated agriculture (Angliss, 2002). The Mokolo River and its tributaries originate in a region of porous consolidated and unconsolidated sedimentary strata and then flow through a region of intercalated arenaceous and argilacoeus strata before reaching the Limpopo River (Angliss, 2002; DWAF, 2008; Sibisi, 2009).

Current water use in the catchment exhaustively contains 87% for agricultural activities and 13% to the mechanical, mining, power generation and besides commonplace water supply administration parts (regions) (DWAF, 2012). A map showing 10 sampling points in the Mokolo River is given in Figure 3.5.
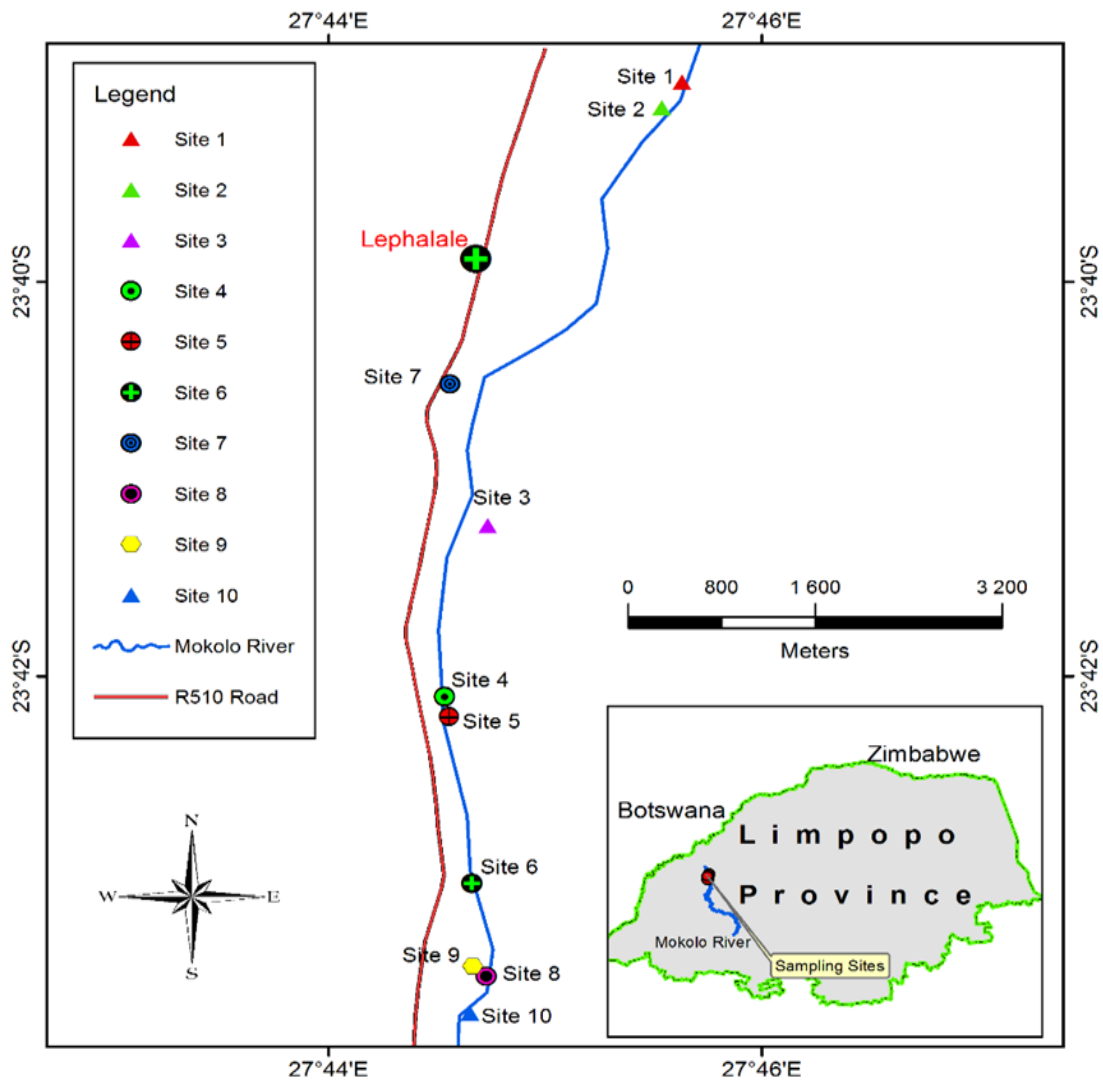
Figure 3.5: Map showing sampling sites at the Mokolo River, Limpopo Province

Water of Mokolo River is also used for irrigation. The irrigation affects the quality and quantity of water for subsequent uses such as industrial, domestic, fisheries and in some cases, recreation. The photo showing irrigation pipeline near sampling point 5 of Mokolo River indicated in Figure 3.6.

Figure 3.6. Sampling site 5 of Mokolo River

3.2 Sample collection, preservation and storage

Sampling was conducted in August 2017. Water and sediment samples were
collected from ten sampling sites in each river. Approximately 2000 mL of water

sample and around 1000 mg of sediment sample were collected at each site. The water samples were collected and kept in heavy wall polyethylene bottles and immediately placed in cooler box. On arrival at the laboratory, water samples were filtered using 0.45 μm membrane filter and acidified with 1% HNO_3 as a preservative. All samples were kept in the refrigerator at 4 °C. The pH of water samples was measured on site. Figure 3.7 shows a picture of pH measurement in water samples.

Figure 3.7: pH measurement in water samples

Sediment samples were collected at a depth of 20 cm using a polyethylene spatula from ten different sampling sites in each river. The sediment samples were labelled and stored in 500 mL polyethylene bottles and transported to the laboratory in a cooler box. Sediment samples were air-dried, sieved through a 100 μm sieve and ground to a fine powder prior to digestion and extraction procedures

3.3 Reagents, reference materials and standards

High purity reagents were used throughout this work for preparation of water and sediment samples. Deionised water was used for dilution of calibration standards and samples. High purity HNO_3 (65%) (Rochelle chemicals, Johannesburg, South Africa) was used for cleaning glassware, digesting sediment samples, column conditioning and elution of Cr(VI). Instrument grade argon air and acetylene (Afrox, South Africa) were used for GF-AAS and FAAS.

For digestion of sediments, 40% HF and 37% HCl obtained from Sigma-Aldrich were used. A 0.1 M Na_2CO_3 solution was prepared by dissolving 1.06 g of Na_2CO_3 in deionised water and diluting to 100 mL. A second reagent of 0.01 M Na_3PO_4 was prepared by dissolving 16.4 g of Na_3PO_4 in deionised and diluting to 1000 mL with deionised water. The content was shaken in a conical flask. Once dissolved, 10 mL of 0.1 M Na_2CO_3 and 10 mL of 0.01 M Na_3PO_4 were used for extraction of Cr(VI) in sediments.

Sodium acetate (CH_3COONa) (Merck, Darmstadt, Germany) and glacial acetic acid (CH_3COOH) (Merck, Darmstadt, Germany) were used for buffer preparation and pH adjustment. Alizarin (BDH Chemicals Ltd, Poole, England) was used for binding Cr(III) to prevent oxidation.

A sediment reference material - SRM 8704 (NIST, USA) and trace elements in water reference material - SRM 1643F, (NIST, USA) were employed to evaluate the accuracy and precision of the method employed for analysis of sediment and water samples, respectively.

Standard solutions were prepared daily, by appropriate dilutions of a stock standard of 1000 mg/L for Cr, Cr(III) and Cr(VI) (Sigma Aldrich, SA). Quantification of Cr and its

species concentrations were performed, establishing calibration curves with external standards prepared in 1% (v/v) ultra-pure HNO_3. The calibration curve was constructed from four points and the blank.

A 100.0 mg/L intermediate standard of analytes were prepared by pipetting 5.00 mL of 1000 mg/L Cr, Cr(III) and Cr(IV) into a 50 mL centrifuge tube and diluting to the mark with deionised water. A blank and four calibration standards were prepared by pipetting 0.00, 0.5, 1.0, 1.5 and 2.0 mL of intermediate standard into 10 mL centrifuge tubes, to prepare 0.00, 5.00, 10.0, 15.0 and 20.0 mg/L standards, respectively. A 1.00 mL of 65% pure HNO_3 were added to each flask, prior to filling to the mark with deionised water.

Preparation of blank and four calibration standards for analysis using GF-AAS were achieved by pipetting 0.00, 250, 500, 750 and 1000 μL of 1000 μg/L intermediate standard into 50 mL centrifuge tubes, to prepare 0.00, 5.00, 10.0, 15.0 and 20.0 μg/L standards, respectively.

3.4 Apparatus and instrumentation

The MARS 5 microwave digestion system (CEM Corporation, USA) was employed for the mineralisation of sediment samples. A Teflon XP-1500 Plus Vessel, allowing a maximum decomposition pressure of 800 psi and temperature of 240 °C, was used for digestion of samples. The High Pressure Digestion Vessel Accessory Sets (CEM Corporation, USA) permits simultaneous processing of up to 12 XP-1500 Plus vessels. At full power, the MARS delivers approximately 1200 W of microwave energy at a magnetron frequency of 2450 MHz.

Solid-phase extraction catridges, Chromabond-NH_2 (3 mL/500 mg) columns (i.e. aminopropyl phase with a 3 mL volume and 500 mg of sorbent) were obtained from Macherey-Nagel (Düren, Germany). These columns were used to separate and concentrate Cr(VI) selectively, both in spikes and in the water samples prior to quantification.

A Thermo Scientific™ Orion™ 520A pH/mV/SE benchtop Meter (USA) was used to measure the pH.

A model 13156 vacuum pump (Gelman Instruments Company, Fort Wayne, Indiana, USA) was used to create a vacuum to dry the column after adsorption of the sample.

A 400 FAAS (PerkinElmer, Waltham, USA) was used for quantification of Cr in sediments. A HGA 900 GF-AAS (PerkinElmer, Waltham, USA) was employed for detection of Cr(VI) and total Cr in water as well as Cr(VI) in sediments.

All glassware was washed with detergent and water. After being rinsed with deionised water (18.2 MΩ cm) three times, it was soaked in 10% HNO_3 (v/v) for 24 h. The glassware was then rinsed three times with deionised water and dried before use.

3.5 Sample preparation

3.5.1 Water samples

Water samples for quantification of total Cr and Cr(VI), were collected from Blood and Mokolo Rivers in Limpopo province.

3.5.1.1 Preparation of water samples for determination of total concentrations of Cr

Filtered water samples in triplicates were transferred directly in to plastic vials and placed on an auto sampler. Determination of total Cr concentrations in water was achieved using GF-AAS.

3.5.1.2 Separation of Cr(VI) in water by SPE technique using chromabond-NH_2 column

Mixed standards of Cr(III) and Cr(VI) containing 0.5 µg/L of each in 100 mL of de-ionised water was mixed with 5 mL 0.001% alizarin sulphonic acid solution. The pH of the sample solution was adjusted to 5.5 using acetic acid and sodium acetate buffer.

Column conditioning was achieved with two column volumes of 1 M HNO_3 followed by two column volumes of deionised water. Selective retention of Cr(VI) in the column was quantitatively achieved by passing the sample through the column at a flow rate of 3 mL/min. After adsorption of the sample, the column contents were dried under vacuum. The Cr(VI) was eluted with two column volumes of 2 M HNO_3.

A 100 mL of water sample was mixed with 5 mL 0.001% alizarin sulphonic acid solution. The pH was adjusted to 5.5 using acetic acid and sodium acetate buffer. Column conditioning was achieved by the same procedure applied for method validation. The SPE sample preparation setup used in this study is given in Figure 3.8.

Figure 3.8: SPE set up for sample preparation and application

3.5.1.3 Stability test of Cr(VI) in water

The concentration of 5 µg/L was used to test the stability of Cr(VI) in water. The separated Cr(VI) solutions were kept in the refrigerator at 4 °C. The stability of Cr(VI) was checked by analysing the solution every seven days for three weeks.

