SPECIATION OF ARSENIC IN WATER AND SEDIMENTS FROM MOKOLO AND GREAT LETABA RIVERS, LIMPOPO PROVINCE

by

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DISSERTATION

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DEDICATION

This dissertation is sincerely dedicated to my dearest parents Letsoalo Raesetja Anna and Morris Molatelo, my lovely grandmother Mochoeneng Makolobe Saeneth, my daughter Thakgatso and siblings Kgabo, Gabriel and Tshepiso Letsoalo for their love, endless support and courage throughout my studies.

DECLARATION

I hereby declare that the dissertation entitled “Speciation of arsenic in water and sediments from Mokolo and Great Letaba Rivers, Limpopo province, South Africa” is my own original work carried out as a Master’s student at the University of Limpopo. All sources that I have used or quoted have been indicated and acknowledged by means of complete references and this work has not been submitted for examination of any other degree at any other educational institutions.

Letsoalo, M.R. (Ms) **Date**

CONFERENCE PRESENTATIONS

Oral presentation

- Mokgehle Letsoalo, Abayneh Ambushe, Takalani Magadzu and Taddese Godeto, Speciation of arsenic in water and sediments using high performance liquid chromatography coupled to inductively coupled plasma-mass spectrometry, SACI Young Chemists Symposium, 23 November 2016, University of Limpopo, South Africa.

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I can do all this through Christ who gives me strength

Philippians 4:13 (NIV)

ABSTRACT

Great Letaba and Mokolo Rivers are major sources of water for domestic use, agriculture and recreational activities in Limpopo Province, South Africa. These Rivers are predisposed to pollution sources from atmospheric deposition of mine dust, emissions from power stations and burning fuel, return flows from agriculture and municipal wastewater discharges and sewage effluents, which may potentially affect the quality of water and the inhabiting biota. Arsenic (As) is an element of prime concern in aquatic systems exposed to such pollution sources due to its toxicity to humans and aquatic life. The quantification and speciation of As in Mokolo and Great Letaba Rivers is important to assess the current levels and predict future trends in the quality of the two rivers. Speciation of As in water and sediments is crucial since the toxicity depends on its chemical forms. In this study, various analytical approaches were explored to precisely identify and quantify different As species in water and sediment samples collected from Great Letaba and Mokolo Rivers.

Sample preparation was carried out with an intensive care to efficiently identify and quantify As species. Identification of each species in the samples was based on matching standard peaks with retention times by simple injection of standards of As species into Hamilton PRP X100 column. The chromatographic separation and determination of As^{3+}, dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and As^{5+} in water and sediment samples were achieved by on-line coupling of high performance liquid chromatography (HPLC) to inductively coupled plasma-mass spectrometry (ICP-MS). A novel extraction method for As species in sediments based on 0.3 M $(NH_4)_2HPO_4$ and 50 mM EDTA showed no species interconversion during extraction. Baseline separation of four As species was achieved in 12 minutes using gradient elution with 10 mM and 60 mM of NH_4NO_3 at pH 8.7 as mobile phases. The analytical figures of merits and validation of analytical procedures were assessed and adequate performance and percentage recoveries ranging from 81.1 – 102% for water sample and 73.0 – 92.0% for sediments were achieved. The As species concentration in water and sediment samples were found in the range 0.224 – 7.70 µg/L and 74.0 – 92.0 ng/g, respectively. The DMA was not detected in both water and sediment samples.

The As content in sediments depends on the solid phase partitioning between inorganic As species and trace elements such as iron (Fe), manganese (Mn) and aluminium (Al). Knowledge of the extent of this partitioning is important to evaluate the distribution and pathways of As in water, aquatic organisms and possible exposure of animals and human beings. Therefore, total concentrations of As, Fe, Mn and Al in water and sediment samples were determined using ICP-MS and inductively coupled plasma–optical emission spectrometry (ICP-OES). The analytical procedures were validated using standard reference materials (SRMs) with percentage recoveries of trace elements ranging 84.0 – 95.6% for water samples and 75.0 – 120% for sediments. The As, Fe, Mn and Al concentrations obtained were further assessed for safe drinking water, irrigation water and for sediments quality about standard guidelines. Moreover, As species concentrations correlated with Fe, Mn and Al and the observed interactions depend on the adsorption capacities between As species and these trace elements.

The inorganic species in water samples were also determined by employing off-line mode of solid phase extraction (SPE) procedure using multi-walled carbon nanotubes (MWCNTs) impregnated branched polyethyleneimine (BPEI) as an adsorbent material. The MWCNTs-BPEI characterised with X-ray powder diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy and Thermogravimetric analysis (TGA) techniques indicated successful modification of the nanomaterial. The MWCNTs-BPEI exhibited selective retention of As^{5+} in the presence of As^{3+} in water samples with the achieved pre-concentration factor of 23.3. The retained As^{5+} was then eluted and detected using ICP-MS. A limit of detection (LOD) of 0.0537 µg/L and limit of quantification (LOQ) of 0.179 µg/L were achieved. The obtained percentage recovery of 81.0% validated the SPE procedure for selective retention of As^{5+}. The As^{5+} concentrations determined after the SPE procedure were found in the range of 0.204 – 7.52 µg/L, which are in good agreement with As^{5+} results obtained using HPLC-ICP-MS.

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ABBREVIATIONS AND ACRONYMS

BPEI	Branched polyethyleneimine
CRM	Certified reference material
DMA	Dimethylarsinic acid
FAO	Food and Agriculture Organisation
FTIR	Fourier transform infrared
HPLC	High performance liquid chromatography
ICP-OES	Inductively coupled plasma-optical emission spectrometry
ICP-MS	Inductively coupled plasma-mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MMA	Monomethylarsonic acid
MPLs	Maximum permissible levels
MWCNTs	Multi-walled carbon nanotubes
RSD	Relative standard deviation
SPE	Solid phase extraction
SRMs	Standard Reference Materials
TGA	Thermogravimetric analysis
XRD	X-ray diffraction
m/z	mass-to-charge
R^2	Square of correlation coefficient

CHAPTER 1

INTRODUCTION

1.1 General background

Speciation analysis has become a scientific topic in the recent years because of the different behaviour of chemical species of a certain element (Rosen and Hieftjie, 2004; Onji *et al*., 2013). For instance, health effects of As not only dependent on the total concentration but also on quantities of its chemical species. In addition, total concentrations do not provide useful information to evaluate the potential health risks associated with As (Akinsoji *et al*., 2013; Muhammad *et al*., 2015). Chemical speciation serves as a vital requisite in water quality assessment because the actual toxicity, availability or accumulation, migration and reactivity of As depend on the existing chemical forms (Plant *et al*., 2003; Rosen and Hieftjie, 2004). Speciation may be described as analytical activities of identifying and measuring the quantities of one or more individual chemical species in a sample (Nordberg *et al*., 2004). The identification of the chemical species in the environmental matrices including water, soil, sediments and air; allows conclusions based on the toxicity of particular species (Pizzaro *et al*., 2003; Terlecka, 2005).

The As occurs naturally in a wide range of minerals and most probably it may be found throughout the environmental matrices including water, soil, sediments and air (Rosen and Hieftjie, 2004). It is widespread in nature and its presence in water may represent the greatest threat for human health as it may be directly introduced to the human body (Koesmawatia *et al*., 2015). The high level in water is attributed to solubility of As salts. Therefore, contamination of water by As has been acknowledged as a global health concern by the WHO due to its toxic prevalence (Rezende *et al*., 2014). There are about 25 As species, which have been identified in water. However, the most prevalent species in the environmental water geochemistry are inorganic species, As^{3+} and As^{5+} and organic species such as MMA, DMA and trimethylarsenate (TMAO) (Terlecka, 2005; Komorowicz and Baralkiewicz, 2011). The rest of the chemical species are adsorbed by the aquatic organisms. The adsorption of As species indicate the capability of As to bind in the tissues of the organisms, thus generating arsenosugars and arsenolipids such as arsenocholine (AsC) and arsenobetaine (AsB), which are considered non-toxic

species (Le *et al*., 1998). Contamination of water by As species could negatively impact the quality of aquatic ecosystem (Rezende *et al*., 2014). In addition, As is usually toxic to plants at concentrations that do not affect animals or human health (Komorowicz and Baralkiewicz, 2011).

1.2 Problem statement

South Africa is regarded as one of the leading countries in mining. As a result, it encounters a severe environmental pollution from mining activities. Pollutants such as potentially toxic elements accumulate into the aquatic ecosystems unnoticed (Terlecka, 2005; Akinsoji *et al*., 2013). One such example is As, which poses a significant risk to both human and aquatic life (Terlecka, 2005; Amman, 2011). The As is ranked amongst top six toxic elements and is often found associated with other valuable minerals including gold (Au), zinc (Zn), copper (Cu) and platinum (Pt) (Oberholster *et al*., 2010). Contamination of drinking water by As has been reported globally in different places including Bangladesh, Argentina, Chile and Gravellote in Limpopo Province, South Africa (Ngai, 2002; Ali, 2010; Amman, 2011). Recent studies on epidemiology have revealed that long term exposure to As may cause adverse health effects to human beings such as dermal changes, neurological development, pulmonary cardiovascular disorder, mutagenic and carcinogenic effects (Kumar and Riyazuddin, 2010; Onji *et al*., 2013). The emanating health conditions were reported to be linked with intake of drinking water having As concentration of greater than 50 µg/L (Ngai, 2002, Amman, 2011; Niazi *et al*., 2011).

The As species determination in water and sediments are essential for water quality assessment. Sediments serve a purpose to trap water pollutants to protect water quality but they also present the potential for further water contamination through dissolution and precipitation processes (Terlecka, 2005). Study areas included Great Letaba River from Tzaneen to Phalaborwa and Mokolo River in Lephalale following the observation of anthropogenic activities related to emission of As in their respective vicinities. There are operational activities of Foskor phosphate mine and Phalaborwa copper mine in the downstream of Great Letaba. Dust containing arsenic is produced during copper and gold smelting, and coal combustion

(Matschullat, 2000). Thus, Great Letaba and Mokolo Rivers are at risk of exposure to As contamination of aquatic ecosystem.

1.3 Rationale of the study

This study was prompted by the industrial activities found in the proximities of the selected study areas. Great Letaba and Mokolo Rivers serve as source of water for domestic use, agricultural activities and recreation in their respective locations. These areas face major challenges which are gradually influencing water quality and aquatic ecosystem through processes such as atmospheric deposition of mine dust, return flows from agriculture and municipal discharge wastewater and sewage effluents (Kumar and Riyazuddin, 2010). The speciation of As in Mokolo and Great Letaba Rivers is a relevant study in order to assess the water quality of the river systems. In addition, As levels in sediments depend on the solid phase partitioning between inorganic As species and trace elements such as Fe, Mn and Al. Hence, total concentration determination of Fe, Mn and Al emerged as of paramount importance since the extraction efficiency of As species depend also on the levels of these trace elements. The obtained analytical data would assist to estimate the likely future changes in the aquatic ecosystem which are induced by anthropogenic activities (Oberholster, 2010).

Numerous studies have been published on the investigation of speciation of As in water and soil or sediments by employing a variety of methods hyphenated to inductively coupled plasma-mass spectrometry (ICP-MS) detection system (Garcia-Manyes *et al*., 2002; Niazi *et al*., 2011; Muhammad *et al*., 2015). The species separation technique such as HPLC coupled to ICP-MS or hydride generation-atomic fluorescence spectrometry (HG-AFS) detection systems were explored (Muhammad *et al*., 2015). This study focused on speciation of As in river water using SPE procedure by employing MWCNTs-BPEI adsorbent material and detection of separated species using ICP-MS (Kavcar *et al*., 2009). The MWCNTs-BPEI nanocomposites offer distinct binding affinity with regard to selective retention of As^{5+} due to high surface area, the competency of forming π-π interaction as well as chemical and thermal stability (Rahman *et al*., 2015).

1.4` Purpose of the study

1.4.1 Aim

The aim of this study is to assess and quantify different species of As in water and sediment samples from Mokolo and Great Letaba Rivers in Limpopo Province, South Africa.

1.4.2 Objectives

The objectives of the study are to:

(i) optimise method for speciation analysis of water samples.

(ii) develop the extraction method for assessment of As species in sediments.

(iii) separate and quantify As species in water and sediment samples using HPLC-ICP-MS.

(iv) determine total concentrations of As, Fe, Mn and Al in water and sediment samples after sediments mineralisation using microwave digestion system and detection by ICP-MS and ICP-OES.

(v) functionalise and characterise MWCNTs-BPEI.

(vi) pre-concentrate levels of As^{5+} in water samples by SPE procedure using MWCNTs-BPEI nanocomposites as adsorbent material.

(vii) validate the developed analytical methods using SRMs of water and sediments.

1.5 Chapters outline of the dissertation

Chapter 1 gives the background and motivation of the study. Research problem is clearly outlined followed by aims and objectives of the project.

Chapter 2 comprises the literature review. This chapter describes in detail the occurrence of As, human threats related to toxicity, health effects, risk assessment, and human exposure to various As species. The biochemistry of As which includes

the bioaccumulation, mechanism of transformation, biogeochemistry, microbial metabolism, detection techniques, adsorbent materials as well as characterisation techniques are clearly outlined.

The methodology used for this research is elaborated in chapter 3. This chapter outlines the justification for selection of study areas, sample collection, preparation and analytical procedures followed to accomplish the purpose of the study.

In chapter 4, results are presented and supported by detailed discussion.

Chapter 5 concludes the study based on the findings. The achievement of the objectives is clearly explained in this chapter. Based on the outcomes of the study, several recommendations were outlined in this chapter.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Arsenic is an element of outmost concern because of its toxicity and health threats it poses worldwide (Le *et al*., 1998; Kumar and Riyazuddin, 2010; Rasool *et al*., 2015). The effect of its toxicity ranges from simple health problems such as skin disease to serious ones such as teratogenesis and mutagenic (Le *et al*., 1998). There are various studies and a large volume of literature on origin of As, its sources and the effects of chemical species on different ecosystems (aquatic and terrestrial) and human beings (Ahsan *et al*., 2006; Winkel *et al*., 2008; Brammer and Ravenscroft, 2009). This chapter clearly outlines factors such as the origin and distribution of As, biochemistry as well as human effects, risk assessment and human exposure to As species. Additionally, this chapter reviews published reports on determination of total concentration of As and various forms of As species; particularly on the environmental matrices using different detection techniques. The sample preparation, detection and characterisation techniques are also outlined.

2.2 Occurrence and distribution of arsenic

Arsenic is a metalloid with an atomic number 33 and atomic weight of 74.9 atomic mass unit (amu). It occurs on the group VA of the periodic table. The allotropic forms of pure As in nature are yellow (alpha), black (beta), and grey (gamma). Elemental As is not soluble in water, however, As salts exhibit a wide range of solubilities depending on pH and the ionic environment (Kavcar *et al*., 2009). The As may occur in crystalline, powder, amorphous or vitreous forms. In the environment, As exists as ^{75}As with various oxidation states (As^{3+}, As^{5+}, As^{0} and As^{3-}). One of the most toxic As species is arsine gas (AsH_3) with As^{3-} oxidation state (Kavcar *et al*., 2009). Elemental As has a specific gravity of 5.73, sublimes at 613 ºC and has a very low vapour pressure of 1 mmHg at 373 ºC. The inorganic As species occur as white, odourless solids with a specific gravity ranging from 1.9 – 5 (Ahsan *et al*., 2006; Kavcar *et al*., 2009).

Arsenic forms a component of about 245 minerals, widely distributed in the earth's crust. Owing to its abundance, it may cycle between the atmosphere, soil, water and sediments (Winkel *et al*., 2008). It is the 47th most abundant element in nature with normal concentrations ranging from 0.5 – 2.5 mg/Kg in most rock materials, 10 mg/Kg in soil, and less than 1 µg/L in aquatic environment (Ahsan *et al*., 2006; Mols, 2016). Different levels of As in the environmental surface are influenced by a variety of geological and hydrological factors, redox nature and pH of the environment matrices (Winkel *et al*., 2008). Moreover, As levels vary notably in the environment, partly in relation to geology and to some degree as a result of anthropogenic activities (Le *et al*., 1998). Its availability on the surface is leached by natural processes like volcanic activities and weathering of As bearing rocks such as realgar (AsS), arsenopyrite (FeAsS), and lollingite ($FeAs_2$) (Ahsan *et al*., 2006). The As containing rocks may be converted to As^{3+} by weathering processes and enters the As cycles as a dust or by dissolution or desorption in the water (Winkel *et al*., 2008).

Arsenic availability in the environment contributed by anthropogenic activities is of great concern. The As could be elevated to toxic levels in the aquatic ecosystem through various anthropogenic activities including, petroleum refining, use of wood preservatives, leather tanning operation, agricultural use of pesticides and herbicides, mining and smelters (Rezende *et al*., 2014; Koesmawatia *et al*., 2015). The natural processes had progressively transferred As from the geosphere into the surface environment and have distributed it through the biosphere, where it poses a potential risk to living organisms (Ahsan *et al*., 2006). Arsenic is quite resistant to degradation and likely to be oxidised or reduced from one species to another. Usually, the oxidised species is easily mobilised through the different media (from soil or sediments to water). For instance, As species in water may precipitate and be adsorbed onto rocks and soil or sediments (Ahsan *et al*., 2006). Aqueous As is of most concern because higher levels have been found in drinking water at various places around the world (Le *et al*., 1998; Winkel *et al*., 2008).

2.3 Health threats related to arsenic

Arsenic is gradually becoming an element of interest in environmental geochemistry and scientific community because of its toxic prevalence (Winkel *et al*., 2008). The

comprehensive toxicity studies on As are of great interest particularly on the speciation inspection since there is a fair correlation between different species and different toxicities (Ahsan *et al*., 2006; Winkel *et al*., 2008; Brammer and Ravenscroft, 2009).

2.3.1 Toxicity of arsenic

The As^{3+} and As^{5+} are considered the most toxic species as compared to DMA, MMA and TMAO. Furthermore, inorganic As species are estimated to be 100 times more toxic than organic As species whereas As^{3+} is estimated to be 60 times more toxic than As^{5+} (Rasool *et al*., 2015). The inorganic As species have comparable bioavailability but differ in their biochemistry. The As^{3+} prefer to bind thiols in proteins and inhibit their activity whereas As^{5+} are known to replace phosphate in several biochemical processes (Le *et al*., 1998; Brammer and Ravenscroft, 2009). The MMA and DMA are less toxic followed by TMAO whereas AsC and AsB are regarded as non-toxic species and commonly found in a wide variety of biological matrices (aquatic and plant tissues) (Le *et al*., 1998). The most common species which are found in the environmental matrices are As^{3+} and As^{5+} and ultra-trace levels of MMA, DMA and TMAO (Le *et al*., 1998). The MMA and DMA occur rarely because they are produced through activities of microbial metabolism (Komorowicz and Baralkiewicz, 2011; Chiban *et al*., 2012). The toxicity of As species could be arranged in the following order from the highest to the lowest toxic: As^{3-} > As^{3+} > As^{5+} > DMA > MMA > TMAO > elemental As (Rasool *et al*., 2015).

2.3.2 Human exposure and possible health impact of arsenic

There is no evidence of a beneficial role for any safe dose of As like other elements such as selenium which their trace levels are essential for human health (Le *et al*., 1998). Furthermore, insignificant amount of As which could possibly be exposed to any living organisms calls for a precise control because of different toxicities of chemical species of As (Le *et al*., 1998). Human exposure to toxic As may be through the environmental matrices such as the inhalation of the contaminated dust, ingestion of contaminated water and agricultural crops (Kavcar *et al*., 2009). Total

concentration determination of As has been used traditionally to assess environmental impact and health risks but it is more recently been recognised that no meaningful interpretation can be made without speciation information (Le *et al*., 1998).

The World Health Organisation (WHO) and International Agency for Research on Cancer (IARC) have classified As^{3+} and As^{5+} as group 1 carcinogens and genotoxic based on findings that these species may cause bladder, lung and skin cancers (WHO, 1996; IARC, 2004). Exposure to As through drinking water may result in acute or chronic health conditions. The symptoms of chronic exposure are related to dermatological symptoms, which include gangrene and skin lesions (melanosis and keratosis) as shown in figure 2.1 (a) and figure 2.1 (b), respectively (Brammer and Ravenscroft, 2009; Rasool *et al*., 2015).

