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

by

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DISSERTATION

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in

CHEMISTRY

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Physical and Mineral Sciences)

at the

UNIVERSITY OF LIMPOPO

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JULY 2021

DECLARATION

I, Matjena Mmakoena Meldred, declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Chemistry to the University of Limpopo, South Africa. It has not been submitted before for any degree or examination to any other University. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references.

Matjena MM (Mrs)

12 July 2021
Date

DEDICATION

To my late father, Mr Koena Kenneth Mokoele, thank you for instilling the love for education in me. May your soul rest in peace.

ACKNOWLEDGEMENTS

TO GOD BE THE GLORY

The completion of this work would not have been possible without the support of a large number of individuals. For starter, my deepest gratitude goes to my supervisor (Prof RM Mampa) and co-supervisor (Dr AA Ambushe).

Prof Mampa, I thank you for agreeing to take over the role of my supervisor (Dr AA Ambushe) when he left the University. I am very grateful to be under your supervision. You somehow seamlessly fulfilled the role of mentor, brother and friend, offering advices and humour where appropriate, both of which were always much appreciated. I can say I learned a lot about research because of you and your advices.

Dr Ambushe, it has truly been an honor to work under your supervision. I am very grateful that you remained active in this project despite your resignation from the University of Limpopo. The advices you have given me, ranging from sample preparation to instrumental analysis has been extremely valuable and I thank you for the time you have spent in guiding me.

My husband, Mr Masilo Stephen Matjena, I can't imagine having done this without you. I am grateful for all the countless times that you stepped in for me during my absence from home. You were always there to listen to my seemingly endless list of complaints, even when I struggled to get a linear calibration curve. Your comfort when I was feeling lost meant a lot. Thank you for the love and support.

I would like to thank my lab mates at UL and UJ, for the knowledge and laughter we shared. I am indebted to you all for the laughs we shared and for your support in everything. We have been through ordeal together and we are all stronger for it. To Refiloe, thank you for welcoming me at UJ and introducing me to everything and everybody in the laboratory. For all my office mate at UJ, thank you for all the discussions about our researches, I have learned a lot from them. I am eternally grateful for the knowledge you imparted on me.

To Connie, Lizzy and Tumelo, thank you for all the sampling trips you accompanied me and for always been there when I need your help. I hope our paths will cross often in the future.

Last but not least, I want to thank my ever-loving family and friends. Thank you for the love, for having faith in me and for encouraging me to never give up. To my children, Boitumelo, Koena and Lefa, thank you for understanding when mommy told you that she has to go to UJ for some time to study. To my sisters, Choene and Tlou and my mother, Rosinah Mokoele, thank you for being mothers to my children when I was on study leave at UJ and thank you for loving me unconditionally. To my friend Tselane, thank you for being there most of the time when I need you.

ABSTRACT

Surface water from the rivers serves as a source of water for many purposes including drinking, irrigation and animal farming. The quality of surface water deteriorates due to human, animals and industrial waste. Amongst these wastes, selenium and other trace elements contaminants are included. Selenium can either be essential or toxic depending on the concentration and oxidation state.

The aim of this current study was to determine the concentrations of inorganic selenium [Se(IV) and Se(VI)] in water and sediment samples collected from Blood and Mokolo Rivers in Limpopo Province, South Africa. Water and sediment samples were collected from 10 different sampling sites from down to upstream of each river. Water samples were acidified with 1% ultra-pure HNO_3 and analysed directly for total selenium concentration. The accuracy of the method was validated using SRM1643f (trace elements in water reference material).

Sediment samples were digested using microwave assisted acid digestion for the determination of total concentration of selenium. The accuracy of the method was evaluated using SRM 8704 (sediment standard reference material). Total concentration of selenium in both water and sediment samples were quantified using ICP-MS. The total concentration of selenium in water samples from Blood and Mokolo River were found to be in the range of 0.0682 to 2.72 µg/L and 0.0851 to 25.4 µg/L respectively. The selenium concentrations in all sediment samples were found to be below instrument detection limit of 0.0571 ng/g in both rivers.

An adopted SPE method using Dowex 1 x 2 resin (chloride form) as an adsorbent material to preconcentrate and separate Se(IV) and Se(VI) was used for the speciation in water samples. Both Se(IV) and Se(VI) were retained on the column. The retained Se(IV) and Se(VI) were eluted using 15 mL 1 M HNO_3 and 3 M HNO_3 respectively at a flow rate of 2 ml/min and diluted to the final volume of 20 mL.

The instrument detection limit was 0.192 µg/L and 0.108 µg/L for Se(IV) and Se(VI) respectively. The validation of the method was performed by using SRM 1643f and solutions of known concentrations. The water samples were adjusted to an optimum pH of 6 throughout the speciation analysis. The Se(IV) had higher percentage recoveries of 95 – 114% than Se(VI) with 53%.

Concentrations of Se(IV) and Se(VI) in Blood River ranged from 0.0411 to 0.820 µg/L and 0.0811 to 1.75 µg/L respectively. Concentrations of Se(IV) and Se(VI) in Mokolo River ranged from 0.135 to 2.79 µg/L and 0.0961 to 14.8 µg/L respectively.

The inorganic selenium species in water samples were also determined by using the adopted online mode of HPLC-ICP-MS with Hamilton PRP-X100 column. The separation of the two species was achieved by using isocratic elution of 100 mM NH_4NO_3 at pH 8.5 in 8 min. The method was successfully validated using SRM 1643f. The LOD of 0.842 µg/L and LOQ of 2.81 µg/L for Se(IV) were achieved. The LOD of 0.690 µg/L and LOQ of 2.30 µg/L for Se(VI) were achieved. The Se(IV) and Se(VI) concentrations determined using HPLC-ICP-MS were found to be in good agreement with Se(IV) and Se(VI) concentrations obtained using SPE in both rivers.

The presence of Se(IV) and Se(VI) in water samples in Blood and Mokolo Rivers indicates that industrial and agricultural activities taking place near the rivers have an effect on the quality of the water. The selenium in water may be due to wastes from industrial, municipal and agricultural runoffs. The absence of selenium in sediment samples suggests that the area where the rivers are located is not rich in selenium.

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ABBREVIATIONS AND ACRONYMS

AEV: Acute Effect Value

CCB: Continuous Calibration Blank

CCV: Continuous Calibration Verification

CEV: Chronic Effect Value

CPE: Cloud Protection Extraction

DWAF: Department of Water Affairs and Forestry

EPA: Environmental Protection Agency

EU: European Union

F-AAS: Flame Atomic Absorption Spectrometry

FAO: Food and Agriculture Organization

GF-AAS: Graphite Furnace Atomic Absorption Spectrometry

GPS: Global Positioning System

HPLC: High Performance Liquid Chromatography

HPLC-ICP-MS: High Performance Liquid Chromatography coupled to Inductively Coupled Plasma-Mass Spectrometry

ICB: Initial Calibration Blank

ICP-MS: Inductively Coupled Plasma Mass Spectrometry

ICP-OES/AES: Inductively Coupled Plasma-Optical Emission Spectrometry/Atomic Emission Spectrometry

ICV: Initial Calibration Verification

LOD: Limit of Detection

LOQ: Limit of Quantification

MAE: Microwave Assisted Extraction

MCL: Maximum Contaminant Level

MCLG: Maximum Contaminant Level Goal

MSPD: Matrix Solid Phase Dispersion

PCRWR: Pakistan Council of Research in Water Resources

SD: Standard Deviation

SPE: Solid Phase Extraction

SQ-ICP-MS: Single Quadrupole Inductively Couples Plasma Mass Spectroscopy

SRM/CRM: Standard Reference Material/Certified Reference Material

TWQR: Target Water Quality Range

USEPA: United States Environmental Protection Agency

WHO: World Health Organization

WWTW: Waste Water Treatment Works

CHAPTER 1: INTRODUCTION

1.1 General background

Selenium is a trace element that was discovered by Berzelium in 1817 (Zhang *et al.*, 2014). It is allocated in the VIA group of the periodic table between sulphur and tellurium, has atomic number 34 and atomic weight 78.94.

Naturally, Selenium is present in earth's crust in low amounts (<0.05 $\mu g.g^{-1}$). However, its components are widely spread throughout the environment from combustion of fossil fuels, improper wastes disposal from activities such as mining, uses in the production of glass, electronic industries as well as agriculture (Camara *et al.*, 2004).

Selenium exists naturally in several chemical forms such as organic, inorganic and methylated derivatives. The inorganic species most frequently found in water and soils are selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) (Pyrzynska *et al.*, 1998). It has been reported that the inorganic forms of selenium are more toxic than the organic forms [selenocysteine ($C_3H_7NO_2Se$)] and [selenomethionine ($C_5H_{11}NO_2Se$)] with toxicity of Se(IV) more severe than Se(VI) (Zhang *et al.*, 2016; Tadayon et al., 2014).

Selenium has become one of the major contaminants of concern in irrigation water and aquatic ecosystems since the 1980s (Zhang *et al.*, 2014). It is an essential element that is required in trace amount for normal health due to its antioxidants activity (Herrero Latorre *et al.*, 2013). It gives protection from several heart diseases, prevents heavy metal toxic effects by binding with them and has anti-carcinogenic activity (Tuzen *et al.*, 2007). Selenium becomes toxic at higher concentration of more than 400 µg/day (Najafi *et al.*, 2010; WHO/HSE/WSH/10.01/14). The interval between the concentration in which selenium is essential and toxic is rather narrow (Torres *et al*., 2011). A concentration of 3 to 5 times higher than the essential concentration is considered to be toxic (Nyaba *et al.*, 2016). The essentiality and toxicity of selenium depends not only on the concentration but also on the oxidation state.

It has been estimated that the ingestion of food stuffs with selenium content above 1 mg/ kg can induce toxicity, while a concentration below 0.1 mg/ kg leads to deficient status (Tadayon *et al.*, 2014). Recent studies revealed that the tolerable upper daily intake limit for selenium is 40 µg/day. When this tolerable daily intake limit is exceeded, a disease called Selenosis may occur (Valencia *et al.*, 1999). Typical symptoms of Selenosis are shown in **Figure 1.1.** Taking these facts into consideration, it is very important to determine the levels of selenium species in water bodies and sediment samples as its concentration may have adverse effect on the environment.

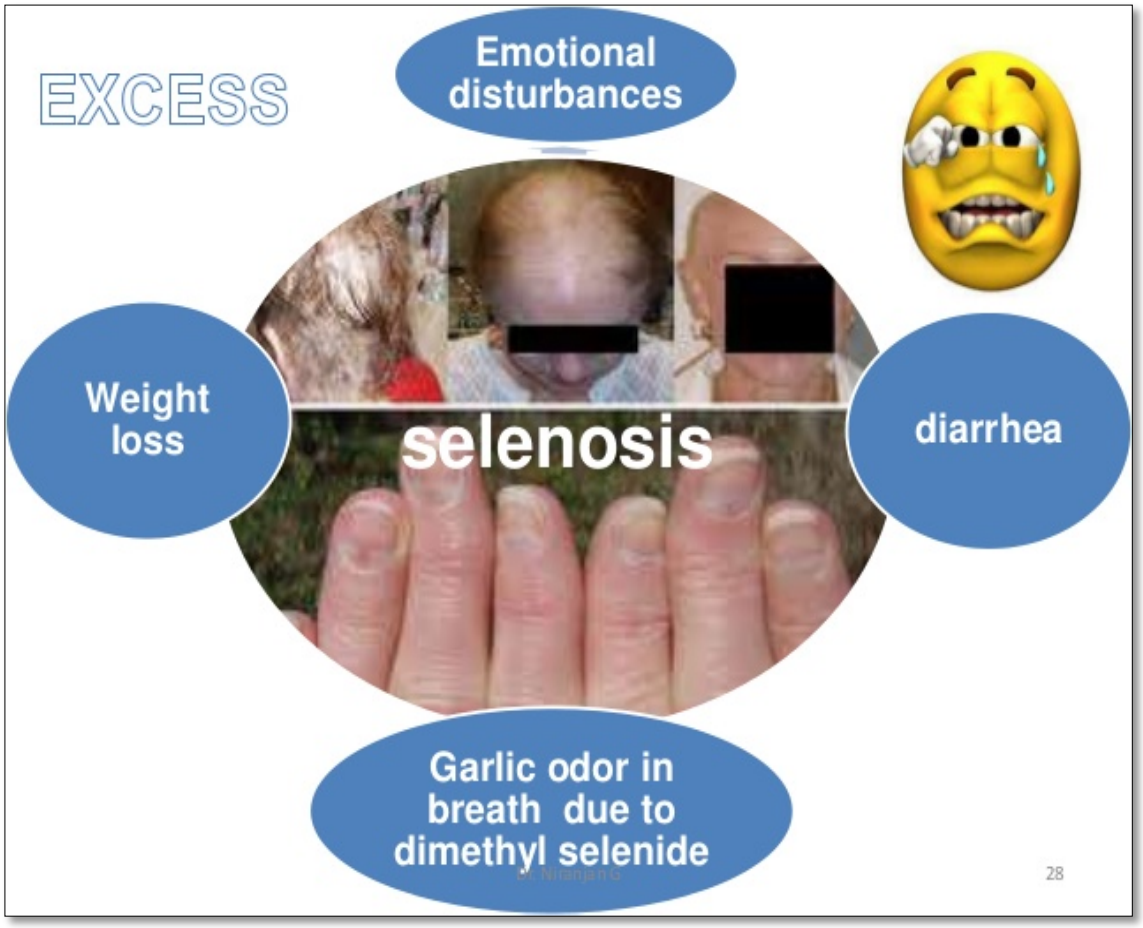

Figure 1.1: Symptoms of Selenosis (too much selenium in the body)

The main area of selenium research is selenium deficiency or excessive consumption as a human health problem although dealing with selenium contamination is also important for monitoring its impact on the environment. However, the determination of total selenium content is not enough. Identification and determination of selenium species by speciation is important since its toxicity, mobility and bioavailability depends on chemical forms of the element in the environmental matrices.

Speciation is defined as an analytical activity of identifying and quantifying the actual chemical species of an element. Speciation thus requires two techniques: a technique to separate chemical species of the element of interest and a technique to detect the separated species (B'hymer *et al.*, 2006).

1.2 Problem statement

Naturally occurring selenium is another trace element of concern in the South African geological environment, and yet little is known about its prevalence and distribution in South Africa. In the last decade, elements like selenium were not on the list of routinely analysed constituents in drinking water (Sami *et al*., 2003).

Selenium has been increasingly recognised as an emerging global metalloid contaminant with potential ecological and human risk second only to mercury (Hu *et al.*, 2009]. Selenium can enter into the ecosystems from natural and man-made sources such as weathering and leaching of rocks, mining activities, volcanic eruption, irrigation drainage water and improper waste disposal (Lemly, 2004). The fact that these trace constituents are relatively common in the above mentioned sources thus suggests the distribution of selenium should be of concern (Sami *et al.*, 2003).

Chronic selenium poisoning of plants, animals and humans has been reported in North-Western India and in some part of China. Poisoning was identified as due to the area being located in the seleniferous region (Dhillon, 2003). Another case of chronic selenium pollution or contamination in the San Joaquin Valley of California where agricultural drain water containing elevated levels being due to adverse reproductive activity and other effects in aquatic birds at the Kersterson National Wildlife refuge (Fan *et al.*, 1990) has been reported. No chronic selenosis has been reported in the Republic of South Africa. Nonetheless, possible toxicity of selenium should be investigated and be known.

The deficiency, toxicity and essentiality of selenium depends on its oxidation state (Kadriye *et al.*, 2007). Each species is absorbed differently from the other species by the human body and has a potential to bio-accumulate in organisms (Tadayon *et al.*, 2014). Speciation of selenium species is therefore essential in order to determine its mode of toxicity (Li *et al.*, 2008).

In South Africa, there are potential sources of inorganic selenium such as mining, improper waste management and agricultural activities, etc. that may contribute to higher levels of selenium in water. As such, elevated levels of selenium will remain unknown for many years until detrimental effects are recognised by health practitioners (Department of water affairs and forestry (DWAF), 1996a). Despite this reality, no selenium speciation has been conducted in the Capricorn and Vhembe Districts of the Limpopo Province despite an increasing economic development within the region which thus suggest a research problem.

1.3 Motivation of the study

This study was prompted by the fact that selenium is an essential nutrient with numerous beneficial effects on health but it becomes toxic at concentration level higher than 5 ppb (Yu *et al.*, 2019). Its toxicity depends not only on the concentration but also on its oxidation state. Thus, it is necessary to determine the concentration of the actual species of the element than the total concentration to assess its toxic effect. Human beings and animals can be exposed to high level of selenium through inhalation of dust contaminated with selenium, ingestion of water contaminated with selenium, agricultural crops irrigated with selenium contaminated water and crops grown on area rich in selenium. So, it is very important and necessary to do speciation of selenium to ensure that human beings and animals are safe from accumulation of high levels of selenium in their systems.

The study is also prompted by the lack of research in the levels of selenium in South African geological environment (Sami *et al*, 2003). Recently, a study on selenium speciation was done in Soweto, Johannesburg, where the highest concentration levels were found to be 84.0 ppb of Se(IV) and 33.0 ppb Se(VI) (Nyaba *et al.*, 2016). For this study, Blood River and Mokolo River systems within Capricorn District have been selected as areas of research.

Blood River (situated in the Capricorn district of Limpopo Province) also serves as a source of water for agricultural activities and livestock farming in the nearby locations. There is no proper waste management system around Blood River residential area.

There are visible dumping sites, sand mines and direct flow of leaking sewage pipes into the river. Some of the wastes are broken glasses, ceramic tiles and paint containers which are sources of selenium. Improper waste management might be due to the exponential population growth in Seshego and the surrounding areas.

These townships are made up of residential and industrial business areas, schools, churches, shopping complexes, etc. The increase in population (estimated population at 508 967 in 2009 – 2011/2016) is attributed to rural-urban migration, as people moves from the rural areas to be close to the city where they can have access to job opportunities and improved social services and infrastructure (Integrated Development Plan- Polokwane, 2011/2016).

Mokolo River is situated in the Waterberg District of Limpopo Province of the Lephalale Area and the river serves as a source of water in the nearby locations for their agricultural activities and livestock farming. The large Mokolo dam, in the Mokolo River catchment, supplies water to the Matimba power station, Medupi power station, Grootegeluk coal mine, the Lephalale Local Municipality (LM) as well as number of downstream irrigators (Report No: PWMA 01/000/00/02914/2, Dec 2015).

The Mokolo River has various tributaries. The Sand River and the Grootspruit, originating in the Waterberg flows into the Mokolo River upstream of the Mokolo Dam. Other tributaries are Tambotie River, Poer se loop and Rietspruit River that flow into the Mokolo River downstream of the Mokolo Dam (DWAF Report No: P/01000/00/0101,2013). The Mokolo River catchment area is surrounded by coal mine (Grootegeluk), power stations (Matimba and Medupi) and agricultural activities. The Mokolo River was selected for this research to investigate effect of the anthropogenic (e.g. Mining and agriculture) activities taking place around it and adverse effects on the water quality. The water in Mokolo River is used by people living around it for domestic requirements. Furthermore, little or no information about the level of selenium in the Mokolo River is known.

Elemental speciation, especially for different oxidation states of a given element, non-chromatographic techniques are more popular than chromatographic techniques because these techniques are time consuming and use a large volume of solvents which are hazardous to the environment and human health (Najafi et al., 2010; Li *et al.*, 2008)

For this study, solid phase extraction (SPE) and high performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) methods were selected. The SPE method has been widely used as a pre-concentration procedure for trace elements due to its high enrichment factor. Appropriate enhancements in sensitivity and adequate elimination of interferences and sample matrix have been achieved with this procedure (Herrero *et al.,* 2013; Bueno *et al.*, 2002). The HPLC-ICP-MS is a technique that has a separating power of HPLC combined with high detection capability of ICP-MS makes speciation to be accomplished efficiently and successfully (Nguyen *et al.*, 2018).

Many modern instrumental techniques have been used for the determination of Se(IV) and Se(VI). Among these techniques, inductively coupled plasma-mass spectrometry (ICP-MS) has been frequently applied for the determination of selenium due to its high sensitivity with a wide linear dynamic range and the capability of isotopic determination (Zhang *et al.*, 2013). For this study, ICP-MS have also been used due to its advantages of high sensitivity.

1.4 Purpose of the study

1.4.1 Aim

The aim of this study is to assess and quantify species of inorganic selenium in water and sediment samples collected from Blood River and Mokolo River systems in the Capricorn and Waterberg districts, Limpopo Province, South Africa.

1.4.2 Objectives

The objectives of the study are to:

(i) optimise a method for the determination of total concentration of selenium in water and sediment samples,

(ii) validate the optimised method for the total determination of selenium in water and sediment samples using standard reference material (SRM) for water and sediments,

(iii) quantify the total concentration of selenium in water and sediment samples using ICP-MS,

(iv) optimise and validate a method for the speciation of inorganic selenium in water and sediment samples,

(v) extract inorganic selenium species in water and sediment samples using the validated method, and quantify inorganic selenium species using ICP-MS and HPLC-ICP-MS.

CHAPTER 2: LITERATURE REVIEW

2. INTRODUCTION

This chapter provides a summarised literature review of selenium in water and sediments, i.e. review on Se(IV) and Se(VI) in water and sediments. Selenium pollution, environmental distribution of selenium, speciation of inorganic selenium in water and sediments, sample preparation methods and analytical techniques for selenium speciation are also included.

2.1 Selenium species and its source in water bodies

Selenium chemistry is complex and its chemical forms vary in their environmental occurrence, biogeochemistry and toxicity. It was discovered in 1817 by Bercelius as a residue while preparing H_2SO_4 (Boyd, 2011). Naturally, it occurs in a few minerals such as sulphide ores (Zane Davis and Hall, 2017). It is placed between sulphur and tellurium as it resembles both elements in some way (Boyd, 2011). In nature, selenium is generally recognised to occur in four oxidation states, selenide (Se^{2-}), elemental selenium (Se^0), selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) (Ramady *et al.*,2014) where each oxidation state has different chemical behaviour. Se^{2-} and Se^0 are insoluble and biologically unavailable (McNeal, 1989). Selenates (Se(VI)) are readily available to plants, or they can slowly reduce to selenites (Se(IV)), which can also be taken up by plants. Selenite is the most common soluble form of selenium under reducing conditions and in acidic soils, which occur typically in higher rainfall areas. Generally, elemental selenium and metallic selenides are not readily bioavailable. They can only be transformed to bioavailable forms under oxidising conditions (Lopes *et al.*, 2017).

Surface water plays an important role in water supply around the world. The majority of people in South Africa live in rural areas where they rely on water from streams and rivers (Momba and Kaleni, 2002). One of the concerns is that the quality of river water is deteriorating due to several natural and anthropogenic activities. Among those water pollutants are trace elements like selenium (Edokpayi *et al.*, 2016). Selenium commonly occurs as a mixture of several different chemical forms in surface water but selenates and selenites are the most common (Quin et *al.*, 2017).

Typical concentrations of selenium in the Earth's crust are < 0.5 mg/kg, but some geological formations are greatly enriched in selenium (Chand *et al.*, 2009). Levels of selenium in ground water and surface water range from 0.06 µg/L to 400 µg/L. Levels of selenium in tap water samples from public water supplies around the world are usually much less than 10 µg/L but may exceed 50 µg/L (WHO/HSE/WSH/10.01/14, 2011). These formations are modified as a result of human activities such as mining, agricultural activities, waste disposal, etc. (Bueno *et al.*, 2002).

The ratio of selenite and selenate in natural water is controlled by several factors such as pH-redox system status and their adsorption kinetics. Selenite, Se(IV) is the dominant oxidation state for selenium at pH and pE (redox potential) values representative of oxygenated surface waters and it is stable under mildly oxidizing conditions. Selenate, Se(VI) represents a significant portion of the selenium in oxygenated surface water and is the dominant oxidation state in highly oxygenated water with pE ≥ 7.72 at pH 7. Selenate is stable under oxidizing and alkaline conditions (Martens, 2003).

Selenium exists naturally with isotopes mass numbers 74, 76, 77, 78, 80 and 82 (Can *et al.*, 2016). The stable selenium isotopes with their natural abundance are shown in **Table 2.1**. Most of the isotopes have been used for the determination of selenium. Interferences on ^{78}Se and ^{82}Se resulting from ^{40}Ar, ^{38}Ar and ^{82}Kr, respectively affect the measurement of selenium on these isotopic masses (Kleckner *et al.*, 2017).

Table 2.1: Stable selenium isotopes with natural abundance

Nominal mass	Accurate mass	%Natural abundance	Chemical form	Enrichment available %
^{74} Se	73.9224746	0.89	metal	31 – 98+
^{76} Se	75.9192120	9.37	metal	74 - 98+
^{77} Se	76.9199125	7.63	metal	68 - 99+
^{78} Se	77.9173076	23.77	metal	89 - 97+
^{80} Se	79.9165196	49.61	metal	96 - 99+
^{82} Se	81.9166978	8.73	metal	73 – 92+

Selenium originates from all natural materials on earth including water, soils, rocks, air, plants and animal tissues. Major source of selenium naturally is weathering of rocks (Fordye, 2013). Selenium in water occurs naturally at low concentration but it gets increased by human activities from industrial and domestic waste (Conde *et al.*, 1997). Groundwater contains higher level of selenium than surface water due to its contact with the rocks. Selenium exposure to humans and animals is through the food consumption. Since diet is the only source of selenium in humans, understanding its availability and mobility is very important (Conde *et al.*, 1997).