3.5.2 Sediment samples

3.5.2.1. Preparation of sediment samples for determination of total Cr

A 0.25 g portion of homogenised SRM 8704 and sediment sample were weighed and transferred in to pre-cleaned and dried microwave digestion vessels. Then 2 mL of 65% HNO_3, 1 mL of 37% HCl and 2 mL of 40% HF were added to each sample and vessels were allowed to stand open until the initial reaction subsided. The samples were mineralised in the MARS 5 microwave digestion system (CEM Corporation, USA), employing the following programme: pressure control, 20 min ramp, 20 min hold, maximum pressure of 800 psi and maximum temperature of 200 °C. A blank solution was prepared by digesting 2 mL of 65% HNO_3, 1 mL of 37% HCl and 2 mL of 40% HF using the same digestion procedure. After cooling, the digested samples and reagent blank were transferred to 50.0 mL volumetric flasks and diluted to 50.0 mL with deionised water then filtered through a hydrophilic Millipore PVDF 0.45 µm filter prior to determination of Cr by FAAS. All digestions were prepared in triplicate.

After each digestion, cleaning of vessels was conducted in the microwave digestion system using 2 mL of 65% HNO_3, 1 mL of 37 % HCl and 2 mL of 40% HF, following the heating programme: 20 min ramp from ambient temperature to 200 °C, holding for 10 min at 500 psi. After cooling, the acid residues were discarded. The vessels were thoroughly rinsed with deionised water and dried in the oven at 100 °C over night.

3.5.2.2 Extraction of Cr(VI) in sediments using Na_2CO_3

A 0.25 g portion of homogenised sediment sample was weighed and transferred into pre-cleaned and dried microwave digestion vessels. Thereafter, 10 mL of 0.1 M Na_2CO_3 was added to each sample in the vessels. The samples were extracted using the MARS 5 microwave extraction system (CEM Corporation, USA), employing the following programme: pressure control, 15 min ramp, 15 min hold, maximum pressure 700 psi and maximum temperature 120 °C. A blank solution was prepared using 10 mL of 0.1 M Na_2CO_3 and applying the same extraction procedure. After cooling, the extracted samples and reagent blank were filtered through a Whatman No: 540 filter papers and diluted to a final volume of 50 mL with deionised water. The sample

solutions were filtered through a hydrophilic Millipore PVDF 0.45 µm filter prior to determination of Cr(VI) using GF-AAS. All digestions were prepared in triplicate.

After each extraction, cleaning of vessels was conducted in the microwave extraction system as detailed in the previous section.

3.5.2.3 Extraction of Cr(VI) in sediments using Na_3PO_4

A 0.25 g weighed portion of homogenised sediment samples were weighed in to pre-cleaned and dried microwave digestion vessels. Then 10 mL of 0.01 M Na_3PO_4 was added to each sample in vessels. The extraction of Cr(VI) was carried out using the MARS 5 microwave extraction system (CEM Corporation, USA), employing the programme described in Section 3.4.2.2. A blank solution was prepared using 10 mL of 0.1 M Na_3PO_4 and applying the same extraction procedure. After cooling the vessels, the extracted samples and reagent blank were filtered through a Whatman No: 540 filter papers and diluted to a final volume of 50 mL with deionised water prior determination by GF-AAS. Sample solutions were prepared in triplicate.

Cleaning of vessels was conducted in the microwave extraction system using 10 mL of 0.01 M Na_3PO_4 following the heating programme detailed in Section 3.4.2.1.

3.6 Sample analysis

3.6.1 Determination of Cr in sediments

Chromium concentrations in sediment samples were determined using the A 400 FAAS operated using the conditions in Table 3.1. Concentrations of Cr in all samples were determined using the external calibration curve. Blank, standards and all sample solutions were nebulised and each solution of standard or sample was followed by introduction of deionised water for at least 1 min, to rinse the sampling system in order to avoid contamination of subsequent solutions. Three independent replicates of each sample were measured and the concentrations were calculated using the average of each value. The blank solutions were also measured, and the intensity of each analyte in the blank sample was subtracted from that of the sample.

Table 3.1: Instrumental conditions for the FAAS

Parameter	Setting
Flame type	Air-C_2H_2
Wavelength (nm)	357.9
Slit width (nm)	0.7
Lamp current (Ma)	20
Mode	Peak height
Air flow rate (mL/min)	6
Acetylene flow rate (L/min)	2

3.6.2 Determination of Cr in water, quantification of Cr(VI) in water and sediments

The HGA 900 GF-AAS was employed for quantification of Cr(VI) in water and sediments as well as for the quantification of total Cr in water samples. The GF-AAS equipped with a transversely heated graphite tube atomizer (THGA) using longitudinal Zeeman-effect background correction and an AS-80 auto-sampler were used. The operating conditions of the GF-AAS are summarised in Table 3.2.

Table 3.2: Instrumental operating conditions for GF-AAS

Parameter	Setting
Wavelength (nm)	357.9
Two step drying, temperatures (^{o}C)	110 and 160
Ashing temperature (^{o}C)	1500
Atomization temperature (^{o}C)	2350
Read time (s)	4.5
Read delay (s)	0.0
Sample Injection volume (μL)	20
Inert gas	Argon
Background correction	Zeeman
Measurement mode	Peak Area
Linear range (μg/L)	up to 20
Slit width (nm)	0.7

3.7 Determination of limit of detection and limit of quantification

To determine the limit of detection (LOD) of the entire analytical procedure, reagent blanks were prepared following the same procedures for the quantification of total Cr and Cr(VI) in the samples. The absorbance of six blanks were measured. The LODs for the species under study, based on three times the standard deviation (3σ) of the average of six individually prepared blank solutions, were calculated. The limit of quantification (LOQ) was calculated as ten times the standard deviation of average of six individually prepared blank solutions.

3.8 Validation of methods for the determination of total Cr and Cr(VI)

For the assessment of the accuracy of the concentration of Cr determined in sediment and water samples, a sediment reference material (SRM 8704) and trace elements in water standard reference material (SRM 1643f), respectively, were used for quality control purposes.

Accuracy of hexavalent Cr in water was ascertained by spiking mixed standards of Cr(III) and Cr(VI) containing 0.5 µg/L of each in deionised water. For the assessment of the accuracy of the concentration of Cr(VI) quantified in sediments, a recovery study was conducted by spiking sediment samples with 3.5 µg/L of Cr(VI).

CHAPTER 4: RESULTS AND DISCUSSION

This chapter discusses the results from all the experiments and procedures described in the previous chapter. The main purpose of the study was to speciate Cr in water and sediments collected from Blood and Mokolo Rivers. Total concentration of Cr and Cr(VI) in water and sediments, comparison of Cr levels and Cr(VI) levels between the two rivers, quantification of Cr(VI) in sediments using two different leaching reagents and stability of Cr(VI) are discussed in depth.

4.1 pH Measurements

The pH is very important because it has a significant effect on the water quality. The pH of the water can be altered by pollution. Extremely high and low pHs can be inconvenient for the utilisation of water. High pH causes an intense taste, water pipes and water-utilising machines wind up encrusted with deposits, and it discourages the viability of the purification of chlorine, in this way causing the requirement for extra chlorine when pH is high. Low pH water will corrode or break up metals and different substances (Shammas and Wang, 2016).

The pH values for this study were observed to be slightly lower at Mokolo River (6.59 - 7.00) than Blood River (6.60 - 10.5). Each sampling site at Mokolo River has the pH value that is close to neutral and lies within acceptable limits for natural water (6.5 - 8.5). Mokolo River passes in the vicinity of Grootegeluk coal mine. The water from coal mines is known to be very acidic decreasing the pH of the river water. By using the logarithm scale, the Grootegeluk mine-drainage water would be 100000 times more acidic than neutral water. Water with a pH>8.5 could indicate that the water is hard. Sites 1, 2, 5, 7 and 8 of Blood River have pH level above 8.5 which indicates the presence of high level of alkalinity minerals. This is because the Seshego WWTW discharges its effluent directly into the Blood River (DWA, 2014; Dombo and Du Plessis, 2000). The ideal pH level of alkaline ionised water is between 8.5 and 9.5 (and no greater than 10.0). This means that water at site 7 and 8 of Blood River is not good for direct consumption. The US EPA recommends that public water systems maintain pH levels of between 6.5 and 8.5 (US EPA, 2014). Figure 4.1 shows pH measurements in water samples of Blood and Mokolo Rivers.
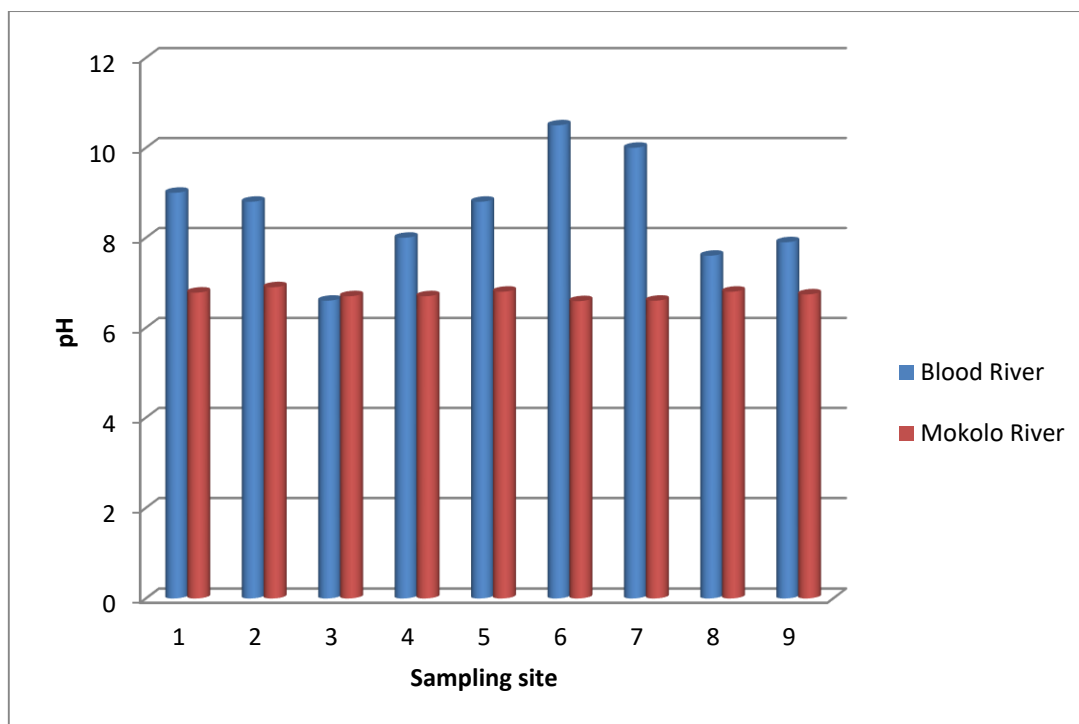
Figure 4.1: pH of water samples collected from Mokolo and Blood Rivers