Figure 2.1 (a): Gangrene symptom of arsenic exposure

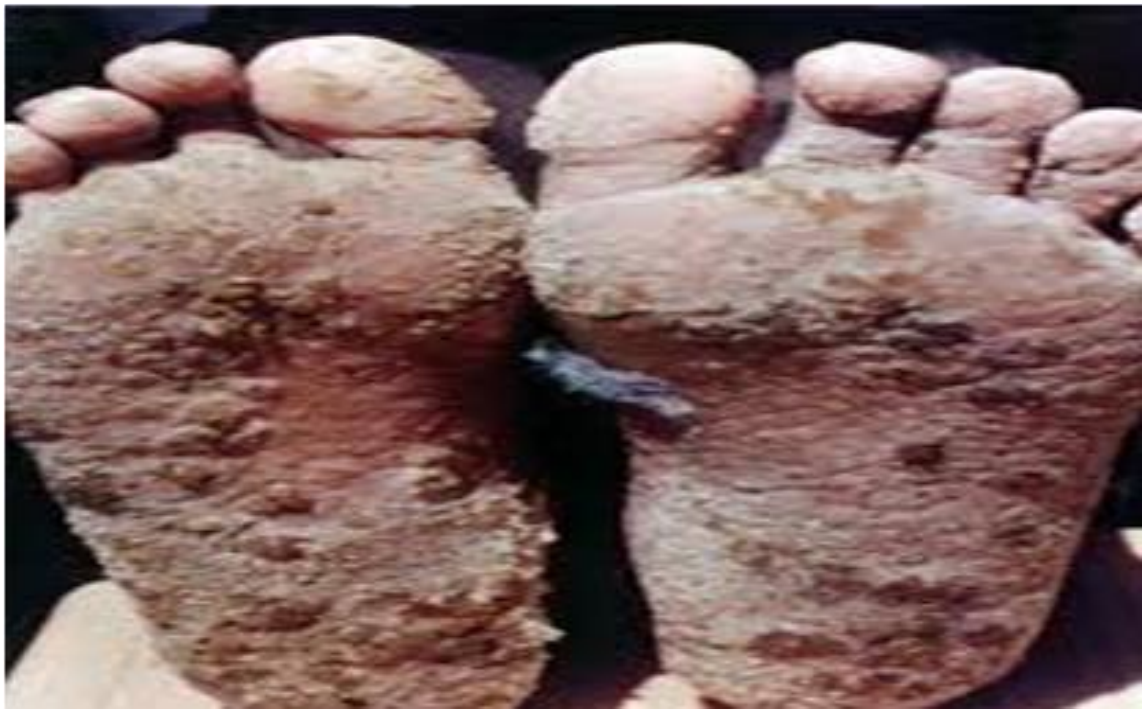

Figure 2.1 (b): skin lesion (keratosis) symptom of arsenic exposure

The other chronic health conditions due to prolonged exposure to toxic levels include increased risk of carcinogenicity conjunctivitis, non-pitting swelling, hepatomegaly, splenomegaly, cardiovascular disease and respiratory problems (Rasool *et al*., 2015). These health conditions are related to the intake of drinking water containing As concentrations of greater than 50 µg/L (Rasool *et al*., 2015). Research studies on epidemiology have revealed that MMA and DMA are toxins classified as group 2B carcinogens, which implies that their exposure circumstances entail exposures that are possibly carcinogenic to humans (Rasool *et al*., 2015; Muhammad *et al*., 2015; Rahma *et al*., 2015). The carcinogen risk (CR) is the probability for cancer risk assessment (Rahma *et al*., 2015). Arsenic ranked first on each of the hazardous substances priority lists compiled for the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) between 1997 and 2007 at national priorities list sites due to its toxicity (McCarthy, 2011; WHO, 1996).

The possible medical effects linked to different levels of As in contaminated water are presented in table 2.1 (EPA, 2005). The As concentrations within the range from 0 – 10 µg/L could be tolerated by human vulnerable to As exposure without any health risks provided the assimilative capacity of water resources are efficient in

maintaining stipulated range. Any concentration above 10 µg/L could lead to simple health problems skin disease to serious ones such as teratogenesis (EPA, 2005).

Table 2.1: The human health threats associated to exposure to variety of arsenic concentrations

Target water quality range of As (µg/L)	Health effects related to exposure to various As concentrations
0 – 10	No health effects expected; ideal concentration range
10 – 200	Tolerable concentration but low risk of skin cancer in highly sensitive individuals over a long term
200 – 300	Increasing possibility of mild skin lesion over long term. Slight possibility of induction of skin cancer.
300 – 600	Possibility of adverse effects in sensitive individuals; brief exposure has no effect; skin lesions, including hyper pigmentation, will appear on long term exposure
600 – 1000	Symptoms of chronic poisoning such as skin lesions including hyper pigmentation, will appear on long term exposure
1000 – 10, 000	Cancer or death will result from chronic poisoning
> 10, 000	Death will result from acute poisoning

2.4 Health risk assessment and regulations for arsenic in environmental waters

The investigation of As species, total As concentration and evaluation of human health risk from intake of As contaminated water are critically important existing gaps to resolve the emerging issue of As contamination (Chakraborty *et al*., 2010). The health risk assessment model is used to evaluate toxic impacts of As in drinking water on the health of people to estimate probability of individual exposed to As poisoning (Muhammad *et al*., 2015). The major As entry route into human body is through ingestion of contaminated food and water. Therefore, health risk assessment in food may be assessed by the estimation of daily intake (EDI) (Muhammad *et al*., 2015; Rahma *et al*., 2015). This is done by evaluation of the exposed amount of As

through consumption of food crops. Human health risk assessment based upon the consumption of As contaminated water has high significance due to the direct, intense and continuous exposure to As. Generally, As concentrations in water could be used to calculate potential health risk assessment either for chronic or carcinogen effects such as average daily dose (ADD), hazard quotient (HQ) and CR (Rahma *et al*., 2015). The HQ and CR are considered to be present when HQ > 1.00 and CR > 10^{-6} (EPA, 2005; Muhammad *et al*., 2015; Rahma *et al*., 2015). The HQ is a ratio of exposure intake or dose to reference dose or concentration at which effect of As is known to occur (EPA, 2005).

The accumulating evidence on the multiple toxicities of As species had led to the reduction of the regulatory limit to be curbed down from 50 – 10 µg/L (Garcia-Manyes *et al*., 2002; Ruiz-Chancho *et al*.; 2005; Pillay and Kindness, 2016). The WHO, South African Standard Specification (SASS) and other worldwide legislature organisations have recommended maximum permissible level (MPL) of As to be 10 µg/L or even less (Sami and Druzynski, 2003; McCarthy, 2011). For instance, Australia and New Jersey State have recommended MPLs of 7 µg/L and 5 µg/L, respectively whereas American Natural Resource Defense Council have suggested maximum standard be set at 3 µg/L (Smedley and Kinniburgh, 2002; Sombo, 2009). However, Africa is behind on this regard since not much data have been collected on As contamination (Akinsoji *et al*., 2013). The recommendation of MPL for As contamination in Africa is based on the outcomes from WHO (Sami and Druzynski, 2003; McCarthy, 2011).

These recommended MPLs of As are contribution of the existing As species which together yield total concentration. The MPLs of native species in the environmental matrices have not yet been established. Generally, accepted worldwide guideline standards for As in water of some selected countries are presented in table 2.2 (Sombo, 2009).

Table 2.2: Accepted worldwide standards for arsenic in water

Guideline values	Country (year adopted) limit in µg/L
Countries whose guideline value is lower than 10 µg/L	Australia (7 µg/L, 1996–2016), New Jersey State (5 µg/L, 2013), American Natural Resource Defense Council (3 µg/L, 2010)
Countries whose guideline value is 10 µg/L	South Africa (2007) European Union (1998), Japan (1993), Jordan (1991), United States (1990), Laos (1999), Mongolia (1998), Namibia (2004), Syria (1994) and WHO (2000),
Countries whose guideline value is lower than 50 µg/L but higher than 10 µg/L	Canada (1999) 25 µg/L
Countries considering to lower the guideline value from 50 µg/L	Mexico(1994)
Countries whose guideline value is 50 µg/L	Bahrain and Bangladesh (1990), Bolivia (1997), China (1991), Egypt (1995), India and Indonesia (1990), Oman and Philippines (1978), Saudi Arabia and Sri Lanka (1983), Viet Nam (1989), Zimbabwe (1994)

2.5 Chemistry of arsenic species

The prevalence of particular As species in the environmental matrices is influenced by variety of factors such as the varied anthropogenic activities, reduction-oxidation (redox) nature, pH, adsorption and desorption as well as solid phase precipitation and dissolution processes and effects of microbial activities (La Force *et al*., 2000; Rakhunde *et al*., 2012; Jablonska-Czapla, 2014).

2.5.1 The effects of reduction–oxidation potential and pH

The most prevalent As species in the environmental matrices are As^{3+}, As^{5+}, DMA and MMA (Le *et al*., 1998; Komorowicz and Baralkiewicz, 2011). Anthropogenic activities could influence the levels of As^{3+} and As^{5+} in water sources, sediments, soil and air (Inskeep, 2001; Komorowicz and Baralkiewicz, 2011). The toxicity, mobility and accumulation of a dominant As species particularly in water, sediments and soil is strongly influenced by processes occurring in that matrices (Inskeep, 2001; Gao, 2012). Amongst these chemical processes, redox nature which indicate the oxidation or reduction environmental condition and pH of that environmental matrices are the most important parameters, which control presence of As species (Rakhunde *et al*., 2012). The redox conditions also depend on the amount of protonation (pKas). The degrees of protonation for both species at oxidising and reducing conditions, in a wide range of pH, are as follows (La Force *et al*., 2000; Gao, 2012; Rakhunde *et al*., 2012):

For oxidising conditions (As^{3+}),

$H_3AsO_3 \rightarrow H_2AsO_3^- + H^+$ $\quad pKa_1 = 9.22$

$H_2AsO_3^- \rightarrow HAsO_3^{2-} + H^+$ $\quad pKa_2 = 12.13$

$HAsO_3^{2-} \rightarrow AsO_3^{3-} + H^+$ $\quad pKa_3 = 13.40$

For reducing conditions (As^{5+})

$H_3AsO_4 \rightarrow H_2AsO_4^- + H^+$ $\quad pKa_1 = 2.20$

$H_2AsO_4^- \rightarrow HAsO_4^{2-} + H^+$ $\quad pKa_2 = 6.97$

$HAsO_4^{2-} \rightarrow AsO_4^{3-} + H^+$ $\quad pKa_3 = 11.53$

The relationship between redox potential and pH at ambient temperature is presented in figure 2.2 (Rakhunde *et al*., 2012). Based on figure 2.2, reducing conditions at pH range of 2 – 6.9 favours the prevalence of $H_2AsO_4^-$ whereas extremely acidic conditions of pH < 2, H_3AsO_4 predominates. The extremely alkaline reducing conditions at pH > 11.5 are dominated by AsO_4^{3-}. The oxidising conditions according to figure 2.2, H_3AsO_3 is likely to predominate wide range up to pH 9.2 as neutral species whereas the extreme alkaline conditions favour prevalence of $H_2AsO_3^-$, $HAsO_3^{2-}$ and AsO_3^{3-} at pH > 9.2 – 14.0.

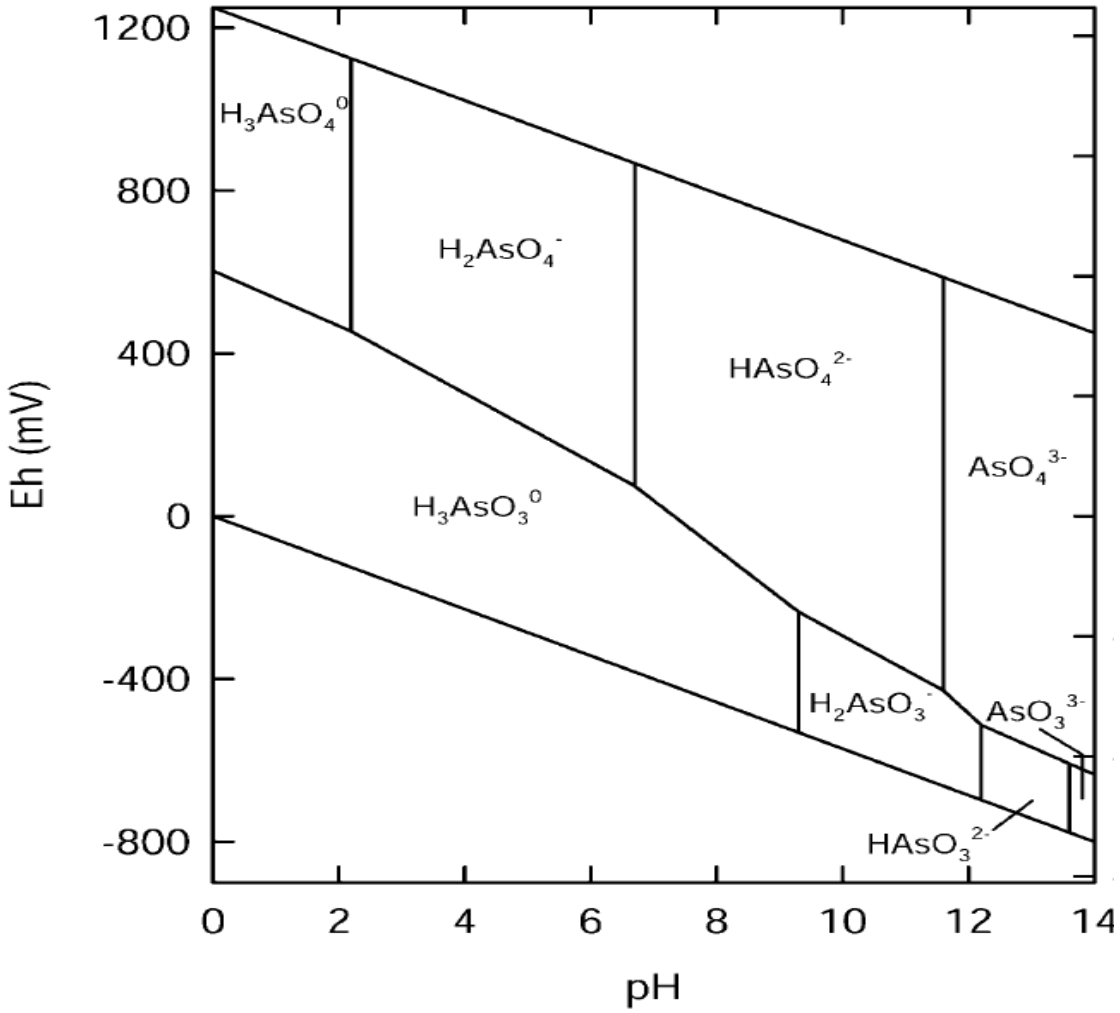

Figure 2.2: Eh-pH diagram of aqueous arsenic species at 25 ^{o}C

2.5.2 Adsorption and desorption processes and the effects of pH

The factors such as reductive and oxidative dissolution as well as ligand exchange and ligand enhanced dissolution, contribute differently to adsorption and desorption as well as mobility of As species. For instance, reductive dissolution involves the dissolution of soil or sediments Fe-oxides as a consequence of flooding or submersion of soil, which affect the release of As through the reduction process (O'Reilly *et al*., 2001). The oxidative dissolution, particularly of ferrous sulphide mineral undergoes oxidation process as a result of soil or sediment exposure to air or increased dissolved O_2 concentration leading to the release of As in the environment (O'Reilly *et al*., 2001). Additionally, natural organic matter plays a crucial role in As speciation due to the ability to affect the adsorption processes by

Fe-oxides, which then controls the mobility and bioavailability of As on the environment (Basu *et al*., 2014).

A comprehensive molecular investigation of the sorption mechanism has been conducted to estimate the long term stability of As associated with various Fe minerals (O'Reilly *et al*., 2001). The As^{3+} and As^{5+} exhibit strong interaction with Fe-oxides surfaces, which also depends on the pH of the surrounding. The sorption of the species to Fe-oxides varies greatly between As^{3+} and As^{5+} due to difference in pH (Jablonska-Czapla, 2014). The optimum As^{3+} adsorption on to mineral surface occurs within range of pH 7 – 8.5 whereas As^{5+} adsorption prevails at pH 4 (O'Reilly *et al*., 2001; Jablonska-Czapla, 2014). The geological studies indicate that As^{5+} forms an inner sphere surface complex on amorphous Fe-oxide by the ligand exchange with hydroxyl group (–OH) in the mineral surfaces (O'Reilly *et al*., 2001). An inner sphere complex is commonly formed by ionic or covalent bonding and no water of hydration is interposed between the adsorbed ion groups (O'Reilly *et al*., 2001). The As^{3+} forms both inner and outer sphere complexes have at least one molecule between the surface functional group and the bound ions. Hence, it involves electrostatic bonding mechanism, which results in weaker bonds that makes As^{3+} the most labile species (O'Reilly *et al*., 2001). The As^{5+} strongly adsorbed to Fe-oxide due to stronger inner sphere surface complex interaction (O'Reilly *et al*., 2001; Jablonska-Czapla, 2014).

The adsorption by Al and Mn-oxides and clays has not yet been studied much. The amorphous $Al(OH)_3$ is known to bind As^{3+} strongly over pH range of 6 – 9.5 and Mn-oxides play a role in the reduction of As^{3+} to As^{5+} (Buschmann *et al*., 2007). The pH and redox nature co-interact to affect the binding sites of the metal-oxides (Niedzielski *et al*., 2002; Famah, 2012). This information is also useful when designing effective As removal technologies as well as when determining the As speciation by ion exchange separation techniques (Basu *et al*., 2014).

2.5.3 Solid phase precipitation and dissolution processes

Solid phases which include organic matter and amorphous oxides of Fe, Mn and Al may occur in a variety of thermodynamic states. Solid phase precipitation may be

defined as the formation of a solid phase from components present in aqueous matrices (O'Reilly *et al*., 2001; Sorg *et al*., 2014). For instance, As content within solid phases as a primary structural component of solid phases, is released to groundwater during dissolution processes (O'Reilly *et al*., 2001). Similarly, As is removed from groundwater when solid phases with As precipitate from aqueous matrices (Jablonska-Czapla, 2014; Sorg *et al*., 2014). In most instances, As co-precipitates with Fe-oxides and in such case Fe-oxide acts as an As source during dissolution and a sink in case of precipitation for groundwater (Sorg *et al*., 2014). Solid phase dissolution contributes not only As content within the phase, but also any As adsorbed to the solid phase surface (O'Reilly *et al*., 2001; Niedzielski *et al*., 2002; Famah, 2012).

2.5.4 Effects of microbial activities

There are some bacteria that can endure and grow in the groundwater environment because of the use of organic carbon as food materials (Jablonska-Czapla, 2014). They have the ability to influence As mobility in water directly by accelerating the oxidation of As^{5+} to As^{3+}. Micro-organisms are also capable to methylate inorganic species to become the organic species in particular MMA and DMA (Gao, 2012; Niedzielski *et al*., 2002). Moreover, they are likely to reduce Fe^{3+} on surfaces to Fe^{2+}, which is then released into water coupled with As which was attached to the Fe^{3+} on the surfaces. However, reduction of As^{3+} to As^{5+} and oxidation of Fe^{3+} to Fe^{2+} may be limited by the amount of organic carbon present in water to sustain micro-organisms activities (O'Reilly *et al*., 2001).

2.6 Recent studies based on arsenic

There has been a steady increase worldwide in the number of publications dealing with arsenic in the environment primarily to assess possible human exposure. The review on As studies conducted in Africa is given below.

2.6.1 Arsenic studies in Africa

Fatoki *et al*. (2013) reported that Africa is the least continent affected by As contamination and the findings may be due to the limited research work on As. Some cases of As contamination of groundwater and soil have been reported in different parts of Africa (Huntsman-Mapila *et al*., 2006; Afolabi *et al*., 2011; Clemens *et al*., 2013). Smedley and Kinniburgh (2002) have unravelled the documented cases in Burkina Faso, Ghana, South Africa, Botswana, Ethiopia and Nigeria. In Burkina Faso, concentrations of As in water were found to be ranging from 0.5 – 1630 µg/L. Arsenic concentrations of groundwater in Ghana were dominant in two areas, Obuasi area in Ashanti region and Bolgatanga area of the upper East region varying from less than 1 – 64 µg/L and less than 1 – 141 µg/L, respectively (Smedley and Kinniburgh, 2002). The As source was found to be sulphide mineral rocks such as AsS and FeAsS present in the basement rocks of both areas (Smedley and Kinniburgh, 2002; Fatoki *et al*., 2013). Other reported study by Clemens *et al*. (2013) showed that out of 138 wells samples taken from Ethiopia in the area of the East African Rift Valley, 6.5% indicated As concentration higher than 10 µg/L. The As concentrations in wells from Southern Nigeria were reported to greater than the recommendation of WHO (Smedley and Kinniburgh, 2002). The high concentration resulted from mechanic and panel beaters workshops operations (Smedley and Kinniburgh, 2002).