Selenium in soils is high in seleniferous areas. Seleniferous soils are characterised by alkaline soils (pH > 7.5) that develop from a sedimentary rock such as shales, condition also observed in South Africa (Davis *et al.*, 2011). The availability of selenium in soils is determined by physio-chemical parameters such as pH and redox conditions (Fordye, 2007). Under the most natural redox conditions, selenite and selenate predominate with selenite being the most stable (Hogberg and Alexander, 2007). Selenium concentrations in crops depends on the amount of available selenium in soil where crops are grown (Gerla *et al.*, 2011). Understanding the mobility and distribution of selenium is important in managing agriculture for optimal selenium in crops.

Selenium in sediments increases because it settles from air and from wastes. When selenium reacts with oxygen, it becomes mobile and dissolves in water. The dissolved selenium in an aquatic ecosystem can bind to suspended sediments and accumulates though it does not stay permanently bound on sediments, it gets cycled back into the aquatic system due to the dynamic flow in the system (Lemly and Smith, 1998).

2.2. Pollution of river water systems by Se(IV) and Se(VI)

Pollution of selenium in the river systems can be through sewage sludge, industrial waste, nuclear waste- by product of nuclear fission and agricultural practices- through application or by harvesting methods. In groundwater, selenium occurs due to weathering and leaching of rocks, or by either dissolution or oxidation of solute salts in soils. Selenium concentration may also increase due to improper waste disposal (Ramesh, 2011).

Aquatic environment can be contaminated by selenium due to agricultural drainage, mine drainage, residues from fossil fuel thermoelectric power plants, oil refineries and metal ores. High levels of selenium in waste water and surface water can cause serious environmental problems. Selenium in soils can be taken to toxic levels by plants, thus entering food chain and reaching animals and humans. Groundwater generally contains higher selenium level than surface water, due to the contact with rocks (Rick Arnold *et al.*, 2016).

Environmental contamination by selenium may occur due to natural and anthropogenic sources. Natural sources include weathering of selenium-containing rocks, soils and volcanic eruption. Human activities include coal combustion, mining, agriculture, oil refining, etc. During rainfall, runoffs from the mines and industrial areas end up into river system increasing levels of selenium in the river. Selenium can be consumed by human beings from different sources (He *et al.*, 2018). Seleniferous agricultural drainage wastewater has become a relevant diffuse pollution source of selenium around the world (Busheina *et al.*, 2016). **Figure 2.1** shows how human beings can be exposed to selenium through inhalation from mining activities, drinking directly from groundwater and eating food that contains high level of selenium.

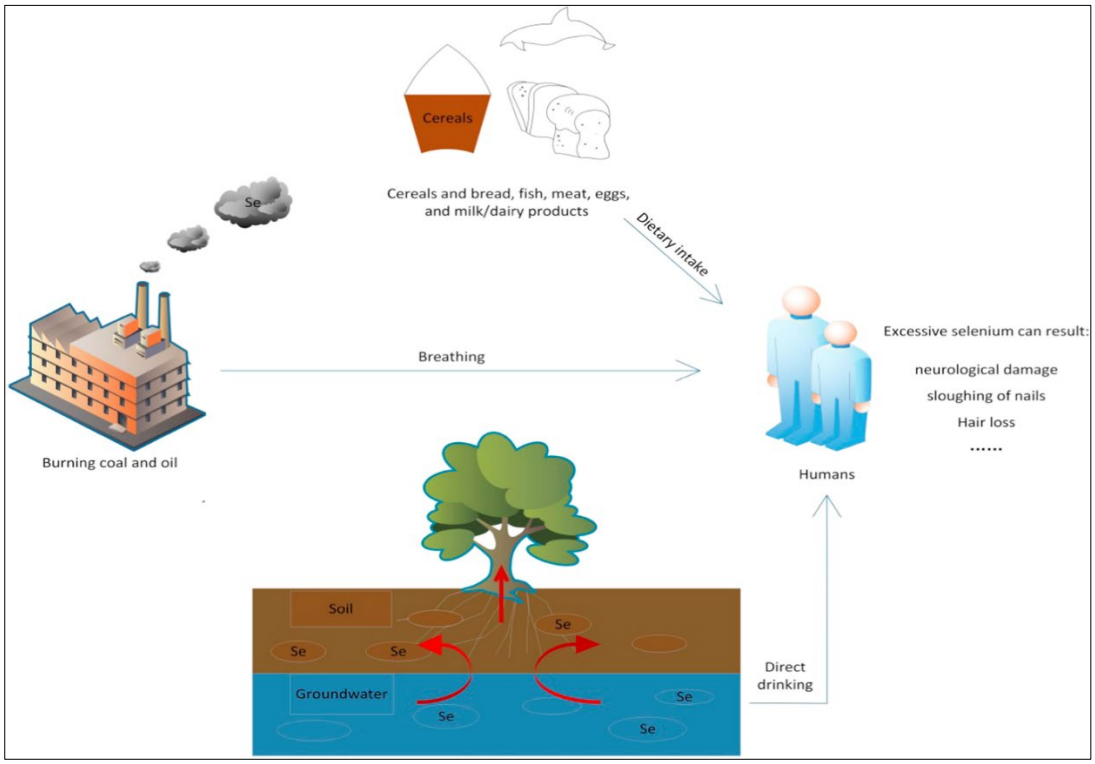

Figure 2.1: Different pathways of exposure to selenium on human beings

2.2.1 Sewage Sludge

Sewage is a type of wastewater that is produced by a community. Selenium is among the potentially toxic metalloid found in wastewater. Selenium is present in faeces and urine. Selenium also comes from food products and supplements, shampoo, other cosmetics and old paints and pigments (Saha, 2017). Selenium discharged into the river system through sewage sludge is becoming a matter of increasing concern because of its toxicity if the concentration is higher than the essential (Staicu *et al.*, 2017). The presence of selenium in sewage sludge can also give rise to hazardous level to the health of plants, humans and animals (Oldfield, 1992; Heninger, 1998).

There are social concerns of uncontrolled use of sewage sludge for irrigation due to potential risk posed to human health from food chain (Wang *et al.*, 2003). Application of sewage sludge for agricultural purposes may lead to increased concentration of toxic metals, which lead to food chain contamination (Selivanovskaya and Latypova, 2003). Many municipalities are currently facing challenges of disposing wastewater sewage into the rivers due to rapid urbanization and population growth and this will in a long term elevate the levels of trace elements in the rivers (Seanego *et al.*, 2013). Most rivers in South Africa are heavily polluted with faecal matter (Barnes et *al.*, 2014; Britz *et al.*, 2013). Sand River like most river systems receives sewage effluent from Polokwane wastewater treatment works (WWTW) and its water is used by farmers downstream for irrigation. Blood River also receives sewage effluent from Seshego WWTW and its water is similarly also used by farmers (Seanego *et al.*, 2013). This is expected to pose threat to humans and animals through food chain.

2.2.2 Agricultural activities

Agriculture is both the cause and the victim of water contamination in the river system. It is both the direct and indirect cause of human negative health impact because pesticides and fertilizers used during plantation and harvesting are the source of water contamination. Generally, agriculture is the cause of water pollution through its discharge of pollutants to the surface and ground water and it is a victim through the use of polluted surface and/or groundwater, which contaminates crops that are consumed by humans and animals (FAO, 1990).

Selenium pollution is becoming a worldwide problem as it is associated with a lot of human activities including agricultural practices (Lemly, 2004). Irrigation water is applied to protect crops from drying and at the same time it can also cause build-up of selenium, which can harm reproduction of crops. Chemicals such as pesticides, insecticides, herbicides and fungicides are applied to the crops to control plant diseases. Some of them contain selenium, which enter and contaminate water through run-off and atmospheric deposition (EPA 841-F-05-001).

Soil is another natural source of agricultural water pollution. Rain water carries sediment and dumps them into the nearby streams. Too much sediment on the river bed reduce the amount of sunlight to reach the aquatic plants. Other pollutants like pesticides attached to the soil particles often get into the river system through run off (EPA 841-F-05-001).

2.2.3 Mining activities

One of the major release of selenium into the environment is through mining activities. The mining of coal, gold, silver and metal sulphides are the main contributors of selenium pollution. The burning of coal produces ash that is rich in selenium which then contaminates aquatic system when it is leached out by rain from the disposal site. Waste rock disposal and tailings produced during mining and processing of metallic ore deposits also contribute to the release of selenium into the environment (KhamKash *et al.*, 2017). Selenium is found in metal sulphide ores, it got released to the environment when processing metallic ore deposits (Kyle *et al.*, 2011).

Figure 2.2 shows how selenium enters the aquatic systems through mining. This release of selenium into the environment increases the risk of its bioaccumulation in the aquatic life. The concentration of selenium in the mining wastewater ranges from 3 to 12 µg/L (KhamKash *et al.*, 2017). Coal mining and waste rock can mobilize selenium into the aquatic ecosystem and increase the level of selenium that exist naturally (Stefaniak *et al*, 2018).

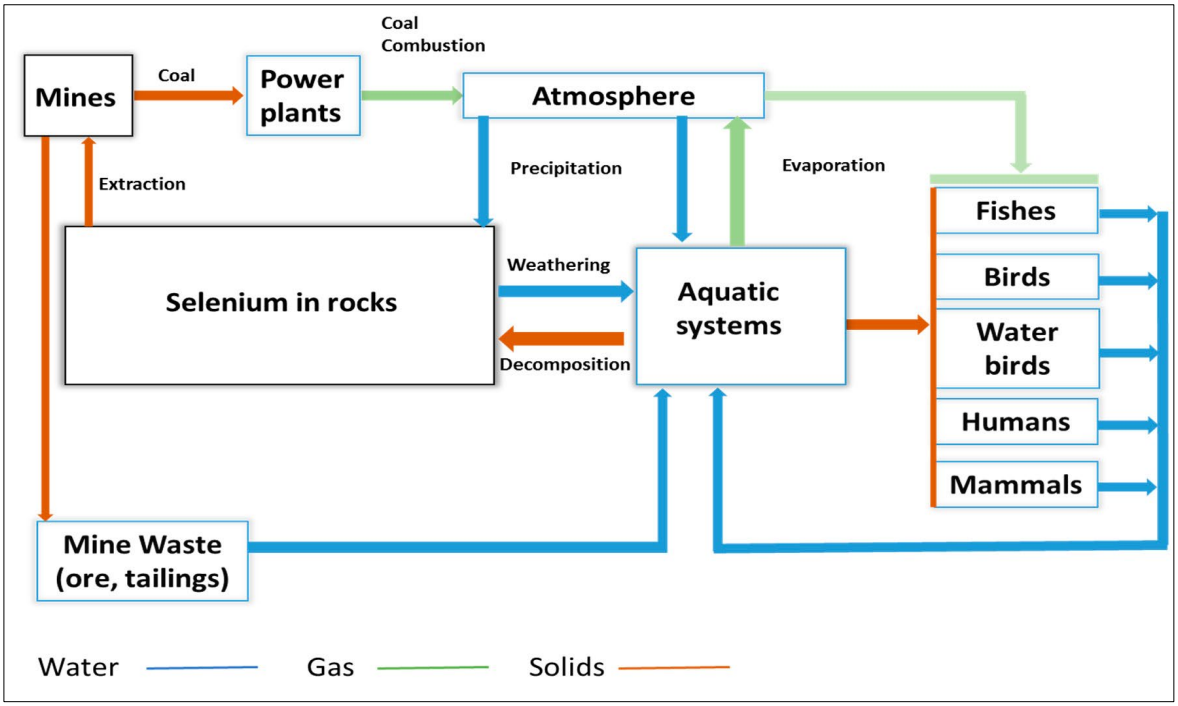

Figure 2.2: Selenium cycle in the environment from mining

2.3 Effects and properties of Se(IV) and Se(VI)

Selenium exists in several oxidation states in environmental matrices but its occurrence and distribution is affected by several factors. The major factors that affect the distribution of the valence states of selenium in water and sediments are microbial activity, pH and redox conditions (Martens, 2003).

2.3.1 Effects of Oxidation-reduction potential and pH

The most prevalent selenium species in the environmental matrices within a normal pH and redox conditions are elemental selenium, Se(0), selenite, Se(IV) and selenate, Se(VI) (Ramady *et al.*, 2014). Anthropogenic activities could influence the levels of Se(IV) and Se(VI) in water bodies, sediments, soil and air (Camara *et al.*, 1995). The toxicity of selenium depends on its oxidation state (Erdogan *et al.*, 2016]. To determine different oxidation state of selenium, speciation needs to be performed. Selenium speciation is highly dependent on pH and redox potential (Eh) (Martens., 2003).

The pH of the sample plays a very important role in the absorption of the analytes as well as its effect on metal binding capacity and surface charge of adsorbent. Different sorbent materials adsorb selenium species at different pH (Erdogan *et al.*, 2016).

At typical groundwater pH values of 7.0 to 9.5 only anionic forms of selenious, Se(IV) or selenic, Se(VI) acid are found. Se(IV) Selenious Acid Dissociation Equilibria is represented as follows: $H_2SeO_3 \rightleftharpoons H^+ + HSeO_{3^-}$ and Se(VI) Selenic Acid Dissociation Equilibria is $H_2SeO_4 \rightleftharpoons H^+ + HSeO_{4^-}$ (https://www.wqa.org/learn-about-water/common-contaminants/selenium, accessed 2020).

Figure 2.3 shows major thermodynamically stable selenium species as a function of pH and Eh.

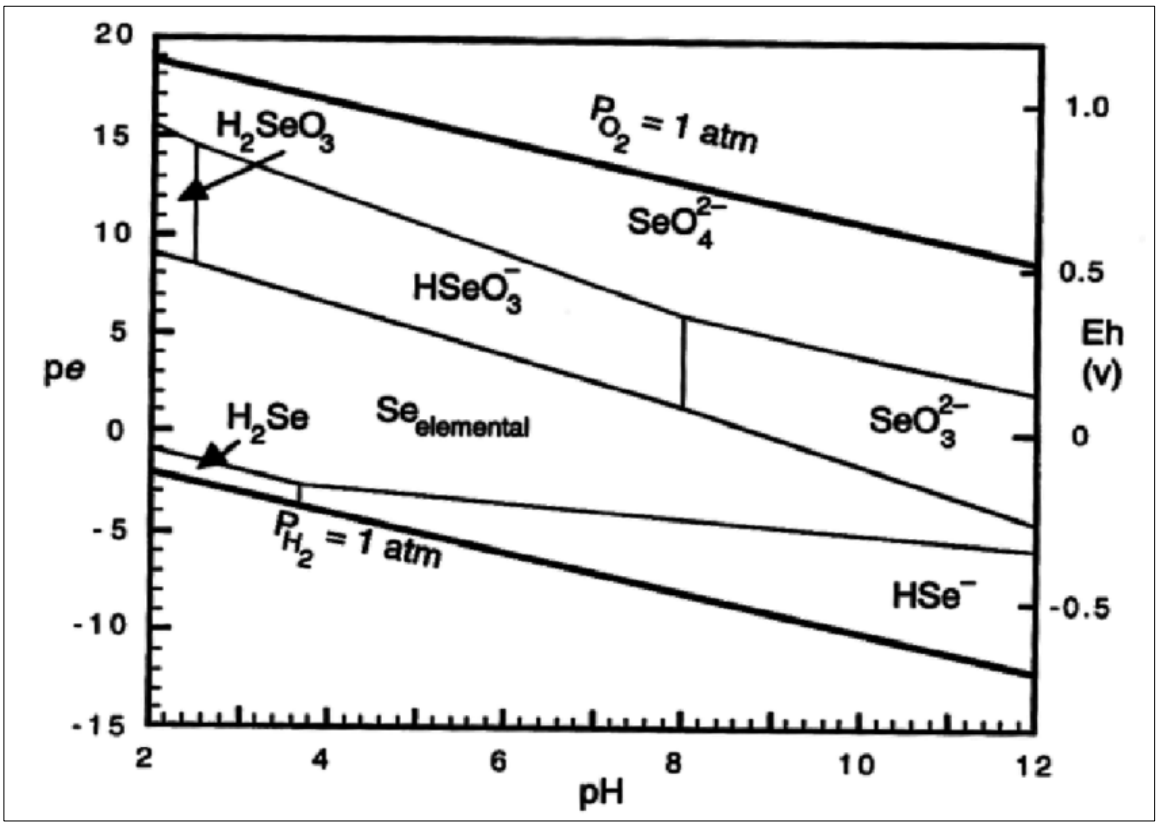

Figure 2.3: Effect of pH and Eh on selenium species in an aqueous system.

Based on **Figure 2.3**, it can be seen that selenate (SeO_4^{2-}) species is found to be more dominant at a pH range of 4 to 9 (which is the range under which most natural systems exist) and strongly oxidizing conditions (pH> 8). Selenate is reduced to selenite (SeO_3^{2-}) at alkaline pH of 8 to 12 under more reducing environment. It also exists in the -2 oxidation state as selenides (Se^{2-}) at a very acidic pH.

In groundwater, Se(IV) is the predominant form of selenium, whereas Se(VI) is the most stable oxidized form of selenium in alkaline and oxidizing solutions (Barceloux *et al.*, 1999). In aqueous solutions, the oxidation of Se(IV) to Se(VI) is kinetically slow, and Se(IV) can thus be found under nonequillibrium conditions (Torres *et al*, 2010).

2.4 Recommended levels of selenium for intake

Selenium is an essential element, and as a result various national and international Organizations established recommended daily intakes of selenium. The joint WHO, Food and Agriculture Organization (FAO) consultation on preparation and use of food-base dietary guidelines as detailed in **Table 2.2**.

Table 2.2: Guidelines for intake of selenium by WHO and FAO

Age	**Recommended intakes of selenium per day**
Infants and children	6-21 µg/day
Adolescent females and males	26-30 µg/day
Adult females and males	26-35 µg/day

The United States National Academy of Sciences panel on dietary oxidants and related compounds revised the recommended selenium levels to the values indicated in **Table 2.3**.

Table 2.3: Guidelines for daily intake of selenium by United States National Academy of Sciences

Age	**Recommended intakes of selenium per day**
Infants (0-6 months)	15 µg/day
Children (4-8 years)	30 µg/day
Men and Women	55 µg/day
Pregnant and lactating women	70 µg/day

In drinking water, a WHO provisional guideline for selenium was set at 40 µg/L. For countries that don't have a legislative framework for selenium in drinking water, the WHO guidelines can be used. European Union (EU) selenium limit in drinking water is 10 µg/L (European Commission, 1998) and maximum contamination level in the USA is 50 µg/L. United States Environmental Protection Agency (USEPA) established 50 µg/L as a chronic aquatic life criterion (an estimate of highest concentration of selenium in surface water to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect).

South African maximum selenium concentrations allowed in aquatic environment are detailed in South African water quality guidelines volume 7: Aquatic ecosystems (DWAF, 1996a). **Table 2.4** provides the amount required for total concentration of selenium in water as set in South Africa.

Table 2.4: Maximum selenium concentrations allowed in aquatic ecosystems.

Criteria	**Selenium concentration (µg/L)**
Target Water Quality Range (TWQR)	2
Chronic Effect Value (CEV)	5
Acute Effect Value (AEV)	30

2.5 Speciation of selenium in water and sediments

Selenium is known for both essentiality and toxicity character. It is essential at low amounts and toxic at high concentration. Its toxicity depends not only on the concentration but also on the oxidation states (Belzile *et al.*, 2000). To determine different oxidation states of selenium, speciation needs to be performed. Speciation is the process for identifying and determining the concentration of various chemical forms of an element in a matrix where these species together form the total concentration of that element in the sample (Diaz *et al.*, 2019).

The inorganic selenium [(Se(IV)) and (Se(VI))] dominating in water is the most toxic form compared to any other forms of selenium. Knowledge of selenium species in natural water and sediments is essential to understand its toxicity and bio-availability (Kucukbay and Demir, 2001). Speciation is an essential tool in evaluating contamination of trace elements in environmental samples (Rezende *et al.*, 2014).

Selenium is adsorbed into the solid phase of sediment through precipitation and adsorption onto sediment surfaces. Elemental selenium and selenides are insoluble in sediments while inorganic selenium are soluble. The adsorption of selenate and selenite on the sediment depends on the following sediment characteristics: sediment minerals, ligand exchange and salinity (Pattanan, 2006). Selenite is strongly adsorbed to sediment surfaces than selenate (Pezzarossa *et al.*, 2011).

Selenium speciation data are limited due to the very low concentrations of total selenium in water and sediments. This low concentration makes it difficult to do speciation as all individual species are below the detection limits of most analytical techniques (Wake *et al.*, 2004). The current limit of quantification is 0.03 µg/L in both fresh and marine waters. Therefore, speciation should be considered if the total concentration of selenium exceeds the limit of quantification value (Anzecc and Armcanz, 2000).

Number of studies on the speciation of selenium in environmental matrices have been done globally but few studies were done in South Africa. Numerous studies on total concentration of selenium in water have been reported in South Africa.

Genthe et al. (2018) determined total selenium in water samples in the lower Olifants River catchment, Limpopo province using ICP-OES. Olifants River has been named one of the most polluted river in South Africa due to anthropogenic activities affecting its water quality. Those anthropogenic activities include mining, coal-fired power stations, industrial and agricultural activities. Mean concentration of selenium in water sample exceeded the WHO guidelines of 10.0 µg/L for safe levels of intake. Selenium mean was found to be 207.82 µg/L (Genthe *et al*., 2018).

Gilbert *et al*. (2017) investigated the level of trace elements and metals such as As, Cu, Fe, Mn, Se, Cr and Pb in fish tissues and sediment samples of Vaal dam in Vereeniging using ICP-MS. The trace elements in sediment samples were leached out using aquaregia in a microwave. Selenium concentration in sediment samples was found to be 0.346 mg/kg. No speciation of selenium was conducted in this study. Contamination of water in the Vaal dam has been linked to several human activities including waste water and pollutants from nearby industries (Gilbert *et al*., 2017).

Munyangane *et al*. (2017) conducted the study to evaluate the occurrence and distribution patterns of toxic trace elements of As, Cd, Cr, Se and Pb in the boreholes water of Greater Giyani area, Limpopo, South Africa. The borehole water is used for human consumption. The detection of the concentration of trace elements was done by ICP-MS. The average concentration of selenium in the dry season was found to be 7.1 µg/L and 4.2 µg/L in wet season.

The findings of this investigation will assist the communities drinking from the affected boreholes to be aware of the water quality regarding harmful trace elements (Munyangane *et al*., 2017).

The study by Nomngongo (2017) on the speciation of inorganic selenium in water samples was conducted using micro solid phase extraction and HG-ICP-OES. Titanium dioxide/multiwalled carbon nanotubes (TiO_2@MWCNTs) were used as a sorbent material. Total concentration of selenium was performed using an ICP-OES spectrometer equipped with charge injection device detector. It was found to be 44.8 µg/L for river 1 and 71.1 µg/L for river 2. Speciated Se(VI) and Se(IV) from river 1 were found to be 19.4 and <LOD respectively. From river 2, Se(VI) and Se(IV) were found to be 45.5 and 25.6 µg/L respectively. The LOD of Se(IV) was 1.6 µg/L.

Nyaba *et al*. (2016) determined the levels of Se(IV) and Se(VI) in water samples from Soweto Area, Johannesburg, South Africa. The speciation of inorganic selenium was performed using suspended dispersive solid phase microextraction (SDSPME) and inductively coupled plasma optical emission spectrometry (ICP-OES). The adsorbent material used was alumina nanoparticles functionalized with Aliquat-336. Nyaba *et al.* (2016) also investigated the effect of pH of the sample on SPE. It was found that the speciation of inorganic selenium depended on the pH of the sample. The method showed an excellent percentage recovery of more than 90% for Se(IV) and 5% for Se(VI). Total selenium concentration in water samples was ranging from 2.43 to 117 mg/L. The concentration of Se(IV) ranged from 0.631 to 84.0 mg/L.

The concentrations of Se(VI) was calculated using the difference between total selenium concentration and concentration of Se(IV). It was found to be ranging from 1.80 to 33.0 mg/L (Nyaba *et al*., 2016).

Crawford (2010) determined the total concentration of selenium in water using ICP-MS and speciation of inorganic selenium using HPLC-ICP-MS. The ICP-MS was also used to determine total concentration of selenium in sediments along with sequential extraction procedure to speciate inorganic selenium. The selenium speciation analysis was also achieved by using LC-ICP-MS and the results were very satisfactory (Ochsenkuhn-Petropoulou *et al.*, 2003).

2.6 Sample preparation methods for speciation

There are numerous published papers on selenium analysis methods which yield a range of sensitivity and precision depending on how the procedures are combined and which sample matrices are being analysed (Kleckner *et al.*, 2017).

Sample preparation is one of the most important steps in trace elements determination and methods involving digestion with oxidant acids and leaching are often employed. However, several of these producers are time consuming and consume large volume of reagents (Bianchi *et al.*, 2017).

Commonly used methods are microwave based extraction and simple dilution (Shinohara *et al.*, 1998). Because of the usually low concentration of selenium in sample matrix, it is necessary to apply sensitive and selective analytical techniques for its determination (Shaltout *et al.*, 2011).

The development of reliable techniques to study the speciation of selenium in environmental and biological sample is necessary to understand the biochemical cycle, mobility and uptake of selenium, as well as its toxicity (Pyrzynska, 1998).