4.2 Calibration curves for the determination of Cr and Cr(VI)

Six point calibration curves with five standards and one blank were constructed under the optimised conditions, resulting in linear calibration curves with correlation coefficients (R^2 values) of more than 0.999. A calibration curve for the determination of total Cr by FAAS is given in Figure 4.2. Figure 4.3 illustrates the calibration curve for the determination of Cr(VI) using GF-AAS.
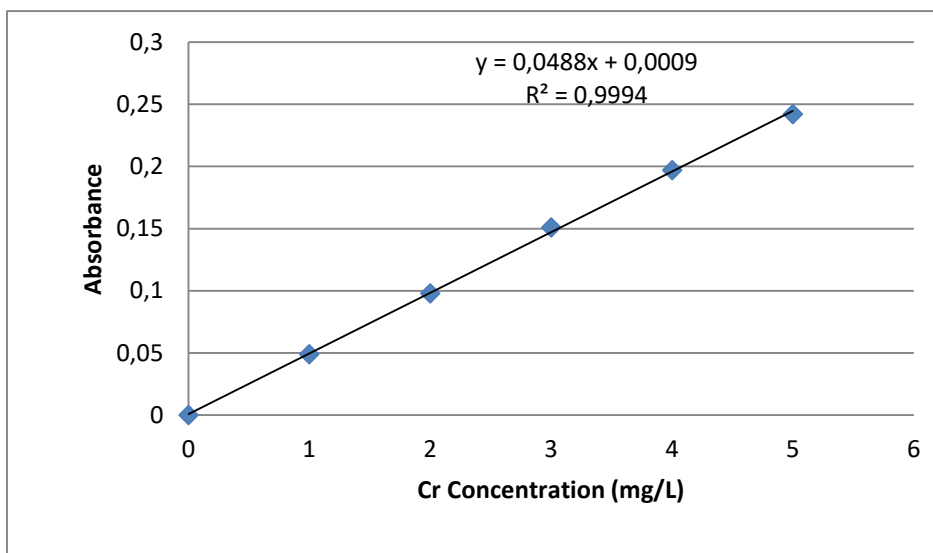
Figure 4.2: Calibration curve obtained from determination of Cr by FAAS
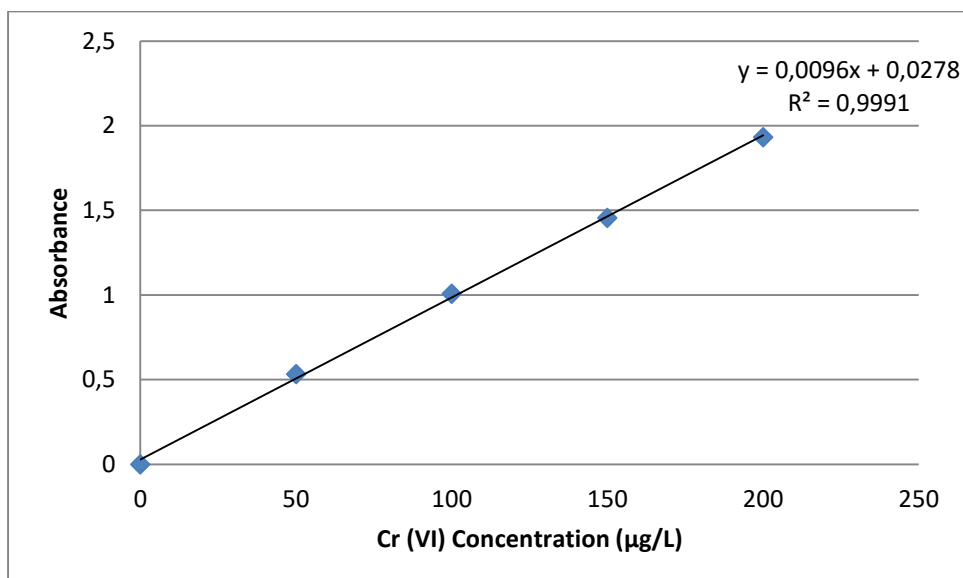
Figure 4.3: Calibration curve obtained for determination of Cr(VI) by GF-AAS

4.3 Determination of limit of detection and limit of quantification

The LOD for Cr and Cr(VI) in water and sediment samples was calculated as three times the standard deviation of the measured concentrations for the six reagent blanks. The LOQ for Cr and Cr(VI) in water and sediment samples was calculated as ten times the standard deviation of the measured concentrations for the six reagent blanks. The LODs and LOQs for total Cr and Cr(VI) in water and sediments are given in Table 4.1.

Table 4.1: Limit of detection and limit of quantification obtained for the detection of Cr and Cr(VI) in water and sediments

Analyte	Sediment LOD (µg/g)	Sediment LOQ (µg/g)	Water LOD (µg/L)	Water LOQ (µg/L)
Cr	0.02	0.07	0.32	1.1
Cr(VI)	0.03[a] 0.06[b]	0.10[a] 0.20[b]	0.11	0.35

[a]denotes Na_2CO_3 extraction procedure; [b]denotes Na_3PO_4 extraction procedure

In water, the LOD and LOQ for measuring total Cr using GF-AAS were 0.32 µg/L and 1.06 µg/L, respectively. Lower LOD and LOQ of 0.11 µg/L and 0.35 µg/L, respectively, were obtained for Cr(IV) using GF-AAS. Jayasinghe et al. (2003) reported LOD of 0.4 µg/L and 0.5 µg/L for Cr and Cr(VI), respectively, in drinking water samples employing GF-AAS. This value is higher than the LODs obtained in our study. These differences in LODs could be attributed to the differences in instrumental conditions and the purity of reagents used during analysis. In another study, the LOD of Cr in river water samples which had undergone co-precipitation with terbium hydroxide prior detection with GF-AAS, was found to be 0.3 µg/L (Minami *et al.*, 2005). A LOD for Cr(VI), of 50 µg/g in sediments was reported by Mamatha *et al.* (2014). This value is higher than the values obtained in the current study. Baig *et al.* (2013) reported lower LOD of 0.037 µg/L for Cr(VI) in river water using GF-AAS after cloud point extraction with ammonium pyrrolidinedithiocarbamate. This value is lower than the LOD determined in this study.

In sediment, the LOD and LOQ for total Cr were 0.02 µg/g and 0.07 µg/g, respectively. The LOD and LOQ, obtained for Cr(VI), extracted using Na_2CO_3 were 0.03 µg/g and 0.06 µg/g, respectively. Higher LOD (0.10 µg/g) and LOQ (0.20 µg/g) for Cr(VI) were observed when using the Na_3PO_4 extraction procedure. The Na_2CO_3 extraction procedure produced better LOD and LOQ than extraction using Na_3PO_4. Mandiwana, (2008) reported a LOD of 0.07 ug/g in soil employing Na_3PO_4 extraction procedure. This value is nearly closer to the one reported in this study.

4.4 Validation of analytical procedures for determination of Cr and Cr(VI) in water and sediments

A sediment reference material (SRM 8704) and trace elements in water reference material (SRM 1643F) were analysed to test the validity of the developed methods for total Cr determination. The results of method validation for total concentration determination of Cr in water by GF-AAS and in sediment by FAAS are presented in Table 4.2.

Table 4.2: Validation of analytical procedure employed for total concentrations quantification of Cr in water and sediment samples

SRM	Measured value	Certified value	Percentage recovery
Water SRM 1643F	3.09 ± 0.35 µg/L	3.60 ± 0.10 µg/L	85.8
Sediment SRM 8704	105 ± 0.25 µg/g	122 ± 3.8 µg/g	86.4

A student's t-test was used to determine statistical differences between the measured and certified mean values of Cr in water (Appendix 1) and sediments (Appendix 2). It was found that there is no significant difference at 95% confidence level between the SRM 1634F mean and the certified SRM 1634F mean. For sediments, there is a significant difference at 95% confidence level between the measured SRM 8704 mean and the certified SRM 8704 mean.

The percentage recovery was evaluated with reference to the standard guidelines recommended by US EPA, which are guidelines for method development and validation (US EPA, 2010). The US EPA recommended suitable percentage recoveries to be within 75 to 125% (US EPA, 2010). The quantitative percentage recoveries of 85.8% and 86.4% indicate that the methods for determination of total Cr in water and sediment, respectively, are validated.

The results of the percent recovery study conducted employing Alizarin to ensure that there is no oxidation of Cr(III) to Cr(VI) at pH 5.5 are given in Table 4.3. Good percentage recoveries of between 88.7 and 91.8% were obtained. These values

indicate that the validation of method for determination of Cr(VI) in water was successful, in reference with standard guidelines recommended by US EPA (US EPA, 2010). Chromate can be sorbed distinctively in the NH_2 segment than dichromate (Koivuhuhta *et al.*, 2008), which brings about some loss of percent recoveries of Cr(VI) standard solutions.

Table 4.3: Results obtained in the spike recovery studies

Replicates	Replicate 1	Replicate 2	Replicate 3	Mean
Concentration of prepared Cr(III)/Cr(VI) solution (µg/L)	5.00	5.00	5.00	5.00
Measured Cr (VI) concentration (µg/L)	4.48	4.59	4.44	4.50
%Recovery	89.6	91.8	88.7	90.0

The percentage recoveries obtained for validation of the analytical procedure for the determination of Cr(VI) in sediment samples are given in Table 4.4. The extraction of Cr(VI) was performed by spiking the sediment samples with 3.5 ppb Cr(VI). The two methods separately used 0.1 M Na_2CO_3 and 0.01 M Na_3PO_4 digestion procedure for extracting Cr(VI) from soluble, adsorbed and precipitated forms of Cr compounds in sediments. As shown in Table 4.4, the percentage recoveries were within 94.9 – 105 % range using the Na_2CO_3 extracting procedure.

Table 4.4: Validation of the Na_2CO_3 analytical procedure employed determination of Cr (IV) in sediment samples

Sediment	Cr(VI) (ng/g)	Cr(VI) (ng/g)
Spiked	12.6	16.8
Unspiked	8.95	13.5
% recovery	105	94.9

The results for Cr(VI) extraction in sediments using 0.01 M Na_3PO_4 were inconsistent and resulted in significantly lower levels of Cr(VI) compared to the Na_2CO_3 method. To quantify total Cr(VI) in solid matrices, these criteria must be satisfied: the extracting solution must solubilise all forms of Cr(VI); and the extraction must not cause oxidation of native Cr(III) contained in the sample to Cr(V) (US EPA, 2003). Due to homogenous distribution of Cr(VI) in the contaminated sediments, the 0.1 M Na_2CO_3 extraction procedure met these criteria and the percent recoveries were consistent with the recommendations of US EPA guidelines for method development and validation (US EPA, 2010).

4.5 Determination of Cr in river water samples

4.5.1 Total Cr concentration in river water samples

The total Cr concentrations in water samples from Blood and Mokolo Rivers are presented in Table 4.5. Concentrations of Cr in water samples were measured using GF-AAS. The Blood River water samples contained generally higher concentrations of Cr than the Mokolo River water samples.

The concentrations of Cr in water of Blood River ranged from 1.56 to 6.11 µg/L. However, concentrations of Cr in sites 6, 7 and 8 were below LOD value of 0.32 µg/L. Jayasinghe *et al*. (2003) detected Cr of 2.86 to 8.59 µg/L in river water samples employing GF-AAS. The outcomes show that the industrial processes and the local and municipal wastes are by all account not the only source of Cr in water but also the fact that Cr happens in nature. In another study, Coetzee *et al*. (2002) recorded lower concentrations of Cr in the Klein Olifants River, Mpumalanga province. Furthermore, Alam *et al*. (2003) reported that, higher concentrations of Cr were observed in rainy seasons (3 to 13 µg/L) than in the dry season (1.2 to 8 µg/L). The concentrations of Cr obtained in this study are lower than those detected in Nyl River systems by Greenfield *et al*. (2012).

The Cr levels in water samples obtained from Mokolo River ranged from 1.34 to 3.53 µg/L with site 1 below LOD. These concentrations fall within the Cr concentration range (1.98 to 19.13 µg/L) detected in Tinishu Akaki River, Ethopia (Melaku *et al*., 2005). Barker (2008) reported total Cr concentrations in Letaba, Levubu and Sabie

Rivers to be 53.5, 57.0 and 54.0 µg/L, respectively. These concentrations are higher than those reported in the current study. Significantly higher dissolved concentrations of Cr were found in the waters of the Olifants River, Kruger National Park (Avenant-Oldewage and Marx, 2006). De Klerk (2011) conducted a baseline study of Mokolo and Lephalala rivers in the Waterberg area. They reported that the Mokolo and Lephalala rivers have relatively good water quality (confirmed through the presence of certain sensitive macro invertebrate and fish species), with both the macro invertebrate and fish populations appearing relatively intact. Trace elements level in water bodies can be affected by alterations in the weather condition of a river catchment and the water chemistry.