In Botswana, six of the twenty water samples collected from wells analysed for total As, were reported to have concentration greater than the MPL stipulated by the WHO (Huntsman-Mapila *et al*., 2006). One of the highest As concentrations was found in Zimbabwe with 20 µg/g measured in gold ore deposits (Sami and Druzynnski, 2003). The As concentrations in water around the Ebenezer Dam in Limpopo province were found to be less than 1.0 mg/L which was attributed to the weathering of rocks such as granites and pegmatites which formed domes in the area (Ogola *et al*., 2011).

2.6.2 Arsenic contamination existence in South Africa

South Africa is rich in mineral resources found in diverse geological formations which compete globally to sustain meaningful transformed minerals and mining sector to ensure that South Africans derive sustainable benefit from the country's mineral wealth (WBG, 1998). It is amongst the countries that own a significant proportion of the world's minerals and has been acknowledged to be leading producer of Pt, Cu, Au, Zn and Cr (WBG, 1998; Sami and Druzynnski, 2003). These minerals are usually hosted or forms metal affinity with As like Au-FeAsS, Cu_3As, and ZnAs. Therefore, during the extraction of these valuable metal ores, As is released as by-product (Gao, 2012). In exception to economic wealth accumulated efficiently as a result of mining activities, heavy metal pollution emitted on the environment is of major concern to human health (WBG, 1998; George and Gqaza, 2015). The environmental contamination with As occurs rapidly since it is released as exchangeable ions (As^{3+} or As^{5+}) which adsorb onto the surface of clay, organic matter or oxides with weak bonds that are easily moved and dispersed into the ecosystem (O'Reilly *et al*., 2001; Jablonska-Czapla, 2014). Mining activities are the most source of As in the environmental matrices. In South Africa, severe As contamination has been reported in Northern Cape and levels of As were reported to be as high as 1000 µg/g in soil and sediment samples (Sami and Druzynski, 2003).

2.6.3 Analytical approaches for investigation of arsenic concentrations

Numerous studies on determination of As in different environmental matrices have been reported globally but few studies were carried out in South Africa. Moremedi and Okonkwo (2007) conducted study on total As determination and sequential extraction of As from soil contaminated historically by cattle tick dip operations. The study was based in Ka–Xikundu village in Limpopo province using sequential extraction technique as described by Tessier *et al*. (1979) and McLaren *et al.* (1998). Sequential extraction scheme is usually carried out to perform fractionation with the use of unbuffered salts, weak acids, reducing agents, oxidising agents and strong acids. The fractions obtained when applying this technique are related to exchangeable metals mainly bound to carbonates, metals released in reducible conditions such as those bound to hydrous oxides of Fe and Mn, metals bonded to

components such as organic matter, sulphides and residual fractions (O'Reilly *et al*., 2001; Jablonska-Czapla, 2014). The phases considered relevant in heavy metals adsorption in sediments are oxides, sulphides and organic matter. Moremedi and Okonkwo (2007) found total As concentration in soil samples in the range 0.15 – 1369 mg/kg using HG-AAS. No speciation was reported.

Ramudzuli and Horn (2014) determined total As in soil of cattle dip tanks in the Vhembe district, Limpopo Province and no As species information was reported in their study. The As studies reported by Moremedi and Okonkwo (2007) and Ramudzuli and Horn (2014) were prompted by the application of As that was previously used as sodium salts of arsenous acid in cattle dipping dips to control ticks, which may have resulted in soil contamination. Although the use of As for cattle dipping has been halted, it is important considering environmental point of view, for authors to assess the type, amount, mobility and bioavailability of As at these cattle dipping sites. Arsenic concentration at cattle dip tanks was found ranging between 0.40 – 47 µg/L (Ramodzuli and Horn, 2014).

Ali (2010) determined levels of toxic elements on the food chain exposure pathways to infants in the selected areas of Limpopo province. The author was concerned with establishing the extent to which various components of the environment were exposed to the three toxic elements namely As, mercury (Hg) and lead (Pb). The concentrations of these three elements were determined in groundwater, surface water, soil, plants, goat's milk and women's breast feeding milk in potentially contaminated areas in Limpopo province. In actual facts, the study was prompted by the numerous lead deposits mines occurring in the dolomitic regions of Limpopo province. Ali (2010) highlighted that all three of the most dangerous minerals are found in high concentrations in parts of South Africa and it is possible that these elements might enter the food chain and affect the health of many South Africans. Amongst the selected study areas conducted by Ali (2010), Gravellote samples were reported to have highest level of As in the range of 14 – 18 µg/L. Based on the outcomes of the study in comparison to the mineral content of water at the study sites, with international and national drinking water standards, it shows that water from Rooiberg, Leeupoort and Gravellote areas are not fit for human consumption. The As, Hg and Pb levels above MPLs of drinking water is an indication of polluted water. However, no speciation studies have been conducted.

Botes *et al*. (2007) and Teclu *et al*. (2009) conducted respective biological studies on hyper-resistance of As in bacteria isolated from Antimony mine based at Gravellote in South Africa and bioremoval of As species from contaminated waters by sulphate-reducing bacteria, respectively. Musingarimi *et al*. (2010) studied the characterisation of the As resistance genes in Bacillus species isolated from maturing fly ash acid mine drainage neutralised solids in various regions. Botes *et al*. (2007) aimed to isolate and identify bacteria from As impacted environment to determine the level of As resistance and establish if the resistance is due to the reduction of As^{5+} to As^{3+}. The authors conducted the biological studies to speciate major As species using HPLC-ICP-MS with isocratic mode of elution.

Gilbert and Avenant-Oldewage (2014) addressed the accumulation of trace elements such as As, Cr, Se and Zn in the water, sediments and tissues of *Labeobarbus Kimberleyensis* collected from the Vaal Dam in South Africa. The authors leached out trace elements in sediments and tissues of *Labeobarbus Kimberleyensis* using microwave acid digestion and concentrations of trace elements were detected using ICP-MS. Total As concentrations in water samples were below LOD of 0.045 µg/L whereas As concentration in sediment ranged from 15 – 29 µg/g. No speciation of As was investigated in the study.

The study conducted by Ogola *et al*. (2011) investigated the origin and distribution patterns of Pb, Zn, Cu, As and Cr of water and sediment samples of Ebenezer Dam in Tzaneen, South Africa. The atomic absorption spectrometry (AAS) and x-ray fluorescence (XRF) spectrometry were used to detect the concentrations of trace elements. The reported maximum concentrations of 57 (mg/g) for Pb, 157 (mg/g) for Zn, 313 (mg/g) for Cu, 73 (mg/g) for As and 888 (mg/g) for Cr in sediment samples. The As concentrations in water around Ebenezer Dam were found to be less than 1.0 mg/L. The findings by Ogola *et al*. (2011) confirmed that the distribution of trace elements in that area were affected by the weathering of rocks.

2.7 Sample preparation methods

The application of leaching As procedures vary according to the nature of the study. For instance, leaching procedures for As analysis in soil mostly focused to ascertain

the potential availability and mobility to relate soil-plant transfer of As and to study its migration in a soil profile which is usually linked with groundwater problems (Rauret, 1998). The study investigates speciation of As in river water and sediment samples to assess long term effects and distribution of As among the geochemical phases trapped by underlying sediments. River sediments serve as a reservoir to protect water quality whereas water mobilise heavy metals contaminants. However, sediments may also become a source of pollution under certain conditions like in heavily contaminated areas or in drastically changing environments (Rauret, 1998).

2.7.1 Sample preparation for total concentration determination

Most commonly used methods for mineralisation of As concentration and other heavy metals from soil and sediments involve one step extraction techniques such as wet acid digestion, microwave acid digestion and sequential extraction. These methods of extraction or digestion are usually carried out with the use of one or combination of mineral acids like H_2SO_4, HNO_3, HF, H_2O_2, HCl and H_3BO_3 (Rauret, 1998; Garcia-Manyes *et al*., 2002). Leaching of As in complex matrices by the use of dry-ashing technique entails subjecting certain amount of powered sample to furnace at temperature of 500 ^oC prior to the wet ashing. Liquid-liquid extraction may also be employed for leaching out As in soil or sediment samples (Rauret, 1998). High extraction efficiencies could be attained when the procedure is repeated twice or more to ensure complete transition between the phases (Rauret, 1998). In common practice, powdered sample is transferred into extraction tubes followed by addition of HCl and then centrifuged until become slurry (Murray *et al*., 2001). The use of acids during extraction processes serves a purpose to achieve a complete destruction of heavy metals bearing phases of soil or sediments (Rauret, 1998). However, the loss of analytes is likely to occur when the time consuming extraction techniques like sequential extraction, wet acid and dry acid digestions are exploited. The quantities of analytes in sediments are detected with the use of various techniques such ICP-MS, AAS and ICP-OES.

2.7.2 Sample preparation for speciation analysis

The importance of speciation analysis is due to evoked well documented toxicities. The quantification of total As in various samples matrices is not sufficient to reflect the risk of As to the environment and human health. In this respect, it is crucial to perform speciation analysis. The methods for determining different As species have become increasingly important, because the toxicity, bioavailability, physiological and metabolic processes depend on a distinct chemical species (Hall *et al*., 1999). Consequently, various speciation procedures have been proposed and reviewed worldwide and the latter scientific studies have indicated that there are no universal extraction procedures for different species and different matrices (Ruiz-Chancho *et al*., 2005). However, for each application and target species, a relevant sample treatment procedure has to be developed. Routine analyses involve separation techniques such as HPLC, ion chromatography (IC) and SPE coupled to highly sensitive detection system such as ICP-MS and GF-AAS.

2.7.3 The conditions of analytical procedures for speciation analysis

Sample preparation of the complex matrices is the most critical steps in speciation analysis. The complexity of the sediment matrices should be accounted since the extraction procedure involve selective separation of target species from matrices. This is of paramount importance because the integrity of species should be maintained through the entire analytical procedure. In addition, extraction solutions of weak acids, buffers and complexing agents are known to be extracting the inorganic and organic forms of least molecular weight. The extractant solution should solubilise all As species from complex matrices such as sediments or soil. The operational conditions for extraction should not induce any species interconversion and extractant reagent should not cause oxidation or reduction of native As species (Hall *et al*., 1999; Ruiz-Chancho *et al*., 2005). The use of mineral acids such as HCl, HNO_3 and H_2SO_4 are not recommended for extraction of As species in the complex matrices. The HNO_3 is a strong oxidising agent whereas H_2SO_4 is poor in preserving species in natural samples (Chappell *et al*., 1995). The HCl is not suitable where the method for detection is ICP-MS due to possible chloride based interference of $^{40}Ar^{35}Cl^{+}$ at ^{75}As m/z ratio (Chappell *et al*., 1995).

2.7.4 Sample preparation for speciation analysis of complex matrices

A quite number of studies have been reported to investigate the extraction efficiency of several extractant solutions suitable for leaching out of As species in soil or sediment samples using microwave assisted extraction system (Garcia-Manyes *et al*., 2002; Ruiz-Chancho *et al*., 2005; Pillay and Kindness, 2016). The use of microwave assisted extraction system is convenient and efficient for extraction purpose because of various advantages. The application of low temperature and reduced extraction time are likely to inhibit species interconversion (Lou *et al*., 2014). It also offers enhancement of kinetic of the sample dissolution with high recoveries, good reproducibility with minimal sample manipulation (Lou *et al*., 2014; Rezende *et al*., 2014). The study by Garcia-Manyes *et al*. (2002) investigated extractant reagents such as 0.5 M NaH_2PO4, 0.6 M H_3PO_4 and 0.5 M EDTA, which were independently adjusted to pH 7.0 for the optimum extraction efficiency with no species interconversion. The use of H_3PO_4 yielded maximum extraction efficiency as compared to the other extractant reagents investigated. Subsequently, H_3PO_4 was assayed at different concentrations to adopt suitable extraction conditions of the microwave system which yield excellent extraction procedures without species loss or transformation. The investigations proceeded with the aid of preservation agents such as NaBr, $C_2H_2O_4$, NH_4OCl and $C_6H_8O_6$ to enhance the stability of the As^{3+} during extraction.

Ruiz–Chancho *et al*. (2005) further investigated the extractant solutions of NH_4OCl against H_3PO_4 for leaching As species in sediments with high content of iron oxides. The extractant solution comprised of H_3PO_4 and $C_6H_8O_6$ yielded the optimum extraction efficiency. Hudson-Edwards *et al*. (2004) outlined the importance of using acid phosphate group for the extraction of As species in complex matrices. The acid-phosphate group has emerged as a suitable extraction reagent for As species in complex matrices due to ability to allow ligand exchange between phosphate (PO_4^{3-}) and As^{5+} during extraction. The ligand exchange process for the extraction of As species involve the exchange of a specifically adsorbed Fe-As^{5+} by the PO_4^{3-} group at the surface of soil or sediment leading to the optimum extraction efficiency (O'Reilly *et al*., 2001). The ligand exchange between As^{5+} and PO_4^{3-} group is facilitated by their competition for binding the adsorption site in the surface of the matrices. The imposed competition is attributed to similar chemical characteristics

between As^{5+} and PO_4^{3-} which may include symmetry, ion size, pKa's and molecular structure (O'Reilly *et al*., 2001; Hudson-Edwards *et al*., 2004). An example is the exchange of adsorbed ligand As^{5+} by PO_4^{3-} at an Fe-oxide surface hosted by soil or sediments.

Fe–oxide–As^{5+} + PO_4^{3-} ⟶ Fe–oxide–PO_4^{3-} + As^{5+} (O'Reilly *et al*., 2001).

The other challenge related to extraction of As species is to stabilise the inorganic species. Garcia-Manyes *et al*. (2002) and Ruiz-Chancho *et al*. (2005) reported $C_6H_8O_6$ to be reliable preservative for As^{3+} species when applying the optimised microwave extraction conditions. The As^{3+} remained stable for 6 days at 4 ^oC without showing the effect of species transformation. The latest research study by Pillay and Kindness (2016) opposed the research findings of Garcia-Manyes *et al*. (2002) and Ruiz-Chancho *et al*. (2005) particularly on the use of $C_6H_8O_6$ as preservation agent. The $C_6H_8O_6$ is a reducing agent hence it is likely to induce the reduction of As^{5+} to As^{3+} (Pillay and Kindness, 2016). Pillay and Kindness (2016) capitalised on the known fact that EDTA is the most commonly used chelating and preservation agent. The authors found out that solution containing no EDTA shows an immediate loss of As^{3+}. The use of EDTA preserved As^{3+} and the data showed loss of As species over 2 months of analysis period while DMA and MMA remained stable months over analysis period. The EDTA complexes with Fe, Al and Mn to prevent interaction with As species thus preserving the native As species. The EDTA is the preferred preservation agent when using chromatography (Pillay and Kindness, 2016).

The reported studies by Garcia-Manyes *et al*. (2002), Ruiz-Chancho *et al*. (2005) and Pillay and Kindness, (2016) employed different mobile phases and chromatographic separation techniques hyphanated to different detection systems. These authors have adopted gradient mode of elution to separate and quantify As species. The investigated study by Tziaras *et al*. (2015) achieved a complete separation and quantification of species of interest (As^{3+}, DMA, TMAO, MMA, As^{5+}) using 10 mM $(NH_4)_2HPO_4$ mobile phase by employing isocratic mode of elution. The investigation was based on overcoming analytical methods to explore the occurrence and environmental significance of methylated As species in atmospheric particles. Martinez-Bravo *et al*. (2001) observed various disadvantages related to isocratic elution when using NH_4NO_3 mobile phase. At lower concentrations of NH_4NO_3,

retention time of As^{5+} was achieved in almost 20 minutes and higher concentration led to poor resolution of the less retained species (As^{3+} and DMA). The gradient mode of elution offers the capability to carry out ion exchange chromatography by the application of different ionic strength of the mobile phases (Martinez-Bravo *et al*., 2001).

2.7.5 Sample preparation for speciation analysis in aqueous samples

The HPLC-ICP-MS procedures are widely used for speciation analysis in aqueous samples. However, reliability of the results obtained depends on the sample pre-treatment prior to analysis and initial levels of analytes to be detected. The ultra-trace levels of As species, which occasionally may not be detected when using HPLC-ICP-MS procedure led to speciation analysis by enrichment and separation of analytes. The innovation of simple, ecologically safe, sensitive and selective methods for the determination of trace components is trending in the modern analytical chemistry (Olazabal and Madariaga, 2006). A wide variety of analytical procedures have been advanced for pre-concentration to enhance low concentration of trace analytes in order to meet LODs of the detection techniques (Terada, 1999; Olazabal and Madariaga, 2006). The pre-concentration refers to a process in which the ratio of the concentration or the amount of trace components to the concentration of macro-components is increased (Olazabal and Madariaga, 2006). In common practice of pre-concentration techniques, retained analytes are accompanied by elution or separation as well as detection and quantification of the analytes. Techniques suitable for detection of analytes of interest include ICP-MS, GF-AAS and HG-AAS (Olazabal and Madariaga, 2006). The low sensitivity of ICP-OES hinders its ability of executing ultra-trace levels of analytes.

Pre-concentration and separation SPE technique based on the sorption appear to be convenient and rapid with the ability to attain sufficient pre-concentration factor (Terada, 1999). In SPE, species of elements of interest which have been selectively retained by sorption on different solid-phases are eluted with acids or other reagents (Olazabal and Madariaga, 2006). Therefore, in order to achieve the higher pre-concentration factor the eluent volume should be as small as possible and the volume of sample solution should be as high as possible. The mechanism of

retention of analytes on solid phases depends on the nature of the solid phase and the nature of the species to be retained (Terada, 1999; Olazabal and Madariaga, 2006). The retention process mostly involves adsorption of the metal ions at the surface of the sorbent by interactions with functional groups, or ion exchange, chelation and ion-pair formation processes. Moreover, it also depends on the experimental conditions, such as pH, temperature and metal ion concentrations (Terada, 1999; Olazabal and Madariaga, 2006). Throughout the sorption process, equilibrium is established between the species adsorbed on the surface of sorbent and the species remaining in solution (Olazabal and Madariaga, 2006).

2.7.6 Solid phase extraction procedure

Recently reported studies on speciation of As deal with HPLC or capillary electrophoresis (CE) coupled to ICP-MS or other detection techniques. However, often when handling ultra-trace levels of As in aqueous samples the direct determination of As species is difficult. The preliminary sample pre-treatment with separation and pre-concentration is highly recommended to enhance favourable enrichment factor and recoveries of analytes of interest while eliminating matrix interferences (Türker, 1991). The SPE is an effective sample pre-treatment technique for the extraction and pre-concentration of analytes from complex matrices, with the merits of satisfactory recovery and pre-concentration efficiency, low cost and reduced reagent consumption as well as environmental friendliness (Türker, 1991).

In exception to the enhancement of analytical detection techniques to overcome matrix interferences and to improve sensitivity and selectivity of the method, pre-concentration techniques are further required to advance the determination of trace analytes. Various efforts have been attempted to improve selectivity, rate and capacity of sorption particularly for heavy metals in ground water. Terada (1999) investigated the use of silica gel as a support for loading immobilised complex agent. The author further reviewed the preparation and characterisation of complex-forming adsorbents for the sorption of heavy metals which lay stress on the utilization of silica gel as a supporting material. Subsequently, pre-concentration of trace elements in the environmental matrices were followed by the use of prepared

complex-forming adsorbent. The analytes adsorbed were leached out by heating with nitric acid prior to detection by ICP-OES (Terada, 1999).

The conventional sorbents such as ion exchange resin, glass and modified mesoporous silica have been proposed for As speciation (Chen *et al*., 2014). The effective on-site As separation was achieved by SPE cartridges packed with anion exchange resin in which As^{5+} is retained on the column. However, there is no interaction with As^{3+}, hence it passes through the column. Chen *et al*. (2014) reviewed a highly efficient separation and pre-concentration technique for As speciation based on ionic liquid (IL) dispersive micro-extraction technique implemented in a flow analysis system. The dispersed IL phase was retained by a microbore glass column filled with florisil resin and followed by elution with acidified methanol. The As^{3+} was determined in eluent solution by electrothermal – atomic absorption spectrometry with LOD of 0.05 µg/L (Chen *et al*., 2014).

Chen *et al*. (2013) reported that the emergence of functional nanomaterials such as carbon nanofibers (CNFs) and carbon nanotubes (CNTs), provide excellent sorbent materials in the field of sample pre–treatment. The carbon nanotubes (CNTs) have been used widely and effectively as solid phase extraction sorbent materials to separate and pre-concentrate the target element species for speciation analysis (Chen *et al*., 2013). The discovery of crystalline carbon nanotubes by Iijima (1991) have attracted the worldwide interest due to their exceptional properties such as high mechanical strength, high electrical conductivity, high surface area, competency of forming π-π interaction and high thermal conductivity. Carbon nanofibers have similar chemical properties as carbon nanotubes. They play a significant role in a diverse range of research and application.