In order to determine selenium species like Se(IV) and Se(VI), a preconcentration and separation, methods of liquid-liquid extraction, solid phase extraction, ion –exchange, cloud point extraction, etc. have been used (Tuzen *et al.*, 2006; Vanzhang *et al*., 2013).

Until now, SPE, (**Figure 2.4**) has been generally used as a preconcentration procedure for trace elements due to known advantages of flexibility, economical and environmental friendly, absence of emulsion, speed and simplicity (Saygi *et al*., 2007). The use of SPE as preconcentration procedure offers the possibility of high detectability (Bueno *et al.*, 2002).

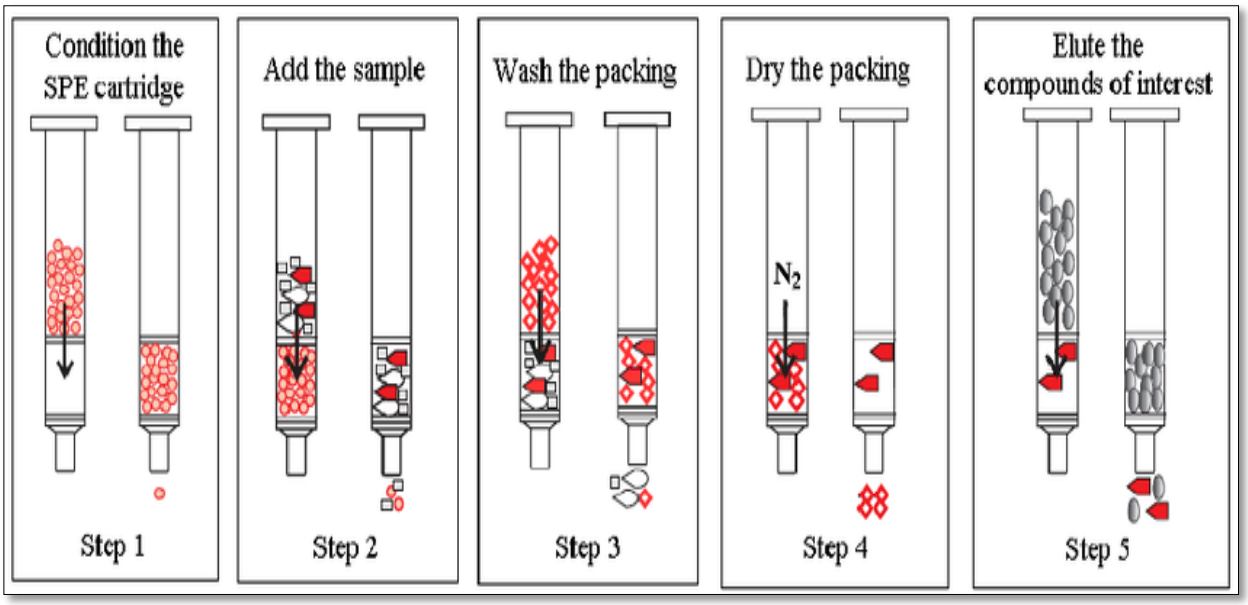

Figure 2.4: Typical Solid Phase Extraction Procedure

Cloud Point Extraction (CPE) (**Figure 2.5**) has also been used for the extraction and preconcentration of many metal ions including selenium (Li *et al*, 2007). The CPE using non-ionic surfactants have been popular over traditional liquid-liquid extraction. The CPE is simple, inexpensive, uses less organic solvents and has high recoveries and high concentration factors (Yang *et al.*, 2017).

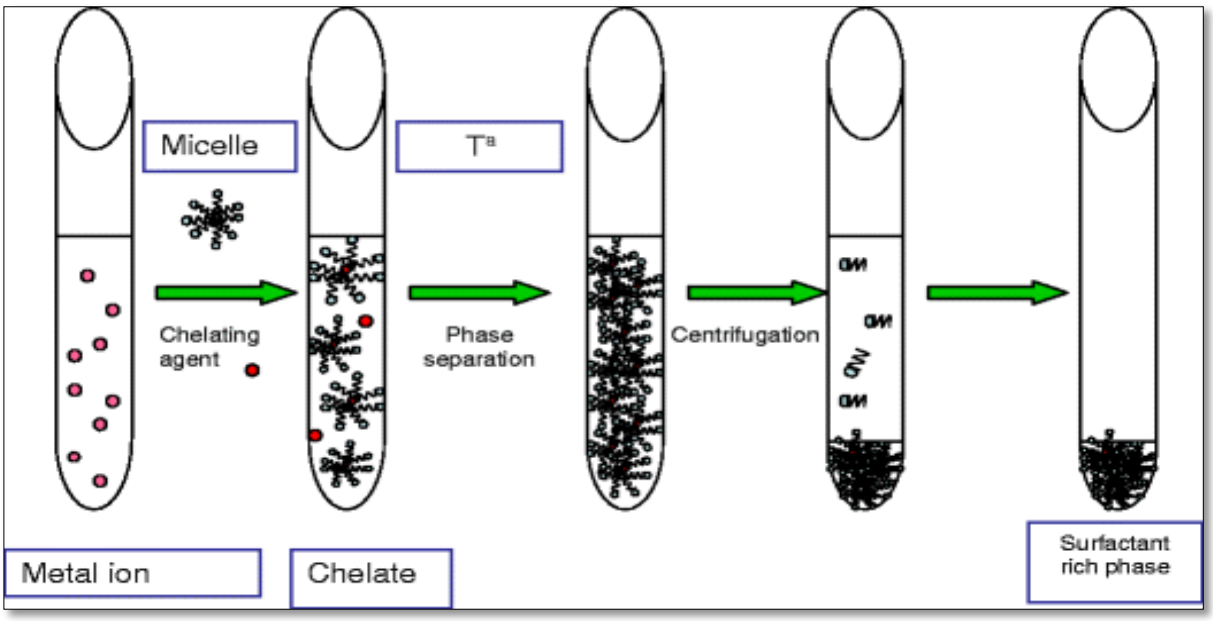

Figure 2.5: Typical Cloud Point Extraction Procedure

In the present study, the speciation of selenium in water samples was performed using SPE technique and HPLC-ICP-MS. The method of SPE was used as a separation and pre-concentration technique prior to the determination of selenium species using analytical instruments (Herrero Latorre *et al.*, 2013).

The coupling of HPLC with a highly sensitive and selective detector has also been chosen as an alternative method for speciation of inorganic selenium. An HPLC has the ability to deal with non-volatile compounds and separation of species. An HPLC was used in conjunction with ICP-MS due to the fact that ICP-MS is highly sensitive and it can read or determine concentration up to µg/L.

Liquid Chromatography is commonly used for selenium separation when coupled with a highly sensitive technique (Sentkowska, 2019). The IEC method is an efficient method which can be used to separate selenium species in a solution based on the anionic and cationic characteristics of selenium species in different pH solutions. In basic solution, Se-amino acids behave as anions and show cation behaviour in acid solutions. Selenite and selenate are anions (Zhang and Frankenberger Jr., 2001). Zhang and Frankenberge speciated selenium species in a plant extracted using anion exchange resin column. Reverse-phase liquid chromatography is mostly used for the speciation of selenium but the inorganic selenium was not well separated. The IEC method is the most suitable technique for separation of selenium species (Ayouni *et al.*, 2006).

2.6.1 Solid phase extraction procedure

The SPE method was introduced as a better option for separation and/or pre-concentration technique prior to the determination of selenium species as compared to liquid-liquid extractions (Herrero Latorre *et al.*, 2013). The SPE method does not use large volume of high purity solvents, which may cause health and environmental hazard. The SPE method is an efficient technique and best in achieving higher recovery of the analyte (Poole *et al*., 2006). The SPE technique has shown to be effective for different sample matrices such as drinking water, river water, beverages, fruits, vegetables, soils, etc. (Wells, 2000).

Different strategies for SPE speciation of inorganic selenium species have been used. These strategies were based on different chemical behaviour of Se(IV) and Se(VI), different sorption materials for SPE retention of Se(IV) and Se(VI), different chemicals for SPE elution of Se(IV) and Se(VI) and the analytical technique for the final measurement of Se(IV) and Se(VI). Different chemical behaviour of the two inorganic species is based on the type of the sorbent material used (Herrero Latorre *et al.*, 2013).

Off-line separation and pre-concentration is not novel but it is considered the easiest way (Lin, 2007). The SPE procedure is still attracting attention in separating inorganic selenium in samples (Lin, 2007). Solid Phase Extraction coupled with ICP-MS has made speciation analysis easier (Zhang *et al.*, 2007). Some novel SPE adsorbents such as modified silica, resins, metal-oxide materials and carbon-based materials have been explored for SPE. Amongst all of the adsorbents, carbon-based nano materials have been mostly used for many trace elements speciation including inorganic selenium speciation due to their good chemical stability, high adsorption capacity, tuneable surface properties and large surface areas (Peng *et al.*, 2015).

Dowex 1 x 2 resin has been used for separation and pre-concentration of Se(IV) and Se(VI) in groundwater. Dowex 1 x 2-chloride form is a strong basic anion exchange resin. It maintains its positive charge across a wide pH range meaning it will exchange anions in both acid and alkaline solutions (Holl, 2000). **Figure 2.6** shows the structure of Dowex 1 x 2 chloride form used in this study.

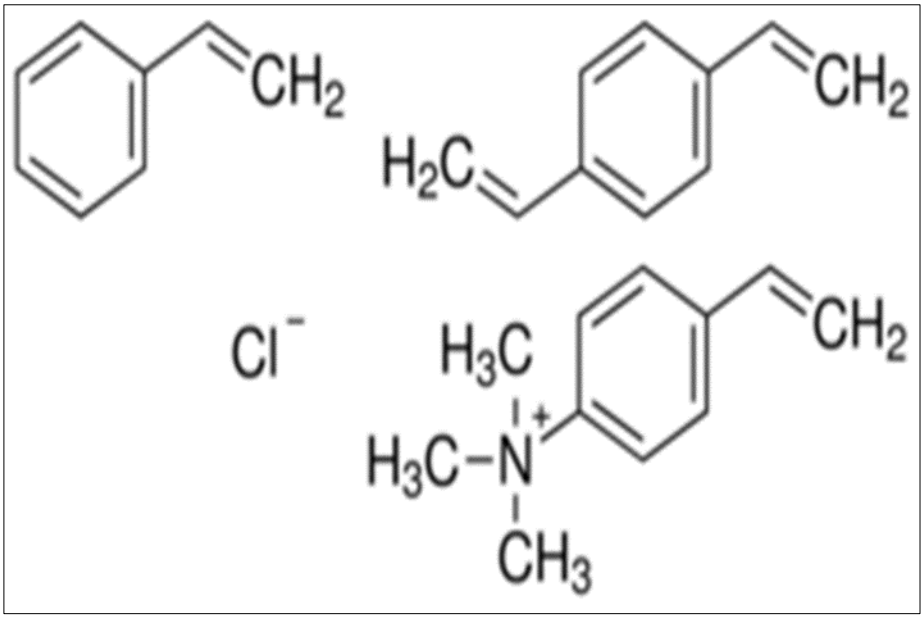

Figure 2.6: Structure of Dowex 1 x 2 resin (chloride form)

In this study, the inorganic species (Se^{6+} and Se^{4+}) were both retained on the resin column and eluted using nitric acid of different concentration (Lin, 2007). From **Figure 2.6,** it can be seen that the anionic Cl^- is a counter ion responsible for neutralising the cationic amide nitrogen (N^+) structure. The structure will no longer attack any inorganic selenium species. The Se(VI) which is more electrophillic than Se(IV) will be adsorbed first on the 1.4-divinylbenzene. The monomeric styrene will be the last one to adsorb the remaining inorganic selenium species. Solid phase extraction technique **(Figure 2.4**) has mostly been used for speciation of inorganic selenium in environmental samples using different adsorption materials and elution solutions (Sahin *et al.*, 2003; Nyaba *et al.*, 2016, Wang *et al.*, 2013; Skorek *et al.*, 2012; Kocot *et al.*, 2015).

In SPE, the analyte of interest is in between the sample matrix and modified solid surface. There are various factors that affect SPE including the choice of sorbent material, selection and volume of conditioning, sample volume, rinse and elution solvents, flow rate, etc. (Huang *et al*., 2011).

2.6.1.1 Effect of sample volume in SPE

In pre-concentration studies, to achieve a high pre-concentration factor, it is desirable to use the maximum volume of sample and a minimum volume of eluent. It is necessary to separate and concentrate lower concentration of selenium in natural water samples to achieve a high pre-concentration factor (Wei *et al.*, 2014).

2.6.1.2 Effect of foreign ions in SPE

In pre-concentration, separation, and/or speciation studies with SPE, foreign ions can usually interfere with the determination of SPE step and detection step. In the first step, other ions can also be retained on the sorbent material, which may decrease the retention of the analytes. In the second step, other ions eluted together with the analytes may interfere with the concentration determination. In SPE, most of the other ions are being separated from the analytes. Thus, interferences in the detection step after SPE procedure is generally low. However, other ions at high concentrations may prevent the retention of the analytes on the sorbent material.

Foreign ions such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Al^{3+}, Fe^{3+}, Mn^{2+} and Co^{2+} as their chloride or nitrate salts should be investigated for their effect on the recovery of Se(IV) and Se(VI) and their tolerance limits should also be investigated. Ions are considered as not interfering if they have an error of less than 5% (Erdogan *et al.*, 2016; Valencia *et al.*, 1999).

2.6.1.3 Effect of temperature and flow rate in SPE

Selenium species separation takes place at room temperature. The samples must be stored in the refrigerator at 4°C after sampling in order to maintain the stability of the analyte (Dietz, 2003). The flow rate of the sample solution affects both the retention of the analyte on the sorbent material and the duration of the experiment. While increasing the flow rate to shorten the duration, retention of the analyte decreases. However, when decreasing the flow rate in order to increase retention, duration increases. Therefore, the effect of flow rate must be considered (Erdogan *et al.*, 2016).

2.7 Sample preparation methods for total concentration of selenium

The behaviour of trace elements in water, soils and sediments is determined by their specific physicochemical forms rather than their total concentrations. Several chemical speciation methods have been developed and applied. Some are still being developed. These methods include chemical extractions, ion-exchange/gel chromatography, filtration, centrifugation and sieving (Tack and Verloo, 1995).

Chemical extraction involves separating a substance from a matrix. The process includes solid phase extraction and liquid-liquid extraction for liquid matrices. The main extraction for solid matrices are matrix solid phase dispersion (MSPD), Soxhlet, sonication and microwave-assisted extraction (Dean and Xiong, 2000).

Matrix solid phase dispersion (**Figure 2.7**) is used for preparation of environmental matrices for the determination of trace elements such as selenium (Rezende *et al.*, 2014). In MSPD, a small amount of the sample and solid support are homogeneously mixed with pestle and mortar. The choice of solid support depends on the polarity of the analyte (Massarolo *et al*., 2018).

The MSPD method is chosen over many extraction techniques due to the fact that it is simple as it accepts the use of green solvents and renewable materials besides being able to extract and purify in one step (Hoff *et al.*, 2018). The difference between MSPD and SPE is that in MSPD sample is dispersed throughout the column while in SPE sample is retained in the column (Subhash *et al*., 2015).

Lu *et al*. (1996) used Soxhlet method to extract selenium in Se-enriched garlic powder to investigate its efficacy on cancer prevention (Lu *et al*., 1996). Soxhlet extraction (**Figure 2.8**) has a disadvantage of using large amount of solvents (300-500mL per sample) and long extraction time up to 24-48 hours (Majors, 2006). Soxhlet extraction is time consuming and its processes are not good for the environment. Vale *et al*. (2007) used enzymatic sonication method for the extraction of selenium in biological samples. The extractants were detected for the total concentration of selenium using electrothermal-atomic absorption spectrometry (Vale *et al*., 2007). Sonication extraction (**Figure 2.9**) operates similar to Soxhlet except that it is faster in operation time (30-60 min) and it uses small amount of solvents. Sonication extraction has a disadvantage of being labour intensive (Majors, 2006). In this study, MAE has been used for the extraction of selenium in sediments for the determination of total concentration.

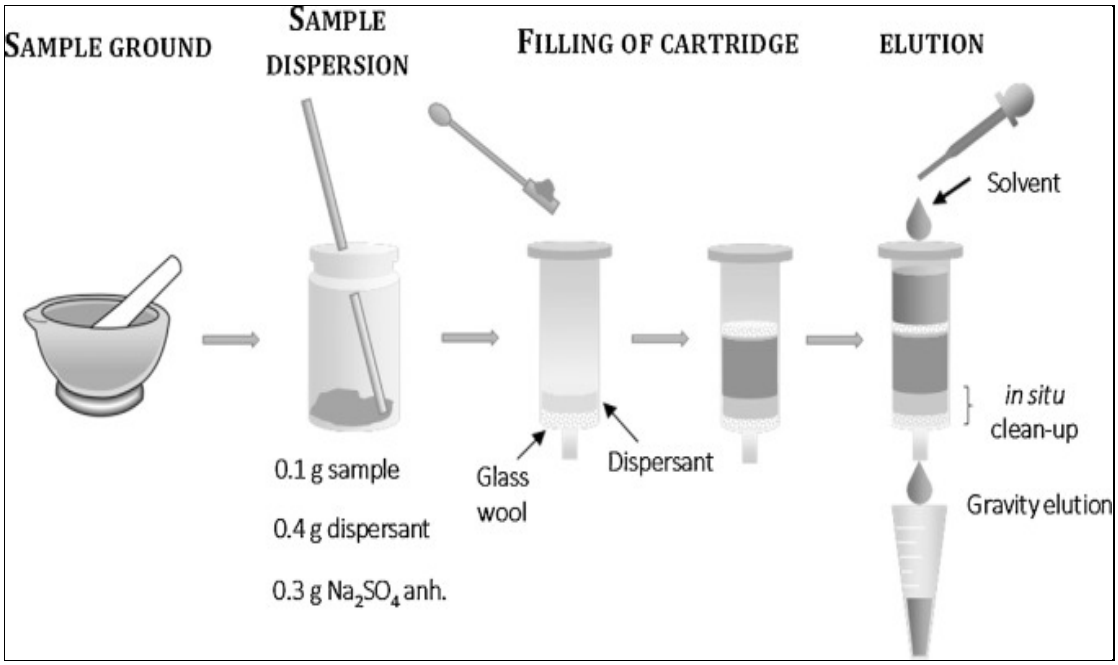

Figure 2.7: Typical matrix solid phase dispersion

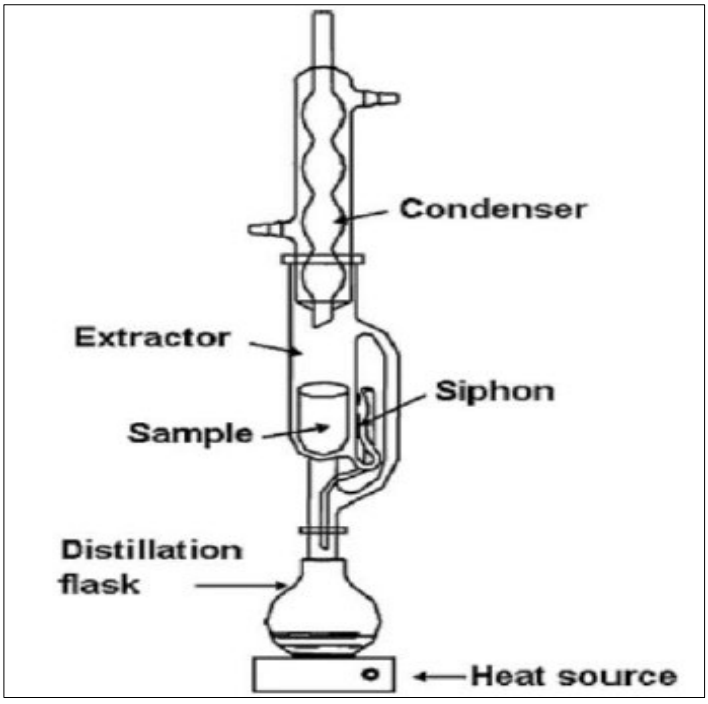

Figure 2.8: Typical Soxhlet extraction process.

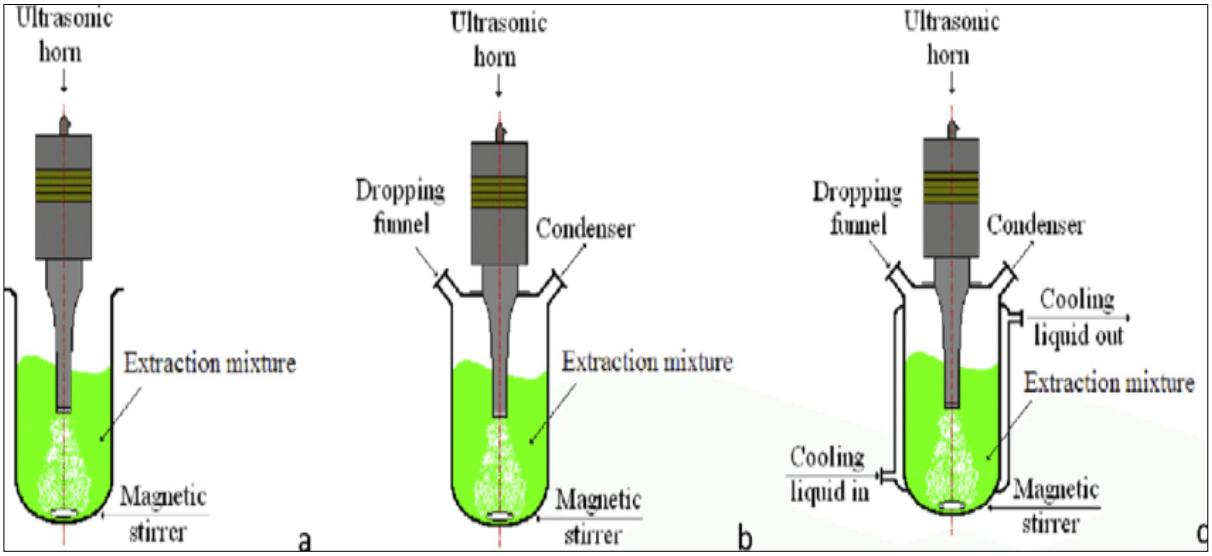

Figure 2.9: Typical sonication extraction process

2.7.1. Microwave Assisted Extraction

The microwave assisted extraction (MAE) method has been used widely as a sample preparation technique for different analytical purposes such as agricultural sample analysis, environmental detection and food inspection. The MAE method is popular due to the fact that it is faster, uses less amounts of solvents and is environmentally friendly.

It uses microwave system to generate the heat that can easily penetrates into the sample pores causing the solvent to be trapped into the pores (Su *et al.*, 2017). The MAE method is faster as compared to conventional heating since the heat is transferred directly to the solvent that absorbs the microwaves. It uses small amounts of solvents as compared to sonication and Soxhlet extraction. The main disadvantage of MAE is extracts must be filtered after extraction, which slow down the operation and also clean-up of extracts is needed because everything gets extracted with MAE. The microwave equipment is moderately expensive (Lopez-Avila, 1999).

In MAE, microwave energy and traditional solvent extraction are combined to heat polar solvents and sample to increase the mass transfer rates of the solutes from the sample matrix into the solvent. Extraction occurs due to electromagnetic waves causing changes in the cell structure (Gagaoua, 2018)]. In MAE, the heat and mass gradients are working in the same direction to accelerate the extraction process (Devgun *et al.*, 2009). **Figure 2.10** indicates the difference between conventional and microwave extraction in relation to mass and heat transfer.

In MAE, the steps that occurs during extraction process include solvent penetration into the sample matrix, breakdown of components, transport of solute out of the sample matrix, migration of the extracted solute into the solution, separation and discharge of the extract from the solid (Veggie *et al.*, 2012).

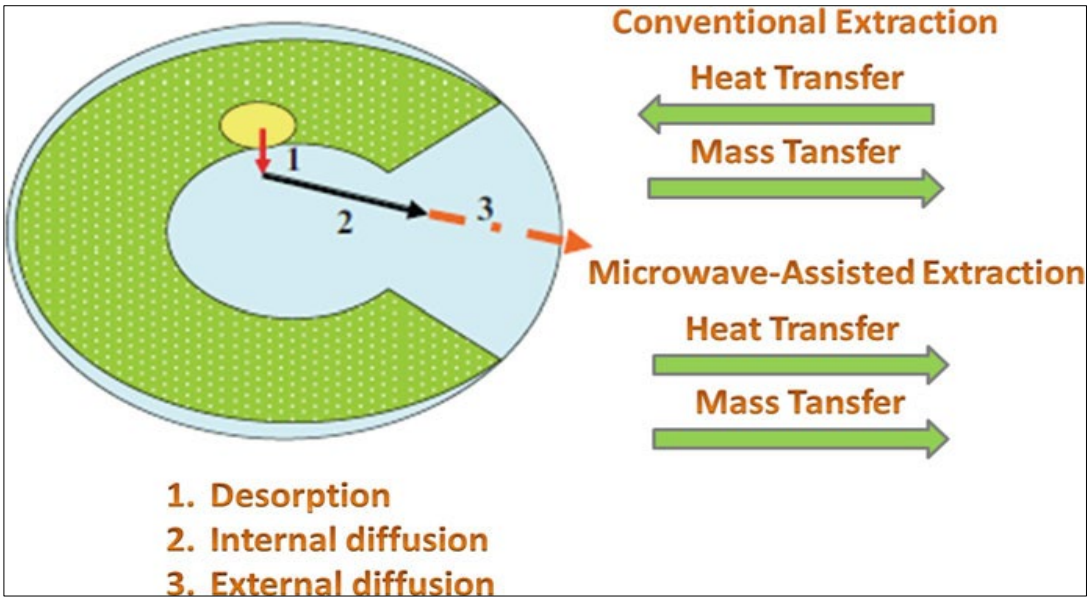

Figure 2.10: Heat and mass transfer mechanisms in microwave and conventional extraction.