Table 4.5: Concentration (µg/L) of Cr in water samples collected from Blood and Mokolo Rivers

Sampling site	Mean Cr concentration (µg/L) ± SD		Standard guidelines Cr (µg/L)
	Blood River	Mokolo River	
1	3.74 ± 0.21	<0.32	50 (WHO, 2017)
2	1.56 ± 0.12	3.53 ± 0.31	100 (Standards
3	4.57 ± 0.05	1.34 ± 0.09	South Africa,
4	6.11 ± 0.48	2.26 ± 0.09	2005)
5	5.68 ± 0.20	1.37 ± 0.07	100 (FAO and
6	<0.32	2.35 ± 0.06	WHO, 2003)
7	<0.32	3.43 ± 0.12	
8	<0.32	2.55 ± 0.10	
9	1.84 ± 0.16	1.63 ± 0.13	
10	3.55 ± 0.09	2.49 ± 0.15	

4.5.2. Comparison with maximum permissible levels in drinking water and irrigation water

Total concentrations of Cr quantified in water samples collected from Blood and Mokolo Rivers systems were assessed for safe drinking and irrigation with reference to the

standard guidelines. The FAO and WHO established acceptable levels of Cr in irrigation water (FAO and WHO, 2003). To guarantee safe drinking water and measure the nature of treated drinking water, guidelines and standards, as far as concentrations of determinants, are generally characterised with which to assess the safety of drinking water (South African National Standards (SANS), 2005; WHO, 2017). Maximum permissible levels (MPLs) of Cr in drinking and irrigation water are given in Table 4.6.

Table 4.6: Maximum permissible levels of Cr in drinking and irrigation water

Guide	Cr (µg/L)	Reference
WHO	50	(WHO, 2017)
SANS for drinking water	100	(Standards South Africa, 2005)
Irrigation	100	(FAO and WHO, 2003)

Chromium concentrations in natural waters are usually very low (Tuzen and Soylak, 2006). According to the guidelines of WHO and SANS the maximum concentration of Cr in drinking water should be 50 and 100 µg/L, respectively. All the samples from S1 to S10 of the two rivers had Cr concentrations below the MPLs stipulated by WHO and SANS. The recommended operational limit for Cr in irrigation water quality standards is 100 µg/L (FAO and WHO, 2003). Poor quality water may influence irrigated crops by causing amassing of salts in the root zone, by causing loss of permeability of the soil because of overabundance heavy metals leaching, or by containing pathogens or contaminants which are specifically dangerous to plants or to those consuming them. Therefore, the water of Blood and Mokolo Rivers can be considered safe for irrigation use since the concentrations of Cr in all sites were all below MPLs recommendations for irrigation water.

4.5.3. Comparison of total Cr levels in waters from the Blood and Mokolo rivers

The Blood River water samples consisted of significantly higher concentration of total Cr in sites 1, 3, 4, 5, 6, 9 and 10 than those of Mokolo River. The Cr concentrations were high in sites 2, 6, 7 and 8 of Mokolo River than those detected in Blood River. In the past decades, the high utilisation of Cr in several anthropogenic activities (Gu *et*

al., 2012; Wang *et al.*, 2013; Qiu *et al.*, 2014) caused water contamination. Blood River is surrounded by more Cr-containing anthropogenic activities as compared to Mokolo River. Waste material from production of stainless steel deposited into Blood River during rainy days could have led to the elevation of Cr concentration. Blood River serves also as a dumping site making it to be more polluted than Mokolo River. Chromium may be present in domestic waste from various synthetic materials. The SWWT plant discharges its effluents directly into Blood River (Dombo and Du Plessis, 2010; DWA, 2014). Discharging Cr polluted wastewater in rivers causes the concentration of Cr to increase. Agricultural activities within the vicinity of Mokolo River are many as compared to Blood River. The detected levels of Cr in Mokolo River may be due to the presence of pesticides (chromated arsenicals) (Morrell and Huffman, 2004). Chromated arsenicals, which incorporates chromated copper arsenate is a mixture of pesticides containing Cr, Cu as well as As that secure wood against termites, growth and different pests that can degrade or threaten the integrity of wood products (Chou *et al.*, 2007). Graphical representation of Cr levels in the Blood and Mokolo rivers is given in Figure 4.4.
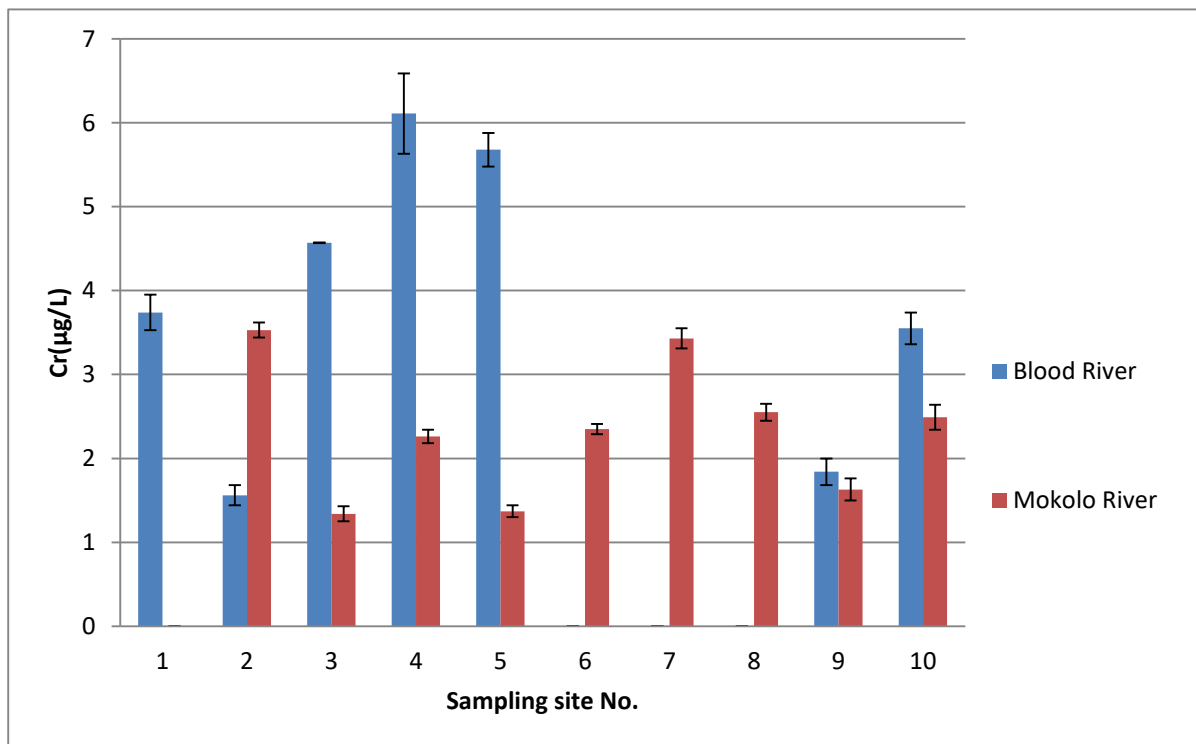
Figure 4.4: Total Cr levels in the water of Blood River and Mokolo River

The highest Cr concentrations of 6.11 µg/L and 3.53 µg/L were detected in site 4 of Blood River and site 2 of Mokolo River, respectively. These values are lower than those detected from Yesilimak River, in Turkey (Cimen *et al.*, 2013). Mandina and Mugadza (2013) reported concentrations range 4.20 µg/L to 8.0 µg/L for Cr using FAAS in waste water samples collected from the metals recovery plant at the slag in Gweru, Zimbabwe. Concentration of Cr as high as 225 µg/L at pH=3 was reported by Djane *et al.* (1999) in surface water samples collected from an abandoned tannery site of Dalalven River, Sweden. The Cr concentrations differ per site and are affected by various factors. The concentrations of Cr obtained in this study were comparable to those found in Sungai Jarak, Sungai Kerian, Sungai Kongsi, Sungai Muda Rivers in Penang, Malaysia (Alsaffar *et al.*, 2016). Current study levels are much lower than those from other rivers given in Table 4.7.

Table 4.7: Comparison of Cr levels in some rivers in South Africa

River	Cr (mg/L)	Reference
Dzindi River	0.03 - 0.10	Edokpayi *et al.* (2014)
Mvudi River	0.01 - 0.59	Edokpayi *et al.* (2016)
Blood River	0.00032 - 0.00611	This study
Mokolo River	0.00134 - 0.00353	This study

4.6 Determination of total Cr concentration in sediment samples

Chromium may enter the aquatic body through the small-scale tanneries, mining sites, natural origins, etc. (Howarth *et al.*, 2005). The total Cr determination in sediments is generally done to assess the level of aquatic and terrestrial environments. In this study, the Cr concentration in sediments were quantified to assess the water quality of the river. Total concentrations of Cr in samples were analysed from each sampling site. Concentrations of Cr in sediment samples from Blood and Mokolo Rivers are presented in table 4.8.

Table 4.8 Total concentration of Cr in sediments of Blood and Mokolo River

Sampling site	Mean Cr concentration (µg/g) ± SD		Standard guidelines for sediment quality Cr (µg/g)
	Blood River	Mokolo River	
1	163 ± 2.6	73.7± 2.1	ERM = 81 (MacDonald *et al*. (2000) Fairey *et al*. (2001) ERL = 370 MacDonald *et al*. (2000) Fairey *et al*. (2001) TEL = 37.3 (CCME, 2001) PEL = 90 (CCME, 2001)
2	146 ± 6.4	91.9 ± 5.4	
3	136 ± 2.1	108 ± 5.8	
4	219 ± 3.0	25.7 ± 1.1	
5	183 ± 2.4	63.4 ± 0.60	
6	129 ± 7.2	52.6 ± 0.87	
7	183 ± 0.60	193 ± 0.80	
8	253 ± 4.7	156 ± 2.3	
9	151 ± 2.7	99.2 ± 0.40	
10	132 ± 3.5	53.0 ± 0.60	

A similar trend to the concentration of Cr in water samples was observed in sediment samples. Sediments collected from the Blood River contained significantly higher levels of Cr compared to those from Mokolo River.

The detected Cr concentrations in Blood River sediments were in the range 129 to 253 µg/g. The maximum value was determined in a sample from site 8. It was observed that this site receives sewage sludge from municipal wastewater treatment plants. Higher concentrations and fractions of Cr were detected in sewage sludge from municipal and industrial wastewater treatment plants (Wang *et al*., 2005). This reveals that the Cr contents in sediments are relatively higher near the discharge point of the sewage facilities. The lowest Cr concentration detected in the sample from site 6 (129 µg/g) is higher than Cr concentrations in the sediment of the Ghaghar River, India (Singh *et al*., 2017). Extremely high Cr concentrations in the range of 1726 to

10299 µg/g were detected in marine sediments of Laryman Bay (Louropoulou *et al.*, 2015).

The Mokolo catchment is in the dominant coal mining area. Therefore, the elevated Cr concentrations in this river were attributed to the fact that Mokolo River is situated near a mining area. Chromium was detectable in all sampling sites. The concentrations of Cr ranged between 25.7 to 193 µg/g. This river is most contaminated at site 7. The lower concentration is found at a very sandy site 4.

Rivers going through urban and rural areas transport metals, halfway dissolved and somewhat adsorbed on suspended material. This suspended material settles on the bottom of rivers and accumulates in the sediment (Pintilie *et al.*, 2007). Accumulation of contaminants in sediments may affect the bio-community through food chain for a long period of time (Yujun *et al.*, 2008). Contaminated sediments have a negative impact on the water quality. Due to land degradation and sheet erosion, the top soil is carried into the water bodies resulting in excess levels of turbidity (Tundu *et al.*, 2018). Turbid waters disturb the growth of aquatic plants and algae. Once the water environmental conditions change, the dynamic equilibrium of the water sediment interface will be broken, so that the contaminants in the sediment will be transferred and transformed, and released to the overlaying water, which will lead to pollution of water quality (Yi *et al.*, 2011; Tao *et al.*, 2012; Ting *et al.*, 2012 and Islam *et al.*, 2015). In addition, the flushing operation caused by flow may not only cause the re-suspension of sediment, but also affect the spatial distribution of heavy metals in the sediment (Schleiss *et al.*, 2016).