Carbon nanotubes exist as a pack of bundles largely hampered by their poor dispersibility due to strong van der Waals forces within the tubules. To improve the chemical reactivity and interaction of CNTs with foreign molecules it is necessary to modify their surface (Su-Tae *et al*., 2015). Furthermore, CNTs are hollow nanometer size tubes of graphitic carbon, resembling a graphene sheet rolled in the form of seamless cylinder. Two forms of CNTs are mainly available; singled-walled carbon nanotubes (SWMCTs) and multi-walled carbon nanotubes (MWCNTs) (Su-Tae *et al*., 2015). The MWCNTs are described as a number of concentric SWMCTs having

different diameters (Iijima, 1991; Su-Tae *et al*., 2015). Modified nanomaterial exhibit highly specific binding affinities to effectively separate and pre-concentrate the native species of interest in total content analysis. The MWCNTs-BPEI nanocomposites were reported to be an excellent adsorbent material for the selective retention of As^{5+} in the presence As^{3+} (Chen *et al*., 2013). The retained As^{5+} is stripped off using diluted NH_4HCO_3 as an eluent. Carbon nanofibers modified with ammoniumpyrroine-dithiocarbarnate (APDC) have analytical capabilities to selectively remove As^{3+}; hence Karimi *et al*. (2014) reported on the use of SPE procedure for As speciation in water samples using micro column packed surface modified CNFs.

Nanomaterials such as modified magnetite nanoparticles have shown the ability to separate and pre-concentrate As species based on molybdenum blue using spectrophotometric technique (Karimi *et al*., 2014). The application of the method was based on adsorption of As^{5+} species on cetylmethylammonium bromide immobilised on alumina-coated magnetite nanoparticles on (CTAB@ACMNPs) after the oxidation of As^{3+} using $KMNO_4$. In this case, total As in water samples is determined as As^{5+} using UV-Visible spectrometric technique based molybdenum blue method. The quantity of As^{3+} was obtained from subtraction of As^{5+} from total concentration (Karimi *et al*., 2014).

2.7.7 Modification of carbon nanotubes

There are several procedures reported to modify the surface of nanomaterials (Chen *et al*., 2013; Karimi *et al*., 2014; Su-Tae *et al*., 2015). The surface modification is usually carried out by acid treatment to introduce surface oxides, carboxylic acids (-COOH), alcohol (-C-OH) and ketone (-C=O) (Chen *et al*., 2013; Karimi *et al*., 2014). The surface oxides act as anchoring sites for the active surface species (polymers, chelating resins, peptides and proteins) to meet the demand for retaining a target analytes of interest attributed to its orientation and specificity (Chen *et al*., 2013). The aqueous oxidising agents' solutions such as H_2SO_4, HNO_3, $H_2SO_4 + H_2O_2$, $H_2SO_4 + HNO_3$, HCl, HF, $KMNO_4 + H_2SO_4$ are generally used to oxidise carbon nanotubes (Malikov *et al*., 2014). The techniques typically used for characterisation of carbon nanotubes include XRD for determination of crystallinity, FTIR, for demonstration of functional groups and TGA for thermal stability analysis.

2.7.8 Factors that affect extraction of As species in complex matrices and aqueous samples for speciation analysis

The process in As speciation not only covers the techniques for species separation, identification and quantification but also the methods for extracting and stabilising the As species from the complex matrices (Garcia-Manyes *et al*., 2002). In South Africa, As speciation studies were reported by Pillay and Kindness (2016). The authors conducted a preliminary investigation into the stability of inorganic As species in laboratory solutions simulating sediment pore water. Pillay and Kindness (2016) reported that due to the complexity of most speciation measurements, on-site analysis is generally not possible and there is some delay before measurements may be carried out. Therefore, successful method to preserve metal speciation, particularly with As, prior to analysis is important as the integrity of species should be maintained throughout the entire analytical procedures to ensure the accuracy of the results obtained (Ruiz-Chancho *et al*., 2005; Pillay and Kindness, 2016).

Various solvent and acid extraction for As species in biological samples (food and plants) have been evaluated with the aid of techniques such as mechanical stirring, ultrasound extraction, supercritical fluid extraction, pressurised liquid extraction and microwave assisted extraction to reduce extraction time and volume of extractant (Chappell *et al*., 1995). Therefore, extraction efficiency depends on the sample matrices of the species extracted, type of solvent, extraction time and temperature. Most of As species present in biological tissues are water soluble and may be extracted with water alone or with a mixture of water and methanol. Solvent extraction has been reported to have low extraction efficiency for the As species in soil or sediment samples (Chappell *et al*., 1995). The author reported preservation of As species in water samples by acidification at pH 4 using H_3PO_4. On contrary, acidifications tend to affect pH of water samples which is likely to induce species interconversion. In order to identify the potential risks in the environmental conditions, As species present in the samples should not be affected during analytical process.

2.8 Characterisation techniques

The analytical procedures for solid phase extraction required the characterisation of adsorbent material using different techniques, which should complement each other in ensuring successful modification of the adsorbent material. The successful modification of MWCNTs with BPEI is able to execute analytical performance characteristics for the intended use.

2.8.1 X-ray diffraction spectroscopy

X-ray diffraction is a widely used analytical technique primarily for phase identification of a crystalline material (Moore and Reynolds, 1997). This technique can also be used for studying particles in liquid suspensions or polycrystalline solids and provide information of crystallite size particularly in nanomaterials (Dutrow, 1997; Hovis, 1997). X-ray diffractometers consist of three basic components namely, an X-ray tube, a sample holder, and an X-ray detector. X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray patterns are produced. The specific wavelengths are characteristic of the target material (Cu, Fe, Mo, Cr). Copper is the most common target material for single-crystal diffraction, with CuKα radiation = 1.5418 Å. The diffracted X-rays patterns are presented as peak positions at two theta (2θ) in degrees and X-ray intensity counts in the form of an x – y plot (Moore and Reynolds, 1997).

2.8.2 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy is an analytical tool used to identify functional groups of the sample material. With an FTIR spectroscopy, infrared radiation is passed through a sample and the resulting spectrum represents the molecular absorption and transmission is measured as a function of its wavelength. Molecular fingerprint of the sample material represents infrared absorption peaks

corresponding to the frequencies of vibrations between the bonds and functional groups of the atoms making up the material. An FTIR absorption peaks are usually represented as a plot of intensity against wavenumber (cm^{-1}) (Thermo Nicolet Corporation, 2001). The absorption bands can confirm the identity of a pure compound or to detect the presence of specific impurities (Thermo Nicolet Corporation, 2001).

2.8.3 Thermogravimetric analysis

Thermogravimetric analysis is an analytical technique used to investigate weight changes in a sample with variations in heating temperature (Willard *et al*., 1974). The weight change exhibit mass loss or gain influenced by decomposition, oxidation or loss of volatiles. Weight gain of the material is usually observed when a foreign molecule is added to enhance the stability. This technique is useful for the study of polymeric materials, carbon black content and decomposition temperature, moisture content of organic and inorganic materials. A TGA comprises an automated balance on to which the sample is loaded and pan containing sample is encapsulated by furnace. A TGA analysis is performed by gradually raising the temperature of a sample in a furnace to about 1000 ^{o}C at a heating rate of 5 – 10 ^{o}C per minute. The sample weight is measured continuously to higher temperatures and mass loss is observed provided thermal events involve loss of a volatile component. The mass loss is recorded as a function of temperature (Sichina, 2001).

2.9 Detection techniques

Different analytical techniques such as ICP-MS, HG-AAS, GF-AAS, ICP-OES and HPLC-ICP-MS techniques are widely employed to carry out assessment of trace elements.

2.9.1 Inductively coupled plasma-mass spectrometry

The ICP-MS recognised as one of the most powerful multi-element analytical technique which is capable of detecting ultra-trace levels of elements (D'llio *et al*., 2012). The samples are nebulised into inductively coupled plasma with temperature of approximately 9000 K. At such high temperature, the nebulised solution is vaporised and then analytes species are atomised and ionised. The ions generated in the high temperature argon plasma are subsequently accelerated into a quadrupole mass analyser for both elemental and isotopic analysis. Intensity measurements are converted to elemental concentration by comparison with calibration standards. This technique has greater speed, precision, and sensitivity, which allows rapid sample processing due to its capability to scan for all elements simultaneously (D'llio *et al*., 2012).

One of the major disadvantages of ICP-MS is the occurrence of interferences caused by atomic or polyatomic species having the same m/z ratio of the analytes (D'llio *et al*., 2012). The presence of interferences may affect the analytical results if they are not properly removed or corrected. The interferences may be classified as spectral and non-spectral interferences. The spectral interference usually occurs as isobaric elemental interferences in ICP-MS caused by isotopes of different elements forming atomic ions with the same nominal m/z ratio (Sheppard *et al*., 1991). Non-spectral interferences are associated with the sample nebulisation and transport processes as well as with ion-transmission efficiencies. Nebulisation and transport processes may be affected if a matrix component causes a change in surface tension or viscosity. Therefore, changes in matrix composition may cause significant signal suppression or enhancement (Sheppard *et al*., 1991). The most stable isotopes for each element with less or no polyatomic or isobaric interferences are monitored for quantification. For instance, suitability for monitoring the isotopic mass ^{58}Fe is usually based on free interferences provided ^{58}Ni isotope of Ni is not determined (Eagles *et al*., 2010). Furthermore, ^{56}Fe isotope is likely to encounter the isobaric interference at signal 56 m/z resulting from $^{40}Ar^{16}O^{+}$. Additionally, monoisotopic elements such as ^{55}Mn and ^{27}Al encounter no interference (Eagles *et al*., 2010). The isotopic ^{75}As is likely to encounter molecular interference from argon (Ar) and chloride (Cl) particularly at $^{40}Ar^{35}Cl^{+}$ because of the similar m/z ratio as ^{75}As (Ruiz-chancho *et al*., 2005; Murray *et al*., 2001).

Generally, application of dynamic reaction cell (DRC) and collision cell technology (CCT) modes are incorporated in the ICP-MS to reduce polyatomic interferences. The DRC is capable of removing the interferences before they reach the mass spectrometer by using controlled gas-phase reaction chemistry inside an enclosed cell containing a second quadrupole mass filter (Murray *et al*., 2001; D'llio *et al*., 2012).

The successful application of DRC is significantly observed when the interference is known and the magnitude of the interference contribution is far greater than the analytes signal. However, DRC-ICP-MS application is limited to reduce polyatomic interferences of the reactive molecular ions (D'llio *et al*., 2012). Alternatively, the use of collision cell technology (CCT) mode consolidated in the ICP-MS may overcome limitations of DRC-ICP-MS. Collision cell technology is efficiently used when the interference is not known or the interference contribution is not more than five orders of magnitude greater than the analytes signal and the element of interest is less than100 amu (Murray *et al*., 2001). The figure 2.3 shows Perkin Elmer Sciex Elan 6100 ICP-MS.

Figure 2.3: Perkin Elmer Sciex Elan 6100 ICP-MS

2.9.2 Inductively coupled plasma-optical emission spectrometry

The ICP-OES is one of the most powerful and popular analytical tools for the determination of trace elements in various matrices types (Murray *et al*., 2001). The technique is based upon the emission of photons from atoms and ions that have been excited in inductively coupled plasma. Similarly to ICP-MS, the sample solution is introduced into the inductively coupled plasma with temperature of approximately 9000 K. However, in ICP-OES the analytes atoms and ions become thermally excited at this temperature and emit light at their characteristic wavelengths. This light is focused on to the entrance of the slit of the spectrometer and passes through a diffraction grating that resolves the light into a spectrum of its constituent wavelengths. Within the spectrometer, the diffracted light is then separated and collected as emission line and amplified to yield an intensity measurement that can be converted to an analyte concentration by comparison with calibration standards (Murray *et al*., 2001).

The elemental analysis using ICP-OES usually encounter spectral interferences when measured element is erroneous due to the presence of another element also detected at that wavelength. This may be compensated by background correction or use of alternative emission lines (Thompson, 2002). Analytes emit energy at specific wavelengths and the intensities of energy emitted is proportional to concentration of the analyte (Thompson, 2002). In most cases, primary wavelengths show less interferences and are recommended to carry out the analysis. The figure 2.4 shows Spectro Arcos ICP-OES.

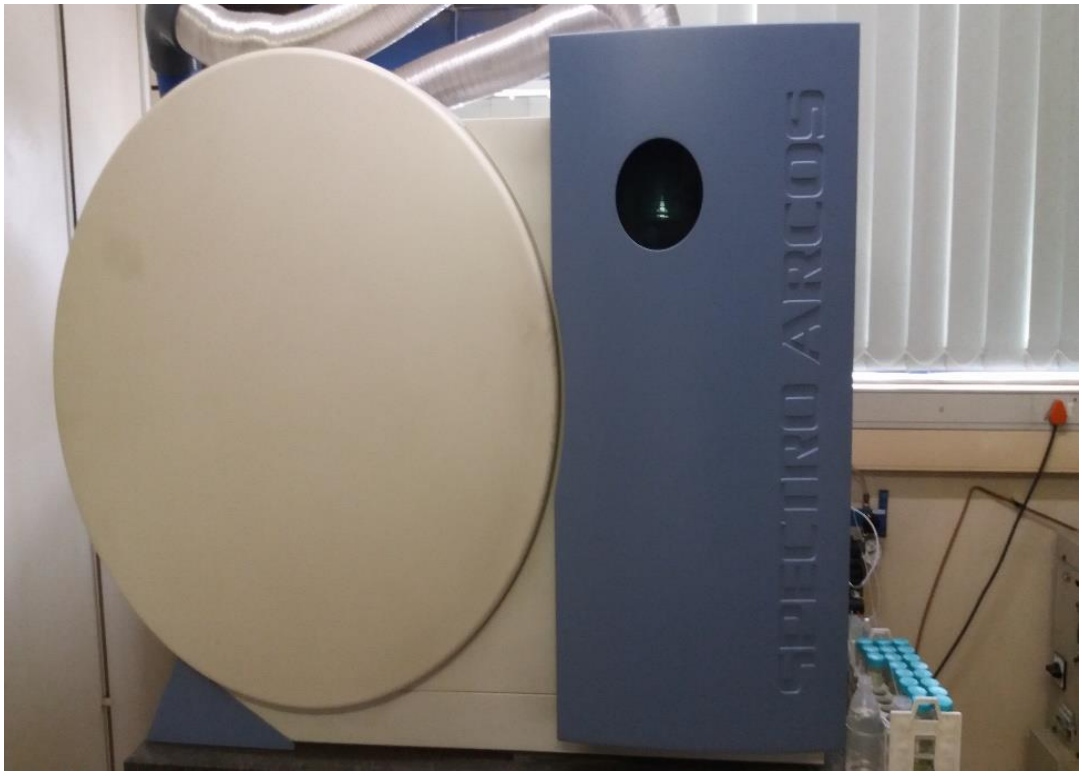

Figure 2.4: The Spectro Arcos ICP-OES

2.9.3 Atomic absorption spectrometry

The other detection techniques which may be used for determination of As, Fe, Mn and Al are flame–atomic absorption spectrometry (F-AAS), HG-AAS and GF-AAS. The HG-AAS is a very effective analytical technique developed to separate hydride forming elements such as Se and As from a range of matrices and varying concentrations. This is due to low absorption wavelengths, which are below 200 nm which makes analytes less accessible to intense interference from flame radicals that may significantly affect detection limits (Hineman, 2012).

The GF-AAS is an analytical technique designed to perform the quantitative analysis of elements in a wide variety of samples. It exhibits high sensitivity primarily because there are no flame gases to dilute the free, gaseous atoms during analysis (Hergenreder, 2011). Unlike ICP-MS, HG-AAS and GF-AAS the low sensitivity of F-AAS often encounters some drawbacks when are to execute trace levels of As in the environmental samples (D'llio *et al*., 2012). The figure 2.5 shows Perkin Elmer Pinaacle 500 F-AAS.

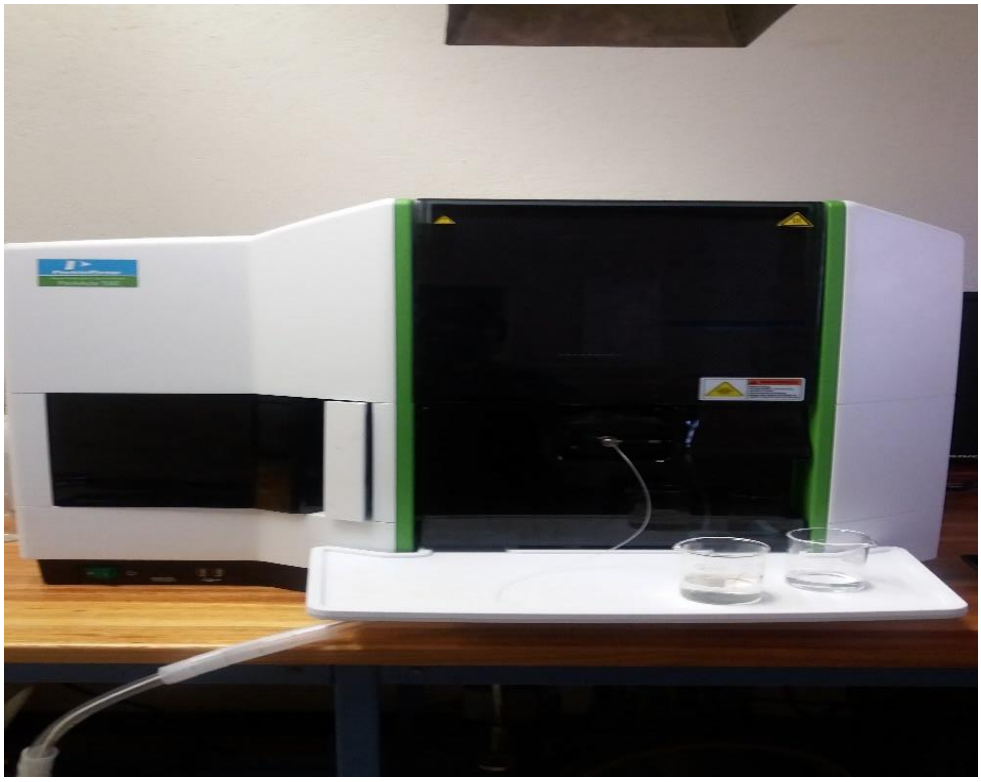

Figure 2.5: Perkin Elmer Pinaacle 500 F-AAS

2.9.4 High performance liquid chromatography coupled to inductively coupled plasma-mass spectrometry

The HPLC-ICP-MS is a technique, which provides nearly all combinations necessary to execute speciation studies. For speciation analysis, the HPLC application is based on the separation of compounds and subsequent detection and identification are possible by aid of the coupled detection system with the ICP-MS (D'llio *et al*., 2012). The use of ICP-MS give great capabilities to attain high sensitivity and wide linear dynamic range. The HPLC-ICP-MS is an established best combination technique that provides simple and reliable way to monitor speciation of trace element in the environmental matrices.

2.10 Analytical figures of merit

Analytical figures of merit are performance characteristics also known as quantitative terms useful for evaluation of the performance of analytical procedure (Komorowicz

and Baralkiewicz, 2011). The quantitative terms link the analytical procedures with analytes of interest and are not limited to LOD, limit of quantification LOQ and linearity. Moreover, analytical figures of merit relate the presentation of analytical results by means of assessment of precision and accuracy. Prior to quantitative terms investigation of the analytical procedure, operational conditions of the analytical instruments employed are calibrated for efficient operation. The acceptance of the analytical procedures is based on optimum performance of the analytical figures of merit and method validation (Komorowicz and Baralkiewicz, 2011).

2.10.1 Limit of detection and limit of quantification

The LOD is the lowest amount of analytes in a sample which may be detected but not necessarily quantified as an exact value (Shrivastava and Gupta, 2011). Generally, lowest limits are evaluated as the signal-to-noise ratio (S/N) equivalent to 3 times the standard deviation of the noise. The LOQ may be defined as the lowest amount of analytes in a sample which may be quantitatively determined with suitable precision and accuracy (Sanagi, 2009; Shrivastava and Gupta, 2011). The LOQ could be estimated by using the proper standard measurement or standard sample and it may not be extrapolated (Shrivastava and Gupta, 2011; Cuadros-Rodriguez *et al*., 2007). The LOD and LOQ procedure are calculated as the concentration corresponding to 3 times and 10 times the standard deviation of the average of at least 6 individually prepared reagent blank solutions, respectively (Shrivastava and Gupta, 2011).