2.7.2 Microwave assisted digestion for total selenium determination in sediments

Most trace analytical techniques require a sample in a liquid form to operate. Hence, solid samples should be digested prior to analysis (Gholami *et al.*, 2016). Microwave-assisted digestion technique involves the use of concentrated acids to convert solid samples to liquid samples at high temperature and high pressure. Digestion of solid samples for total concentration analysis is now established as a normal sample preparation method. Microwave sample digestion technique became popular since 1980s due to the fact that it provides rapid, safe and efficient digestion. The use of low volume microwave digestion is recommended to allow the determination of analytes in small samples. In this way, the decrease of sensitivity due to loss of volatile analytes is avoided (Shirdam *et al*., 2008).

Microwave-assisted digestion is recommended as the best method for digestion compared to open vessel digestion based on the recovery rate, safety, cost and time. Acid digestion reaction of solid material depends on the following: acid used and its concentration, reaction time and external forces such as microwave, heat, ultra-sound, etc. The choice of acid mixture in digestion depends on the nature of the metals present in the solid material. HCl, H_2O_2, HF and HNO_3 are often used for digestion. HCl extracts metal associated with carbonates, phosphates, borates and some oxides while HNO_3 extract metals from a vast range of metal salts. To extract metals bound to silicate, HF is used. H_2O_2 is used to improve metal recovery and increase reaction kinetics in wet acid digestion (Das and Ting, 2017).

Possible contamination of the digested material is minimized in a close vessel microwave-assisted digestion. Concentrated acids are often used in the digestion process, however diluted acids has also been successfully employed. Diluted acids generate low blank values and low relative standard deviation. H_2O_2 is required as an oxidant agent when using dilute acids. The feasibility of using dilute acids and small amount of H_2O_2 comes from the high temperature and high pressure involved in the close-vessel microwave-assisted digestion (Araujo *et al.*, 2002).

For the determination of selenium in sediments, a mixture of HCL, HNO_3 and HF with microwave digestion rather than conventional hotplate gave the best recoveries (Crompton, 2001]. The EPA method 3052 allows the specific reagents for a specific matrices and analyte of interest to avoid interferences. The use of HCl in the digestion involving selenium should be limited due to Cl interferences when using HPLC-ICP-MS with isotope ^{77}Se. H_2O_2 can be used instead but in a small amount (Mangum, 2009).

2.8 Detection techniques

A variety of analytical methods have been used to determine trace concentrations of selenium in environmental water but some have become obsolete recently as they are time-consuming and does not have low detection limit (Niedzielski *et al.*, 2003). These analytical techniques include Fluorometry, Neutron Activation Analysis (NAA), Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma - Mass Spectroscopy (ICP-MS), Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES), Graphite Flame Atomic Absorption Spectrometer (GFAAS), X-Ray Fluorescence analysis etc.

Analytical methods that can distinguish different chemical forms of an element without pre-treatment are now preferred (Bueno *et al*., 2002). Despite the very sensitive analytical techniques available for selenium, due to its low levels in environmental water, it requires pre-concentration to improve its detectability and to remove matrix interferences (Gomez- Ariza *et al.*, 1998).

Although several instrumental techniques allow information on the isotopic composition of target elements to be obtained, MS is without a doubt the most versatile and the most powerful (Vanhaecke and Degryse, 2012). Amongst these analytical techniques, ICP-MS has proven to be effective due to its high sensitivity and relative ease of interfacing with common chromatographic and separation techniques (B'hymer *et al.*, 2006).

2.8.1 Inductively coupled plasma-mass spectrometry

An ICP-MS was introduced in 1983. It was initially used for determination of total concentration of selenium in liquid samples. An ICP-MS has matured into detecting all kinds of compounds via their different characteristic element content. Elements such as phosphorus, sulphur, selenium or even halogens can now easily be detected by ICP-MS (Vanhaecke *et al.*, 2012).

An ICP-MS is the preferred method for elemental speciation when coupled with any different methods for separation because it can attain low detection limits. It can also measure several elements at once and it gives information on isotopes. Compared to ICP-AES, ICP-MS detection limits are 2-3 orders of magnitude lower.

There are different types of ICP-MS but for this study single quadrupole (SQ) - ICP-MS is used. **Figure 2.11** shows a typical SQ-ICP-MS used in this study. An ICP-MS uses liquid samples. A sample is introduced into a plasma via combination of nebulizer, spray chamber and a torch. A sample is delivered to a nebulizer using a peristaltic pump. The sample is converted into an aerosol by the nebulizer and gets ionized by the Argon gas. The ions are then separated based on their m/z (mass to charge) ratios and gets detected individually at any given time by the MS (Beauchemin, 2017; Zhang and Cresswell, 2016).

Figure 2.11: Typical ICP-MS from Perkin Elmner

This instrument faces some challenges regarding interferences on selenium. The instrument uses Argon gas to generate plasma. Argon has isotopes at mass 36, 38 and 40, however at extremely hot temperature (>5000 °C) of the plasma, Ar converts to Ar_2 and overlap with isotopes of selenium. **Table 2.5** below shows interferences between Ar_2 and Se (Nelms, 2016).

Table 2.5: Ar_2 and selenium interferences

Se isotope	**Ar_2 interference**
^{74}Se (0. 9%)	$^{36}Ar^{38}Ar$ (0.0004%)
^{76}Se (9.4%)	$^{36}Ar^{40}Ar$ (0.67%)
^{77}Se (7.6%)	No Ar_2^+ interference
^{78}Se (23.8%)	$^{38}Ar^{40}Ar$ (0.13%)
^{80}Se (49.6%)	$^{40}Ar^{40}Ar$ (99.2%)
^{82}Se (8.7%)	No Ar_2^+ interference

From the table, it is clear that all isotopes except ^{74}Se are sufficiently abundant for selenium analysis. ^{80}Se being the highest abundance with Ar_2 interference posing a problem of large interferences. ^{76}Se and ^{78}Se will also suffer Ar_2 interferences although the interference is not that large as compared to ^{80}Se (Nelms, 2016). To avoid this spectra interference with ^{80}Se isotope (the largest natural abundance of 49.6%), ^{82}Se and ^{77}Se are often monitored (Holl., 2000).

^{77}Se and ^{82}Se also suffer interferences from typical sample components and Ar impurities. **Table 2.6** shows those interferences.

Table 2.6: ^{77}Se and ^{80}Se interferences

Se isotope	**Interference**
^{77}Se (7.5%)	$^{40}Ar^{37}Cl$
^{82}Se (8.8%)	$^{81}Br^{1}H$, ^{82}Kr

To correct these interferences, the following mechanisms can be used: mathematical correction, collision cell operation, collisions by gases introduction (gases such as H_2, He, O_2 and NH_3). However mathematical correction becomes less accurate if the signal from the interferences is higher than the concentration of selenium (Neubauer,

2004; Bianchi *et al.*, 2017; Goldberg *et al.*, 2006). Selenium detection using ICP-MS suffers from ionization potential of selenium which is responsible for low ionization in Ar plasma. An ICP-MS equipped with reaction cell has been proven as a solution to overcome these interferences (Darrouzes *et al.*, 2007). The specific element to be analysed can be obtained by the hyphenation of ICP-MS with different separation techniques such as non-chromatographic methods SPE or CPE as well as chromatographic methods such as HPLC (Pröfrock and Prange, 2012).

2.8.2 HPLC coupled with ICP-MS

Speciation of inorganic selenium is accomplished most easily using HPLC-ICP-MS. HPLC separate the inorganic selenium species and ICP-MS detect them individually. HPLC is an established technique and require minimal sample preparation. ICP-MS measures concentrations of elements at a wide range from ng/L to mg/L. Coupling HPLC with ICP-MS offers the following advantages, simple sample preparation, separating power of HPLC, low detection capability of ICP-MS and automated analysis (Neubauer, 2008). **Figure 2.12** shows the setup for HPLC-ICP-MS used in this study.

Figure 2.12: HPLC-ICP-MS setup

HPLC measures samples that are in liquid form. Aquatic samples such as river water, ground water do not require any pre-treatment other than filtration with 0.45 µm filter membranes. However, direct determination of selenium species at concentration level present in environmental samples is difficult or rather impossible. Many studies have confirmed that a hyphenated technique of coupling chromatographic separation techniques with ICP-MS is powerful and sensitive technique for speciation of selenium (Tie *et al.*, 2017). HPLC method development involves selecting the correct column and mobile phase. Chromatographic separation in HPLC is affected by components such as: the concentration of species, mobile phase and its pH and column stationery phase (Neubauer, 2008). Both isocratic and gradient elutions can be used for selenium species separation.

The different types of HPLC techniques are classified as size-exclusion chromatography (SEC), ion-exchange chromatography (IEC), cation-exchange chromatography, reversed-phase chromatography and hydrophilic interaction liquid chromatography. Amongst all of these types, anion-exchange chromatography is mainly used for selenium speciation analysis. Cation-exchange chromatography is only suitable for organic selenium speciation. Anion exchange column (Hamilton PRP-X100) is commonly used for selenium species separation (Tolu *et al.*, 2011; Guerin *et al.*, 1997; Fitzpatrick, 2003; Yu *et al.*, 2019). Hamilton PRP-X100 is highly stable and has inert material for a variety of anion analysis (Yu *et al.*, 2019). **Figure 2.13** shows a structure for Hamilton PRP-X100. The packing inside the column is stable from pH 1 to pH 13. Single column can be used for analysis of many anions (www.hamiltoncompany.com/hplcapplicationindex , accessed 2020)

Mobile phases commonly used in anion-exchange chromatography contain small amount (2-5%) of methanol (organic modifier) and buffered salt solution (e.g. acetate, phosphate and citrate) (Sentkowska, 2019). Buffer solutions used in IEC should not exceeds 25 mM in concentration. Higher concentration of the buffered salt solution will lead to the blockage of the nebulizer as the salt is deposited on the cones of the ICP-MS resulting in the instability of the plasma and shifting the retention time of the individual species (Sentkowsa, 2019).

The use of NH_4NO_3 as a mobile phase has shown to have good signal stability and less salt is deposited on the cones of the ICP-MS (Yu *et al.*, 2019).

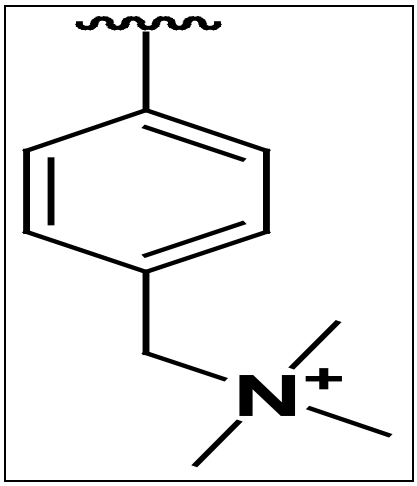

Figure 2.13: Hamilton PRP-X100 anion exchange column stationary phase structure

2.8.3 Other techniques that can be used for selenium speciation.

2.8.3.1 ICP-OES

An ICP-OES like ICP-MS uses a sample solution. Sample solution is introduced into a core of ICP through argon gas. Argon plasma generates extremely high temperature of about 8000 °C when the sample is introduced into the instrument for analysis. At this high temperature, all elements become excited and emits light at their characteristics wavelengths. The light is then collected by the wavelengths and yields an intensity measurement. This measurement is then converted to an elemental concentration by comparison with the calibration standards. To determine how much of an element is in the sample, a plot of the calibration curves of emission intensity versus the concentration and extrapolated concentration of the species of interest from the graph is constructed (Boss and Freedeen, 2004).

An ICP-OES is used to complement other techniques due to its moderate sensitivity. An ICP-OES is not sensitive enough for selenium analysis due to selenium levels being very low in the environmental samples. Preconcentration and separation step is often required for selenium analysis (Dai *et al*, 2011).

2.8.3.2 Flame and Furnace Atomic Absorption Spectrometries (FAAS and GFAAS)

Flame Atomic Absorption Spectrometry detection limits for many elements are comparable to those of ICP-OES. Graphite Furnace Atomic Absorption Spectrometry has greater sensitivity as compared to F-AAS and ICP-OES. The F-AAS and GF-AAS measure the reduction in the intensity of optical electromagnetic radiation. The difference between them is how the samples are atomised. The F-AAS uses flames while GF-AAS uses a graphite furnace to atomize samples (Wilson, 2010). The F-AAS has less sensitivity compared to GF-AAS especially in the analysis of some environmental samples (soil, water and plant material) where concentrations of trace elements may be very low (Hayes, 2017). The F-AAS is not sensitive enough for speciation of elements at a trace level of the environmental samples. Direct determination of selenium in water and sediments by F-AAS is not feasible due to instrument's sensitivity (Michalke *et al.*, 2013). The GF-AAS may be used for the speciation of inorganic selenium provided that chemical reactions such as SPE and CPE are done prior to analysis (Pyrzyn'ska, 1998).

2.9 Calibration methods

One of the most important aspects of any analytical method is the calibration of the instrument with respect to the concentration of the analyte. Calibration is a process of assessment and refinement of the accuracy and precision of a method used for the qualitative and quantitative analysis of an analyte. The calibration done in the analytical determination is the process of validating the method. Typical parameters of method validation are accuracy, precision, limit of detection and quantification and limits of linearity of the calibration curve (Stauffer, 2018).

The assessment of the analytical method and the related instrument is done by analysing the standard/certified reference material (SRM/CRM) containing a known and established quantities of the analytes to be determined. The SRM/CRM are an important tool for measurement and method validation. They are used as a control material, as some calibration/verification materials to calibrate/verify processes and development of new methods (Stauffer, 2018).

In the absence of SRM/CRM, a method of spiking using the analyte in question (standard addition) is used with the recovery studies. Recovery studies, R, can be used to check the trueness of the method (Cuadros-Rodriguez *et al.*, 2001). By doing recovery studies, both the accuracy can be tested and the precision can be estimated. Recovery studies is when the concentration obtained is compared to the true value of the added standard (Danzer *et al.*, 2004).

2.9.1 Calibration curve, linearity and sensitivity

Calibration curve defines the relationship between the analyte of interest in the sample matrix and the response of the detector of the instrument. Calibration curve for each analyte when analysing multiple elements should be generated. The calibration curve may be linear or non-linear. The linear calibration curve with correlation coefficient (R^2) of close to 1 is preferred as it minimizes the percentage errors. The linearity of an analytical method is capable of showing results that are directly proportional to the concentration of the analyte in the sample within a given range. The calibration curve should consist of at least five points that cover the range of the expected concentration of the analyte of interest in the sample matrix (McMillan, 2016).

2.9.2 Limit of Detection and limit of Quantification

Limit of detection (LOD) and Limit of quantification (LOQ) are two of the most important parameters in method validation. They are used to describe the lowest concentration of an analyte that can be detected reliably by an analytical procedure (Armbruter and Pry, 2008). Limit of detection is the lowest concentration of an analyte in a sample that can be detected but not quantified. Limit of quantification is the lowest concentration of an analyte in the sample that can be quantified with acceptable precision and accuracy (Shrivastava and Gupta, 2011). There are three methods in analytical chemistry to determine the LOD and LOQ. Those methods are method based on S/N ratio, slope of calibration curve (CCS) and laboratory fortified blank (LFB). Amongst these methods, detection limits based on LFB is commonly used and showed lower detection limits. The LOD is often defined as 3 times the signal to noise (S/N) ratio while LOQ is 10 to S/N ratio. If the noise is approximate to the standard deviation (SD) of the blank, LOD is 3 times SD of the blank and LOQ is 10 time SD of the blank (Saadati *et al.*, 2013).

CHAPTER 3: EXPERIMENTAL

This chapter outlines sampling, sample pre-treatment, sample preparation methods and sample analysis. This chapter also details the chemicals used and their purity, standards and standard reference materials. It also gives details of settings and parameters on various instruments used for analysis of samples.

3.1 Sampling sites description

Samples were collected from Blood River and Mokolo River at ten (10) different sites. Samples were collected from downstream of the river to the upstream of the river based on the accessibility of the river. Water and sediment samples were collected from the sites shown on the maps **(Figure 3.1** and **Figure 3.4).**

3.1.1 Blood River

Blood River passes through Seshego Township, North-West of Polokwane City in the Limpopo Province. The water from the Blood River joins the Sand River near Seshego and some of the boreholes situated along Sand River to supply water to the city. Polokwane Municipality Report (IDP, 2012-2013/15) indicated that the municipal and domestic wastes contribute to the pollution of the river water. The pollution is attributed to lack of waste removal system and illegal dumping of waste close to river banks (**Figure 3.2**). Wastes such as broken bottles, broken construction material, computer parts, plastic bags, dead animals lie along the banks of the Blood River and finally enter into the river system.

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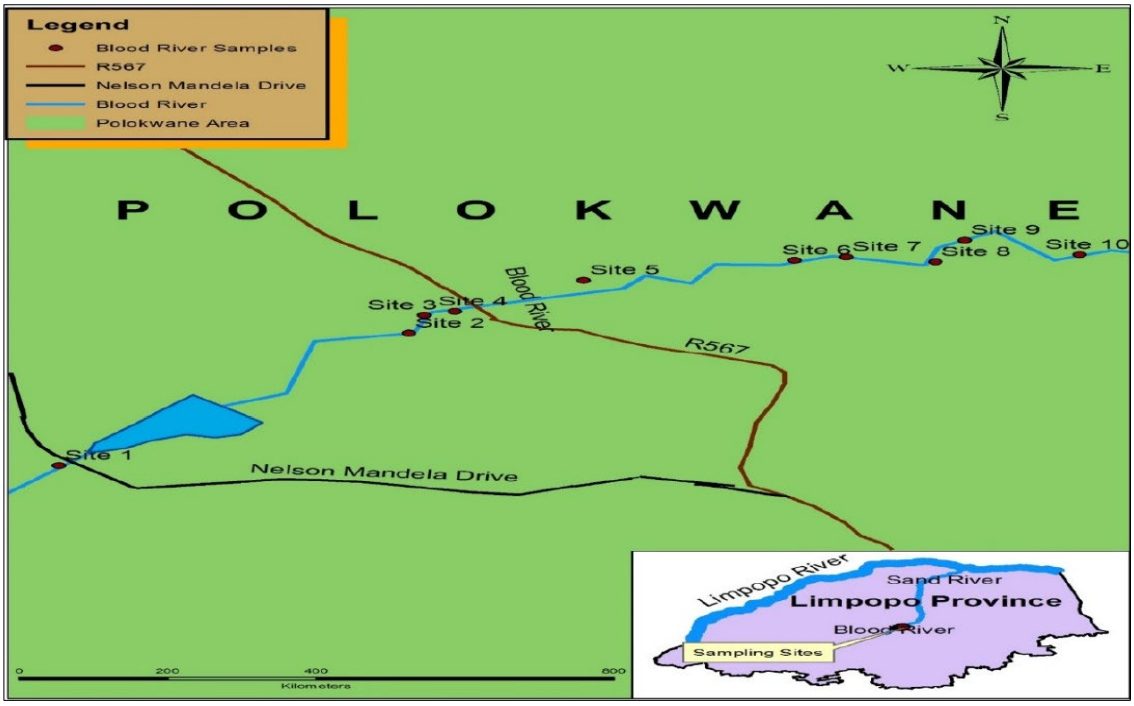

Figure 3.1 The map of the location of the river and selected sampling sites.

Figure 3.2: Illegal dumping site close to Blood River bank.

The River also flows alongside Seshego wastewater treatment plant. The untreated sewage flows into the river from damaged pipes of the sewage plant (**Figure 3.3**). This causes the water from the river to be polluted. The water from Blood River is used by local farmers downstream for irrigation of agricultural produce.

Untreated sewage leakage, improper waste management, farming, fishing and sand mining are the most common activities carried out around Blood River. The improper waste management is due to the increase in population (estimated population at 508 967 in 2016, Integrated Development Plan- Polokwane) which is attributed by rural-urban migration, as people moves from the rural areas to be close to the city where they can have access to job opportunities and improved social services and infrastructure (Integrated Development Plan- Polokwane, 2011/2016). Description of the sampling sites at Blood River is outlined in **Table 3.1**.

Table 3.1: Description of sampling sites at Blood River

Sampling site	Description
S1	Next to the road adjacent a crop farm, a close distance from Nelson Mandela drive
S2	Adjacent to Seshego Dam
S3	Adjacent to Seshego Dam
S4	Adjacent to illegal dumping site
S5	Adjacent to abandoned fishery around R567 road
S6	Adjacent to wastewater treatment plant
S7	Leaking untreated sewage from Seshego wastewater treatment plant
S8	Adjacent to the leaking sewage and illegal industrial dumping site
S9	Direct sand mining
S10	Next to Sand mine

Most rivers in South Africa like Blood River are facing challenges of water pollution due to sewage waste from treatment plants. These river water are used for irrigation of crops that are being consumed by animals and human-beings. **Figure 3.3** shows conditions of untreated sewage waste flowing into the River at sampling site 7.

Figure 3.3: Untreated sewage waste flowing into the River

3.1.2 Mokolo River

Lephalale is currently the fastest growing town in South Africa due to industrial and other economic developments accompanied by population growth and urbanisation. These factors are likely to have an effect on the Waterberg's water resources. The main rivers of the Waterberg municipality are Mokolo, Matlabas and Mogalakwena Rivers. These rivers are also tributaries to the main Limpopo River, which forms a border between South Africa and the following countries: Botswana, Zimbabwe and Mozambique. Mokolo River is the main catchment of Waterberg, which is considered to be the biggest in terms of mining activity in SA. Mokolo dam, the main impoundment of the Mokolo River, supply surface water to the Lephalale municipality and farmers for irrigation, who rely mainly on these river system (DWAF, 2012). The Mokolo River is therefore exposed to the following anthropogenic activities: acid-mine drainage, return flows from agriculture and discharge of sewage effluent. Sand mining is also one of the activities posing risk of water contamination in the Mokolo River.

Sampling at Mokolo River was conducted around Lephalale area at different sampling sites as outlined in **Figure 3.4**. The sand mining activity and water abstraction pump systems for irrigation purpose were the major activities taking place in the river observed during sampling.

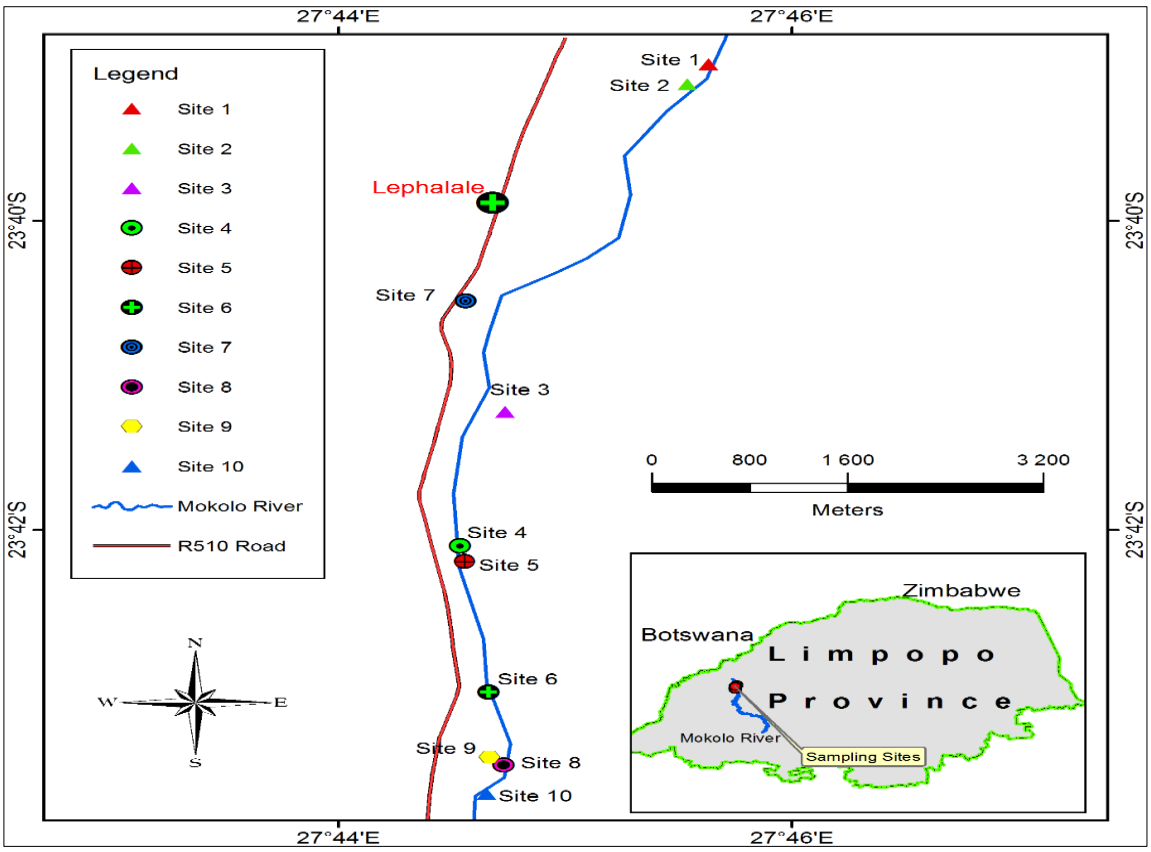

Figure 3.4: Sampling sites at Mokolo River

A photo showing one of the activities taking place in and around Mokolo River (Water abstraction System for irrigation) is indicated in **Figure 3.5.**

Figure 3.5: A photo depicting water abstraction system in Mokolo River for irrigation purpose

Irrigation for commercial farming, sand mining and fishing are the most common activities carried out around the Mokolo River. Summary of the description of the sampling sites at the Mokolo River is outlined in **Table 3.2**.