4.6.1 Comparison of total Cr concentrations in sediment with sediment quality guidelines

The established sediment quality guidelines (SQGs) ensure healthy aquatic ecosystems by setting adequate levels for chemical substances: for instance, metals, natural chemicals, and nutrients that might be found in the aquatic environment. Waters with concentrations of chemicals below the guidelines are the most part viewed as "protected", and are not expected to prompt negative consequences for the environment or aquatic organisms (WHO and UNICEF, 2011). The two general forms

of SGQs are empirical and mechanistic. Empirical SQG approaches are based on the statistical analysis of large data bases of matched sediment chemistry and toxicity data to identify chemical concentrations associated with various levels of biological effects. Examples of this type of SQG include the effects range-low (ERL) and effects range-median (ERM), which are concentrations corresponding to the 10th and 50th percentile of the distribution observed in toxic samples, respectively (MacDonald *et al.*, 2000; Fairey *et al.*, 2001). The mechanistic approach for the SQGs focuses on understanding the bioavailability of anthropogenic contaminants in sediments and determining whether these bioavailable contaminants will be available in quantities sufficient to cause adverse effects (Di Toro *et al.*, 2005). Two SQGs (Table 4.9) reported by MacDonald *et al.* (2000) and Fairey *et al.* (2001) for marine water and estuarine sediments and that of Canadian Council of Ministers of the Environment (CCME, 2001) for freshwater sediments were used to describe the possible toxicity levels of heavy metals in this study.

Table 4.9: Standard guidelines for sediments quality

Guideline range	Cr (µg/g)	Reference
ERL	81	MacDonald *et al.* (2000) Fairey *et al.* (2001)
ERM	370	MacDonald *et al.* (2000) Fairey *et al.* (2001)
TEL	37.3	CCME (2001)
PEL	90	CCME (2001)

Where ERL= effect range-low; ERM = effect range-medium; TEL = threshold effect level; PEL = probable effect level.

The total Cr concentrations in sediments of Blood River were found to be higher than the recommended ERL. The concentrations (132 - 253 µg/g) of Cr in Blood River exceeded the TEL and PEL of CCME SQGs (CCME, 2001). The determined data were lower than the ERM reported by MacDonald *et al.* (2002) and Fairey *et al.* (2001). The highest total Cr concentration found in site 8 of Blood River was also lower than ERM. This means the level of Cr in Blood River has no adverse effects on aquatic organisms.

Field *et al*. (2002) used a less stringent definition of toxicity which stated that there is no concentration limit above which toxicity is possible and below which toxicity is impossible. Zhang *et al*. (2012) derived empirical SQGs of TEL and PEL of heavy metals to protect native benthic organisms of China. There is also a wide range of uncertainty associated with the effects of similar dry weight concentrations in sediments from different locations and with different properties (Wenning and Ingersoll, 2002).

The concentrations of Cr in the sediments of Mokolo River were determined and compared with the values of ERL, ERM, TEL and PEL (Table 4.9). The concentrations of Cr in sediment samples in Mokolo River were in the range of 25.7 to 193 μg/g. The determined values of Cr in site 1, 4, 5, 6 and 10 were found to be below ERL. These sites also had Cr concentrations lower than PEL with TEL lower than Cr concentration in site 4. The remaining sites 2, 3, 7, 8 and 9, Cr concentrations exceeded ERL. The concentrations of Cr of these sites exceeded PEL. Conversely, the determined Cr concentrations were lower than the ERM reported by MacDonald *et al*. (2000) and Fairey *et al*. (2001). The obtained Cr levels in sediments of Mokolo River pose no threat to sediments dwelling and aquatic ecosystem. Despite SQGs derived by MacDonald *et al*. (2000) and Fairey *et al*. (2001), limitations have been used to interpret sediment data from various global regions (Birch and Taylor 2002; Muniz *et al*., 2004; Hyun *et al*., 2007). Garcia *et al*. (2011) assessed trace metals pollution in estuarine sediments using Acid Volatile Sulfide/Simultaneously Extracted Metals (AVS/SEM) and ERM-ERL predictions. In their study, Cr concentrations (3.54 - 74.7 μg/g) in sediments were all found to be below ERL and ERM.

4.6.2 Comparison of Cr levels in sediments from the Blood and Mokolo rivers

Sediments assume an essential part in physico-chemical and biological elements, any change in concentrations of heavy metal residues in the sediments will influence the natural aquatic life support systems (Jumbe *et al*., 2009). Chromium in Blood and Mokolo rivers was detectable in all the samples and their concentrations ranged from 129 to 253 μg/g and 25.7 to 193 μg/g, respectively. The levels of Cr in sediment samples of Blood River were higher than those of Mokolo River. The presence of Cr in Blood and Mokolo Rivers resulted from various anthropogenic activities or from natural

processes as the weathering of ultramafic rocks (Polti *et al.*, 2007). The Mokolo River sediments are sandy in nature while those of Blood River are more like clay.

The highest concentration of Cr in sediment samples reported by Esen and Balci, (2008) was 235.9 µg/g. This concentration is higher than those detected in Mokolo River. Baysal and Akman (2018) detected 0.50 µg/g in sediment sample using GF-AAS which is extremely lower than Cr concentrations reported in this study. A concentration of 1390 µg/g was reported in sediments of Vaal Dam (Gilbert and Avenant-Oldewage, 2014). The Cr levels in sediments of Blood and Mokolo Rivers are lower than this concentration. The comparison of Cr concentration in sediment samples of Blood and Mokolo Rivers is presented in Figure 4.5.
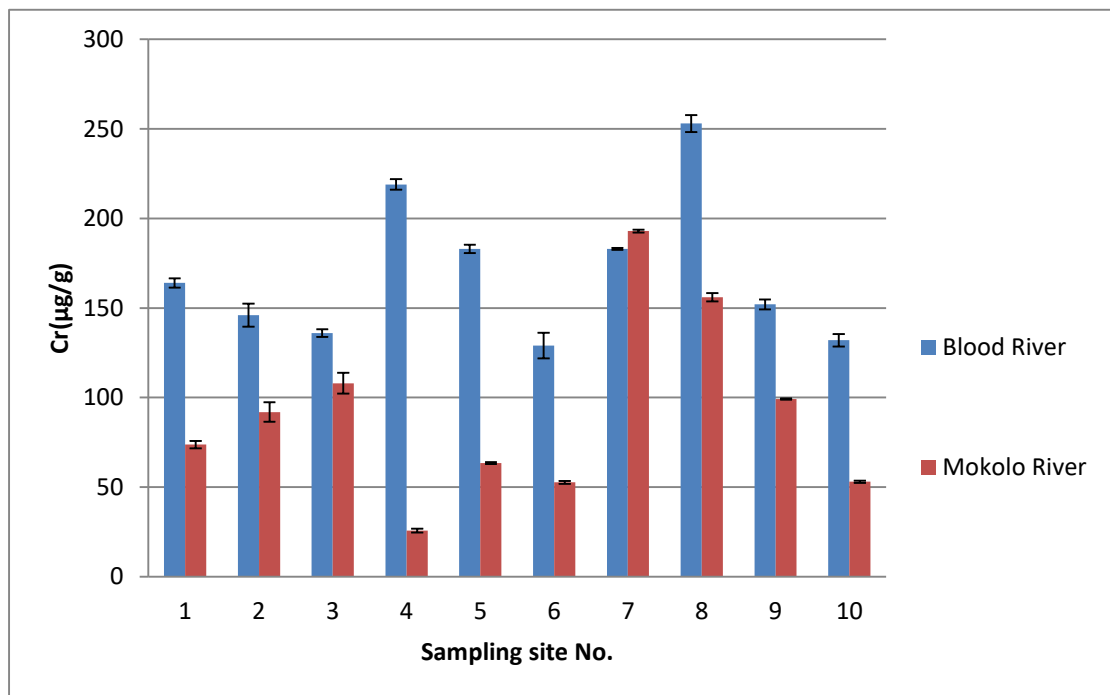
Figure 4.5: Total Cr levels in sediments of Blood River and Mokolo River

The levels of Cr in sediments of Blood and Mokolo Rivers were also compared with the levels of Cr in sediment samples from other countries. Caccia *et al.* (2003) reported Cr levels in the range of 57.0 to 357 µg/g in sediments from Florida Bay, USA. A low concentration (8.15 µg/g) of Cr was detected in sediments of Gonti River, India (Sing *et al.*, 2005). This concentration is lower than Cr levels reported in the current study.

Sediments of Bohai Bay in Brazil consisted of Cr level range from 60.1 to 224.5 µg/g (Gao and Chen, 2012). The concentration (178 µg/g) of Cr in sediments from Briganga River (Bangladesh) reported by Ahmad *et al.* (2010) falls within the Cr concentration range of Blood and Mokolo Rivers.

Ogola *et al.* (2011) investigated the origin and distribution of heavy metals around Ebenezer Dam in Limpopo Province. They found that the Cr concentrations were higher in soils, sediments but lower in rocks. A comparison of Cr levels in sediments with related studies in other countries is given in Table 4.10.

Table 4.10: Comparison of Cr levels in sediments with related studies in other countries

Sampling area	Cr (µg/g)	Reference
Florida Bay, USA	57.0 – 347	Caccia *et al.* (2003)
Buriganga River, Bangladesh	178	Ahmad *et al.* (2010)
Bohai Bay, Brazil	60.1 – 224.5	Gao and Chen (2012)
Gonti River, India	8.15	Sing *et al.* (2005)
Wilge River, South Africa	189 – 213	Moyo *et al.* (2015)
Swartkops River, South Africa	10.8 – 20.3	Binning and Baird (2001)
Blood Rivers, South Africa	129.2 – 252.9	Current study
Mokolo River, South Africa	25.7 – 193.4	Current study

4.6.3 Comparison of Cr concentrations in water and sediments of Blood River

The results of the analysis of water and sediment samples collected from Blood River revealed that the concentration of Cr in sediments was significantly higher than that of water (Table 4.11). At sites 6, 7 and 8 Cr could not be detected in water but only in sediments. The highest Cr concentrations found in water and sediments were from sites 4 and 8, respectively. Sediments of the aquatic environment act as a source of contaminants (Caccia *et al.*, 2003) and as a major reservoir of metals (Kwok *et al.*, 2014). Chromium adsorbs on finer sediments, hence higher Cr levels in sediments than in water.

Ali *et al.* (2016) reported high Cr concentration in water and sediments to be 86.93 µg/L and 160.3 µg/g, respectively. They further indicated that the higher Cr input might have originated from the urban and industrial wastes. High Cr was observed in both water and sediments at sites 1, 3, 4, 5, 9 and 10 of Blood River. The highest Cr concentrations were reported in sediments (2.31 µg/g) than in water (1.40 µg/L) in a subtropical Brazilian River (Weber *et al.*, 2013). Burbridge *et al.* (2012) reported that the depth of maximum total Cr pore water concentration for peeper 1 corresponds with the depth at which the core sample reached its maximum sediment Cr concentration at 83000 mg/kg at 25 – 30 cm.

Table 4.11: The concentration of Cr in water and sediments of Blood River

Sampling site	Mean Cr concentration	
	Sediment (µg/g) ± SD	Water (µg/L) ± SD
1	164 ± 2.6	3.74 ± 0.21
2	146 ± 6.4	1.56 ± 0.12
3	136 ± 2.1	4.57 ± 0.05
4	219 ± 3.0	6.11 ± 0.48
5	183 ± 2.4	5.68 ± 0.20
6	129 ± 7.2	<0.32
7	183 ± 0.60	<0.32
8	253 ± 4.7	<0.32
9	152 ± 2.7	1.84 ± 0.16
10	132 ± 3.5	3.55 ± 0.09

4.6.4 Comparison of Cr concentrations in sediments and water of Mokolo River

The results of the analysis of water and sediment samples collected from Mokolo River are presented in Table 4.12. Similarly, sediment samples contained significantly higher Cr than water samples in all sampling sites. Sites 2 and 7 contained highest Cr concentrations in water and sediments, respectively. The lowest Cr concentration in water and sediments were detected in site 2 and 4, respectively. Precipitation,

regardless of whether slight or heavy can influence flow of water and sediment transport in this manner causing variations in Cr concentrations. The mean annual rainfall of Mokolo is 558 mm (Midgely *et al.*, 1994), which is low. Hence, low Cr concentrations in water and sediments measured in the samples collected in the Mokolo River.