2.10.2 Linearity and method validation

The linearity exhibit the ability within a given range of calibration standards to obtain analytical results which are directly proportional to concentrations of the analytes in the sample. The range of calibration standards may be plotted against the corresponding concentrations to deduce the linear calibration curve with the correlation coefficient (Stone, 2016).

The method validation is indispensable for the development of analytical procedures since it provides proper characterisation for the assessment of the validity of the method (Komorowicz and Baralkiewicz, 2011). Method validation verifies the effectiveness of the applicability of analytical procedure for the intended use. Generally, validation is performed to evaluate the percentage recoveries with reference to certified values of the analytes on the certification of analysis of the SRMs (Komorowicz and Baralkiewicz, 2011). The quality assurance and control are also monitored to evaluate the precision and accuracy of the results obtained.

CHAPTER 3

EXPERIMENTAL

This chapter outlines the justification of selected study areas, sample collection, sample pre-treatment, and sample preparation procedures. The chemicals and their purity, standards and standard reference materials are mentioned in this chapter. This chapter also includes the instrumentation employed for analysis of samples and instrumental conditions.

3.1 Description and justification of the study areas

Limpopo province has been recognised as one of the biggest provinces in mining due to the availability of wide ranges of mineral deposits (Ali, 2010). The major mining towns are Phalaborwa and Thabazimbi followed by Burgersfort where five platinum mines have been established (Ali, 2010). The other mining town is Lephalale where coal mines such as Grootegeluk and Boikarabelo are located (RHP, 2006; Ali, 2010). Mining industries significantly contribute to the country's economic growth (RHP, 2006). However, mining and other industries such as smelters, steel operations and power stations have increased the prevalence and occurrence of toxic As species through dust emissions, mine tailing and wastewater effluents (RHP, 2006). The rural communities are likely to be exposed to harmful levels of As produced as a by-product because mining operations are spread mostly in the vicinities of rural areas. Despite the mineral wealth, Limpopo province is one of the poorest in South Africa exhibiting strong rural basis (Ali, 2010; DWA, 2011). The majority of the province comprise of rural areas where surface water serves as a water resources for plants, animals and humans, especially during the times of water scarcity (DWA, 2011).

3.1.1 Great Letaba River

Great Letaba River is one of the perennial rivers that flow through the Lowveld region of South Africa. It originates in the area of high rainfall catchment in

Drakensberg escarpment. It flows through one of the largest citrus-growing area around Tzaneen and Phalaborwa regions and then to Kruger National park (DWA, 2011; RMP, 2015). Great Letaba River has a rocky bed with a complex geology varied between sedimentary rocks in the North and metamorphic and igneous rocks in the South (DWA, 2011; RMP, 2015). The photo showing Great Letaba River during high flow sampling season is indicated in figure 3.1. The depletion of potable water resources prompted the initiation of strategic adaptive management (SAM) to sustain the capacity for adaptation of water resource management in the Great Letaba River (RMP, 2015). The implementation of SAM is facilitated by the institutional interaction between dam operators and stakeholders including Kruger National Park. The SAM approach led Great Letaba River to have a reduced flow of water during low rainfall season due to number of dams such as Tzaneen, Magoebaskloof and Ebenezer built within the river (RMP, 2015).

Figure 3.1: Photo showing Great Letaba River during high flow sampling season

Water and sediment samples were collected in Great Letaba River upstream starting from the Tzaneen dam descending through the North of Phalaborwa to the downstream before Kruger National Park. The map showing 10 sampling sites in Great Letaba River is indicated in figure 3.2. The sample collection was based on the river accessibility. The agricultural activities were observed alongside of the river throughout the upstream. The agricultural activities tend to negatively affect the

quality of water by the use of perhaps As containing herbicides washed out into river during heavy rainfall. The mining and other industries were found in the proximity of the upstream of Great Letaba River. The activities of swimming, fishing, water collection for irrigation and household purposes and sand mining were observed during sampling campaign. Sand mining tends to slightly destroy natural habitat of the river. The photo showing fish obtained from Great Letaba River by local fisher is indicated in figure 3.3. The hippos and crocodiles had inhabited the river whereas domestic animals (cattle, goats and donkeys) were observed drinking water directly from Great Letaba River.

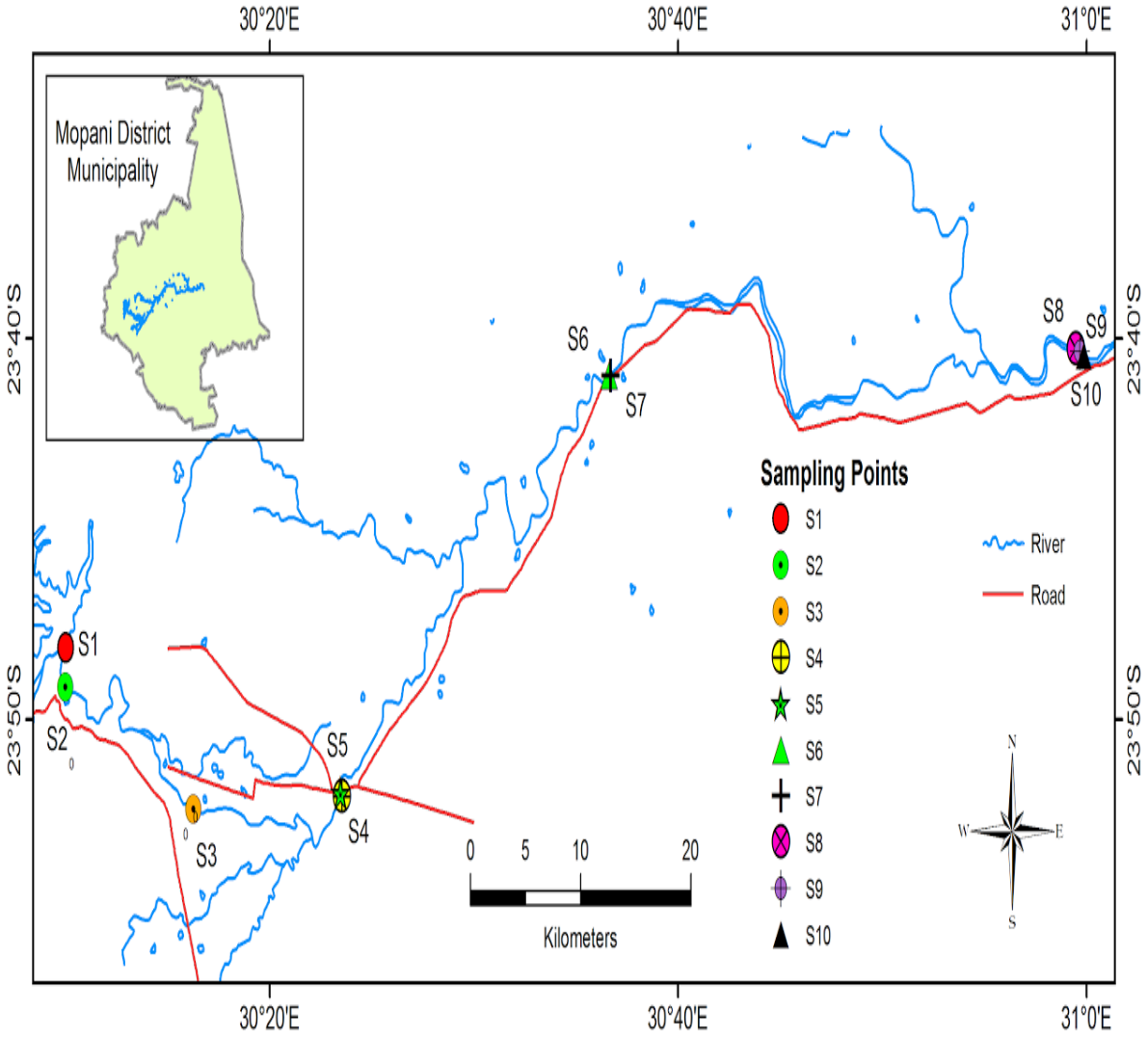

Figure 3.2: Sampling sites in Great Letaba River

Figure 3.3: Fish obtained from Great Letaba River

3.1.2 Mokolo River

Mokolo River originates in the western part of Waterberg District near Modimolle (Nylstroom) flowing to the North passing through Lephalale area to Limpopo River (Sibisi, 2009). The variety of anthropogenic activities observed surrounding Mokolo River are likely to impact negatively on the water quality of the river system. Lephalale area, previously known as Ellisras, is a small coal mining town adjacent to Mokolo River. It is the host of the Grootegeluk and Boikarabelo coal mines as well as Matimba and Medupi power stations which are found few miles from Mokolo River (Sibisi, 2009). The water scarcity issue had led Medupi power station to operate with air-cooled condenser to generate electricity (Oberholster, 2010). Water source for Lephalale area is Mokolo River flowing into Mokolo dam constructed to supply water to Lephalale municipality, power stations and coal mines (DWAF, 2012; Sibisi, 2009; Oberholster, 2010).

Sampling at Mokolo River was conducted around Lephalale area during different flow seasons of the river on the same sampling sites. The photos showing Mokolo River during high and low flow sampling season are indicated in figure 3.4 and figure 3.5, respectively.

Figure 3.4: Photo showing Mokolo River during high flow sampling season

Figure 3.5: Photo showing Mokolo River during low flow sampling season

Samples were collected at 10 sites based on the ability to access the river. The first sampling season was done during high flow and a second sampling season was during low flow as indicated in figure 3.5. The water and sediment samples were collected from site 1 to site 10 shown by sampling map in figure 3.6. Mokolo River is a tributary of Limpopo River which is found in the North of Limpopo province. Hence, it flows towards the North of the river (Site 1). The sand mining and water abstraction

pump systems for irrigation purpose were the major activities taking place in the river observed during sampling campaigns. The photo showing water abstraction from Mokolo River for irrigation of farmlands is indicated in figure 3.7.

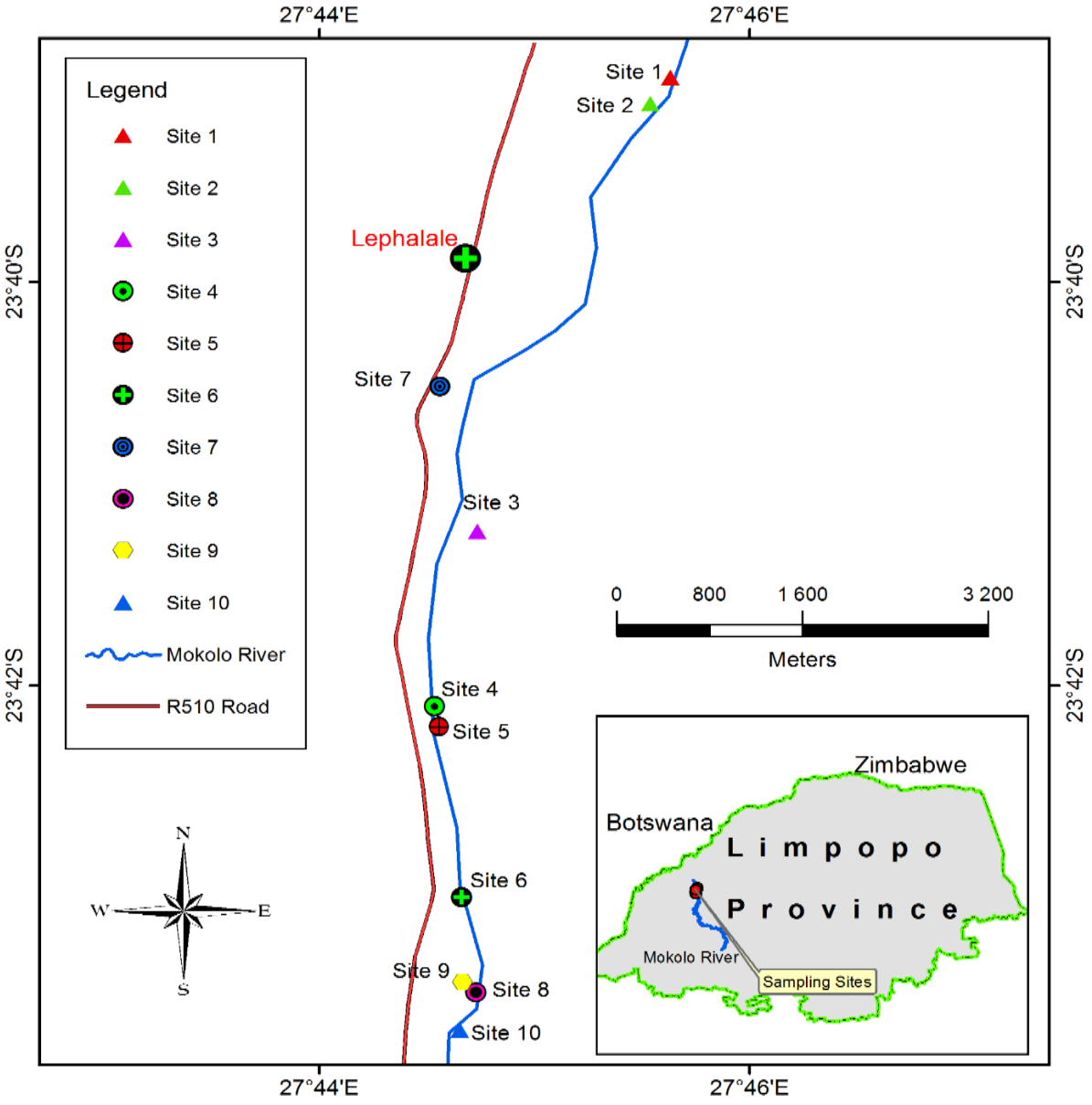

Figure 3.6: Sampling sites in Mokolo River

Figure 3.7: Photo depicting water abstraction system in Mokolo River for irrigation purpose

3.2 Chemicals, standards and standard reference materials

High purity chemicals were used throughout this work for preparation of water and sediment samples. De-ionised water obtained from laboratory ultra-pure water purification unit (Milli-Q® Reference, Merck) with a resistivity of 18.2 MΩ.cm at 25 °C was used for dilution of calibration standards and samples. To attain complete digestion of sediment samples for concentration determination of As, Fe, Mn and Al, the following mixture of acids were used HCl (puriss p.a, 37%, Sigma Aldrich), HNO_3 (puriss p.a, 65%, Sigma Aldrich), H_2O_2 (puriss p.a, 30%, Sigma Aldrich) and HF (40 – 45%, Sigma Aldrich). Extraction of As species in sediment samples was conducted using $(NH_4)_2HPO_4$ (dibasic, 99%, Chemies reagent), NH_4OH (ACS reagent, 28 – 30% NH_3 basis, Sigma Aldrich) and EDTA (Analytical grade ≥ 99%, Sigma Aldrich). Mobile phase used for separating native As species was prepared by NH_4NO_3 (ACS reagent ≥ 98%, Sigma Aldrich) and pH was adjusted with NH_3 solution (7 N CH_3OH, Sigma Aldrich). Calibration standards used for determination of As, Fe, Mn, Al and native species in water and sediment samples were As^{5+} standard (1000 µg/mL, Aldrich Chemistry), Fe (1000 mg/L Fe in HNO_3, Sigma Aldrich), Mn (1000 mg/L Mn in HNO_3, Sigma Aldrich), Al (1000 mg/L Al in HNO_3, Sigma Aldrich), As^{3+} standard (1000 µg/mL, Aldrich Chemistry), DMA standard (500 mg DMA analytical grade,

Sigma Aldrich) and MMA (500 mg MMA analytical grade, sigma Aldrich). Gallium (Ga) (99.99%, Sigma Aldrich) was used as internal standard. The SPE procedure was carried out using the following chemicals: MWCNTs (95% carbon 6 – 9 nm x 5 µm, Aldrich Chemistry), BPEI (Mw 25 kDa, Sigma Aldrich), NaCl (ACS Reagent ≥ 99%, Sigma Aldrich), NH_4HCO_3 (Reagent Plus ≥ 99%, Sigma Aldrich), H_2SO_4 (Reagent grade ≥ 95%, Sigma Aldrich), HNO_3 (Reagent grade ≥ 55%, Sigma Aldrich) and $AgNO_3$ (ACS reagent ≥ 90%, Sigma Aldrich). The post-digests, extracts and water samples were filtered through laboratory filtration system using nylon 66 filter membranes (pore size of 0.45 µm, diameter 47 mm) obtained from Sigma Aldrich. The standard reference materials (SRMs) used for validation of the analytical methods for water and sediment samples were SRM 1643f – Trace elements in water (NIST, USA), SRM 8704 – Buffalo River sediment (NIST, USA) and BCR 280R – lake sediment certified reference material (CRM) (IRMM, European Commission).

3.3 Apparatus and instrumentation

The following analytical instruments were employed to carry out a successful study. The extraction of As species and digestion of trace elements were carried out using microwave reaction system (Anton Paar Multiwave Pro, Canada) and microwave digestion system (Mars 5, CEM Corporation, United States of America). The HPLC (Flexar Solvent Manager, Perkin Elmer, Singapore) coupled to ICP-MS (Sciex Elan 6100, Perkin Elmer, Germany) was used for separation and detection of As species. The concentration determination of trace elements was carried out using ICP-MS (Sciex Elan 6100, Perkin Elmer, Germany) and ICP-OES (Spectro Arcos, Metek Material Analysis Division, Germany). The characterisation of nanomaterial was conducted using XRD (PW 1830 X-ray diffractometer, PANalytical, Philips), FTIR spectroscopy (Spectrum BX, Perkin Elmer, Germany) and TGA technique (Mettler Toledo, United States of America).

3.4 Sample collection and pre-treatment

Water and sediment samples were collected at Mokolo River in different flow seasons of the river and Great Letaba River. At each sampling point, global

positioning system (GPS) coordinates to allow the exact sample collection location were recorded to plot the sampling sites maps. The pH of water samples from both river systems were found in the range 7 – 8.5. High-density polyethylene sampling bottles of 500 mL and 1000 mL were soaked in 10% (v/v) HNO_3 for 24 h and rinsed thoroughly with distilled water before sampling campaigns. Bottles were further rinsed with de-ionised water and left to dry. At the sampling sites, bottles were rinsed with river water before collection of water samples. Water samples were collected at the bottom of water surface and bottles were closed while still kept at the bottom of water surface to minimise sample exposure to air. Sediment samples were collected at the point where water samples were collected. Collections of sediments were based on the ability to trap sediments beneath the surface of water using plastic scooped spoon. Samples were kept in the cooler box containing ice and transported to laboratory. Water samples for speciation analysis were filtered through 0.45 µm nylon 66 filter membranes and kept in a refrigerator at 4 ^oC. For total concentration determination, water samples were filtered, preserved with 1% (v/v) HNO_3 and kept in the refrigerator. Sediment samples were air-dried, ground to fine powder using agate mortar and pestle and were sieved using Labotec sieve with the opening aperture of 150 µm.

3.5 Sample analysis

Water and sediment samples were analysed for determination of As species and total concentrations of As, Fe, Mn and Al using suitable techniques.

3.5.1 Determination of arsenic species in water and sediment samples

Filtered water samples were transferred into plastic vials and loaded to HPLC (Flexar Solvent Manager, Perkin Elmer) coupled to ICP-MS (Sciex Elan 6100, Perkin Elmer) for species separation and quantification. Mobile phases were prepared in sterilised glassware by dissolving appropriate amount NH_4NO_3 in de-ionised water. Mobile phases were adjusted to a pH of 8.7 using NH_3 solution. The powdered sediment samples of 500 mg were transferred into pre-cleaned Teflon vessels for extraction of As species using microwave extraction system (Anton Paar Multiwave Pro, Canada).

A 10 mL of extractant solutions was then added into Teflon vessels. The extractant solution was prepared from 0.3 M $(NH_4)_2HPO_4$ and 50 mM EDTA. The EDTA was dissolved in 5% NH_4OH before preparation of extractant solution.

Table 3.1: Heating conditions for microwave extraction system

Parameters	settings
Power	100 W
Pressure	30 bar
Maximum pressure rate	0.5 bar/s
Internal temperature limit	150 ^oC
Ramping time	20 min
Holding time	15 min

Table 3.2: The HPLC-ICP-MS operating conditions for separation and detection of arsenic species

Parameters	Settings
HPLC parameter and setting instrument	Perkin Elmer Flexar Solvent Manager
Analytical column	Hamilton PRP-X100
Column dimension	4.6 x 250 mm, 5 µm
Guard column dimension	4.6 x 150 mm, PEEK
Pump flow rate	1 mL/min
Pump pressure	2320 psi
Injection volume	100 µL
Mobile phase A	10 mM NH_4NO_3 at pH 8.7
Mobile phase B	60 mM NH_4NO_3 at pH 8.7
ICP-MS parameter and setting instrument	Perkin Elmer Sciex Elan 6100
Nebulizer gas flow	1.0 L/min
Auxiliary gas flow	1.2 L/min
Plasma gas flow	14 L/min
ICP RF power	1350 W
Lens voltage	14 V

The vessels were mounted in the rotor and placed in the microwave extraction system. Extracts were allowed to cool and were filtered using Acrodisc syringe filters. Later, 5 mL of extracts were diluted to 10 mL and kept in the refrigerator at 4 ^{o}C for maximum of 3 days before analysis using HPLC-ICP-MS. The optimised microwave heating conditions for extraction and HPLC-ICP-MS parameters are indicated in table 3.1 and table 3.2, respectively.