Table 3.2 Description of sampling sites at the Mokolo River

Sampling site	Description
S1	Irrigation pump and sand mining
S2	Under the bridge close to a farm
S3	Distant from the bridge
S4	Irrigation pumping system and cattle grazing
S5	Agricultural activity close to an industrial complex
S6	Direct irrigation pump and sand mining
S7	Under the bridge
S8	Fishing and swimming activities
S9	Sand mining
S10	Sand mining

3.2 Chemicals, standards and standard reference materials

High purity chemical reagents were used throughout this work. De-ionised water obtained from the laboratory ultra-pure water purification unit (Milli-Q® Reference, Merck) with a resistivity of 18. 2 MΩ.cm at 25 □C was used for preparation and dilution of calibration standards and samples. To obtain a complete digestion of sediments for the determination of the concentration of selenium, the following reagents were used: HNO_3 (puriss p.a., 65%, Sigma Aldrich), HF (40 – 45%, Sigma Aldrich) and H_2O_2 (puriss p.a., 30%, Sigma Aldrich).

Calibration standards used for the determination of total selenium and inorganic selenium species in water and sediment samples were prepared daily using 1000 mg/L Se(IV) and 1000 mg/L Se(VI) from Sigma Aldrich. Calibration curves were prepared in 1% pure HNO_3 for all the analysis of samples. The SPE procedure was carried out using Dowex 1 x 2 (chloride form, 50-100 mesh) as a sorbent material and HNO_3 (puriss p.a., 65%, Sigma Aldrich) as an elution solution.

Water samples were filtered through filtration system using nylon 66 filter membranes (pore size of 0.45 µm, diameter 47 mm obtained from Lasec (Pty) Ltd). Standard reference materials (SRMs) used for validation of the analytical methods for water and sediment samples were SRM 1643f of trace elements in water (NIST, USA) and SRM 2709a (San Joaquin Soil, Nist®).

3.3 Apparatus and Instrumentation

All the apparatus were cleaned with soap, rinsed with water, soaked in 10% HNO_3 and finally rinsed 3 times with de-ionised water and dried in the lab under ambient environment. The following instruments were used to carry out analysis in this study: (i) a Thermo Scientific 520A pH/mV/SE benchtop Meter (USA) was used to measure the pH, (ii) microwave digestion system 9 mars 5, CEM Corporation, United States of America) was used for the digestion of selenium in sediment samples, (iii) a model 13156 Vacuum pump (Gelman Instruments Company, Fort Wayne, Indiana, USA) was used to control flow rate and dry the column during SPE procedure, (iv) ICP-MS (Sciex Elan 6100, Perkin Elmer, Germany) for total concentration of selenium and (v) HPLC-ICP-MS for the determination of concentration of inorganic selenium.

3.4 Sample collection and pre-treatment

Water and sediment samples were collected from Blood River and Mokolo River. The samples were collected at 10 different points from uphill to downhill. Global Positioning System (GPS) coordinates was used at each sampling site to record the exact sample collection location. Clean polypropylene bottles were used to collect and store water and sediment samples. Water and sediment samples were collected at the same point at each sampling site. Sediment samples were collected with a plastic scooped spoon under the water surface. Samples were kept in a cooler box filled with ice blocks and transported to the laboratory. Water samples were filtered through 0.45 µm nylon 66 filter membranes and kept in a refrigerator at 4 □C.

Filtered water samples from each site were acidified with 1% (v/v) HNO_3 and kept in the refrigerator until analysis, for the determination of total concentration of water. Sediment samples were air-dried, ground to fine powder and sieved using a sieve of 100 µm pore size.

3.5 Sample Analysis

Water and sediment samples were analysed for the determination of total concentration of selenium and concentration of inorganic selenium species using suitable techniques.

3.5.1 Determination of total concentration of selenium in water and sediment samples

3.5.1.1 Water samples

Total concentrations of selenium in water samples were determined using ICP-MS. Acidified water samples were used for total concentration analysis.

A 1.0 mL of pure HNO_3 acid was added to 100 mL of filtered water samples and the samples were then quantified using ICP-MS. The parameters for ICP-MS are indicated in **Table 3.3**.

Table 3.3: The ICP-MS operating conditions

Parameters	**Settings**
Nebulizer gas flow	1.0 L/min
Auxiliary gas flow	1.2 L/min
Plasma gas flow	14 L/min
ICP RF Power	1400 W
Lens voltage	10 V
Analogue Stage Voltage	-2550 V
Pulse Stage Voltage	1050 V
Torch box temperature	30 □C
Cooling system:	
1. Main water temperature	18.0 □C
2. Interface water temperature	32.6 □C

3.5.1.2 Sediment samples

The finely ground sediment samples were digested using the CEM MARS 5 microwave digestion system shown in **Figure 3.6** for the determination of total concentration of selenium. Sediment samples were completely digested with 4 mL HNO_3, 2 mL H_2O_2 and 1 mL HF using microwave digestion system (Mars 5, CEM Corporation).

Figure 3.6: Microwave digestion system used for sediments digestion

A 250 mg of finely ground sediment samples were weighed and put in a clean Teflon vessel, digestion reagent (4 mL HNO_3, 2 mL H_2O_2 and 1 mL HF) were added, and the vessels were closed and kept at room temperature for 15 minutes to reduce pressure from organic reaction. The vessels were then put in the rotor, which was then placed in the microwave digestion system. The heating conditions of microwave digestion system are indicated in **Table 3.4.**

Table 3.4: Microwave digestion heating conditions

Parameters	Settings
Power	1350 W
Pressure	800 psi
Temperature	200 □C
Ramping time	10 min
Holding time	20 min

The digested samples were transferred into 50 mL calibrated centrifuge tubes and diluted to 30 mL mark with de-ionised water. The samples were then kept in the refrigerator until analysis. The samples were analysed by ICP-MS together with the blanks and SRM. The ICP-MS conditions used are indicated in **Table 3.3**.

3.5.2 Determination of the concentration of inorganic selenium species

3.5.2.1 Solid phase extraction method

The SPE method for pre-concentration and separation of inorganic selenium in water samples was achieved by the use of Dowex 1 x 2 (chloride form, 50-100 mesh) purchased from Sigma Aldrich. The SPE method is a five step method as shown in **Figure 2.4** involving selection of proper sorbent material, conditioning the sorbent material, loading of sample, washing the sample after loading, eluting the species of interest.

A 6 mm diameter SPE tube was used for the procedure. A small amount of cotton wool was put at the end of the SPE tube to avoid sorbent material to pass through. A 3.0 g of the Dowex 1 x 2 was added to the SPE tube. The SPE column was conditioned with 150 mL de-ionised water, 100 mL 1M HNO_3, 3M HNO_3 and finally with 50 mL de-ionised water to wash away any excess of HNO_3. The SPE setup is indicated in **Figure 3.7**.

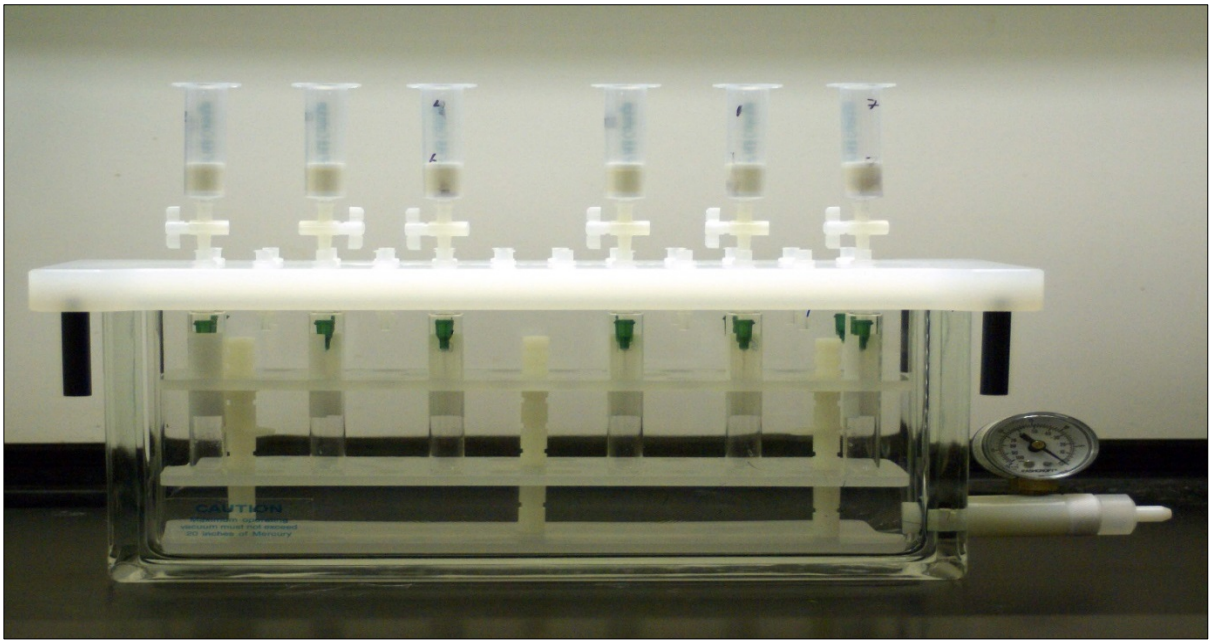

Figure 3.7: The SPE setup

A 100 mL water sample was passed through a packed column of the SPE setup shown in **Figure 3.7**. A 20 mL de-ionised water was passed through the column after the addition of the sample to remove the interferents. The SPE column was dried under vacuum before the elution step. The retained inorganic species, Se(IV) and Se(VI) in all the columns were eluted by 15 mL of 0.1 M HNO_3 and 15 mL of 1 M HNO_3, respectively. The eluents were then transferred into 50 mL calibrated vials, diluted to 20 mL mark with de-ionised water and kept in the refrigerator at 4 □C until analysis. The samples were then analysed using ICP-MS. The ICP-MS conditions used are indicated in **Table 3.3**. All water samples were adjusted using 0.5 M HNO_3 and 0.5 M NH_4NO_3 to a pH of 6.

3.5.2.2 HPLC-ICP-MS

Filtered water samples were transferred into vials and loaded to HPLC (Flexar Solvent Manager, Perkin Elmer) for inorganic selenium species separation coupled to ICP-MS (Sciex Elan 6100, Perkin Elmer) for the species quantification. The HPLC-ICP-MS setup used is shown in **Figure 3.8**. Mobile phase was prepared by dissolving appropriate amount of NH_4NO_3 in de-ionised water. Mobile phase was adjusted using NH_3 solution to a pH of 8.5. Mobile phase was then loaded to HPLC operating under the conditions detailed in **Table 3.5**.

Table 3.5: HPLC-ICP-MS operating conditions for separation and detection of inorganic selenium species.

ICP-MS Conditions	
ICP RF Power	1 400 W
Plasma gas flow	14 L/min
Auxillary gas flow	1.2 L/min
Nebulizer gas flow	1.0 L/min
Lens voltage	10 V
Isotope monitored	^{78}Se and ^{82}Se
HPLC Conditions	
Analytical Column	Hamilton PRP-X100 (4.6 x 250 mm, 5 µm)
Mobile phase	NH_4NO_3
Mobile phase composition	100 mM
Mobile phase pH	8.5
Mobile phase flow rate	1 ml/min
Injection volume	1.00 µL
Run time	8 min

3.6 Analytical method validation

Methods for total concentration determination of selenium and speciation of inorganic selenium were validated using the analytical figures of merit such as LOD, LOQ and linearity. The accuracy of the methods was checked using standard reference materials.

3.6.1 LOD and LOQ determination

Reagent blanks were prepared by following the same procedure for total concentration determination of selenium and speciation analysis of inorganic selenium in water and sediment samples. Reagent blanks were analysed by ICP-MS using the same ICP-MS conditions stated in **Table 3.3**. Standard deviations were calculated from the concentration of reagent blanks. The LODs were calculated as three (3) times the standard deviation of the mean of the eight (8) reagent blanks and LOQs as ten (10) times the standard deviation of the mean of the eight (8) reagent blanks.

3.6.2 Linearity and calibration curves

An intermediate standard solution of 10 mg/L of Se(IV) was prepared each day of analysis by pipetting 1 mL of 1000 mg/L of Se(IV) into a 50 mL centrifuge tube and diluting it to the mark with de-ionised water. Series of calibration standards were made from an intermediate standard solution. A six-point calibration curves were prepared by pipetting 0.00 (Blank), 25 µg/L, 50 µg/L, 100 µg/L, 150 µg/L and 200 µg/L from intermediate standard into 50 mL calibrated centrifuge to prepare 0.00 µg/L (Blank), 5.00 µg/L, 10.00 µg/L, 20.00 µg/L, 30.00 µg/L and 40.00 µg/L standards, respectively. In each standard, 550 µg/L of pure 65% HNO_3 were added prior to filling the tube with de-ionised water to the mark. The blanks were prepared by adding 550 µg/L of pure HNO_3 into 50 mL calibrated centrifuge tubes and diluted to the mark with de-ionised water. The intensity values for each standard were used to plot the calibration curves.

3.6.3 Accuracy, precision and monitoring of instrument performance

Analytical procedures were validated to ensure accuracy and precision of the results. Accuracy for the procedure of the total concentration of selenium in water samples was verified using SRM 1643f and for total concentration of selenium in sediments, SRM 2709a was used. The SRMs were prepared the same way as the sample preparation procedures for water and sediments. The obtained results of the SRMs were compared with the certified values to assess percentage recoveries.

The SPE procedure was validated by using the solution of known concentration of selenium species followed by the percentage recovery studies. All the procedures were done in triplicate. The mixed standard solutions of 5 µg/L of Se(IV) and Se(VI) were prepared in 100 mL volumetric flask. The solution was then adjusted with 0.5 M HNO_3 and 0.5 M NH_4NO_3 to a pH 6. The mixed solution of Se(IV) and Se(VI) was then passed through the conditioned column. The Se(IV) was eluted using 15 mL of 0.1 M HNO_3, Se(VI) was eluted using 15 mL 1 M HNO_3 and analysed using the ICP-MS**.** The percentage recoveries were assessed for method validation.

The HPLC-ICP-MS method was validated using SRM 1643f and the percentage recoveries were calculated to check the accuracy of the method.

The performance of analytical instrument (ICP-MS) was monitored by analysis of initial calibration blank (ICB) and initial calibration verification (ICV) solution at the start of the running of the samples. After running 10 complete set of samples, a continuous calibration blank (CCB) and continuous calibration verification (CCV) solution were analysed. All the analysis of samples was done in triplicate to check the precision of the results in terms of percentage relative standard deviation (% RSD).

Chapter 4: RESULTS AND DISCUSSION

This chapter details the results and discussion from all the experiments and procedure described in Chapter 3. The main purpose of the dissertation was to speciate inorganic Se(IV) and Se(VI) in water and sediment samples from Blood and Mokolo River. The total concentration of selenium and inorganic species from Blood and Mokolo River were determined and discussed. The chapter also includes comparison of the obtained results with other studies.

4.1 The pH measurement

Rivers are open systems where the pH of the water changes regularly. The organisms that depend on river water require some stability of the water pH or some reasonable tolerance. Quality of river water is measured by several indicators including pH. The pH level plays an important role in metal toxicity. Solubility of toxic metals increases with the decrease in pH (from surface to depth, from alkaline to acid) (Radulescu *et al.*, 2014). In general, water with a pH lower than 7 is acidic and with a pH greater than 7 is basic. The normal pH of surface water is 6.5 to 8.5. The highest desirable level according to PCRWR is 7 to 8.5. Unfortunately, the pH of river water can be changed by anthropogenic activities such as acid-mine drainage (AMD), agricultural run-off, fossil fuel emissions, industrial effluent discharge and improper waste management. Low pH levels cause organisms like fish to be more vulnerable to diseases (Rogers, 2009).

A pH level of the water is measured by using pH meter which should be calibrated with standard buffer solution of pH 4, pH 7 and pH 10 (South African Water Quality Guidelines, 1996). The generally accepted pH for irrigation water is between the range of 5.5 and 7.5. (Department of Primary Industries, 2011).

The pH values for this study were observed to be slightly lower at site 6 of Blood River where pH is 5.98. The value is below the normal pH range of the surface water but it is acceptable as a pH for irrigation water. Site 9 of Mokolo River is also lower than the acceptable pH range of 6.5- 8.5 with the pH value of 4.55.

The low pH value at site 6 of the Blood River may be due to stagnant water, possible rock formation, whereas the low pH value of the Mokolo River maybe due to the fact that the river passes through the vicinity of the Grootegeluk Coal Mine. The water from the coal mine is very acidic and it decreases the pH of the river water (Chen *et al*., 2007).

The highest pH is observed at site 8 of the Blood River with a pH of 8.21. This may be attributed to a pipe leakage of effluents from Seshego wastewater treatment plant. The pH of the water greater than the acceptable limit (6.5 to 8.5) as recommended by PCRWR is observed at site 10 of the Mokolo River. The water is not recommended for irrigation purpose and not good for consumption. All the other sites have the acceptable pH values of between 6.5 and 8.5. **Figure 4.1** shows pH measurements in water samples from Blood and Mokolo Rivers.

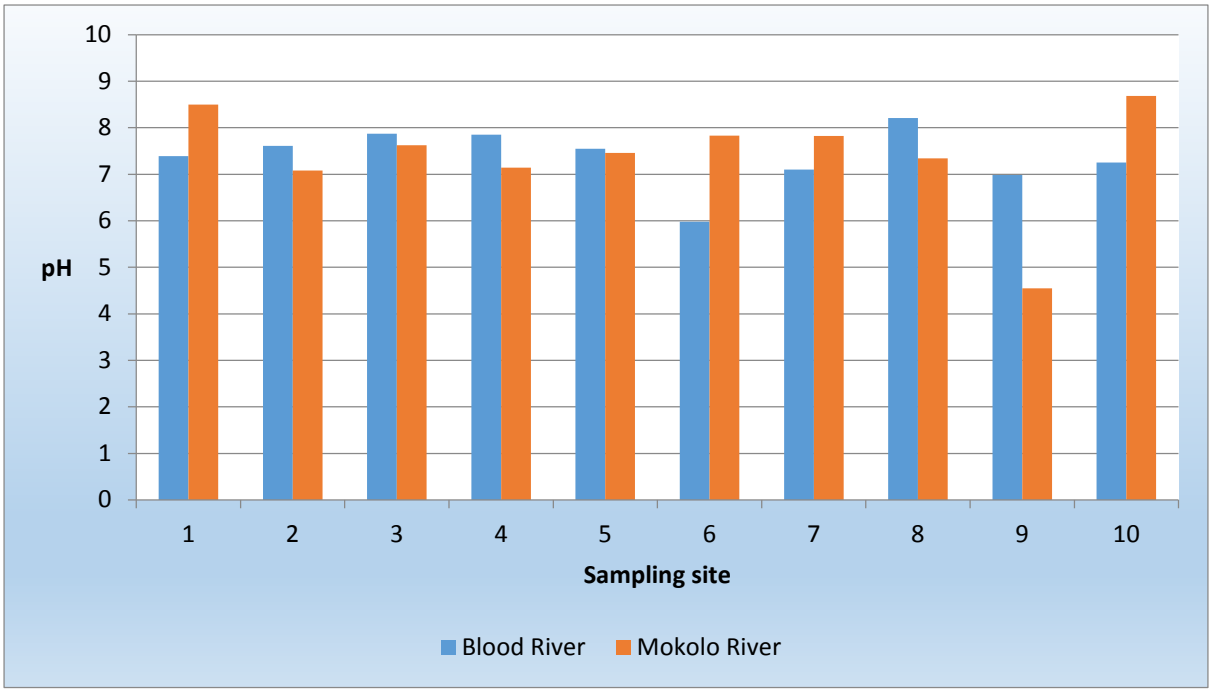

Figure 4.1: pH measurements in water samples from the two rivers

4.2 Temperature measurements

Temperature readings were taken from morning to afternoon, from site 1 to site 10 and during low rainfall season. The highest water temperature measured is 28.4 °C from Blood River and the lowest being 19.3 °C from Mokolo River as presented in **Table 4.1**. The temperatures of inland waters in South Africa range from 5 – 30 □C (DWAF, 1996c) and all the recorded water temperatures fall within suggested ranges during the study.

Table 4.1: Water temperatures from Blood and Mokolo Rivers

Temperature, □C	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Blood River	21.9	21.5	23.9	23.9	23.9	24.4	24.9	27.2	27.6	28.4
Mokolo River	23.9	21.9	21.3	22.6	22.8	19.3	24.7	25.8	23.1	22.9

The temperature of water varies from site to site in both rivers. Thus, the temperature is constantly changing sometimes depending on the time of the day. A higher temperature affects a lot of processes like the amount of dissolved oxygen and toxicity of certain elements. Higher temperatures decrease the solubility of dissolved oxygen in water, decreases the concentration of nutrients and its availability to aquatic organisms as it affects the plant's ability to grow (DWAF, 1996c).

4.3 Validation of analytical methods

Analytical figure of merit such as LODs, LOQs and linearity of calibration curves are useful in method validation. These analytical figures of merit show performance characteristic of the speciation of inorganic selenium species and for the determination of total concentration of selenium.

4.3.1 The LODs and LOQs for the total determination of selenium.

The LOD and LOQ were obtained from analysis of the reagent blanks prepared similar to the water and sediment samples for the determination of total concentration. For water samples, the blank solution of 1% (v/v) ultra-pure HNO_3 solution pure water was prepared. The blank solution for sediment samples was prepared by digestion method defined in Chapter 3. The LOD was calculated as 3 times the standard deviation of the measured concentration of the reagent blanks while the LOQ was measured as 10 times the standard deviation of the reagent blanks.

The LODS and LOQS for total concentration of selenium in water and sediment samples are indicated in **Table 4.2.**

Table 4.2: The LODS and LOQS for analytical procedure for the total concentration of selenium in water and sediments using ICP-MS.

Analyte (Se)	Water (µg/L)	Sediments (ng/g)
LOD	0.008	0.057
LOQ	0.027	0.189

Vanhoof *et al.* (2016) reported LOD of 0.040 µg/L and LOQ of 0.134 µg/L for water samples using ICP-MS. Manecki *et al.* (2017) -Thermo Fischer Scientific, reported LOD of 2.018 ppt of sediment samples derived from the regent blanks by using solutions 1 mL HNO_3, 1.5 mL HF and 1.5 mL $HClO_4$ and quantification using ICP-MS. Pinho *et al.* (2005) also reported a low detection limit of 0.030 µg/g for the determination of selenium in sediments by acid digestion and quantification by ICP-MS using ^{82}Se isotope. The LOD and LOQ for water analysis in this study is lower than those reported which may be attributed to different instrumental setup and different isotopes used. The LOD and LOQ for sediments are higher than those reported because of the different acid digestion used.

4.3.2 Linearity of calibration curves for the total concentration of selenium

To determine the relationship between an instrument response and known concentration of the analyte (standards), the simplest regression model called linearity is used. The linearity is tested using the calibration curve. To obtain a good calibration curve, a series of replicates of the expected range of concentration value for each standard are used including the blank. The regression coefficient should be closer to 1 (Moosavi *et al.*, 2018) where a minimum of 5 standards are recommended for linearity (Chandran *et al.*, 2007).

The linear standard calibration curve used for the determination of the total concentration of selenium is indicated in **Figure 4.2**. The calibration curve was obtained using ICP-MS and it is linear with correlation coefficient, R^2, of 0.9999.

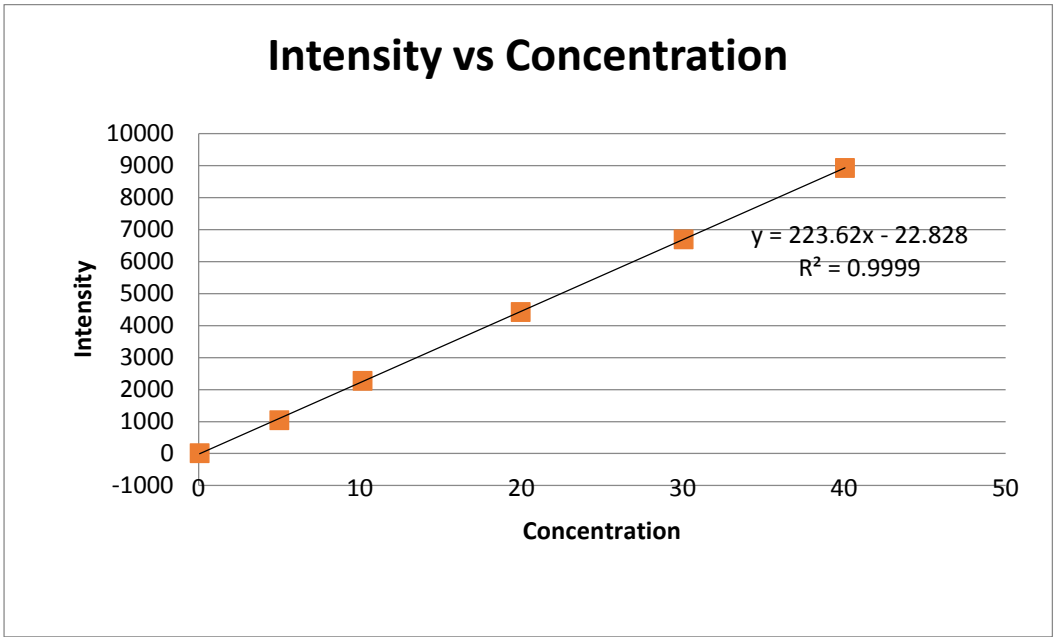

Figure 4.2: Calibration curve used for the determination of the total concentration of selenium

The, R^2 is 0.9999 for the determination of total concentration of selenium and it shows excellent linearity.