In a study by Kumar *et al.* (2015), water samples from Yamuna River in India had higher Cr concentration (10 μg/L) than sediments (9.31 μg/g). The concentration of heavy metals (Cd, Cr, Fe, Ni) in water, plants and sediments of river Yamuna flowing in Haryana through Delhi were reported in selected 14 stations covering upstream and downstream sites of major industrial complexes of the state (Kaushik *et al.*, 2009). In their study, concentrations of Cr were moderate and high enrichment values observed in only at a few sites. Sediments of Hackensack River which is adjacent to a chromite ore processing residue site in Kearny, New Jersey, contained more than 3000 μg/g of total Cr and Cr in pore water in the range of <2.0 to 5.3 μg/L (Driscoll *et al.*, 2009).

Table 4.12: The concentration of Cr in water and sediment samples of Mokolo River

Sampling site	Mean Cr concentration	
	Sediment (μg/g) ± SD	Water (μg/L) ± SD
1	73.7± 2.1	<0.32
2	91.9 ± 5.4	3.53 ± 0.31
3	108 ± 5.8	1.34 ± 0.09
4	25.7 ± 1.1	2.26 ± 0.08
5	63.4 ± 0.60	1.37 ± 0.07
6	52.6 ± 0.87	2.35 ± 0.06
7	193 ± 0.80	3.43 ± 0.12
8	156 ± 2.3	2.55 ± 0.10
9	99.2 ± 0.40	1.63 ± 0.13
10	53.0 ± 0.60	2.49 ± 0.15

4.7 Determination of Cr(VI) in water samples

Hexavalent Cr occurs naturally in the environment from the erosion of natural Cr deposits. It can also be produced by industrial processes. There are many sources of Cr(VI) in Seshego township including paints, pigments, soils, sewage and effluents discharged from the SWWT plant. The concentrations of Cr(VI) in Blood River water samples range from 0.13 to 1.99 µg/L (Table 4.13). Jayashinge *et al.* (2003) reported higher Cr(VI) concentration range (1.95 – 5.65 µg/L) in water employing GFAAS. They used a similar procedure to our study. The difference in Cr(VI) concentrations might be due to the different geological setting, weather and environmental conditions. The low RSD values (0.05 to 4.10%) indicate the good precision of the methodology during practical analysis. The highest Cr(VI) concentration detected in this study was 1.99 µg/L. Systematic investigations in Asopos river, Eastern Sterea Hellas, Greece reported Cr(VI) concentrations as high as 80 µg/L in surface and ground waters used for public water supply (Vasilatos *et al.*, 2008). Water samples of Blood River contained 10.9 to 34.5 % of Cr(VI). This indicates the presence of Cr(VI) in relatively low concentrations with reference to the total Cr. High percentages (86.3 and 90.0%) of Cr(VI) were quantified in groundwater of Oropos and Schimatari Municipalities, Eastern Sterea Hellas, Greece (Vasilatos *et al.*, 2008). The percentage Cr(VI) reported by Vasilatos and co-workers (2008) are higher than the ones obtained in the current study. The percentage of Cr(VI) in drinking water supply of Ahmedabad City, India was found to be in the range (1.03 – 47.9%) (Zaidi *et al.*, 2014). The reported 1.03 % of Cr(VI) in the Ahmedabad City is lower than the percentage of Cr(VI) in water samples of Blood River.

Table 4.13: The concentration (µg/L) of total Cr, Cr(VI) and % Cr(VI) in water samples of Blood River

Site	Cr(VI) µg/L ± SD	Total Cr µg/L ± SD	% Cr(VI)	Standard guidelines Cr(VI) µg/L
1	0.130 ± 0.05	3.74 ± 0.21	34.5	0.06
2	0.170 ± 0.09	1.56 ± 0.12	10.9	(California
3	0.570 ± 0.06	4.57 ± 0.05	12.5	EPA)
4	1.99 ± 0.004	6.11 ± 0.48	32.6	
5	1.44 ± 0.01	5.68 ± 0.20	25.4	
6	0.320 ± 0.03	<0.32	-	
7	0.440 ± 0.08	< 0.32	-	
8	0.410 ± 0.12	< 0.32	-	
9	0.220 ± 0.03	1.84 ± 0.16	12.0	
10	1.02 ± 0.07	3.55 ± 0.09	28.7	

The detection of Cr(VI) is much more important for environmental impact studies. Hexavalent form is expected to dominate whenever increased levels of naturally leached Cr occur. Associated with Matimba power station is the Grootegeluk Coal Mine which supplies coal to the power station, local users, as well as for export. There is also the thermal Medupi Power Station in the Mokolo catchment. All these could lead to locally elevated levels of Cr(VI) in the Mokolo River. Hexavalent Cr could be released from coal fly ash in aquatic environment when fly ash comes into contact with water. The Cr(VI) concentrations obtained in Mokolo River (0.13 – 0.71 µg/L) (Table 4.14) were lower than the levels reported by Loock *et al.* (2014), in surface water in the proximity of ferrochromium smelters located in the Bushveld Igneous Complex in South Africa. Elevated Cr(VI) concentrations were detected in wells from mafic-rich aquifers in Forsyth County, North Carolina that are located more than 30 km from a coal ash pond (Vengosh *et al.*, 2016). The concentration of Cr in water of site 1 is low almost near to the LOD value. This is in concurrence with the aggregate grouping site. These low concentrations of Cr make it impossible to calculate the percentage of Cr(VI) of this site since it is higher than Cr detected. The percentage of Cr(VI) in water samples of Mokolo River (Table 4.14) were in the range between 9.49 and 28.2%. Izbicki *et al.*

(2015) reported a percentage range from 15.6 to 47.0% of Cr(VI) in water from public supply wells in California. This range is higher than the percentage range of Cr(VI) in water samples from Mokolo River.

Table 4.14: The concentrations (µg/L) of total Cr, Cr(VI) and %Cr(VI) in water samples of Mokolo River

Site	Cr(VI) µg/L ± SD	Total Cr µg/L ± SD	%Cr(VI)	Standard guideline Cr(VI) µg/L
1	0.32 ± 0.08	< 0.32	-	0.06 (California EPA)
2	0.46 ± 0.03	3.53 ± 0.31	13.0	
3	0.32 ± 0.07	1.34 ± 0.09	23.9	
4	0.55 ± 0.001	2.26 ± 0.09	24.3	
5	0.13 ± 0.04	1.37 ± 0.07	9.49	
6	0.44 ± 0.001	2.35 ± 0.06	18.7	
7	0.71 ± 0.02	3.43 ± 0.12	20.7	
8	<0.11	2.55 ± 0.10	-	
9	0.46 ± 0.08	1.63 ± 0.13	28.2	
10	0.24 ± 0.08	2.49 ± 0.15	9.64	

4.7.1 Comparison of Cr(VI) in water from the Blood and Mokolo rivers

Concentrations of Cr(VI) in water samples from Blood and Mokolo Rivers ranged from 0.13 to 1.99 µg/L and 0.13 to 0.55 µg/L, respectively (Figure 4.6). These low concentrations of Cr(VI) pose a low risk to human beings and aquatic plants and animals. Blood River has higher levels of Cr(VI) in sites 3, 4, 5, 8 and 10 than those in Mokolo River. This is in agreement with the findings regarding the levels of total Cr in Blood and Mokolo Rivers. The concentration of Cr(VI) in site 7 of Blood River is equal to the concentration of Cr(VI) in site 6 of Mokolo River.

In this study, using chomabond-NH$_2$ column, the selective adsorption of Cr(VI) was achieved and the separated Cr(VI) quantified in river water samples from Blood and Mokolo Rivers. A different study conducted by Noroozifar and Khorasani-Motlagh

(2003) used activated carbon modified with tris(hydroxymethyl) aminomethane as an adsorbent, to selectively adsorb Cr(VI) in surface water samples. They reported Cr(VI) concentration range of 0.08 – 0.084 µg/L. Wandoyo *et al.* (2006) reported Cr(VI) concentrations in river samples of Yogyakarta, Indonesia in the range 0.03 - 0.04 mg/L. These values are much higher than those detected in the current study. A spectrophotometric method was used for the determination of Cr(VI) in water samples (Nagaraj *et al.*, 2009). Hexavalent Cr concentration as high as 567.2 µg/L was reported using this method. The concentration of Cr(VI) in site 8 of Mokolo River was found to be below LOD value of 0.11 µg/L. This is comparable with the findings reported by Onchoke *et al.* (2015). Ball and Izbicki (2004) reported elevated Cr(VI) levels in groundwater associated with ultramafic aquifers in California. Comparison of Cr(VI) in Blood and Mokolo Rivers is shown in Figure 4.6.
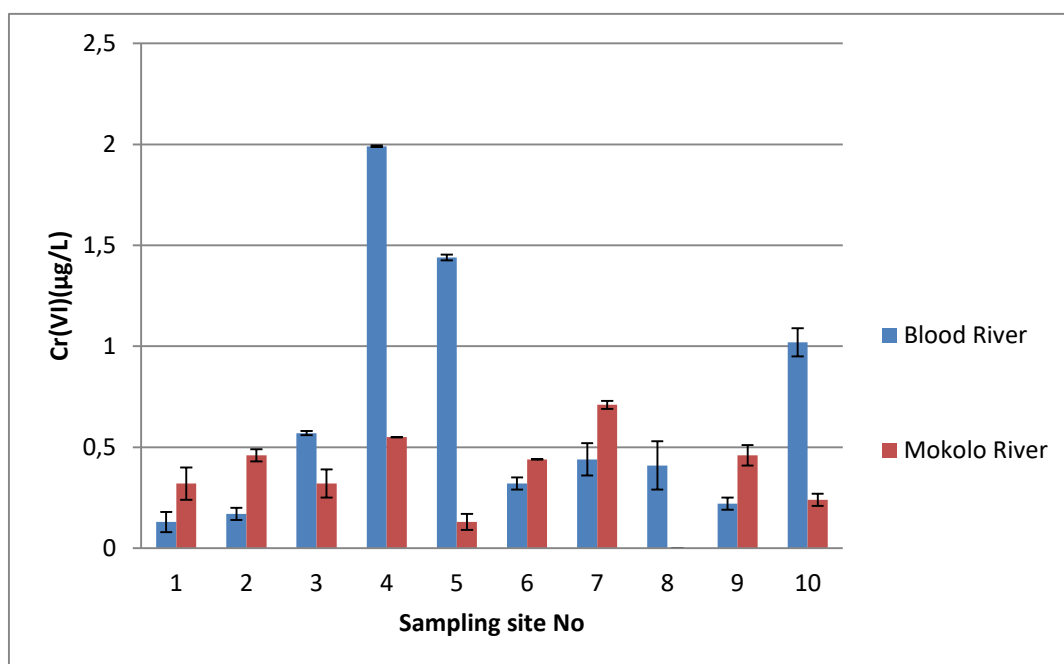
Figure 4.6: Graphical representation of Cr(VI) levels in water of both rivers

4.7.2 Comparison of Cr(VI) in water samples of Blood and Mokolo Rivers with maximum permissible levels in drinking water

The levels of Cr(VI) in water obtained during this study were compared with international drinking water standards recommended by California EPA (1997). According to the guidelines of California EPA, the maximum concentration of Cr(VI) in

drinking water should be 0.00006 mg/L. This value is significantly below all the levels of Cr(VI) detected in this study. Consequently, water from Blood and Mokolo Rivers pose a threat to public health with reference to the Cr(VI) standards recommended by California EPA. The recommended tolerable maximum limit for Cr(VI) in drinking water is not specified by SANS (2005).