3.5.2 Determination of total concentrations of As, Fe, Mn and Al in water and sediment samples

Total As concentrations in water samples were determined using ICP-MS (Sciex Elan 6100, Perkin Elmer). Quantification of Fe, Mn and Al in water samples were conducted using ICP-OES (Spectro Arcos, Germany). Water samples were diluted two times before analysis using ICP-MS. The parameters for ICP-MS and ICP-OES are indicated in the table 3.3 and table 3.4, respectively.

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 V
Lens voltage	10 V
Analogue stage voltage	-2550 V
Pulse stage voltage	1050 V
Torch box temperature	30 ^{o}C
Cooling system:	
1. Main water temperature	18.0 ^{o}C
2. Interface water temperature	32.6 ^{o}C

Table 3.4: ICP-OES operating conditions

Parameters	Settings
Plasma power	1400 W
Coolant flow	13 L/min
Auxiliary gas flow	2 L/min
Nebulizer gas flow	0.95 L/min

The finely ground sediment samples were digested for determination of As, Fe, Mn and Al. Depending on the nature of sediment types, various pools of mineral acids were assayed for the complete acid digestion. Sediment samples of Great Letaba River were digested completely with mixture of 4 mL H_2O_2 and 4 mL HNO_3 using two stages of microwave digestion system (Mars 5, CEM Corporation). A 250 mg of powdered samples were weighed into the pre-cleaned Teflon vessels and after the addition of reagents, the vessels were closed and kept at room temperature for 15 minutes. The vessels were then mounted in the rotor, which was placed in the microwave digestion system. The two stage heating conditions of microwave digestion system are indicated in the table 3.5.

Table 3.5: Two stages microwave digestion heating conditions

Parameters	Settings
First stage	
Power	1600 W
Pressure	800 psi
Temperature	200 ^oC
Ramping time	20 min
Holding time	15 min
Second stage	
Power	1600 W
pressure	800 psi
Temperature	200 ^oC
Ramping time	15 min
Holding time	15 min

Mokolo River sediment samples were digested completely with an acid mixture of 4 mL HCl, 1 mL HNO_3 and 1 mL HF using one stage of microwave digestion system following the same procedure as Great Letaba River sediments. The digested sediments were transferred into a beaker after cooling and further heated at 25 ^oC for 20 minutes on an electric heating mantle (Heating and stirring mantle, Thermo Scientific) to evaporate HF with a boiling point of 19 ^oC. The acidic strength of HF tends to itch ICP-MS torch during samples analysis.

Different reagent combination used for digestion of sediment samples was essential due to different types of sediments found in Great Letaba and Mokolo river systems. Mokolo River is constituted by sandy sediments hence a stronger acid combination was required to achieve complete digestion as compared to mud-like sediments of Great Letaba River. The heating conditions of microwave system adopted for these procedures are indicated in the table 3.6.

Table 3.6: One stage microwave digestion heating conditions

Parameters	Settings
Power	1600 W
Pressure	800 psi
Temperature	250 ^oC
Ramping time	25 min
Holding time	15 min

The post digests were transferred to 50 mL calibrated vials and diluted with de-ionised water to the mark and kept in refrigerator at 4 ^oC until taken out for analysis. The digested samples were further diluted before total concentration determination of Fe, Mn and Al to prevent overloading ICP-MS detector. The Ga internal standard of 50 µg/L was added to blanks, standards, SRMs, water and sediment samples. All samples were filtered using Acrodisc syringe filters before analysis. The ICP-MS conditions indicated in table 3.2 were employed during sample analysis.

3.5.3 Solid phase extraction procedure

The SPE procedure for pre-concentration of As^{5+} in water samples was achieved by use of prepared MWCNTs-BPEI nanocomposites. Prior to the application of SPE, control-MWCNTs were oxidised with acids, modified with BPEI and characterised using various characterisations techniques.

3.5.3.1 Oxidation and modification of multi-walled carbon nanotubes

The oxidation of MWCNTs was achieved by exploring the performance of various acids combinations under the optimised conditions to successfully introduce the functional groups of interest. A 300 mg of MWCNTs were sonicated with a mixture of H_2SO_4/HNO_3 (3:1, v/v) at 30 ^oC for 30 minutes. The oxidised MWCNTs were collected by filtration and repeatedly washed with de-ionised water until the pH 7 reached. Oxidised MWCNTs were dried in an oven at 50 ^oC for overnight before the modification with BPEI polymer. The incorporation of oxidised MWCNTs to BPEI (1:3; w/v) was achieved by stirring a mixture of 500 mg oxidised MWCNTs and BPEI polymer, which was prepared by dissolving 1500 mg of BPEI into 30 mL of 1.0 M NaCl for 12 hours using magnetic stirrer plates at room temperature. The MWCNTs-BPEI nanocomposite were collected by vacuum filtration and washed thoroughly with de-ionised water to remove the impurities. The complete removal of NaCl impurities were tested using 0.1 M $AgNO_3$ solution. The schematic representation of oxidation and modification of MWCNTs is indicated in figure 3.8. Nanomaterials were characterised using XRD technique, FTIR spectroscopy and TGA technique.

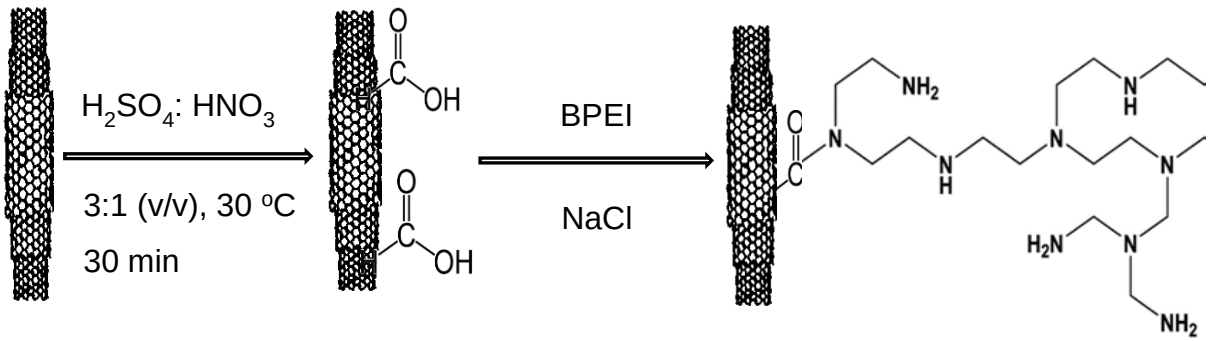

Figure 3.8: Schematic representation of oxidation and modification of MWCNTs

3.5.3.2 The MWCNTs-BPEI preparation and characterisation by XRD

A 1000 mg of the sample was ground to a fine powder using agate mortar and pestle and mounted on an acetone-cleaned Al plate sample holder. In order to achieve sample flatness, a glass slide was used for removal of excess powder. It was then mounted in the sample chamber of the diffractometer. The XRD patterns of the control MWCNTs, oxidised MWCNTs, MWCNTs-BPEI and BPEI polymer were collected on Philips PW 1830 X-ray diffractometer using Cu-Kα radiation (λ = 1.5405 Å), operating at a voltage of 40 kV and a tube current of 40 mA. The scanning speed was 0.025 °/s over the 2θ range of 2 – 63°. The XRD patterns were then recorded on the computer equipped with a PC-APD diffraction software and saved in Microsoft Excel 2010 format for analysis. The results were interpreted using data analyser Origin 6.1 software.

3.5.3.3 The MWCNTs-BPEI preparation and characterisation by FTIR

A 10 mg of finely ground sample was placed between the face of a KBr plate and window plate. The window plates were gently moved in circular, back-and-to evenly distribute the sample between the plates. An FTIR spectra of control MWCNTs and MWCNTs-BPEI nanomaterials were collected on Perkin Elmer Spectrum BX FTIR system as a percentage transmittance of IR radiation. The 32 number of scans were collected per spectrum and resolution was 4.0 cm^{-1} with the wavenumber ranging within 1000 – 4000 cm^{-1}. An FTIR data were exported to ASCII format for the plotting of FTIR spectra using data analyser Origin 6.1 software.

3.5.3.4 Column preparation and application for solid phase extraction

A syringe with a diameter of 12.0 mm was blocked at the end with small amount of glass wool to avoid the leakage of nanocomposites during the adsorption process. A 40 mg of the MWCNTs-BPEI was used to pack the column. Prior to the adsorption process, the column was conditioned with de-ionised water. The SPE packed columns ready for extraction purpose are indicated in figure 3.9.

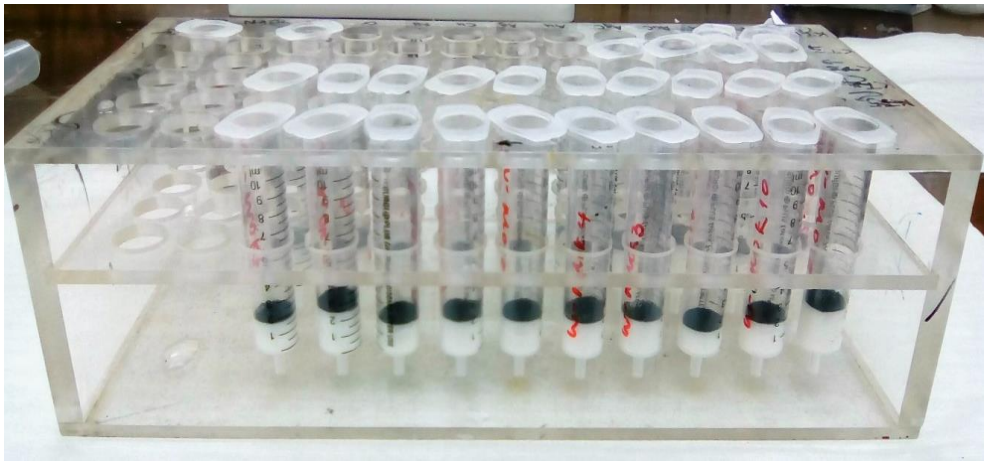

Figure 3.9: The SPE packed columns

Water samples with volume of 350 mL were passed through a packed column of the SPE set up as shown in figure 3.10. The SPE columns were dried completely before the elution step. The retained As^{5+} in all the columns were stripped off from the walls of the nanocomposites by passing 2 mL of 0.6% (m/v) NH_4HCO_3 through the columns. The eluents from all the columns were diluted to 15 mL volumetric flask. The Ga internal standard was added to the eluted samples, calibration standards and blanks for analysis using ICP-MS.

Figure 3.10: Set up for off-line application of SPE

3.6 Analytical method validation

To validate procedures for speciation analysis, total concentration determination and the SPE technique, the analytical figures of merit including LOD, LOQ and linearity were evaluated for each procedure, spiking and recovery experiments conducted and certified reference materials analysed.

3.6.1 Limit of detection and limit of quantification determination

To determine LODs and LOQs of each analytical procedure, reagent blanks were prepared following the same procedure for speciation analysis in water and sediments, total concentration determination of As, Fe, Al and Mn in water and sediment samples and pre-concentration of As^{5+} in water samples. The intensities of 10 blanks were measured for each analytical procedure. Standard deviations were calculated from the concentration of these 6 reagent blanks. The LODs were mainly calculated as 3 times the standard deviation and LOQs were based on the 10 times the standard deviation of the averaged 6 individually prepared reagent blank solutions.

3.6.2 Linearity and calibration curves determination

The linearity for As species quantification was performed in the concentration range of 2 – 20 µg/L of mixed As standards. An intermediate stock solution of 100 µg/L mixture of As^{3+}, DMA, MMA and As^{5+} was prepared each day of analysis. Series of calibration standards were diluted with de-ionised water. A six-point calibration curves were performed in the concentration range of 0.5 – 20 µg/L for total concentration determination of As, range of 5 – 200 µg/L for Fe, Al and Mn determination and range of 1 – 8 µg/L for pre-concentrated As^{5+} detection. An intermediate stock solution of 1 mg/L As and 10 mg/L Fe, Al and Mn were prepared. A 50 µg/L Ga internal standard was added into standards and dilutions were made with 1% HNO_3. However, calibration standards for SPE procedure were prepared with an elution reagents for matrix matching. To each calibration standard, 2 mL NH_4CO_3 and 50 µg/L Ga internal standard was added and diluted with de-ionised

water. The intensity value for each standard was used to construct the calibration curves.

3.6.3 Accuracy, precision and instrument drift

Analytical procedures were validated to ensure quality, precision and accuracy of the results. The speciation analysis using HPLC-ICP-MS and SPE procedure was validated by spiking and recovery studies. The off-line SPE procedure was validated using de-ionised water spiked with As species standards. The procedure was performed in triplicate. A mixed standard solution consisting of 2 µg/L As^{5+} and 1 µg/L As^{3+} prepared in 350 mL volumetric flask. The mixed standard solution was passed through the columns packed with modified MWCNTs-BPEI nanocomposites and the same procedure was followed for water samples analysis. The percentage recoveries were assessed for method validation.

Accuracy of the procedure for total concentration determination of As, Fe, Mn and Al in water samples was checked using SRM 1643f – Trace elements in water and that of sediments checked using SRM 8704 – Buffalo River sediment and BCR 280R – lake sediment CRM. The validation of each analytical procedure was performed the same way as the samples and the obtained results were compared with the certified values to assess the percentage recoveries.

The performance of analytical instruments, ICP-MS and ICP-OES was monitored by analysing initial calibration verification (ICV) solution and an initial calibration blank (ICB) solution at the beginning and end of every run. A continuous calibration verification (CCV) solution was analysed after every 10 samples throughout the run. The carry-over effects from HPLC-ICP-MS were monitored by analysing de-ionised water after 5 injections of the samples. Analyses were performed in triplicate to assess the precision of the results in terms of relative standard deviation (RSD).

CHAPTER 4

RESULTS AND DISCUSSION

This chapter entails a thorough description and discussion of the results obtained for As speciation using HPLC-ICP-MS, total concentration determination and SPE technique using ICP-OES and ICP-MS. The selection of the internal standard, isotope masses for ICP-MS and emission lines for analysis using ICP–OES are summarised.

4.1 Selection of the isotopes for ICP-MS and emission lines for ICP-OES

The ICP-MS has ability to detect various isotopes of particular element. The selected isotopes for total concentration determinations of trace elements were ^{75}As m/z, ^{56}Fe m/z, ^{55}Mn m/z, ^{27}Al m/z and ^{69}Ga m/z. With the ICP-OES sample analysis, several emission lines for determination of Fe, Mn and Al were observed. The emission line with no interference for each analyte was identified having lower concentration as compared to the other emission lines for that particular element. The monitored emission lines for Fe, Mn and Al were 238.204 nm, 294.921 nm, and 167.078 nm, respectively.

4.2 Choice of the internal standard

The total concentration determination and SPE procedures were based on the internal standardisation method. Whenever samples are prepared for analysis, quantitative errors are likely to be introduced from several variables such as volume or weight measurement, analyte loss because of evaporation, contamination as well as instrumental drift. The use of internal standard aimed to compensate these potential sources of errors. A 50 µg/L of Ga was added to the samples, calibration standards and reagent blanks. The variations of internal standard in the methods is indicated in figure 4.1. The percentage recoveries of Ga were observed within 85 – 125%. The United State Environmental Protection Agency (USEPA) guideline advice

the use of results exceeding 65 – 125% recovery of the internal standard and Ga variations were consistent with the recommended percentage recovery (EPA, 2010).

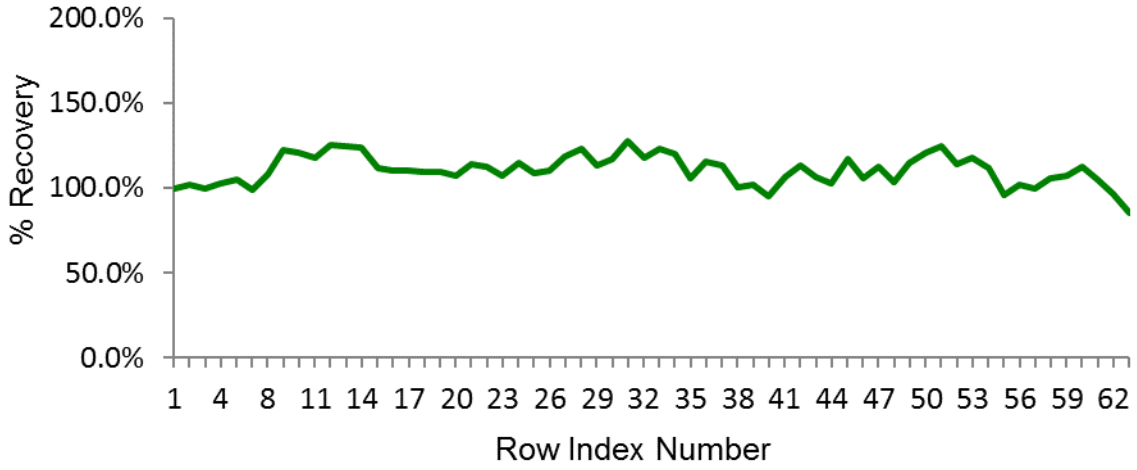

Figure 4.1: The variations of Ga in the analytical procedure

4.3 Validation of analytical methods

The analytical figures of merit such as LODs, LOQs and linearity of calibrations show performance characteristics of the SPE procedure and for determination of As species and total elemental concentrations. Analytical figures of merit are useful in method optimisation and development. The performance characteristics for each analytical procedure were evaluated for acceptance of that particular procedure.

4.3.1 The LODs, LOQs and linearity of calibration curves for determination of As species

The LODs and LOQs for As speciation determination in water and sediments samples are indicated in table 4.1.

Table 4.1: The LODs and LOQs for analytical procedure for water and sediments analysis using HPLC-ICP-MS

Analyte	LOD		LOQ	
	Water (µg/L)	Sediments (ng/g)	Water (µg/L)	Sediments (ng/g)
As^{3+}	0.22	0.11	0.74	0.37
DMA	0.094	0.07	0.31	0.23
MMA	0.13	0.09	0.43	0.30
As^{5+}	0.078	0.03	0.26	0.10

The obtained LOD of As species in water samples are comparable with the LODs reported by Martinez-Bravo *et al*. (2001). The authors reported LODs as 0.19 µg/L for As^{3+}, 0.16 µg/L of DMA 0.067 µg/L of MMA and 0.040 µg/L for As^{5+} in water samples using HPLC-ICP-MS. The obtained LODs suggest that the optimised method could efficiently quantify levels of As species in water samples.

The results show that the LOD of As^{3+}, DMA, MMA and As^{5+} are 0.11, 0.07, 0.09 and 0.03 ng/g, respectively. The LOD obtained in this study are similar to that reported by Garcia-Manyes *et al*. (2002) of soil samples using liquid chromatography ultraviolet irradiation hydride generation inductively coupled plasma mass spectrometry (LC-UV-HG-AFS). Garcia-Manyes *et al*. (2002) reported 0.03 ng/g for As^{3+}, 0.10 ng/g for DMA, 0.06 ng/g for MMA and 0.12 ng/g for As^{5+}. Furthermore, this study obtained lower LODs as compared to that reported by Lou *et al*. (2014). The authors reported 0.82 ng/g for As^{3+}, 2.3 ng/g for DMA, 1.45 ng/g for MMA and 2.31 ng/g for As^{5+} in guano and ornithogenic sediments using HPLC-HG-AFS. The LODs obtained in this study shows the reliable quality of the analytical procedure.

The linearity of calibration curves for As species were assessed in the concentrations ranging from 0.5 – 20 µg/L. The matrix effect of water samples and sediments extract does not affect the calibration curve hence determination of native species was performed by the external calibration. The calibration curve and correlation coefficient (R^2) for quantification of As^{3+} is shown in figure 4.2. The linear equation and R^2 value for DMA, MMA and As^{5+} are indicated in table 4.2.