4.3.3 The LODs and LOQs for speciation of inorganic selenium in water using SPE

Peng *et al.* (2015) reported LOD of 0.016 µg/L for Se(VI) using SPE procedure as a separation/preconcentration method and ICP-MS for a quantification. Lin (2007) used the same sorbent material on SPE procedure as in the current study. Lin (2007) reported LOD of 0.0056 µg/L for both Se(IV) and Se(VI). The LODs for this study is 0.192 µg/L Se(IV) and 0.641 µg/L Se(VI), these LODs are the highest as compared to the reported LODs based on the same method. The higher values might be attributed to instrumental setup and conditions. The LODs and LOQs for the SPE procedure for the speciation of inorganic species of selenium are indicated in **Table 4.3**.

Table 4.3: The LODs and LOQs for the SPE procedure in water samples

Analyte	LOD (µg/L)	LOQ (µg/L)
Se(IV)	0.192	0.641
Se(VI)	0.108	0.361

4.3.4 The LODs and LOQs for speciation of inorganic species using HPLC-ICP-MS

Afton *et al. (*2008) reported the LODs of 0.321 µg/L for Se(IV) and 0.312 µg/L for Se(VI) when speciating using HPLC-ICP-MS. Tonietto *et al. (*2010) reported the lowest LODs of 0.081 µg/L for Se(IV) and 0.075 µg/L for Se(VI) as compared to the LODs in this study when using the same mobile phase and instruments. The difference in the LODs might be due to the usage of different column. Tonietto *et al*. (2010) used MetrosepASupp 10-250/4.0 while in this study Hamilton PRP-X100 (5 µm 4.6 x 250 mm) was used. The LODs and LOQs for selenium speciation in water samples are indicated in **Table 4.4**.

Table 4.4: The LODs and LOQs for selenium speciation in water samples using HPLC-ICP-MS

Analyte	LOD (µg/L)	LOQ (µg/L)
Se(IV)	0.842	2.81
Se(VI)	0.690	2.30

4.3.5 Linearity of calibration curves for speciation of inorganic species using HPLC-ICP-MS

The standard calibration curves for selenium species were assessed in the concentrations ranging from 2 µg/L to 30 µg/L. The linear equation and R^2 value for Se(IV) and Se(VI) are indicated in **Figure 4.3** and **Figure 4.4,** respectively. The R^2 is 0.998 for both Se(IV) and Se(VI) and it falls within the requirements which is closer to 1.

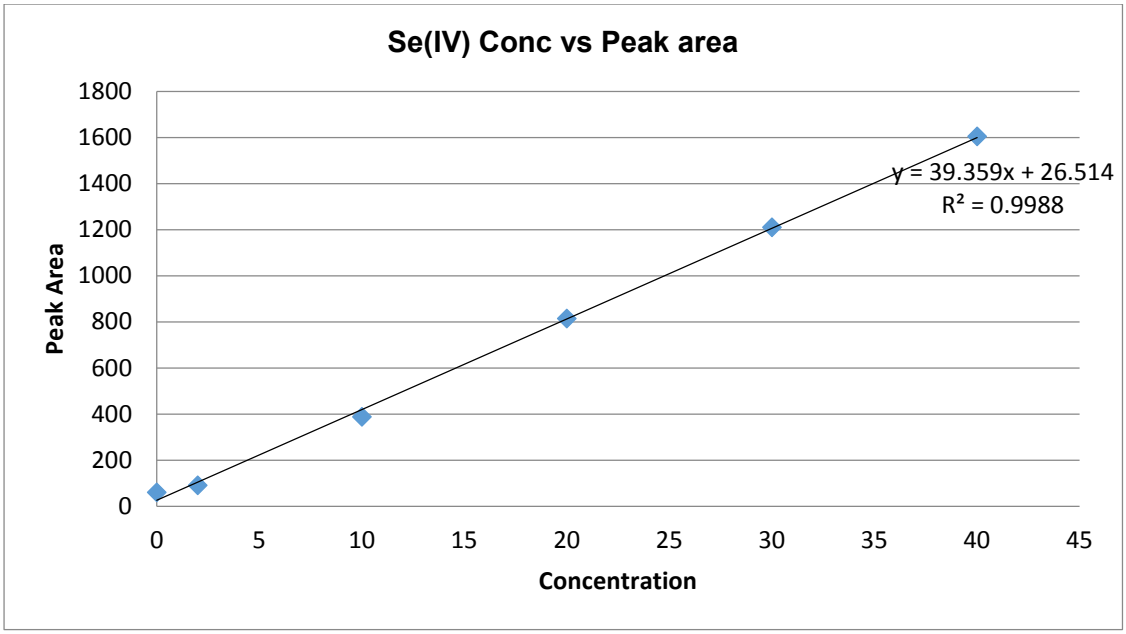

Figure 4.3: Calibration graph for Se(IV)

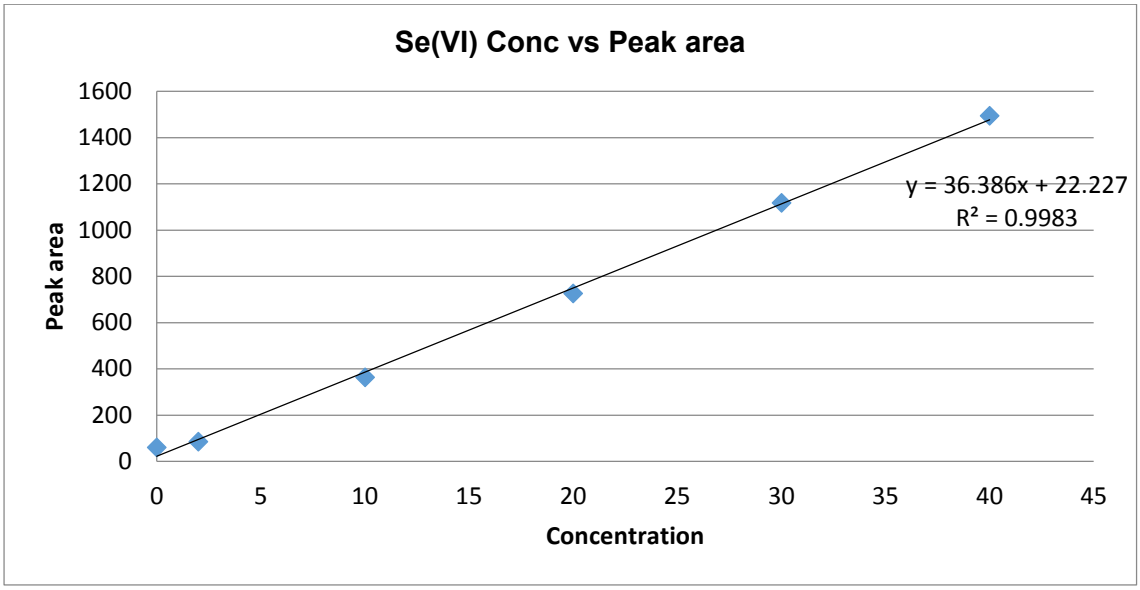

Figure 4.4: Calibration graph for Se(VI)

4.4 Accuracy and Precision of the analytical method

Method validation is a procedure used to confirm the quality, reliability and consistency of the analytical procedure. Tests related to method validation were performed and the results of each procedure are outlined below.

4.4.1 Accuracy of analytical procedures for determination of total concentration of selenium

The method validation for the quantification of total concentration of selenium in water and sediment samples was validated using SRMs for water and sediments, respectively. The analytical procedure for water sample analysis was validated using SRM 1643f and detected by ICP-MS. The analytical procedure for sediment sample analysis was validated using SRM 2709a. These SRMs were prepared the same way as samples were prepared. The results for the method validation for total concentration of selenium in water and sediment samples are outlined in **Table 4.5**.

Table 4.5: Validation of analytical procedures for total concentration of selenium in water and sediment samples

Analyte (Se)	Water samples (µg/L)	Sediment samples (ng/g)
Measured value	10.3	1.57
Certified value	11.700	1.5
Percentage recovery	88.1	105

The results showed quantitative recoveries of 88.1% and 105% for both water and sediment samples, respectively. These values are consistent with the recommendations of USEPA guidelines for method development and validation (US EPA, 2010). Therefore, the analytical procedures for quantification of total concentration of selenium for both water and sediment samples were validated.

4.4.2 Validation of solid phase extraction (SPE) procedure for speciation of inorganic selenium

The analytical procedure for SPE was validated using solution of known concentrations and recovery procedures. The procedure was also validated using SMR 1643f. These method validation solutions were treated the same way as samples to ensure reliability and accuracy of the analytical procedure. The results for the validation of SPE procedure for speciation of inorganic selenium are indicated in **Table 4.6** and **Table 4.7**. The influence of sample pH on the adsorbent material was also investigated.

Table 4.6: Validation of SPE procedure using solution of known concentration

Analyte	Measured value (µg/L)	Known value (µg/L)	Percentage recovery
Se(IV)	5.18	5.00	104
Se(VI)	2.67	5.00	53

Table 4.7: Validation of SPE using SRM 1643f

Analyte	Measured value (µg/l)	Certified Value (µg/l)	Percentage recovery
Se(IV)	9.04	11.700	77
Se(VI)[b]	-	-	-

[b]The SRM 1643f does not contain Se(VI)

Influence of pH and the recovery studies on SPE method was investigated using 5 µg/L of each inorganic species within the range of 2.0 to 10.0. The 5 µg/L of Se(IV) and Se(VI) were loaded on to SPE column containing 3 g of conditioned Dowex 1 x 2 (chloride form) sorbent material. The elution was performed with 0.1 M HNO_3 for Se(IV) and 1 M of HNO_3 for Se(VI) of 5 µg/L. The data corresponding to each species is shown in **Figure 4.5**. Maximum percentage recovery was obtained at pH greater than 5 (95%-114%) for Se(IV) and pH of 6 (53%) for Se(VI). Low percentage recovery was obtained for Se(VI) as compared to the one reported by Lin (2007). The pH of 6 was selected as optimum as it yields an excellent percentage recovery for Se(IV) and an average recovery for Se(VI). Percentage recovery for Se(VI) is lower as compared to the recommended one by EPA. (2010b) but Shabir stated that low percentage recovery is acceptable depending on the needs of the method.

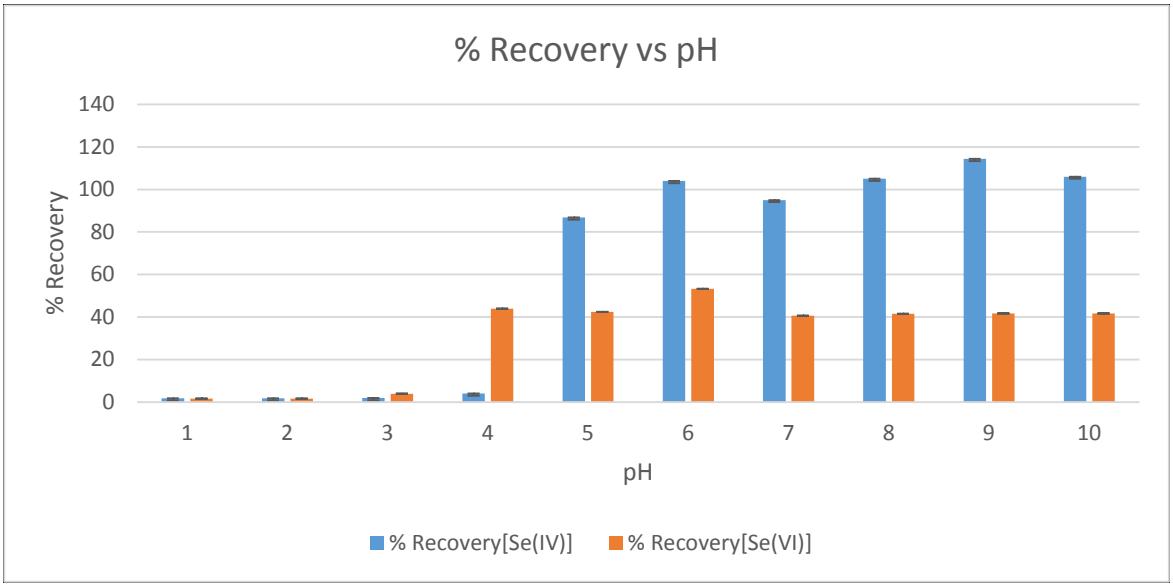

Figure 4.5: Effect of sample pH on the percentage recovery of Se(IV) and Se(VI) using the Dowex 1 x 2 (Chloride form) in SPE

4.4.3 Validation of analytical procedure for HPLC-ICP-MS speciation of water samples

The analytical method for speciation of selenium using HPLC-ICP-MS was validated using SRM 1643f. The SRM 1643f is certified as 11.70 ± 0.081 µg/L Se. All selenium in SRM 1643f being present as Se(IV), was confirmed by HPLC-ICP-MS. The concentration for the Se(IV) and percentage recovery were calculated and the results are indicated in **Table 4.8**.

Table 4.8: Validation of HPLC-ICP-MS using SRM 1643f

Analyte	Measured value (µg/L)	Certified value (µg/L)	Percentage recovery
Se(IV)	9.04	11.70	77.26

The analytical results obtained show a good percentage recovery of 77.26%. The method for the speciation of Se(IV) and Se(VI) using HPLC-ICP-MS will also produce reliable and accurate results based on the outcomes of the method validation results obtained using SRM 1643f.

4.5 Speciation of inorganic selenium using HPLC-ICP-MS

The HPLC-ICP-MS analytical procedure based on external calibration method was employed for quantification of inorganic selenium species in water samples. Prior to the determination of inorganic selenium species in water samples, standard selenium solutions were used to test the sensitivity of the speciation technique using HPLC-ICP-MS to identify retention time for each species. The results for the single injection of a 20 µg/L Se(IV) and Se(VI) are shown in **Figures 4.6** to **4.7.**

Retention time is a measure of the time taken for a solution to pass through a chromatography column. In simple terms, it measures the time taken from injection to detection. Retention time might change irrespective of using same chromatography column and same instruments. Factors such as pump pressure, flow rate, composition and pH of the mobile phase can influence the retention time to shift. Chromatography column in HPLC separates the two inorganic selenium species and ICP-MS produces a chromatogram with the retention time. For this study, the chromatogram will be used to calculate concentration of each inorganic specie.

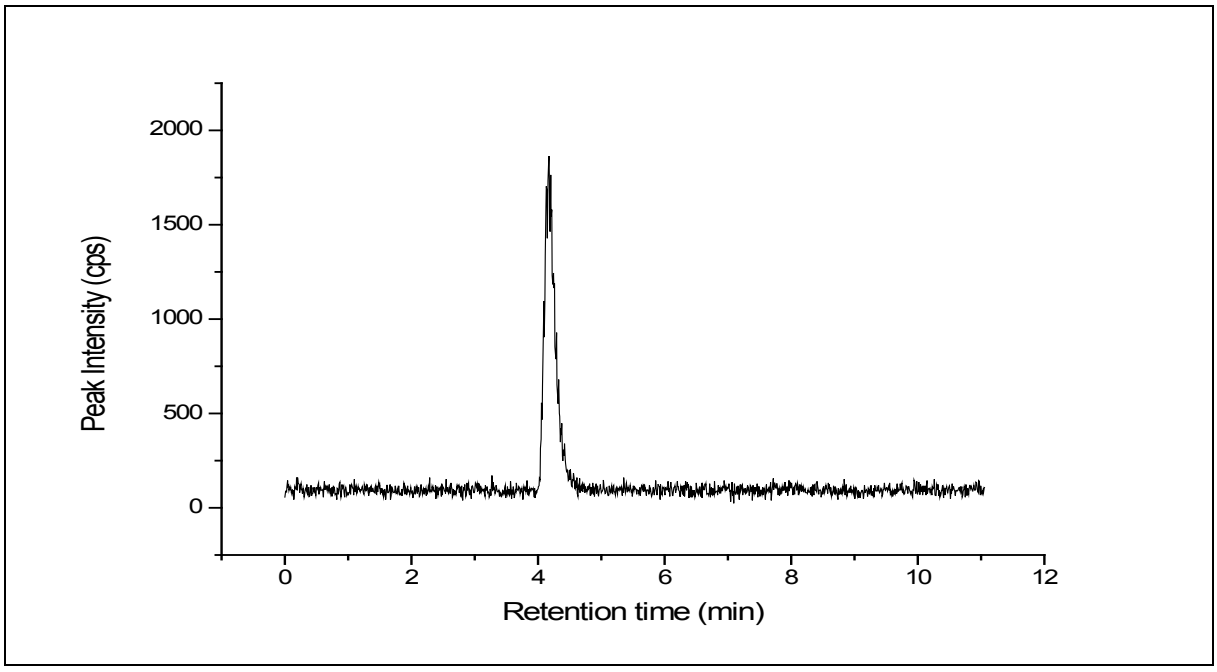

Figure 4.6: Chromatogram for Se(IV), 20 µg/L; Column, Hamilton PRP-X100 column (6.4 x 250 mm, 5 µm); Mobile phase, 100 mM NH_4NO_3; sample injection, 100 µg/L.

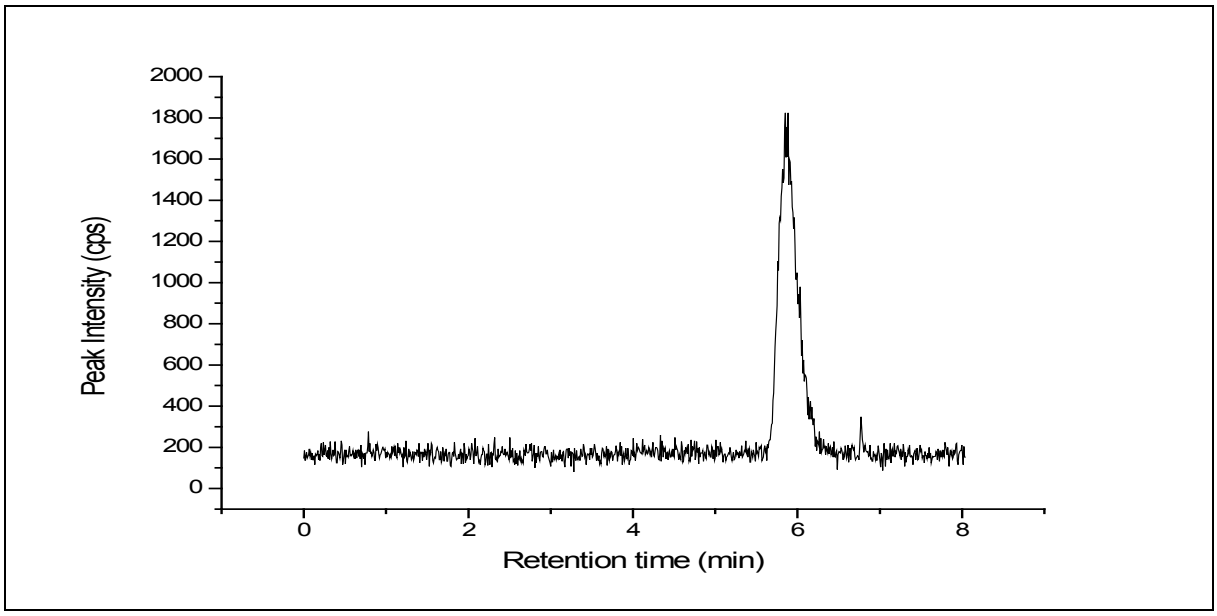

Figure 4.7: Chromatogram for Se(VI), 20 µg/L; Column, Hamilton PRP-X100 column (6.4 x 250 mm,5 µm); Mobile phase, 100 mM NH_4NO_3; sample injection, 100 µg/L.

The mixture of Se(IV) and Se(VI) standards was injected into HPLC column under the same operating conditions as the single standard injections to evaluate the compliance of the retention time in relation to each inorganic species, and the species were able to separate under the same retention time. The less retained Se(IV) was eluted before the strongly retained Se(VI). The chromatogram indicating the separation of the two inorganic species is presented in **Figure 4.8**.

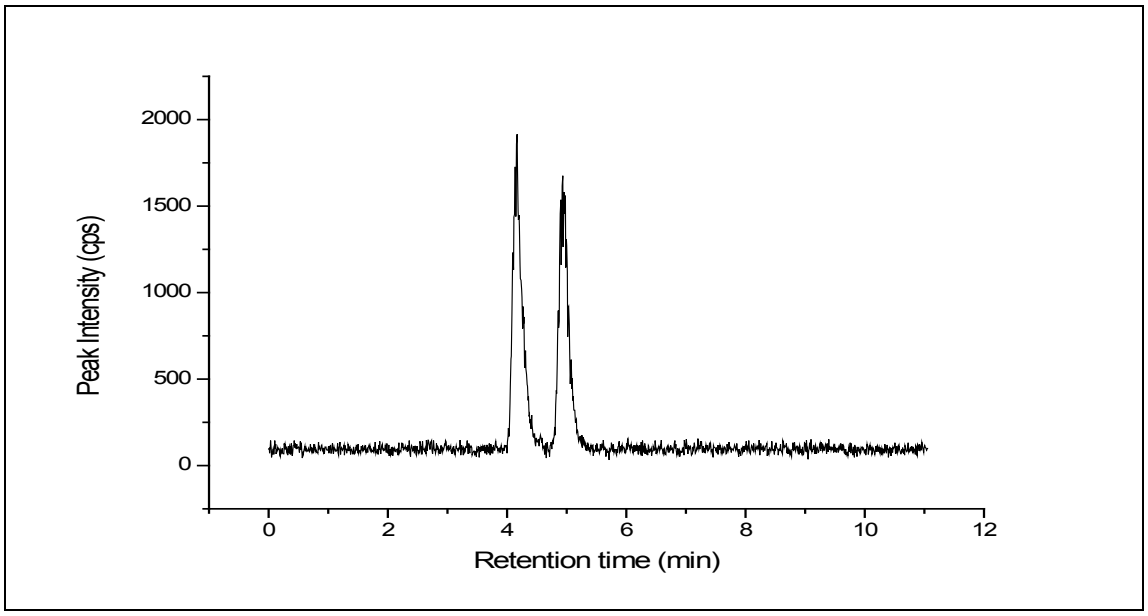

Figure 4.8: Chromatogram for mixed standards, Se(IV) and Se(VI), 20 µg/L; Column, Hamilton PRP-X100 column (6.4 x 250 mm,5 µm); Mobile phase, 100 mM NH_4NO_3; sample injection, 100 µg/L.