4.8 Determination of the concentrations of Cr(VI) in sediments

Sediment samples were analysed by GF-AAS after the leaching procedure using Na_2CO_3 for the determination of Cr(VI). The amount of Cr(VI) leached by 0.1 M Na_2CO_3 represents the concentration of Cr(VI) in a given sediment sample. The results obtained are shown in Table 4.15. Hexavalent Cr content in sediment samples differs significantly in all sites of samples. The concentrations of Cr(VI) in sediment samples from Blood River were within the range of 0.41 to 1.32 µg/g. The highest Cr(VI) concentration was observed in sampling point 8 of the river. It is also where the high total Cr contents were observed. Dahl *et al.* (2013) reported that high levels of Cr(VI) were attributed to the leaching process, since Cr(VI) compounds are known to be mobile in the environment. Hexavalent Cr represented only a little fraction of the total Cr, with the range of 0.32 to 0.97%. This is due to reduction of Cr(VI) to Cr(III) being produced in the sediments due to an alkaline pH (Loo *et al.*, 2012). The Cr(VI) fraction in sediment samples of Blood River is in accordance with a report by Rosales *et al.* (2016) where Cr(VI) in sediment was presented in low fraction (4.13%). Zhang *et al.* (2018) reported the Cr(VI) content of approximately 971.3 mg/kg in soil samples. This concentration is much higher than the Cr(VI) in sediment samples of Blood River. Hauslade and Fendorf (2017) conducted a study based on the generation of Cr(VI) in naturally structured soils and sediments. Their results demonstrate that Cr(VI) is produced in a fixed physical system resembling soils and sediments from both abiotic and biotic Mn oxides.

Table 4.15: The concentration(µg/g) of Cr(VI) and %Cr(VI) in sediments of Blood River

Site	Cr(VI) µg/L ± SD	Total Cr µg/L ± SD	%Cr(VI)
1	1.28 ± 0.05	132 ± 3.5	0.97
2	0.45 ± 0.02	146 ± 6.4	0.32
3	0.61 ± 0.05	136 ± 2.1	0.45
4	0.93 ± 0.01	219 ± 3.0	0.42
5	1.02 ± 0.02	183 ± 2.4	0.56
6	0.41 ± 0.05	129 ± 7.3	0.32
7	0.82 ± 0.04	183 ± 0.60	0.44
8	1.32 ± 0.09	253 ± 4.7	0.52
9	0.72 ± 0.07	152 ± 2.6	0.47
10	0.54 ± 0.10	164 ± 3.5	0.33

The presence of Cr(VI) in sediments represent a permanent environmental risk, therefore, it is important to quantify the level of Cr(VI) in sediments (Elci *et al.*, 2010). Concentrations of Cr(VI) in sediment of the Mokolo River ranged from 0.17 to 0.82 µg/g (Table 4.16). The predominant Cr(VI) concentration was found in site 7 and least abundant Cr(VI) was found in site 9. These values indicate clearly that anthropogenic activities do exist around Mokolo River since Cr(VI) does not exist in nature (Bourotte *et al.*, 2009). Sodium carbonate was used to leach out maximum Cr(VI) concentration in soil at a ferrochrome slag dump in Gweru, Zimbabwe (Mandina and Mugadzu, 2013). A maximum level (1.65%) of Cr(VI) adsorbed to sediments of the Mokolo River have been quantified after leaching it with this reagent. This low levels of Cr(VI) pose no health risk to aquatic plants and animals.

Table 4.16: The concentrations of Cr(VI) and %Cr(VI) in sediments of Mokolo River

Site	Cr(VI) µg/L ± SD	Total Cr µg/L ± SD	%Cr(VI)
1	0.36 ± 0.05	73.7 ± 2.1	0.49
2	0.18 ± 0.03	91.9 ± 5.4	0.20
3	0.33 ± 0.06	108 ± 5.8	0.30
4	0.43 ± 0.08	25.7 ± 1.1	1.65
5	0.31 ± 0.03	63.4 ± 0.60	0.49
6	0.58 ± 0.02	52.6 ± 0.87	1.10
7	1.04 ± 0.06	193 ± 0.80	0.54
8	0.76 ± 0.05	156 ± 2.3	0.49
9	0.20 ± 0.02	99.2 ±0.40	0.20
10	0.82 ± 0.08	53.0 ± 0.60	1.54

4.8.1 Comparison of levels of Cr(VI) in Blood and Mokolo Rivers sediments using the 0.1 M Na_2CO_3 extraction procedure

The levels of Cr(VI) in sediments obtained from both rivers are presented graphically in Figure 4.7, to observe which river was dominated by Cr(VI) levels.
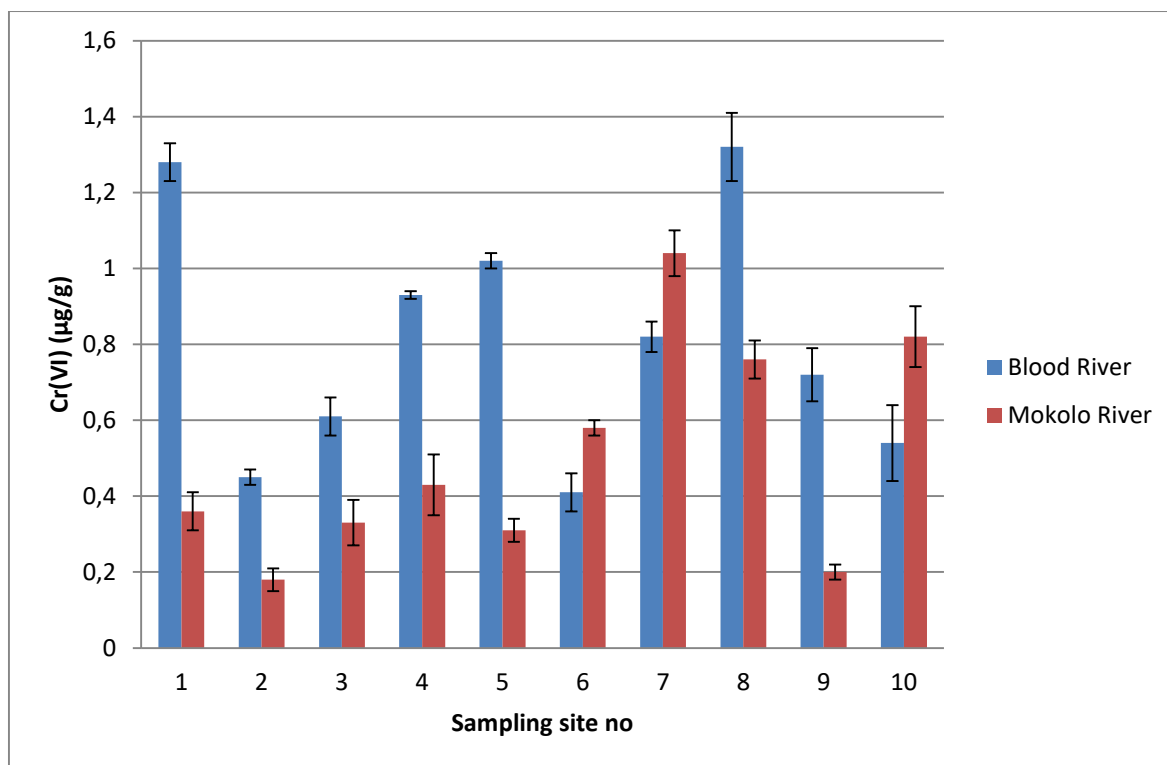
Figure 4.7: Cr(VI) concentrations in the sediments of the Blood and Mokolo rivers

Aquatic ecosystems surrounded by catchments with a known history of mining, near artisanal and small scale mining operations, and expanding agricultural and urban developments are often exposed to varied sources of metal contaminants (Mwamburi, 2016). This is the case of the Blood and Mokolo rivers. Historical trends of Cr(VI) levels in sediments from these rivers are not available. Blood River contained Cr(VI) concentrations ranging from 0.41 to 1.28 µg/g. These reported values are lower than those detected in Mokolo River (0.18 – 1.04 µg/g). Sediments of Mokolo River were sandy while the ones in Blood River were flocculent muddy. A maximum Cr(VI) concentration (79 µg/g) in flocculent muddy sediments of Guadalentin River, Spain was reported in the riverbed contaminated by tannery effluents (Rosales *et al.*, 2016).

The removal of Cr(VI) by serpentine sediments was investigated in order to delineate Cr(VI) sorption behaviour in aquifers with ultramafic geologic background (Mpouras *et al.*, 2017). The adsorption was found to increase and decrease proportionally with increasing initial Cr(VI) concentration of 250 µg/L and particle size, respectively. Kazakis *et al.* (2018) reported that the measurement of concentrations (up to 80 µg/kg)

of environmentally available Cr(VI) in soils and sediments can be attributed to the impact/presence of dispersed fly ash in the soils and sediments of the same area.

4.8.2 Comparison of levels of Cr(VI) in Blood and Mokolo River sediments using the 0.01 M Na$_3$PO$_4$ extraction procedure

The procedure based on Mandiwana's (2008) method was applied for the determination of Cr(VI) in sediments collected from Blood and Mokolo Rivers. Mandiwana (2008) used 0.01 M Na$_3$PO$_4$ to completely leach Cr(VI) in soil. This was achieved by boiling the soil-reagent solution mixture for a period of 5 min. We used microwave assisted extraction method by using the same concentration reagent to extract Cr(VI) in sediments for a period of 15 min. The concentrations of Cr(VI) in sediment samples from Blood River were within the range of 0.06 to 0.09 µg/g with sites 2, 3, 4, 6, 7, and 8 below LOD value of 0.06 µg/g. All sites of Mokolo River had concentrations of Cr(VI) all below LOD value of 0.06 µg/g. Sediments from Mokolo River had lower concentrations of Cr(VI) as compared to those from Blood River. These low levels of Cr(VI) were not affected by conditions set for GF-AAS prior analysis because the high pyrolysis temperature helped to effectively eliminate the majority of the matrix before to atomization and any residual amounts of Na$_3$PO$_4$ had no influence on the release of Cr(VI) during atomisation. It has also been reported that the extraction of Cr(VI) tend to increase at neutral/alkaline conditions (Reddy and Chinthamreddy, 2003; Jensen *et al.*, 2012). The concentrations of Cr(VI) in sediment samples of Blood and Mokolo are compared in Table 4.17.

Table 4.17: Comparison of Cr(VI) levels in sediment samples of Mokolo and Blood River by 0.01 M Na_3PO_4

Site	Blood River	Mokolo River
	Cr(VI) µg/g ± SD	Cr(VI) µg/g ± SD
1	0.09 ± 0.02	< 0.06
2	< 0.06	< 0.06
3	< 0.06	< 0.06
4	< 0.06	< 0.06
5	0.061 ± 0.03	< 0.06
6	< 0.06	< 0.06
7	< 0.06	< 0.06
8	< 0.06	< 0.06
9	0.062 ± 0.001	< 0.06
10	0.07 ± 0.003	< 0.06

4.8.3 Validation of Cr(VI) leaching by comparing the leaching efficiency of Na_3PO_4 with that of Na_2CO_3 in sediment samples

The total concentration of Cr(VI) was determined using the validated analytical procedure. The quantified levels of Cr(VI) in sediment samples of Blood River are presented in Table 4.18 below.

It has been observed that Na_2CO_3 extraction procedure was more effective in extracting Cr(VI) in sediment samples than the Na_3PO_4 extraction procedure. This is supported by the elevated levels of Cr(VI) at different sites obtained by applying Na_2CO_3 extraction procedure. The amount of Cr(VI) leached by 0.01 M Na_3PO_4 were found to be low (0.061 to 0.09 µg/g). The highest Cr(VI) detected was found at site 8 extracted by 0.1 M Na_2CO_3. This value is approximately 22 times higher than the highest Cr(VI) concentration (0.09 µg/g) extracted by 0.01 M Na_3PO_4. Mandiwana (2008) used Na_3PO_4 extracting reagent and reported percentages of Cr(VI) in soil samples between 0.4 and 0.79 µg/g which are comparable with those obtained in sediments of Blood River. The comparison of results of Cr(VI) in sediment samples by

the Na_2CO_3 method and the other method that utilizes Na_3PO_4 showed substantial difference between the two methods.

Table 4.18: The concentrations of Cr(VI) in sediment samples of Blood River using the Na_2CO_3 and Na_3PO_4 extraction procedures

Site	Cr(VI)[a] µg/g ± SD	Cr(VI)[b] µg/g ± SD
1	1.28 ± 0.05	0.09 ± 0.02
2	0.45 ± 0.02	< 0.06
3	0.61 ± 0.05	< 0.06
4	0.93 ± 0.01	< 0.06
5	1.02 ± 0.02	0.061 ± 0.03
6	0.41 ± 0.05	< 0.06
7	0.82 ± 0.04	< 0.06
8	1.32 ± 0.09	< 0.06
9	0.72 ± 0.07	0.062 ± 0.001
10	0.54 ± 0.10	0.07 ± 0.003

[a]denotes the concentration of Cr(VI) extracted using 0.1 M Na_2CO_3; [b]denotes the concentration of Cr(VI) extracted using 0.01 M Na_3PO_4

Similarly, the levels of Cr(VI) in sediments from Mokolo River obtained using the two extraction procedures (Na_2CO_3 and Na_3PO_4) are compared in Table 4.19.