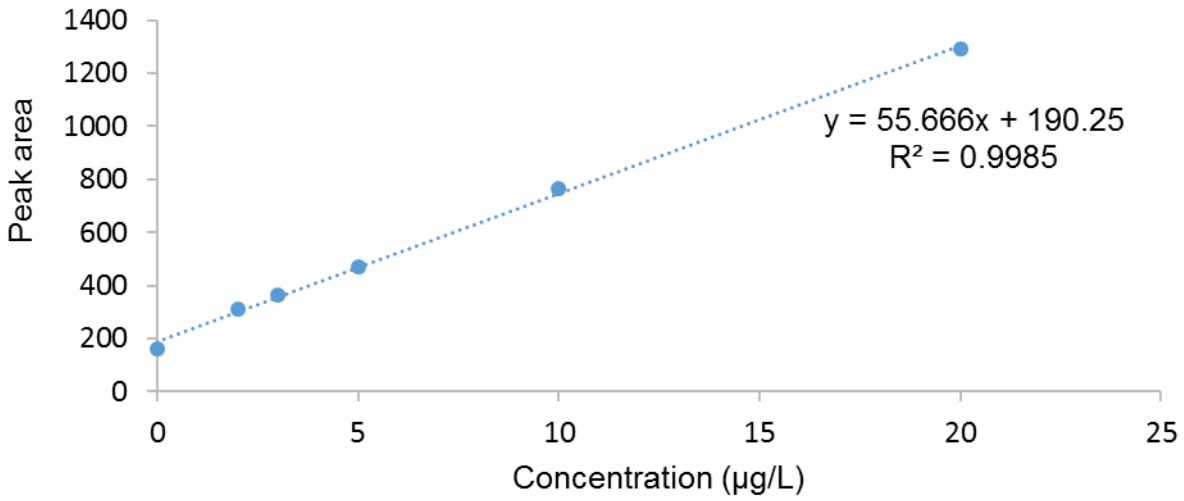

Figure 4.2: Calibration curve used for quantification of As^{3+}

Table 4.2: Linear equations and correlation coefficients for quantification of As species

Analyte	Linear equation	Correlation of coefficient value
DMA	y = 90.151x + 204.55	0.9992
MMA	y = 99.306x + 227.69	0.9995
As^{5+}	y = 108.39x + 204.78	0.9991

4.3.2 The LODs, LOQs and linear calibration curves for total concentration determination

The analytical methods for total concentration determination of As, Fe, Mn and Al in water and sediment samples were conducted by the evaluation of performance characteristics. The LODs and LOQs for As, Fe, Mn and Al in water and sediment samples are indicated in table 4.3.

Table 4.3: The LODs and LOQs for analytical procedure employed for water and sediments analysis

Analyte	LOD		LOQ	
	Water (mg/L)	Sediments (µg/g)	Water (mg/L)	Sediments (µg/g)
As[(a)]	0.0441	0.0796	0.147	0.265
Fe[(b)]	0.0862	0.101	0.287	0.337
Mn[(b)]	0.0574	0.101	0.191	0.337
Al[(b)]	0.0218	0.101	0.0725	0.337

[(a)] obtained using ICP-MS in µg/L
[(b)] obtained using ICP-OES

The LOD for As, Fe, Mn and Al obtained by employing the analytical method for water and sediment samples analysis using ICP-MS are comparable with reported studies. The LOD for As and Fe reported by Garbarino *et al*. (2006) in water samples using ICP-MS were 0.06 µg/L and 1 µg/L, respectively. The LOD of 0.8 mg/L for Al in natural water reported by Tria *et al*. (2007) using ICP-OES is 10 times the one obtained in this study. The difference in LODs between this study and previously reported studies are attributed to sensitivities, which varies with type of the detection technique used. The LOD of 0.016 µg/g for As, 0.510 µg/g for Fe and 0.023 µg/g for Mn reported by Duzgoren-Aydin *et al*. (2011) in soil and sediment samples using ICP-MS are comparable with LODs obtained in this study. The LODs in this study shows adequate sensitivity of the analytical techniques for quantification of analytes in water and sediment samples.

The linear calibration curve used for quantification of total As based on the response of the spiked level of internal standard is indicated in figure 4.3. The performance of Ga in the method as observed in figure 4.3 is excellent, hence instrumental drift and other induced errors were corrected and precision of the results have improved.

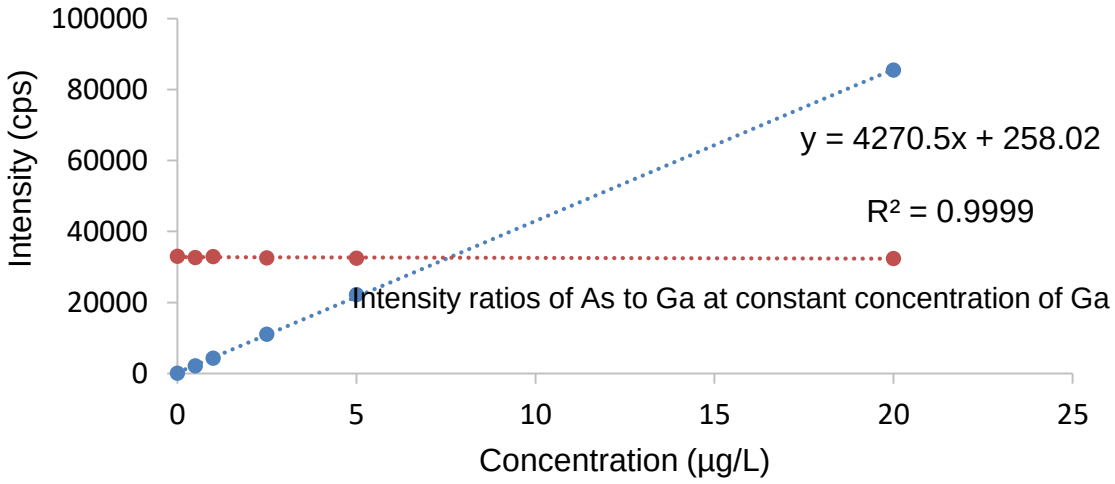

Figure 4.3: Calibration curve used for quantification of total As and intensity ratios of As to Ga at constant concentration of Ga

4.3.3 The LOD and LOQ for solid phase extraction procedure

The SPE procedure for determination of As^{5+} in water samples was also evaluated using quantitative terms. The LOD and LOQ of 0.0537and 0.179 µg/L, respectively were obtained by employing off-line mode procedure after detection using ICP-MS. A previous study conducted by Zhad *et al*. (2009) reported LOD of 0.24 µg/L using ICP-MS which is 4.5 times higher than that obtained in this study. The LOD in this study show adequate sensitivity of detection technique for quantification of As^{5+}.

4.4 Method validation

Method validation is the process used to confirm the effectiveness of the analytical procedure followed for a specific test. The results of the method validation may judge quality, reliability and consistency of the analytical procedure. Method validation is an integral part of any good analytical practice. The results for method validation of each analytical procedure are clearly outlined.

4.4.1 Validation of analytical procedures for speciation analysis

The analytical procedures for As speciation analysis in water and sediment samples were validated based on spiking-and-recovery procedures due to lack of SRMs which have certified values of As species.

Validations were conducted by spiking water and sediment samples with As species standards at 1x LOQs and 10x LOQs levels to investigate percentage recoveries. The concentrations of each species were obtained from the differences of spiked and unspiked samples to assess the percentage recoveries. Quantification was based on the integration of peak area to account for tailing peaks rather than the use of peak heights intensities (using technical graphics software Origin 6.1). The percentage recoveries were evaluated with references to the standard guidelines recommended by USEPA which are guidelines for method development and validation (EPA, 2010). The USEPA recommended suitable percentage recoveries to be within 75 – 125% (EPA, 2010). The percentage recoveries obtained at 1x LOQs and 10x LOQs are presented in tables 4.4 and 4.5.

Table 4.4: Percentage recoveries at 1x LOQ level for water samples

Analyte	Unspiked sample (µg/L)	Spiking at 1x LOQ (µg/L)	Concentration after spiking (µg/L)	Percentage recovery (%)
As^{3+}	0.26 ± 0.001	0.74	0.865 ± 0.035	81.8
DMA	0.096 ± 0.003	0.31	0.241 ± 0.0023	46.8
MMA	0.22 ± 0.005	0.43	0.62 ± 0.007	93.0
As^{5+}	7.7 ± 0.24	0.26	8.0 ± 0.14	115

The HPLC-ICP-MS procedure was applied for analysis of water samples which were spiked at 1x LOQs of As species standards. The data in table 4.4 indicates the satisfactory percentage recoveries of As^{3+}, MMA and As^{5+} in the ranges from 81.8 – 115%. These percentage recoveries are within the acceptable range of USEPA guidelines (EPA, 2010). The percentage recovery of 46.8% for DMA is far below the acceptable range and as a result the analytical findings of DMA would not be reliable. The relative standard deviations (RSDs) for concentrations after spiking were found to be 4% for As^{3+}, 9.5% for DMA, 0.076% for MMA and 0.37% for As^{5+}. The RSDs values of less than 15% demonstrate a high degree of repetitiveness for the analytical procedure (Lou *et al*., 2014). Martinez-Bravo *et al*. (2001) have

reported percentage recoveries of As species ranging from 80 – 97% with adequate precision, under similar conditions.

Table 4.5: Percentage recoveries at 10x LOQ level for water samples

Analyte	Unspiked sample (µg/L)	Spiking at 10x LOQ (µg/L)	Concentration after spiking (µg/L)	Percentage recovery (%)
As^{3+}	0.26 ± 0.001	7.4	6.32 ± 0.51	81.9
DMA	0.096 ± 0.003	3.1	1.64 ± 0.026	49.9
MMA	0.22 ± 0.51	4.3	4.62 ± 0.39	102
As^{5+}	7.7 ± 0.24	2.6	10.1 ± 0.055	92.3

The data in table 4.5 indicates As^{3+}, MMA and As^{5+} percentage recoveries in the ranges from 81.9 – 102%, and are consistent with USEPA guidelines (EPA, 2010). The recovery of DMA has not improved at 10x LOQ level. The RSDs after spiking were found to be 8.1% for As^{3+}, 1.6% for DMA, 1.5% for MMA and 0.5% for As^{5+}. Chen *at al*. (2006) reported percentage recoveries of 89.2% for As^{3+}, 96.4% for DMA, 95.2% MMA and 103.1% for As^{5+} after spiking water sample with 50 µg/L of each species using ion chromatography (IC) coupled to ICP-MS. The percentage recoveries at 1x LOQs and 10x LOQs levels show the efficiency of HPLC-ICP-MS procedure to quantify As^{3+}, MMA and As^{5+} in water samples.

The validation of analytical procedures for speciation analysis in sediment samples was also based on spiking-and-recovery procedures to evaluate percentage recoveries. The extraction efficiency of analytical procedure was assessed at 1x LOQs and 10x LOQs levels and percentage recoveries are indicated in tables 4.6 and 4.7.

Table 4.6: Percentage recoveries at 1x LOQ level for sediment samples

Analyte	Unspiked sample (µg/g)	Spiking at 1x LOQ (µg/g)	Concentration after spiking (µg/g)	Percentage recovery (%)
As^{3+}	0.340 ± 0.0021	0.37	0.640 ± 0.035	73.0
DMA	0.09 ± 0.003	0.23	0.122 ± 0.0023	14.0
MMA	0.311 ± 0.004	0.30	0.570 ± 0.017	86.0
As^{5+}	0.189 ± 0.005	0.10	0.274 ± 0.014	85.0

The analytical results obtained show adequate percentage recoveries at 1x LOQ level of 85.0% and 86.0% for As^{5+} and MMA, respectively. A fair recovery of 73.0%

of As^{3+} may be quantitative and poor recovery of 14.0% DMA will not produce reliable analytical results.

Table 4.7: Percentage recoveries at 10x LOQ level for sediment samples

Analytes	Unspiked sample (µg/g)	Spiking at 10x LOQ (µg/g)	Concentration after spiking (µg/g)	Percentage recovery (%)
As^{3+}	0.35 ± 0.0020	1.2	1.33 ± 0.028	82.0
DMA	0.09 ± 0.002	0.77	0.439 ± 0.04	45.0
MMA	0.312 ± 0.004	1.0	1.18 ± 0.09	87.0
As^{5+}	0.188 ± 0.007	0.33	0.491 ± 0.05	92.0

The data in table 4.7 shows the acceptable percentage recoveries obtained at 10x LOQs level for As^{3+}, MMA and As^{5+}, which are consistent with the recommendations of USEPA guidelines (EPA, 2010). The obtained recoveries show that the developed method is efficient for extraction and quantification of As^{3+}, MMA and As^{5+} in sediment samples. The improvement in DMA percent recovery at 10xLOQ level is not sufficient to obtain reliable results by employing the method. The RSDs after spiking were found to be 2.1% for As^{3+}, 9.1% for DMA, 7.6% for MMA and 10.1% for As^{5+}. The RSD in all cases were monitored to assure the precision of the results which showed a high degree of repetitiveness for the analytical procedure (Lou *et al*., 2014).

4.4.2 Validation for total concentrations quantification procedures

The method validation for total concentrations determination of As, Fe, Mn, and Al in water and sediment samples were validated using SRMs of water and sediments. The detection of As in water samples by ICP-MS and the detection of Fe, Mn, and Al by ICP-OES were validated using SRM 1643f – Trace elements in water. The analytical procedures for sediment samples analysis were validated by ICP-MS using SRM 8704 – Buffalo River sediment and BCR 280R – lake sediment CRM. To ascertain the validity of each analytical procedure, standard reference materials were treated exactly the same as the samples treatment outlined in chapter 3. The method validation results for total concentration determination of As, Fe, Mn and Al in water samples are presented in table 4.8.

Table 4.8: Validation of analytical procedure employed for total concentrations quantification of As, Fe, Mn and Al in water samples

Analyte	Measured value (µg/L)	Certified value (µg/L)	Percentage recovery (%)
As[(a)]	55.9 ± 2.1	57.42 ± 0.38	97.4
Fe[(b)]	78.5 ± 8.2	93.44 ± 0.78	84.0
Mn[(b)]	31.3 ± 2.8	37.14 ± 0.60	84.3
Al[(b)]	128 ± 12	133.8 ± 1.2	95.6

[(a)] obtained using ICP-MS
[(b)] obtained using ICP-OES

The results show good recoveries for all analytes, which are consistent with the recommendations of USEPA guidelines for method development and validation (EPA, 2010). Therefore, analytical procedure for total concentration determination of As, Fe, Mn and Al in water samples was validated.

The percentage recoveries of SRM 8704 – Buffalo River sediment obtained for validation of analytical procedure for determination of As, Fe, Mn and Al in sediment samples is indicated in table 4.9.

Table 4.9: Validation of analytical procedure employed for total concentrations quantification of As, Fe and Mn in sediment samples using SRM 8704

Analytes	Measured value (µg/g)	Certified value (µg/g)	Percentage recovery (%)
As	18.9 ± 0.15	17.0	111
Fe	654 ± 28.6	544 ± 21	120
Mn	29930 ± 170	39700 ± 1000	75.0

The percentage recoveries obtained falls within range of suitable recoveries as recommended by USEPA (EPA, 2010). The BCR 280R – lake sediments CRM has certified value only for As. The measured value was 33.7 ± 1.0 µg/g and certified value of As was 33 µg/g. Therefore, percentage recovery of 101% was obtained. The analytical procedure for total determination of As, Fe and Mn was validated based on the quality, reliability and consistency of the method.

4.4.3 Validation for solid phase extraction analytical procedure

The validation for SPE was conducted by spiking-and-recovery procedures. The evaluation of percentage recovery was based on the assessment of the adsorbed As^{5+} which was eluted and quantified using ICP-MS. Briefly, a mixture of 2.0 µg/L As^{5+} and 1.0 µg/L As^{3+} was spiked in water samples and passed through SPE column packed with MWCNTs-BPEI nanocomposites. After SPE procedure, measured value of 1.62 ± 0.09 µg/L of As^{5+} was obtained leading to percentage recovery 81.0%. The recovery obtained is consistent with standard guidelines recommendation of USEPA (EPA, 2010). Chen *et al*. (2013) validated similar study by spiking snow water and rain water with respective concentrations of 3.2 µg/L and 4.0 µg/L of As^{5+}. They obtained percentage recoveries of 108% and 92.5% for snow water and rain water, respectively after SPE procedure using AFS detection system. The validated SPE procedure is efficient to separate and quantify As^{5+} in water samples in presence of As^{3+}.

4.5 Speciation of arsenic in water and sediment samples

The HPLC-ICP-MS analytical procedure based on external calibration method was employed for quantification of As species in water and sediment samples. Prior to determination of As species in samples, As species standards of 20 µg/L were separately injected into HPLC-ICP-MS to identify retention time for each species.

4.5.1 Chromatographic conditions

The chromatographic conditions such as retention time identification, mobile phase compositions and pH of mobile phase were optimised for adequate peaks separation and resolution.

4.5.1.1 Retention time identification

The retention time measures time taken for a particular compound to pass through a chromatography column (Kormany *et al*., 2014). It is calculated as the time from the

sample injection to the point at which the display shows a maximum peak height for the compound (Kormany *et al*., 2014). The retention time for a compound is not fixed, it could be influenced by factors such as pump pressure, components of stationary phase, flow rate and exact composition of the mobile phase even if the same HPLC and column are used. The operational conditions were attentively monitored because retention times are used to identify the type of As species. The determination of retention times is limited to the availability of standards. Each peak or signal in the chromatogram represent the signal created when a compound elutes from the HPLC column into the ICP-MS detection system. The chromatograms of As^{3+}, DMA, MMA and As^{5+} with the identified retention times are indicated in figures 4.4 (a) – 4.4 (d), respectively.