The peak intensities of both Se(IV) and Se(VI) were examined using a series of mixture of standards at different concentrations. Mixed standards containing equimolar amounts of Se(IV) and Se(VI) were analysed in the ranges of 2 µg/L to 30 µg/L selenium. It was observed that the peak intensity increases as the concentrations of the analytes increases. The chromatogram showing peak intensities at different concentrations and their retention time corresponding to the tested inorganic species are indicated in **Figure 4.9.**

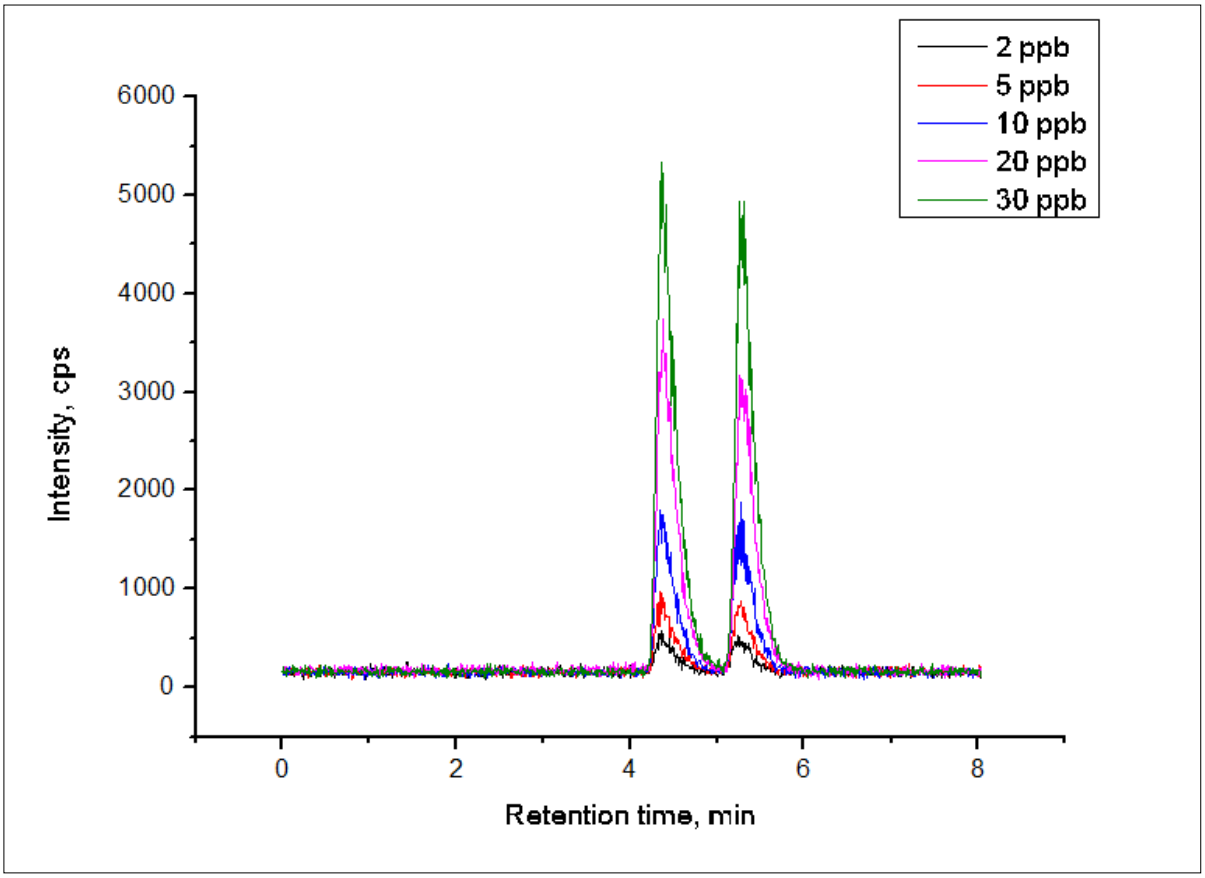

Figure 4.9: Peak intensities related to different concentrations of Se(IV) and Se(VI), 2-30 µg/L; Column, Hamilton PRP-X100 column (6.4 x 250 mm,5 µm); Mobile phase, 100 mM NH_4NO_3; sample injection, 100 µg/L·

4.5.1 Chromatographic conditions

Chromatographic conditions such as retention time, mobile phase and its pH were considered for peaks separation and resolution. In most selenium speciation, phosphate solutions are widely used because they give good chromatographic conditions (Yu *et al.*, 2019).

In this study, ammonium nitrate was chosen as a mobile phase because it has ionic strength necessary to elute the analytes without problems related to interferences of analytes determination, cones clogging and it does not produce a decrease in ionization efficiency of the hot plasma like when sodium is used as a counter cation (Martenez-Bravo *et al.*, 2001). Isocratic elution mode was used with high concentration of NH_4NO_3 (100 mM) at a pH of 8.5 adjusted by NH_3. High concentration resulted in shorter retention time of less than 10 min accompanied by good resolution. It has been reported by Martenez-Bravo *et al.* (2001) that at a pH less than 8.2, Se(IV) interfere with $^{40}Ar^{37}Cl$.

4.6 Total concentration of selenium in water samples

4.6.1 Quantification of selenium in water samples

The total concentrations of selenium in water samples from Blood and Mokolo Rivers are presented in **Table 4.9**. Total concentrations of selenium in water samples were determined using the validated analytical procedures. The water samples from each site were analysed in triplicates.

Table 4.9: Total concentrations of selenium (µg/L) in water samples from Blood and Mokolo Rivers

Name of the rivers	Site 1	Site 2	Site 3	Site4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
Blood	1.14 ± 0.070	1.03 ± 0.14	0.629 ± 0.061	0.678 ± 0.031	0.563 ± 0.030	0.0682 ± 0.020	1.77 ± 0.050	2.72 ± 0.020	1.48 ± 0.050	0.134 ± 0.050
Mokolo	0.309 ± 0.012	0.159 ± 0.0090	0.297 ± 0.0030	1.02 ± 0.031	0.115 ± 0.0030	0.109 ± 0.0070	0.0851 ± 0.0030	0.113 ± 0.012	25.4 ± 0.31	0.275 ± 0.021

The total concentrations of selenium in water samples from Blood River ranged from 0.0682 to 2.72 µg/L. Concentrations at all sites are above the LOD value of 0.0130 µg/L. The highest concentration of 2.72 µg/L was detected at Site 8, this value is higher than TWQR for selenium which is 2 µg/L. This concentration may be high due to waste discharged from Seshego Waste Treatment Plant to the river. Greenfield *et al.* (2012) reported level of selenium in the Nyl River, determined using ICP-MS ranging from 2.00 µg/L to 6.17 µg/L. These values are above the TWQR for selenium which is ≤2 µg/L. These values may be high due to increase use of pesticides in agricultural activities, increased use of fossil fuels in informal settlements and increased weathering of surrounding geology. The study by Shibambu showed lower concentrations of selenium (below instrument detection limit) in Mawoni River (Shamuyarira, and Gumbo, 2018).

The total concentrations of selenium in water samples from Mokolo River ranged from 0.0851 µg/L to 25.4 µg/L. These values are all above the LOD value of 0.0130 µg/L. Dabrowski *et al.* (2014) reported concentration of selenium at Flag Boshielo Dam, which flows into the Limpopo River at below instrument detection limits. Mahlatji (2014) also reported selenium concentrations at below instrument detection limit all seasons at Olifants River except during the spring season. A mean value of 0.1 mg/L was observed during spring. A highest concentration value of 25.4 µg/L is observed at site 9. This value is higher than TWQR value of 2 µg/L for selenium. The main activity taking place at site 9 is sand mining. Sand mining release waste which may contain elements hazardous to the aquatic ecosystem and pose risk to human health (Kosior *et al.*, 2015). High concentration at site 9 may be due to sand mining releasing selenium into the river.

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

Total concentrations of selenium found in water samples collected from Blood and Mokolo Rivers were assessed for safe drinking and irrigation with comparison to the standard guidelines established around the World and within South Africa. The levels of selenium in groundwater and surface water range from 0.06 to 400 µg/L (WHO/HSE/WSH/10.01/14, 2011).

Level of selenium in drinking water from the taps for public water supplies around the world are usually much less than 10 µg/L. High concentration of selenium was reported in China where the concentration ranges from 50-160 µg/L. This concentration was high due to the soil with high selenium concentration (WHO/HSE/WSH/10.01/14, 2011).

The WHO (2011) set a guideline for selenium in water at 0.04 mg/L. The MCLG and MCL for water by EPA (2001c) was set at 0.05 mg/L. South African drinking water guidelines has set its water quality range of selenium for domestic use at a range of 0.00 -0.02 mg/L. The TWQR for aquatic ecosystems in SA is 2 µg/L and 20 µg/L for drinking water (DWAF, 1996c; Mathebula, 2016).

All the samples from S1-S10 from both Blood and Mokolo Rivers, indicate concentrations below the MPLs set by WHO which is 40 µg/L (WHO, 2011). For irrigation water, selenium is toxic to plants at a concentration as low as 25 µg/L but the maximum concentrations recommended for trace elements in irrigation water is 20 µg/L (Gupta *et al.*, 2017). River water from all sites of both rivers is considered safe for irrigation and drinking purpose as far as selenium is concerned, with exception to site 9 of Mokolo River. Concentration of selenium at site 9 of Mokolo River is 25 µg/L, which is above the recommended concentration for trace elements in irrigation water. Therefore, water at site 9 is not recommended for irrigation purpose. However, the concentrations in most sites were found to be in acceptable range.

4.6.3. Comparison of trace element levels in water from different rivers

The selenium concentration was high in site 8 of Blood River as compared to all other sites within the river. Activities at site 8 of Blood River include leaking sewage and illegal industrial dumping. The water from Mokolo River has a high concentration of total selenium at site 9 as compared to all other sites within the River. Site 9 of Mokolo River is where sand mining activity is taking place. Levels of selenium in Blood River are a bit higher as compared to the ones in Mokolo River at most of the sites. Sites 1, 2, 3, 5, 7 and 8 of Blood River are higher as compared to similar sites of Mokolo River. Blood River as compared to Mokolo River has many anthropogenic activities taking place, which could contain selenium. Blood River has several illegal dumping sites as compared to Mokolo River.

Waste material such as broken glasses, building materials etc. may lead to elevated level of selenium concentration when in contact with the river water as some of them are made from materials containing selenium. There is a pipe leak of waste effluent directly into the Blood River which is expected to cause the concentration of selenium to increase. Concentrations at Mokolo River are mostly below 1 µg/L except site 9.

The presence of selenium may come from the use of pesticides during agricultural activities taking place around the river. **Figure 4.10** represent graphical total selenium concentrations in water from Blood and Mokolo Rivers.

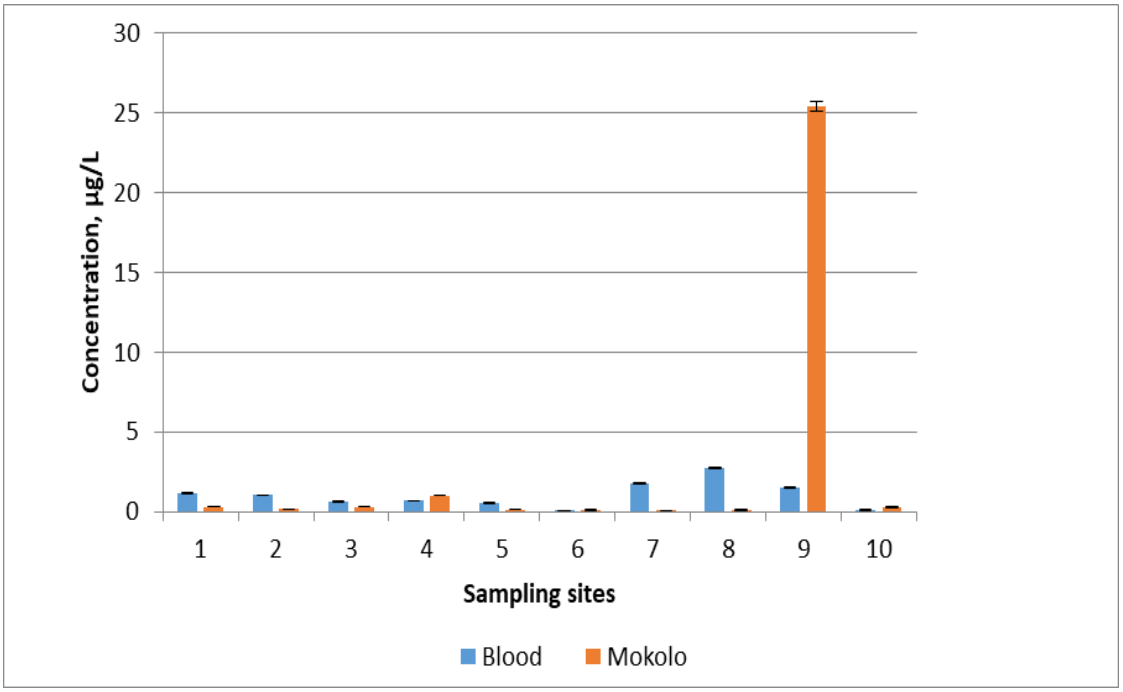

Figure 4.10: Graphical representation of selenium levels in the two rivers

The highest total selenium concentration of 2.72 µg/L and 25.4 µg/L were detected at Site 8 and Site 9 of Blood and Mokolo Rivers, respectively. The total concentrations of selenium differ from site to site due to variety of factors. The total concentrations of selenium in water on this study as compared to other studies done in South Africa are indicated in **Table 4.10.**

Table 4.10: Comparison of selenium levels in water of some rivers in South Africa

Element	Vaal Dam (Gilbert and Avenant-Oldewage., 2014)	Nyl River (Greenfield *et al.*, 2012)	Blood River (current study)	Mokolo River (current study)
Se (µg/L)	<0.253	(2.00-6.17)	(0.068-2.724)	(0.085-25.405)

4.7 Total concentration of selenium in sediment samples

4.7.1 Quantification of total selenium in sediment samples

Sediments can contain elevated levels of selenium due to anthropogenic activities. Selenium accumulation in sediments threatens the ecosystems (Hsu *et al.*, 2016). Accumulated selenium can enter the food chain as bottom sediments in river basin. Total concentrations of selenium in sediments need to be quantified to assess the quality of river water. In this study, total concentrations of selenium were quantified using ICP-MS.

The detected selenium concentration in both rivers were below the LOD value of <0.0571 ng/g. This is probably due to the fact that the soil in that area is not rich in selenium. Selenium that is detected in water does not settle down on the sediments, it flows with the river water. Currently, selenium cannot be detected in the sediments. However, it may accumulate in sediments in future as it can be detected in water.

Sediments are generally seen as a sink for many elements in an aquatic system. Hence, the study of sediment quality based on concentration of elements is extremely important and critical for the protection of the aquatic ecosystem health. Sediment quality has been regulated using sediment quality guidelines (SQGs). National irrigation water quality program guidelines for selenium are as detailed in **Table 4.11.**

Table 4.11: Level of toxicity of selenium in sediments

Selenium	No effect	Level of concern	Toxicity threshold
Concentration, mg/kg	<1	1-2	>2

Canton and van Derveer (1997) mentioned a sediment toxicity threshold of 2.5 µg/g (Hamilton *et al.*, 1999). Concentrations of selenium in Blood and Mokolo Rivers were found to be below the LOD value of 0.0571 ng/g. According to National irrigation water quality program guidelines, sediments from both Blood and Mokolo Rivers doesn't pose any threat to the aquatic system with respect to selenium levels.

Durowoju *et al. (*2016) reported selenium concentrations of [0.55-0.8] and [0.17-0.61] mg/kg in soil at Siloam and Tshipise springs in Limpopo province, respectively. Concentrations of selenium in sediments were also lower than the selenium levels in water samples collected from the sites of Blood and Mokolo Rivers. The spring water at Siloam and Tshipise is used for various domestic purposes like as a source of water for irrigation of subsistence farming. Manecki *et al.* (2017) also reported level of selenium in sediments below LOD (0.17 mg/kg) when using ICP-MS. Low levels of 0.17 mg/kg was also reported from sediments at Danube River by Dediu *et al.* (2016).

4.7.2 Comparison of selenium concentrations in water and sediment samples from Blood River

Blood River has been subjected to input of heavy metals and trace elements from both natural and anthropogenic sources. Water from Blood River receives waste almost every day from Seshego Waste Treatment Plant and waste from improper waste disposal. Some of this waste release pollutants that settle down on the sediments or flowing with the River water. Like most of the metals, elevated level of selenium in both water and sediments pose a risk to the aquatic ecosystems. Potential health risks are also posed to humans eating selenium-contaminated food. It is important to examine

selenium concentrations in water and sediments to test the potential of having elevated levels in the river (Hu *et al*, 2009).

Water and sediments analysis has proven that anthropogenic sources are the basic sources of selenium in Blood River because selenium can only be detected in water samples at all sites but not on sediments. Geologically based on the results obtained from sediments, it can be concluded that the soil in that area is not rich in selenium.

4.7.3 Comparison of selenium concentrations in water and sediment samples from Mokolo River

Mokolo River like Blood River faces almost the same challenges as far as water quality is concerned. Activities in Mokolo River include sand mining, farming, etc. To determine the existing level of risks related to metals from both natural and anthropogenic activities in the Mokolo River, it is important to determine concentrations of metals in both water and sediment samples. Quantification of selenium in both water and sediment samples is crucial. Selenium is essential trace element at low levels occurring naturally but its concentration can be elevated by man-made activities to becomes toxic (Gilbert *et al.*, 2014).

The results indicate the presence of selenium in lower concentrations which are below the detection limit in sediment samples as compared to the water samples. These results are similar to the results obtained from Blood River in this current study. The findings also suggest that the soil in Mokolo River area is not rich in selenium. The recorded selenium levels in both water and sediment samples from Mokolo River pose no threat to the aquatic ecosystems.

4.8 Speciation of inorganic selenium in water samples

The two main inorganic selenium in an aqueous system are Se(IV) and Se(VI) with Se(IV) being more toxic than Se(VI). Selenium (both natural and anthropogenic origin) enters the ecosystem predominantly as Se(IV) and Se(VI) (Gennari *et al.*, 2014). Speciation need to be done in order to determine the different inorganic species in water samples.

4.8.1 Determination of Se(IV) and Se (VI) in water samples using SPE

4.8.1.1 Levels of Se(IV) and Se(VI) in Blood River water samples using SPE

Selenium in waters occurs in higher concentration mainly from anthropogenic activities. Blood River water faces challenges of accumulating more Se(IV) and Se(VI) due to activities happening around the river. Selenite is the main selenium valence for water soluble selenium and the most toxic inorganic selenium. The Se(IV) occurs naturally in a trace amount but its concentration can increase due to anthropogenic activities. The concentrations of Se(IV) in Blood River using SPE range from 0.0411 to 0.820 µg/L. Lin (2007) reported low concentration of 0.0168 µg/L when using the same SPE method and GF-AAS for quantification. The difference in Se(IV) concentrations might be due to different environmental conditions. The highest Se(IV) concentration was detected in site 10. This might be due to sand mining activity taking place next to the river.

High concentrations of Se(VI) can occur in agricultural drainage water in arid areas. Irrigation can elevate the levels of Se(VI) in drainage water. Most of the selenium in the groundwater are present as Se(VI). In general, Se(VI) is more available and more mobile than Se(IV) in the environment (Plant *et al.*, 2003). The study by G. A. Gutter and L. S Gutter. (2001) reported a higher concentration of Se(VI) in marine waters than Se(IV). The Se(VI) is easily leached from the soil to ground waters (Wuilloud *et al.*, 2014). The concentrations of Se(VI) in Blood River using SPE ranges from 0.0811 to 1.75 µg/L. The results for Se(IV) and Se(VI) from Blood river are indicated in **Table 4.12**.

Table 4.12: Concentrations (µg/L) of Se(IV), Se(VI) and total selenium in water samples of Blood River

Site	Se(IV) ± SD	Se(VI) ± SD	Se ± SD	%Se(IV)	%Se(VI)
1	0.323±0.0091	0.966±0.042	1,29±0,070	25	75
2	0.434±0.0071	0.958±0.017	1,39±0,014	31	69
3	0.193±0.017	0.524±0.0081	0,717±0,061	27	73
4	0.0512±0.010	0.476±0.0080	0.526±0,031	10	90
5	0.163±0.012	0.502±0.0081	0,664±0,030	24	76
6	0.0411±0.0012	0.0811±0.0062	0,122±0,020	34	66
7	0.385±0.010	1.35±0.0071	1,73±0,050	22	78
8	0.333±0.0071	1.75±0.047	2,09±0,020	16	84
9	0.358±0.020	1.21±0.014	1,57±0,050	23	77
10	0.820±0.052	1.29±0.014	2,11±0,050	39	61

The proportion of Se(IV) to total concentration of selenium ranges from 10 to 39%. This indicates the presence of low concentrations of Se(IV) in water with reference to total selenium concentration. Lin (2007) reported a higher percentage of 38 to 52% of Se(IV) as compared to the % reported in this study. The highest concentration of Se(IV) was reported by Nyaba *et al. (*2016) in the surface water collected around Soweto area. The concentrations were ranging from 0.631 to 84.0 µg/l. High percentage of Se(IV) as reference to the total concentration of selenium was also reported. These percentages are higher than the ones obtained in this study. The highest concentration is obtained in site 8 which is 1.75 µg/L. This concentration may be high due to illegal dumping of industrial wastes and sewage leakage from Seshego sewage treatment centre. This concentration is still lower than the set limit of 2 µg/L inorganic selenium in water.

The percentages of Se(VI) in water samples from Blood River from all sites are higher than those of Se(IV). The percentages of Se(VI) ranges from 66 to 90%. These higher percentages indicate Se(VI) being in a dominant position as compared to Se(IV) in Blood River water samples. Higher percentage of 92% Se(VI) was reported by Gao *et al.* (2000) in California (Tulare Lake Drainage District). Yee (2012) reported a higher concentration of Se(VI) in municipal wastewater discharge as compared to concentration of Se(IV) where Se(VI) was often 70-90% of the total dissolved selenium. Selenate, Se(VI) concentrations at all sites of Blood River are still lower and doesn't pose any threats to the environment.

4.8.1.2 Levels of Se(IV) and Se(VI) in Mokolo River water samples using SPE

Selenite, Se(IV) and Selenate,Se(VI) concentrations at all sites of Mokolo River in water samples were analysed and the results obtained are shown in **Table 4.13**. Concentrations differ from site to site depending on the activities happening at each site.

Table 4.13: Concentrations (µg/L) of Se(IV) and Se(VI) in water samples of Mokolo River

Site	Se(IV) ± SD	Se(VI) ± SD	Se ± SD	%Se(IV)	%Se(VI)
1	0.157±0.018	0.859±0.0040	1.02±0,012	15	85
2	0.280±0.010	0.695±0.011	0.974±0,0090	29	71
3	0.619±0.015	1.10±0.032	1.72±0,0030	36	64
4	0.590±0.027	2.34±0.016	2.93±0,031	20	80
5	0.174±0.013	0.350±0.0090	0.523±0,0030	33	67
6	0.216±0.0041	0.272±0.0080	0.488±0,0070	44	56
7	0.135±0.012	0.167±0.0050	0.302±0,0030	45	55
8	0.178±0.0071	0.521±0.029	0.699±0,012	25	75
9	2.79±0.013	14.8±0.27	17.6±0,31	16	84

10	0.196±0.0012	0.0961±0.0060	0.292±0,021	67	33

The percentage of Se(IV) in water samples from Mokolo River ranges from 15 to 67%. This range is higher than the percentage range obtained in the current study from Blood River. This indicates that more sites have high concentration of Se(IV) as compared to Blood River sites. Site 10 has 67% of Se(IV), meaning the water is dominated with Se(IV) as the total concentration is very low. Even with higher percentage, the concentration level is still below the recommended limit for inorganic selenium species which is 2 µg/L. The concentration of Se(IV) at site 9 is higher than all other sites within the Mokolo River. The concentration is also higher than recommended levels of inorganic selenium in water of 2 µg/L. Currently, site 9 of the Mokolo River needs a special attention as far as toxic levels of selenium is concerned.

The concentrations of Se(VI) from Mokolo River ranges from 0.0961 to 14.8 µg/L. The highest concentration is obtained at site 9 with the concentration of 14.8 µg/L. Concentration of 2.93 µg/L, slightly higher than the set limit for inorganic selenium in water is obtained at site 4. High concentration of Se(VI) at site 9 may be due to sand mining while Site 4 is where water abstraction system was used to collect the water for irrigation. The percentage of Se(VI) with reference to total dissolved selenium ranges from 33 to 84%. The lowest percentage is observed in site 10 where Se(IV) is dominating but the total selenium concentration is still below the set limit for Se(VI) which is 2 µg/L. The highest percentage is observed at Site 9, with Se(VI) dominating by 84%. With the highest concentration and the highest percentage, water at site 9 pose a high risk to human beings, aquatic plants and animals.

4.8.1.3 Comparison of Se(IV) and Se(VI) in water samples from Blood and Mokolo River

Selenite, Se(IV) was able to be separated from Se(VI) using SPE method with Dowex 1 x 2 (chloride form) as a sorbent material. The separated Se(IV) was quantified using ICP-MS. A different study by Nyaba *et al.* (2016) was conducted in South Africa, Johannesburg using modified nano-Al_2O_3 as an adsorbent material but the material adsorbed only Se(IV). Nyaba *et al.* (2016) used ICP-OES to quantify the adsorbed

Se(IV). They reported a higher Se(IV) concentration of 84.0 µg/L of the surface water in the Soweto Area. This concentration is higher than the levels detected in Blood and Mokolo Rivers.

Concentrations of Se(IV) in Blood and Mokolo Rivers ranged from 0.0411 to 0.820 µg/L and 0.135 to 2.79 µg/L respectively. Graphical representations of the results are shown in **Figure 4.11**.

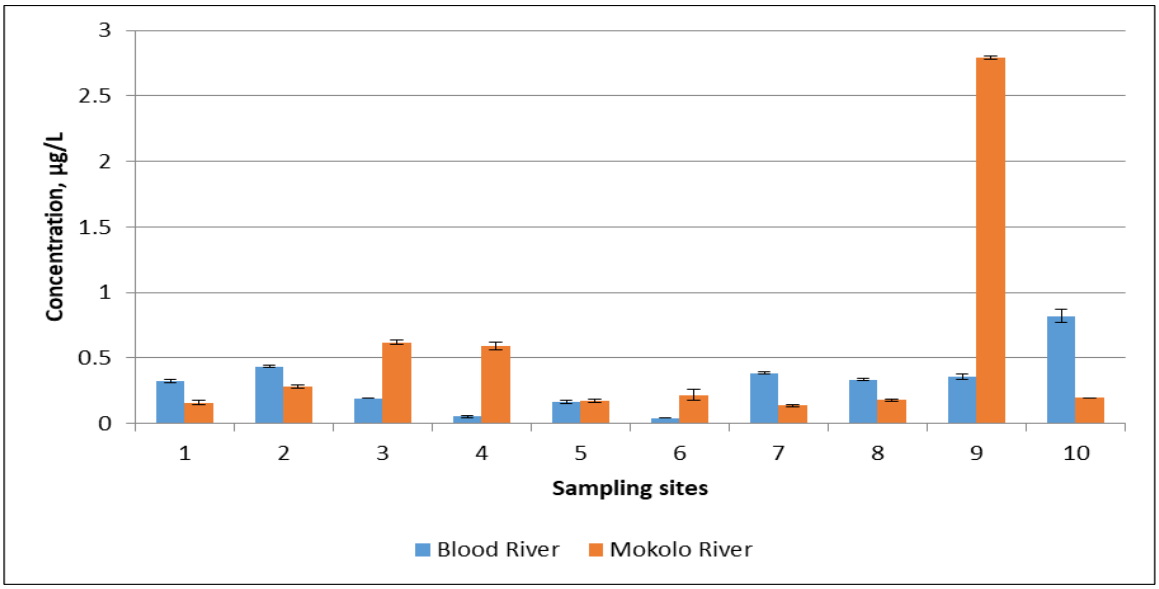

Figure 4.11: Graphical representation of Se(IV) levels in water of both rivers

Higher concentration of Se(IV) in site 9 is in agreement with the findings for the total concentration at that site. The concentration of Se(IV) at other sites are much lower as compared to other studies. The comparison results are shown in **Table 4.14**.