Table 4.19: Comparison of Cr(VI) levels in sediment samples of Mokolo River by Na_2CO_3 and Na_3PO_4 extraction procedures

Site	Cr(VI)[a] µg/g ± SD	Cr(VI)[b] µg/g ± SD
1	0.36 ± 0.05	< 0.06
2	0.18 ± 0.03	< 0.06
3	0.33 ± 0.06	< 0.06
4	0.43 ± 0.08	< 0.06
5	0.31 ± 0.003	< 0.06
6	0.58 ± 0.02	< 0.06
7	1.04 ± 0.06	< 0.06
8	0.76 ± 0.05	< 0.06
9	0.20 ± 0.02	< 0.06
10	0.82 ± 0.08	< 0.06

[a]denotes the concentration of Cr(VI) extracted using 0.1 M Na_2CO_3; [b]denotes the concentration of Cr(VI) extracted using 0.01 M Na_3PO_4

Leachates produced using Na_2CO_3 yielded high concentrations of Cr(VI) than those extracted with Na_3PO_4. Over the course of the 10 leaching tests from 10 different sites, 1.04 µg/g of Cr(VI) was leached from the total Cr 193.4 µg/g of the sediment sample. These concentrations were found in site 7 of Mokolo River. The lowest levels of Cr(VI) in Mokolo River leached by Na_2CO_3 and Na_3PO_4 were 0.18 µg/g and <0.06 µg/g, respectively. The levels of Cr(VI) in sediments leached by the two methods are not the same. This means each method has a different behaviour for Cr(VI) in the sediment samples. The percentage recoveries from 94.9 to 105% obtained by applying 0.1 M Na_2CO_3 for extraction suggest that oxidation state of Cr(VI) was not altered during the extraction process (Panichev *et al.*, 2005). Whereas, the microwave-assisted extraction procedure using 0.01 M Na_3PO_4 did not yield reproducible percentage recoveries. Thus, 0.1 M Na_2CO_3 is more efficient than 0.01 M Na_3PO_4 for extraction of Cr(VI) in sediment samples using microwave-assisted extraction procedure.

4.9 The stability of Cr(VI) in water of Blood River

Hexavalent Cr is a strong oxidant and therefore can be reduced by biological and chemical processes. In this study, the reduction was strongly dependent on time. The lower pH value of 5.5 favoured Cr(VI) reduction (Prasad *et al.*, 2011). The presence of ferrous iron or sulfide in anaerobic conditions might have also caused the reduction of Cr(VI) to Cr(III) (Wielinga *et al.*, 2001). The levels of Cr(VI) extracted from spiked (5.00 µg/L) aqueous solutions applying the developed method were 5.48, 4.71, 3.14 and 2.93 µg/L in day 1, 7, 14 and 21, respectively. Only 94.2, 62.8 and 58.6% of Cr(VI) in water were recovered after 7, 14 and 21 days, respectively. A study by Cadkova and Chrastny (2015) used an isotope approach to study the stability of Cr(VI) in ground water samples. Based on their study, Cr(VI) was found to be unstable over short time periods in anthropogenically contaminated groundwater samples regardless of water treatment. The results in Figure 4.8 shows significantly reduced Cr(VI) levels in water.
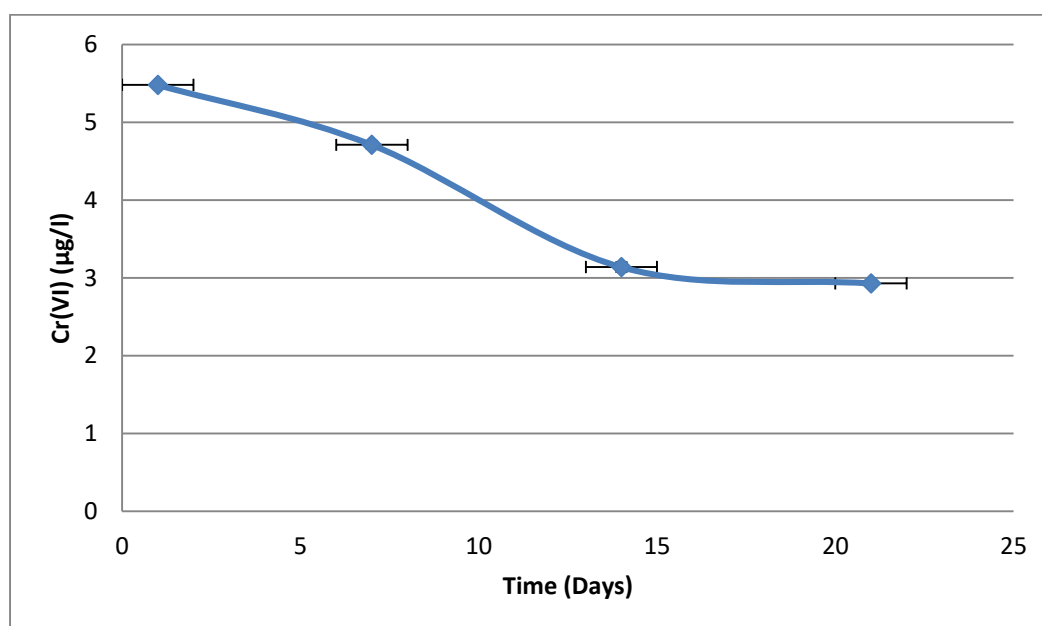
Figure 4.8: The concentration of Cr(VI) in water at different days

The percentage recoveries of Cr(VI) in water were calculated in order to check how much of Cr(VI) has been reduced to Cr(III) over a short period of time. In addition to the reduction there might be loss due to adsorption to the walls of the container too. It has been observed that when time increases, the percentage recovery decreases. The

percentage recoveries of Cr(VI) in water are given in table 4.20. Based on the stability test for Cr(VI), we recommend analysis of the extracts within seven days by keeping the solutions at 4 $^\circ$C in refrigerator.

Table 4. 20: Percentage recoveries of Cr(VI) in water after different number of days

Day	% recovery Cr(VI)
1	109.6
7	94.2
14	62.8
21	58.6

CHAPTER 5: CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

The pH range of 6.00 - 10.5 of Blood River indicates that the river is highly polluted than Mokolo River. The levels of Cr are high due to the welding activities, the sewage and also due to the fact that Blood River serves as a dumping site for domestic and municipality waste. The Mokolo River seems to be slightly acidic (pH= 6.59 - 7.00) due to mining activities that take place around the river.

The levels of Cr in sediments were quantified using FAAS because of its high concentration (ppm range). The microwave digestion procedure was applied for digestion of sediment samples collected from Blood and Mokolo Rivers to evaluate the total concentration of Cr in sediments and also to evaluate how the two rivers are contaminated with Cr. The microwave-assisted digestion of sediments was carried out efficiently, which reduced the risk of external contamination and required smaller quantities of acids, thus improving detection limits and the overall accuracy of the analytical method. Sediments of Blood River are highly contaminated as compared to sediments of Mokolo River. Procedure followed for quantification of Cr in sediments was quantitative according to the percent recovery values obtained (86.4%). The present study reveals that concentrations (132 to 253 µg/g) of Cr in sediments of Blood River are slightly higher than the safe values which indicate that the river is polluted. They exceed TEL and PEL and are lower than ERM. In Mokolo River, the determined levels of Cr in site 1, 4, 5, 6 and 10 were below the recommended ERM and PEL. Sites 2, 3, 7, 8 and 9 of Mokolo River exceeded ERL and PEL.

Water pollution is a major problem in the Limpopo province. Chromium in water was quantified successfully using GF-AAS as validated by analysing SRM1643f. The determined levels of Cr in water of Blood and Mokolo Rivers were in the range 1.56 – 6.11 µg/L and 1.34 – 3.53 µg/L, respectively. It was found that the concentrations of Cr in water of Blood and Mokolo Rivers were below the recommended MPLs for drinking and irrigation water. With regard to the quantified Cr levels in water, water of Blood and Mokolo Rivers is less affected by the industrial and agricultural activities as well as domestic wastes discharged to the rivers.

The NH$_2$ column separated Cr(VI) from Cr(III) successfully. It is precise, and selective towards water samples with low concentration of Cr(VI). It also provides rapid, economical and effective systems for sample preparation. The SPE procedures were preferably used due to their simplicity, selectivity, high enrichment factors safety and ease of automation. The findings suggest that the concentration of Cr(VI) can be quantified successfully by using Alizarin which binds the Cr(III) to prevent oxidation. Adjustment of pH to 5.5 prevented oxidation and reduction of Cr(III) to Cr(VI) and Cr(VI) to Cr(III). Hexavalent Cr concentrations in water samples of Blood and Mokolo Rivers were in the range 0.130 – 1.99 µg/L and 0.130 – 0.710 µg/L, respectively. We can conclude that the water of Blood River is contaminated with Cr(VI) or has high Cr(VI) levels than the water of Mokolo River.

The leaching procedure revealed the presence of Cr(VI) concentrations in sediments of Blood and Mokolo Rivers. Hexavalent Cr in sediments was successfully leached out using 0.1 M Na$_2$CO$_3$ as extractant and microwave-assisted extraction. This method was compared with the microwave-assisted extraction method using 0.01 M Na$_3$PO$_4$. The extraction method based on 0.1 M Na$_2$CO$_3$ leached out Cr(VI) in sediments more efficiently than the extraction procedure based on 0.01 M Na$_3$PO$_4$. The former method also found to be more reproducible compared to the latter method. Low LODs were achieved using the leaching method described in this study. The sediment samples of Blood and Mokolo Rivers were found to contain concentrations that were lower than MPLs. The approach based on microwave-assisted extraction prior atomic spectrometric detector allows the quantitative determination of Cr(VI) in spiked and natural sediment samples.

5.2 RECOMMENDATIONS

The low levels of Cr and Cr(VI) in water of Blood and Mokolo Rivers suggests that the water is fit for domestic and irrigation purposes. Any discharges of liquid, waste and hazardous substances to the water of Blood and Mokolo Rivers should be reported to Council. This will improve the water quality of Blood and Mokolo Rivers.

The developed SPE technique using Chromabond-NH$_2$ is recommended for a successful determination of Cr(VI) in water.

The use of Na_2CO_3 is advised because it has shown to leach out Cr(VI) best in sediments compared to Na_3PO_4. To determine if Na_2CO_3 is indeed a good leaching reagent than Na_3PO_4, the experiment should be conducted again with the same experimental and instrumental conditions.

Speciation of Cr in water and sediments of Blood and Mokolo Rivers should be conducted regularly in order to reveal the accurate levels of Cr species especially the toxic Cr(VI). This can be done during low and high flow seasons to assess seasonal variations.

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APPENDICES

$$t_{cal} = \frac{|X - \mu|}{\frac{s}{\sqrt{n}}}$$

$$= \frac{|3.60 - 3.09|}{\frac{0.35}{\sqrt{3}}} = 2.52$$

$$t_{\alpha.df} = t_{0.05} \times 2 = 4.30$$

$$t_{cr} = 4.303$$

$t_{cal} < t_{cr}$, at 95% confidence level

Appendix 1: Comparison of measured and certified mean values of Cr in water.

$$t_{cal} = \frac{|X - \mu|}{\frac{s}{\sqrt{n}}}$$

$$= \frac{|122 - 105|}{\frac{0.25}{\sqrt{3}}} = 118$$

$$t_{\alpha.df} = t_{0.05} \times 2 = 4.30$$

$$t_{cr} = 4.30$$

$t_{cal} > t_{cr}$, at 95% confidence level

Appendix 2: Comparison of measured and certified mean values of Cr in sediments.