Selenate, Se(VI) concentration was also determined using SPE method with Dowex 1 x 2 resin (Chloride form) as a sorbent material. The same method was used by Lin (2007) for the determination of the levels of Se(VI) in ground water samples. Ling reported a higher Se(VI) concentration of 0.028 µg/l with concentrations ranging from [0.011-0.028 µg/L]. These concentrations are lower than the concentrations obtained in this study. The study in South Africa was done by Nyaba *et al*. (2016) in Johannesburg, Soweto Area. Nyaba *et al.* (2016) reported a higher Se(VI) concentration of 33.0 µg/L in surface water. These concentrations are higher than the concentrations reported in both Blood and Mokolo Rivers at all the sites. Concentrations of Se(VI) in Blood and Mokolo Rivers ranged from 0.0811-1.75 µg/L

to 0.0961-14.8 µg/L, respectively. Graphical representation of the results is shown in **Figure 4.12**.

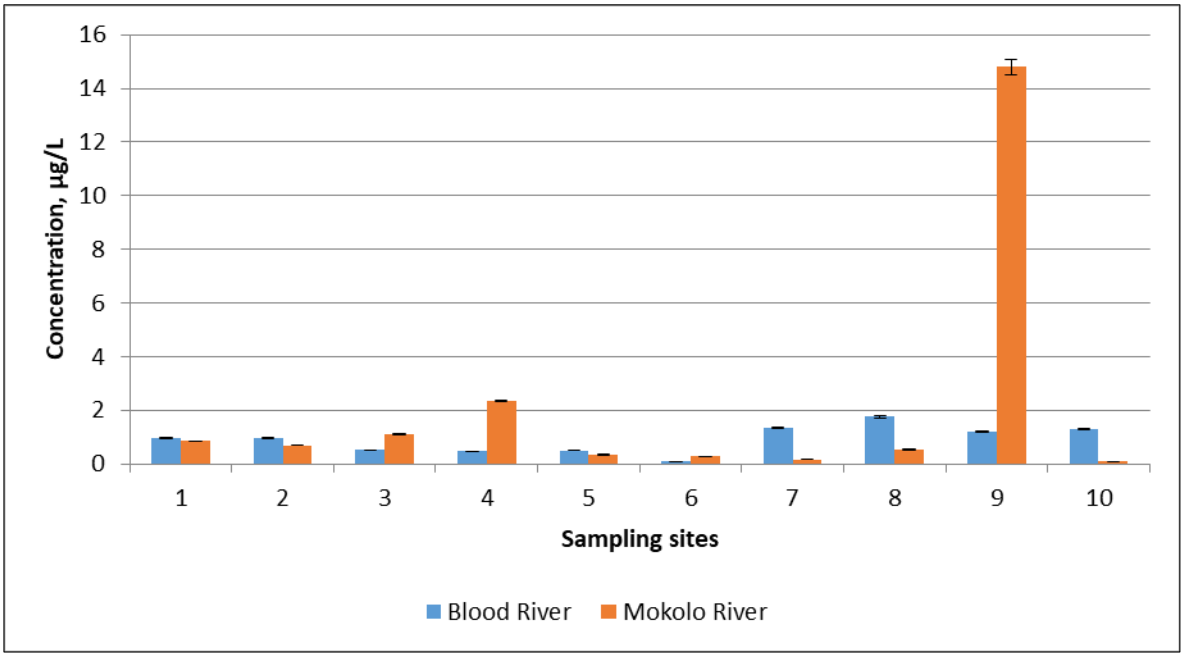

Figure 4.12: Graphical representation of Se(VI) levels in water of both rivers

Highest concentration of Se(VI) is obtained in site 9 of the Mokolo River. The second highest level above 2 µg/L is found in site 4 of the Mokolo River. Concentrations in Blood River are all below 2 µg/L at all the sites. The Se(VI) in Blood River and Mokolo River water as compared to other river differ from site to site depending on the environmental conditions. Comparison results with other studies around the world are shown in **Table 4.14**. Other studies done outside the country reported low concentration of Se(VI) as compared to this study. Where the concentration of Se(VI) is above 2 µg/L, the site need to be continuously assessed for avoiding more and more accumulation Se(VI).

Table 4.14: Comparison results of Se(IV) and Se(VI) in both rivers with other studies

Analyte, Se(IV) and Se(VI)					
Matrix	Adsorbent material	Analytical technique	Concentration,µg/L		References
			Se(IV)	Se(VI)	
Surface water	Modified nano-Al_2O_3	ICP-OES	0.631-84.0	1.80-33.0	Nyaba *et al.*, 2016
River water	DLLM	ETV-ICP-MS	0.12		Liu *et al.*, 2015
River water	Graphene oxide- TiO_2 composite SPE	GFAAS	0.57-0.90ng mL	2.62-4.80ng. mL	Zhang *et al.*, 2016
Tap water	Coprecipitation method	GFAAS	0.11-1.10 µg/L	0.16-1.10 µg/L	Tuzen *et al.*, 2007
River water	Dowex 1 x 2 (Chloride form)	ICP-OES	7.8-30.3 ng/l	11.1-28.0 ng/l	Lin, 2007
Blood River water	Dowex 1 x 2 (Chloride form)	ICP-MS	0.041 – 0.820	0.081-1.753	Current
Mokolo River water	Dowex 1 x 2 (Chloride form)	ICP-MS	0.135 – 2.789	0.096-14.789	Current

The values reported in literatures are mostly lower than other sites of Blood and Mokolo Rivers. The values in the current study indicate that Se(IV) and Se(VI) concentrations in water samples are usually lower with exception to site 9 of the Mokolo River.

4.9 Speciation of inorganic selenium using HPLC-ICP-MS

Speciation of inorganic selenium in water samples from Blood and Mokolo Rivers was achieved successfully by using HPLC-ICP-MS. Water samples were filtered and stored in the refrigerator prior to analysis. Speciation of inorganic selenium in water samples was determined using validated HPLC-ICP-MS method.

4.9.1 Determination of Se(IV) and Se(VI) in water samples from Blood River and Mokolo River

Identification of Se(IV) and Se(VI) was based on the retention time and quantification of peak areas. It was noted that there is a peak shift for both Se(IV) and Se(VI) in water samples. This peak shift is caused by matrix composition of the water sample. The integrated peak areas were used with the slope of the regression line to calculate the concentrations of Se(IV) and Se(VI). Speciation of Se(IV) and Se(VI) in water samples was successfully conducted using HPLC-ICP-MS. The chromatogram of water sample with the detected species is indicated in **Figure 4.13**.

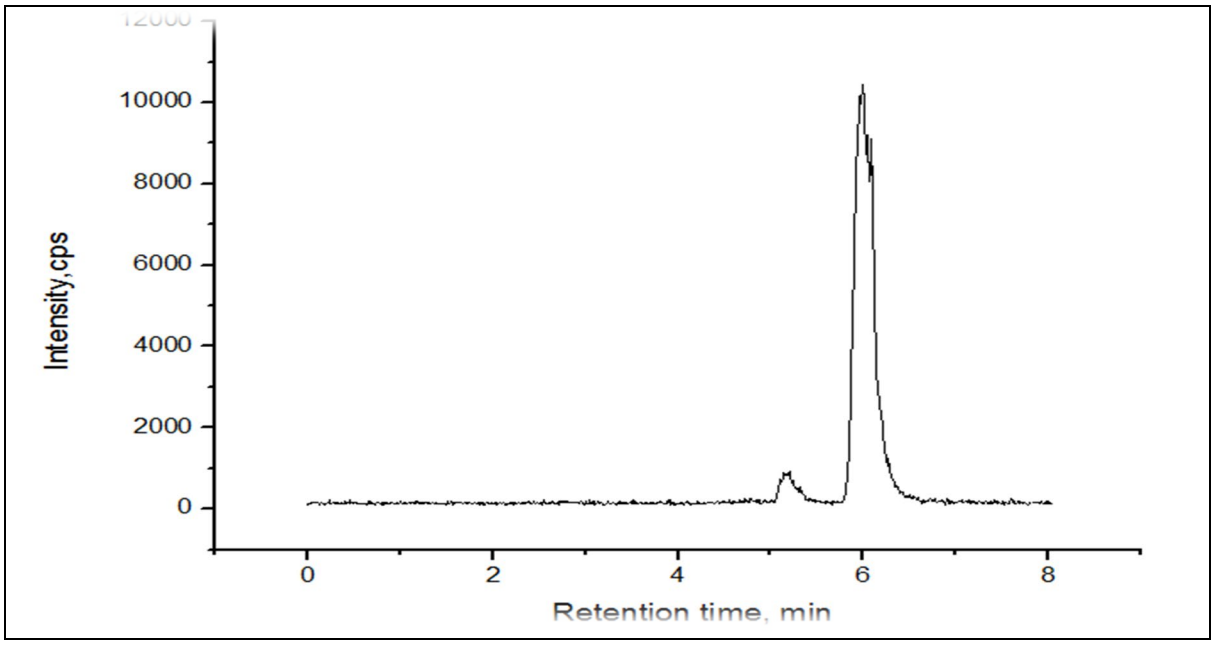

Figure 4.13: Chromatogram of Se(IV) and Se(VI) in water sample. Column, Hamilton PRP-X100 column (6.4 x 250 mm,5 µm); Mobile phase, 100 mM NH_4NO_3; sample injection, 100 µg/L.

The inorganic selenium species in water samples collected from Blood and Mokolo Rivers were determined using validated analytical procedure. The concentrations of Se(IV) and Se(VI) from both rivers are presented in **Table 4.15**.

Table 4.15: Concentrations of Se(IV) and Se(VI) in water samples from Blood and Mokolo River

Sampling site	Blood River		Mokolo River	
Concentration, µg/L	Se(IV)±SD	Se(VI)±SD	Se(IV)±SD	Se(VI)±SD
1	<0.842	<0.690	<0.842	<0.690
2	<0.842	<0.690	<0.842	<0.690
3	<0.842	<0.690	<0.842	<0.690
4	<0.842	<0.690	<0.842	2.63±0.050
5	<0.842	<0.690	<0.842	<0.690
6	<0.842	<0.690	<0.842	<0.690
7	<0.842	<0.690	<0.842	<0.690
8	<0.842	<0.690	<0.842	<0.690
9	<0.842	<0.690	3,11±0.090	33.8±0.10
10	<0.842	<0.690	<0.842	<0.690

The results in **Table 4.15** show that Se(IV) and Se(VI) were not detected from almost all the sites with exception to site 4 and 9 of Mokolo River. Only Se(IV) was detected at site 4 of Mokolo River. These results indicate that the inorganic selenium species in Blood River pose no threat to aquatic ecosystem because the species were not detected. The observed high concentration of Se(IV) and Se(VI) from site 9 of Mokolo River is due to anthropogenic activities such as sand mining taking place at that site.

The fact that the river passes near Grootegeluk mine, Medupi and Matimba power station might also be a factor. The elevated concentrations of Se(IV) and Se(VI) at site 9 of the Mokolo River is also indicated by the very low pH of 4.7 measured at that site. Inorganic selenium concentration in surface waters may be elevated to several hundred µg/L due to waste such as sewage effluent, irrigation, etc. (Plant *et al*., 2003). Both inorganic species are soluble in water with Se(VI) being less adsorbed onto soil surfaces and more tendency to leach into aqueous phase (Meher *et al*., 2019). The Se(VI) is much higher as compared to Se(IV) at Site 9 of Mokolo River. The concentrations are higher than the recommended level of 2 µg/L for both Se(IV) and Se(VI). These findings suggest that site 9 of Mokolo River needs attention as far as selenium water pollution is concerned.

4.9.3 Comparison of Se(IV) and Se(VI) in water samples from Blood and Mokolo River using SPE and HPLC-ICP-MS.

The results obtained from the two methods are in agreement with each other. The highest concentration of both Se(IV) and Se(VI) was observed only site 4 and site 9 of the Mokolo River when using both SPE and HPLC-ICP-MS. Graphical representation of the comparison results obtained using SPE and HPLC-ICP-MS from each river is indicated in **Figure 4.14 and Figure 4.15.**

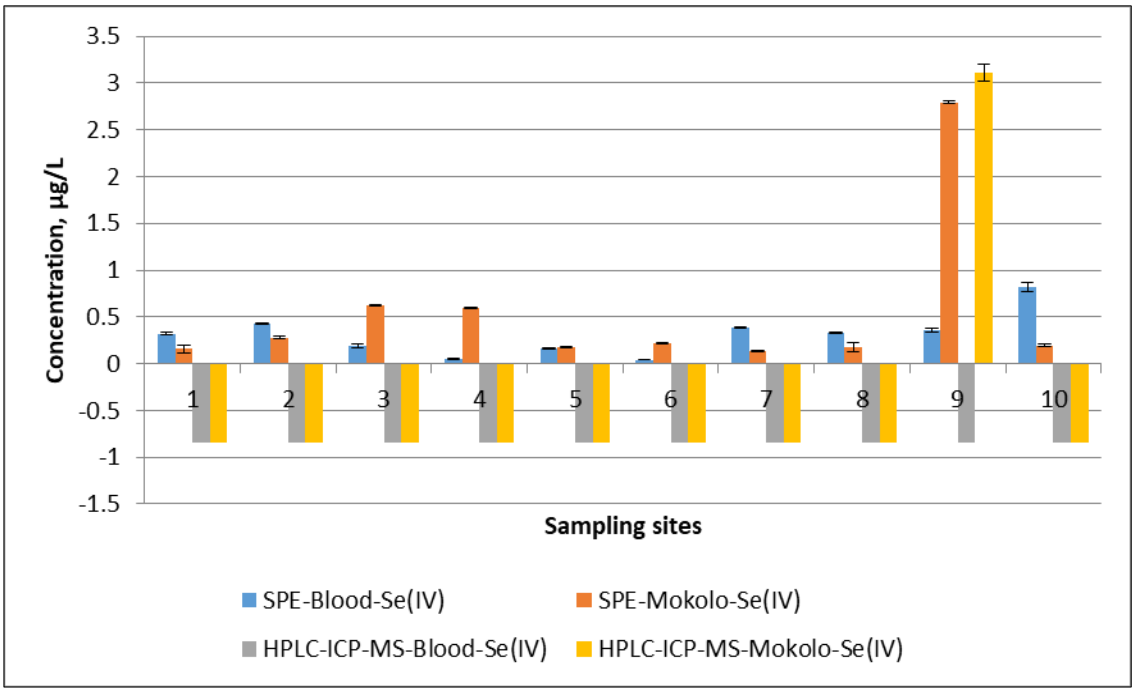

Figure 4.14: Comparison results for Se(IV) using SPE and HPLC-ICP-MS.

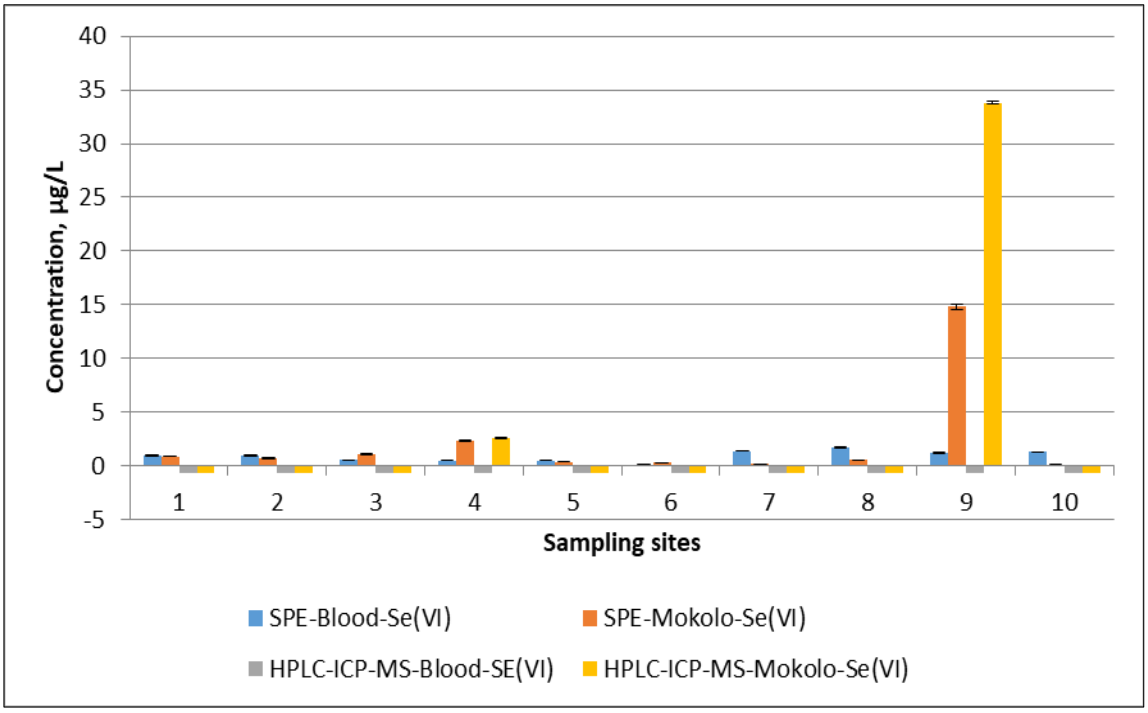

Figure 4.15: Comparison results for Se(VI) using SPE and HPLC-ICP-MS

The results from **Figure 4.14** show that with HPLC-ICP-MS the only detected Se(IV) is from site 9 of the Mokolo River. It is also observed that Se(IV) from site 9 of Blood River is higher in concentration as compared to the other sites when using both methods. The concentration exceeds the required maximum limit of 2 µg/L. The results from **Figure 4.15** shows the detection of Se(VI) only in site 4 and 9 of the Mokolo River when using HPLC-ICP-MS. The fact that selenium occurs in low concentration naturally made it difficult to be detected in most sites when using HPLC-ICP-MS. HPLC-ICP-MS offers online separation which does not require any pre-treatment while SPE was used as a preconcentration and separation procedure. When using SPE, both Se(IV) and Se(VI) were able to be detected in all the sites from both rivers due to samples being preconcentrated. However, the detected concentrations in most of the sites were below the recommended maximum limit of 2 µg/L except for site 4 and 9 of Mokolo River.

Based on the results obtained using SPE and HPLC-ICP-MS method, it can be observed that concentration of Se(IV) and Se(VI) differ with respect to the potential sources of selenium in water samples. The chemistry and pH of water also play an important role in the existence of selenium species in water. Site 9 of Mokolo River being the most dominant in both inorganic selenium species, it was also observed that the pH of water at that site was the lowest as compared to all other sites.

The anthropogenic activities contributing to the change in pH and elevated levels of Se(IV) and Se(VI) at site 9 of Mokolo River might be that the river passes near Grooteluk mine, Medupi and Matimba power station. Sand mining activity is also one of the activity happening at that site.

4.9.5 Comparison of Se(IV) and Se(VI) in water samples from Blood and Mokolo Rivers with maximum permissible levels in water.

Toxic effect thresholds for inorganic selenium in water, food-chain organisms, fish and aquatic bird tissues is 2 µg/L. Concentrations of Se(IV) and Se(VI) above 2 µg/L has an effect on food-chain bioaccumulation and reproductive failure in fish and wildlife (IVL –report B1486., 2002; Lemly, 1993a). Levels of Se(IV) at all sites in both Blood and Mokolo River falls below 2 µg/L except site 9 of Mokolo River. Water from site 9 of Mokolo River indicates high potential of selenium toxicity and reproductive effects. Concentrations of Se(VI) at site 4 and 9 of the Mokolo River exceed the toxicity thresholds for inorganic selenium in water, which is 2 µg/L. Water from these two sites of the Mokolo River pose a threat to aquatic ecosystem and it is not fit for irrigation purpose.

CHAPTER 5: Conclusions and Recommendations

5.1 Conclusions

Pollution has been a great concern in the river systems globally over the years. The state of the rivers deteriorates due to various man-made activities. Blood and Mokolo Rivers are facing challenges of water pollution due to various anthropogenic activities including sand mining, improper waste disposal, direct flow of sewage effluent in to the rivers, etc.

In the present study, the pH of the water and levels of total selenium and inorganic selenium in water and sediments were assessed. The measured pH values were within the target water quality range for aquatic ecosystems suggested by the DWAF guidelines (DWAF, 1996a) with exception of site 9 Mokolo River. The pH of site 9 of the Mokolo River is 4.55 indicating that the water is polluted. The pH range of 4.6 to 8.5 of Mokolo River as compared to the pH range of 6.0 to 8.2 of Blood River indicates that the Mokolo River is more polluted than Blood River. The low pH of the Mokolo River water samples is associated with sand mining activity and also with the fact that the river is passing near the Grootegeluk Mine, Matimba and Medupi power stations. Mining activities makes the water acidic.

Total Concentration of selenium, SPE and HPLC-ICP-MS methods were optimised, validated and applied to quantitative analysis of selenium in water and sediments. Total concentration of selenium in water was quantified successfully using ICP-MS. The method was validated using SRM 1643f. The quantified levels of selenium in water from Blood and Mokolo Rivers were ranging from 0.0681 to 2.72 µg/L and 0.0851 to 25.4 µg/L, respectively. It was found that only at site 9 of Mokolo River, the levels of selenium were above the recommended MPLs for drinking and irrigation water. Water from both Rivers from all other sites is less affected by the contaminants flowing into the River.

The levels of selenium in sediments were quantified using ICP-MS. The microwave system has been applied for the digestion of sediment samples from Blood and Mokolo

Rivers in order to determine its total concentration of selenium. The procedure was carried out using small quantities of the following reagents: HNO_3, HF and H_2O_2.

The method was validated using SRM 8704. Levels of selenium in sediment samples from Blood and Mokolo Rivers were all below the LOD value of 0.057 ng/g. This indicates that the selenium from the anthropogenic activities does not settle on the sediments. It also indicates that the areas where the rivers are located, the soil is not rich in selenium. Due to very low levels of selenium in sediments, speciation of inorganic selenium in sediments was not conducted.

The separation of inorganic selenium, Se(IV) and Se(VI) was achieved successfully by using Dowex 1 x 2 resin (chloride form) on SPE technique and HPLC-ICP-MS. The SPE technique was also used as a pre-concentration method due to low levels of selenium present in water. The findings indicate that Se(IV) was fully separated from Se(VI) using both methods. Levels of Se(IV) from Blood and Mokolo Rivers were ranging from 0.0411 to 0.821 µg/L and 0.135 to 2.79 µg/L, respectively. Levels of Se(VI) from Blood and Mokolo Rivers were ranging from 0.0811 to 1.75 µg/L and 0.0961 to 14.8 µg/L, respectively. A requirement of 2 µg/L has been set as toxic effect thresholds for inorganic selenium in water, food-chain organisms, fish and aquatic bird tissues. Site 9 of Mokolo River has both levels of inorganic selenium species higher than the recommended levels. The water from site 9 of Mokolo River is not recommended for irrigation and drinking purposes. All other sites from Blood and Mokolo Rivers has both levels of Se(IV) and Se(VI) below 2 µg/L.

The adopted HPLC-ICP-MS method for the speciation of inorganic selenium in water samples requires just a filtration of samples using a membrane filter of 0.22 µm pore size followed by a direct injection into HPLC-ICP-MS without further treatment. The findings using this method also indicate a higher concentration of Se(IV) and Se(VI) in site 9 of Mokolo River which are above the recommended maximum level of 2 µg/L. All other sites from both rivers concentrations are below LODs.

Findings of this study on the levels of inorganic selenium species from Blood River indicated that the river is not heavily polluted by pollutants from anthropogenic activities into the river system as the levels are very low.

5.2 Recommendations

The levels of total selenium and inorganic selenium species in water and sediments from Blood and Mokolo River with exception to site 9 of Mokolo River suggests that the water is fit for domestic and irrigation purposes. Site 9 of Mokolo River needs attention due to higher levels of selenium that are higher than the recommended limits. The SPE technique using Dowex 1 x 2 is recommended for a successful determination of Se(IV) in river water samples. Investigation of the elution of Se(VI) fully from the Dowex 1 x 2 resin (chloride form) should still be conducted. The SPE technique is a better method as compared to HPLC-ICP-MS due to its ability to pre-concentrate where the levels are too low to be detected by ICP-MS.

Speciation of selenium in water and sediment samples from Blood and Mokolo Rivers should be conducted regularly as it can be observed that there is a potential accumulation of selenium (especially in water) due to discharge of wastes into the rivers. The study should be conducted during low and high flow season to assess seasonal variation and to assess the level of contamination in relation to the MPLs guidelines for trace elements in the environmental samples. The inorganic selenium species contamination in water is a matter of concern due to its health hazards.

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