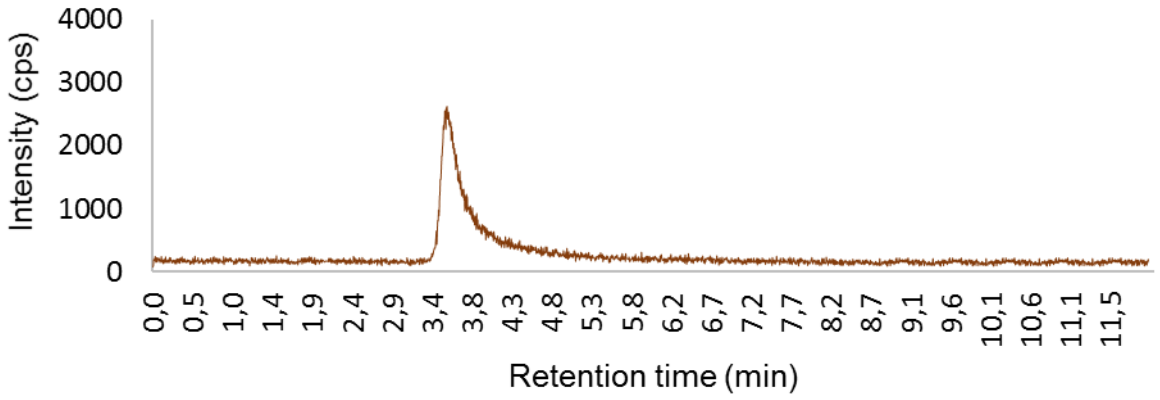

Figure 4.4 (a): Chromatogram showing retention time for As^{3+} at 3.52 minutes

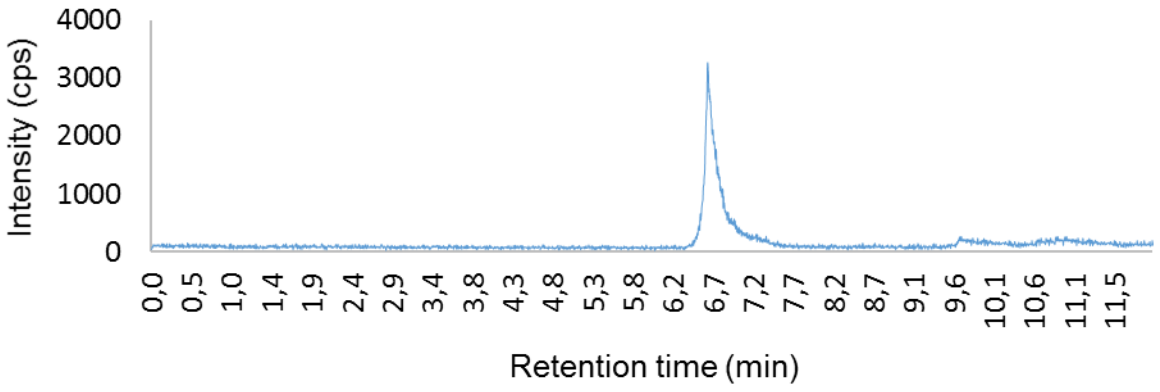

Figure 4.4 (b): Chromatogram showing retention time for DMA at 6.71 minutes

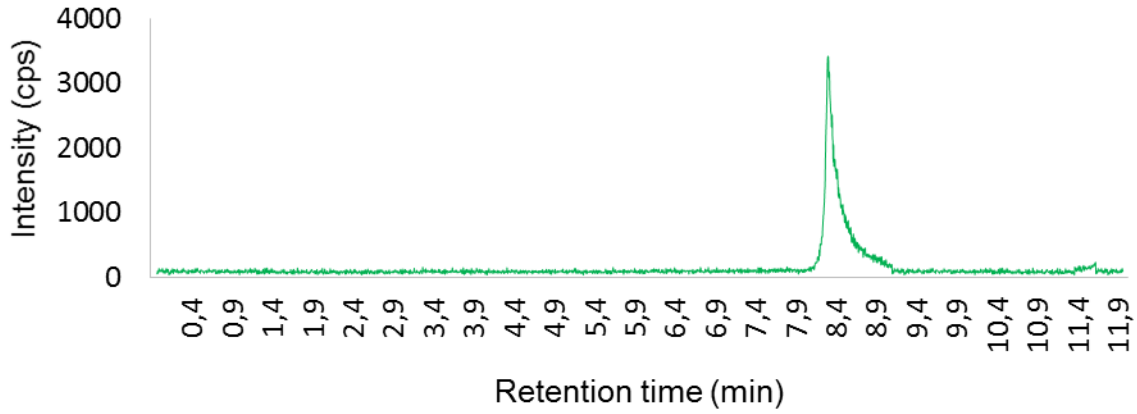

Fig 4.4 (c): Chromatogram showing retention time for MMA at 8.31 minutes

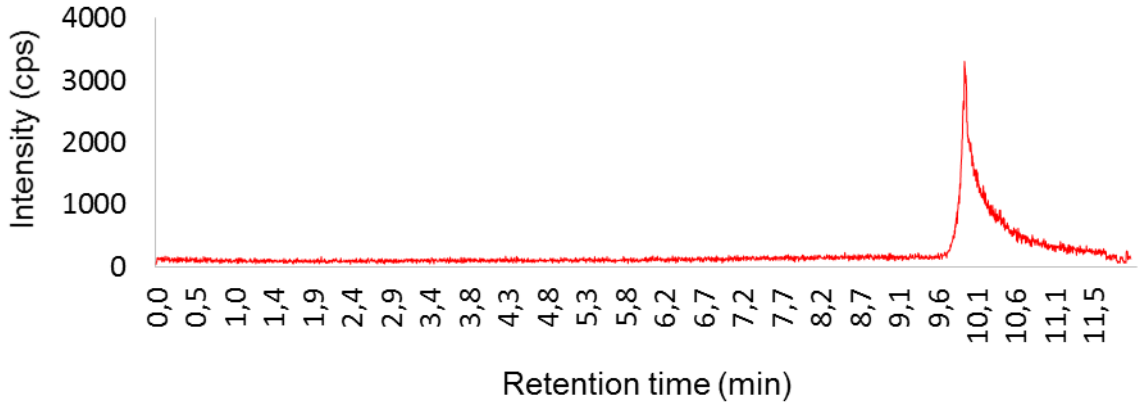

Figure 4.4 (d): Chromatogram showing retention time for As^{5+} at 9.95 minutes

The sequentially separated species of mixture of standards (Stds) were investigated to evaluate the compliance of the retention time with the relative native species. The mixture of standards was injected into HPLC column under the monitored operating conditions and the species were separated according to their identified retention times. The order of elution of a standard mixture indicates that As^{3+} species is the least retained species in the column while As^{5+} species is strongly retained. Moreover, the chromatogram indicates the efficient communication of HPLC and ICP-MS by allowing synchronous separation and detection of the species. The

chromatogram that shows sequentially separated As species is presented in figure 4.5.

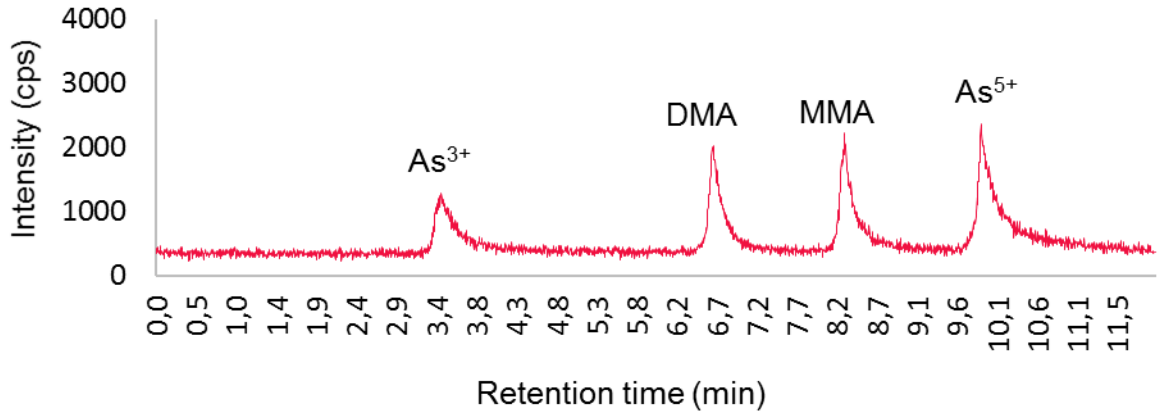

Figure 4.5: Sequential separation of As species in a mixture of standard solutions

The peak intensity is proportional to the increasing concentrations of the analytes. The peaks intensities of native As species were examined at series of mixture of standards after the injection into HPLC column. The observed enhanced signals and intensities clearly demonstrate agreement of the proportionality. The chromatogram (Figure 4.6) shows the relationship between the peak intensities and corresponding different concentrations of As species standards at a constant order of elution. The increasing peak areas were used to plot the linear calibration curves.

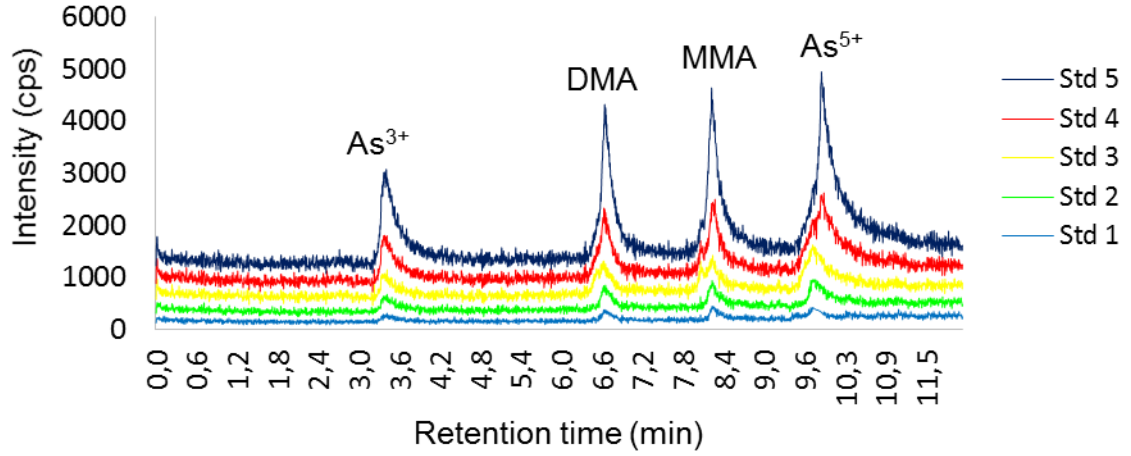

Figure 4.6: Intensified peaks relative to As species standard concentrations

4.5.1.2 Effect of mobile phase compositions

The optimisation of chromatographic conditions led to baseline separation of four As species in 12 minutes employing gradient elution with 10 mM NH_4NO_3 (mobile phase A) and 60 mM NH_4NO_3 (mobile phase B) adjusted to pH 8.7 using NH_3. The adoption of NH_4NO_3 mobile phase is due to competency in providing the ionic strength essential in eluting the target ions without any interference as compared to NaH_2PO_4 mobile phase (Martinez-Bravo *et al*., 2001). Martinez-Bravo *et al*. (2001) observed that NaH_2PO_4 decrease ionisation efficiency of the plasma because of the presence of Na counter-cation, which is readily ionisable. Thus, NaH_2PO_4 or other eluents containing Na are not ideal for the separation of As species based on the reported findings.

The application of different concentration of NH_4NO_3 mobile phase for As species determination was observed with no challenges related to clogging of nebuliser, the sampler and skimmer cones of the ICP-MS. Furthermore, lower concentration of mobile phase A was able to allow the least retained components to be separated while strongly retained components are adsorbed by the stationary phase of the column. However, increasing the concentration to 60 mM NH_4NO_3 (mobile phase B) led to desorption of strongly retained components due to steady increase of competition for the adsorption site (Sun *et al*., 2015). The stabilised HPLC pump plays a major role in allowing on-line mixing of the mobile phase composition. Thus, making gradient mode of elution most suitable for separation and detection of As species. The gradient elution programme employed for As species determination is indicated in table 4.10.

Table 4.10: Gradient elution programme at 1 mL/min flow rate with the mobile phase A of 10 mM NH_4NO_3 and mobile phase B of 60 mM NH_4NO_3 at pH 8.7

Time (min)	0	1	1.5	3	3.5	10	12
% A	100	100	50	50	0	0	0
% B	0	0	50	50	100	100	100

4.5.1.3 Effect of pH of the mobile phases

The peak resolution and retention times of the analytes may be optimised through adjustment of pH and ionic strength of the mobile phase. Martinez-Bravo *et al*. (2001) investigated the effect pH of NH_4NO_3 mobile phase within the range of 7.7 – 10.2 using NH_3 solution. The authors varied pH of the mobile phase for the adoption of stable pH which compromised chloride interference yet yielding reasonable peak resolutions for As^{3+}, DMA, MMA and As^{5+}. The MMA was observed subjected to interference from $^{40}Ar^{35}Cl$ at pH 8.2 whereas decreased peak resolution of As^{3+}, DMA and As^{5+} were observed at pH greater than 8.7. At pH greater than 9.2, As^{3+} is present as an anionic species (Rakhunde *et al*., 2012; Chen *et al*., 2013). Therefore, at pH 10.2, As^{3+} was retained by stationary phase whereas MMA and As^{5+} were overlapped (Martinez-Bravo *et al*., 2001). The adopted composition of mobile phases at pH 8.7 and gradient mode of elution programme with optimised 10 mM NH_4NO_3 mobile phase A and instrumental operational conditions yielded excellent peak resolutions, low LODs and reduced analysis time.

4.5.2 Speciation of arsenic in water samples

The identification and quantification of As species in water samples were achieved by matching the retention times to the standard peaks. The integrated peak areas were employed to plot calibration curves and subsequent determination of As species.

4.5.2.1 Concentrations of As species in water samples

Speciation of As in water samples collected from Great Letaba and Mokolo Rivers was successfully conducted using HPLC-ICP-MS. Water samples were preserved by filtration and analysed without acidification. The preservation of As species by acidification cause the interconversion of species. The chromatogram of water sample with most detected species is indicated in figure 4.7. The detection of As^{3+}, MMA and As^{5+} in the water sample shows the successful preservation of the As species by the proposed method.

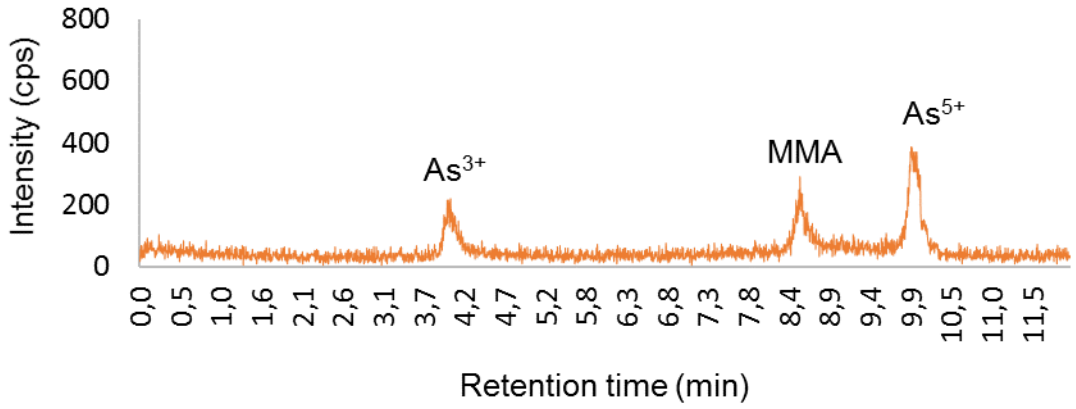

Figure 4.7: The chromatogram showing detected As species in water sample

The As species in water samples collected from the respective rivers were determined using validated analytical procedure. The difference in As species concentrations were influenced by the different factors such as water chemistry, geological area and varied anthropogenic activities. The concentration of As species are presented in tables 4.11 – 4.13.

Table 4.11: Concentrations of As species in water samples of Great Letaba River

Sample Id	As^{3+} (µg/L)	DMA (µg/L)	MMA (µg/L)	As^{5+} (µg/L)
GLR-1	< 0.22	< 0.094	< 0.13	< 0.078
GLR-2	< 0.22	< 0.094	< 0.13	< 0.078
GLR-3	< 0.22	< 0.094	< 0.13	< 0.078
GLR-4	< 0.22	< 0.094	< 0.13	0.537 ± 0.0099
GLR-5	< 0.22	< 0.094	< 0.13	< 0.078
GLR-6	< 0.22	< 0.094	< 0.13	0.274 ± 0.013
GLR-7	< 0.22	< 0.094	< 0.13	0.653 ± 0.023
GLR-8	< 0.22	< 0.094	0.459 ± 0.022	1.02 ± 0.057
GLR-9	< 0.22	< 0.094	0.546 ± 0.014	1.05 ± 0.0014
GLR-10	< 0.22	< 0.094	0.437 ± 0.0053	1.27 ± 0.012

The concentrations of As species obtained in Great Letaba River water samples vary per site of samples collected. The results in table 4.11 show that As^{3+} and DMA were not detected, MMA was detected at site 8 to 10 and As^{5+} was detected at site 4 and site 6 to 10. The MMA concentration was found to be within the range of 0.437 – 0.546 µg/L whereas As^{5+} was less than 1.5 µg/L. The RSDs were less than 10%, thus the precision of the results is adequate. Additionally, these results indicate that

As species in Great Letaba River pose a threat to aquatic ecosystem because As is not an essential plant nutrient at any level of concentration (DWAF, 1996). The hippos and fish inhabited in the river and domestic animals (cattle and goat) which drink water directly from Great Letaba River may accumulate As species to toxic level (Le *et al*., 1998; Rezende *et al*., 2014). There is no evidence of a beneficial role for any safe dose of As consumption (Le *et al*., 1998). Therefore, humans may be exposed to As species through the food chain by consumption of As contaminated food like fish as the fisher man was observed during sampling campaign.

The observed concentrations of MMA and As^{5+} may have attributed to the weathering of rocks. Great Letaba River has a rocky bed with a complex geology and effect of weathering of such rocks may have resulted with the minor levels of As^{5+} as revealed by the analysis results. The As contamination as a result of weathering processes does not rapidly elevate As in the environment since the process takes longer time to occur (Winkel *et al*., 2008). The activities of mining and industries found in the proximity of the downstream of Great Letaba River may have also affected the concentration of inorganic As species indirectly. The MMA and DMA concentrations usually affected by microbial metabolism through methylation of inorganic species (Komorowicz and Baralkiewicz, 2011; Chiban *et al*., 2012).

Table 4.12: Concentration of As species in water samples of Mokolo River during high flow sampling season

Sample Id	As^{3+} (µg/L)	DMA (µg/L)	MMA (µg/L)	As^{5+} (µg/L)
MOK-1	0.301 ± 0.011	< 0.094	< 0.13	0.951 ± 0.029
MOK-2	< 0.22	< 0.094	< 0.13	1.55 ± 0.21
MOK-3	0.299 ± 0.028	< 0.094	< 0.13	0.681 ± 0.024
MOK-4	< 0.22	< 0.094	0.224 ± 0.01	7.70 ± 0.24
MOK-5	< 0.22	< 0.094	< 0.13	< 0.078
MOK-6	< 0.22	< 0.094	< 0.13	< 0.078
MOK-7	< 0.22	< 0.094	< 0.13	0.0895 ± 0.0036
MOK-8	< 0.22	< 0.094	< 0.13	< 0.078
MOK-9	< 0.22	< 0.094	< 0.13	< 0.078
MOK-10	< 0.22	< 0.094	< 0.13	0.118 ± 0.016

The As species concentrations detected in high flow sampling season also differ per site of samples collected. The As^{3+} was detected in site 1 and site 3 with respective concentrations of 0.301 µg/L and 0.299 µg/L. The MMA was detected in site 4 with

concentration of 0.224 µg/L. The As^{5+} was predominating and concentrations of the detected sites were alternating within the range of 0.681 – 7.70 µg/L. The precision of the results is adequate since RSDs were found to be less than 10%. The DMA was not detected in all the sites. Mokolo River is found at the area dominated by variety of anthropogenic activities which may result with the elevated level of As species in the environment. The varied concentration of As species per sampling sites are also affected by the direct exposure of that particular site to As source. For instance, the astonishing concentration of As^{5+} at site 4 may have attributed to the direct deposition of burning fuel released by the motor vehicles. The deposition of burning fuel influenced the rapid accumulation of As at sampling site 4 which samples were collected under the bridge. The As species is not essential nutrient for plants and animals at any concentration so the aquatic ecosystem of Mokolo River is threatened by As contamination.

The sampling campaign at Mokolo River was conducted twice at different rainfall seasons to monitor the concentration of As species at each season. The As species concentrations obtained during low flow sampling season are presented in table 4.13.

Table 4.13: Concentrations of As species in water samples of Mokolo River during low flow sampling season

Sample Id	As^{3+} (µg/L)	DMA (µg/L)	MMA (µg/L)	As^{5+} (µg/L)
LEP-1	< 0.22	<0.094	< 0.13	< 0.078
LEP-2	< 0.22	<0.094	< 0.13	0.449 ± 0.005
LEP-3	< 0.22	<0.094	0.645 ± 0.018	2.03 ± 0.020
LEP-4	< 0.22	<0.094	0.451 ± 0.030	4.99 ± 0.027
LEP-5	< 0.22	<0.094	0.348 ± 0.033	3.24 ± 0.044
LEP-6	< 0.22	<0.094	0.391 ± 0.013	0.920 ± 0.013
LEP-7	< 0.22	<0.094	0.315 ± 0.0051	0.529 ± 0.010
LEP-8	< 0.22	<0.094	< 0.13	0.324 ± 0.027
LEP-9	0.304 ± 0.027	<0.094	< 0.13	0.733 ± 0.031
LEP-10	0.881 ± 0.019	<0.094	0.512 ± 0.072	1.15 ± 0.0013

The As species concentrations obtained in low flow sampling season show the detected As^{3+} site 9 and 10 with the varied concentrations of 0.304 µg/L and 0.881 µg/L, respectively. The MMA was detected at wide range of the sites with the concentrations varied from 0.315 – 0.645 µg/L. The As^{5+} was predominant at all site

with the maximum concentration of 4.99 µg/L found at site 4. The excellent precision of the results was proven by the RSDs which were found below 10%. The DMA was not detected in all sites. The low flow sampling season show higher concentrations of As species as compared to that obtained during high flow sampling seasons. This observation shows that As species in Mokolo River is gradually been elevated. This may have affected to the various factors such as the pH, redox potential as well as the bacterial effects. The predominating As^{5+} may have been influenced by the oxidising environmental condition of the river system which occurs at wide range of pH and it favours the dominance of As^{5+} (Rakhunde *et al*., 2012). The As^{3+} is likely to dominate in the reducing conditions at pH < 9.2, hence As^{3+} was not in favourable conditions and was found at low concentration (Rakhunde *et al*., 2012). The MMA concentrations show higher microbial activities in Mokolo River during low flow sampling seasons. The microbial activities are known to methylate the inorganic As species and facilitate the oxidation of As^{3+} to As^{5+} resulting with the increased As^{5+} concentration (O'Reilly *et al*., 2001). The microbial activities are likely to be enhanced by sewage effluents directly or indirectly deposited into the river.

4.5.2.2 Comparison of arsenic species in water samples

Different As species found in water samples of Great Letaba and Mokolo Rivers varies in concentrations. The MMA and As^{5+} were detected in Great Letaba River whereas As^{3+}, MMA and As^{5+} were detected in Mokolo River at different concentrations of each sampling season. The DMA was not detected in all river systems. The graphical representation of As species concentrations in water samples of Great Letaba and Mokolo River during high and low flow sampling seasons are indicated in figures 4.8, 4.9 and 4.10. The concentrations are represented from the LOD values and error bars on the graphs represent ± standard deviations.

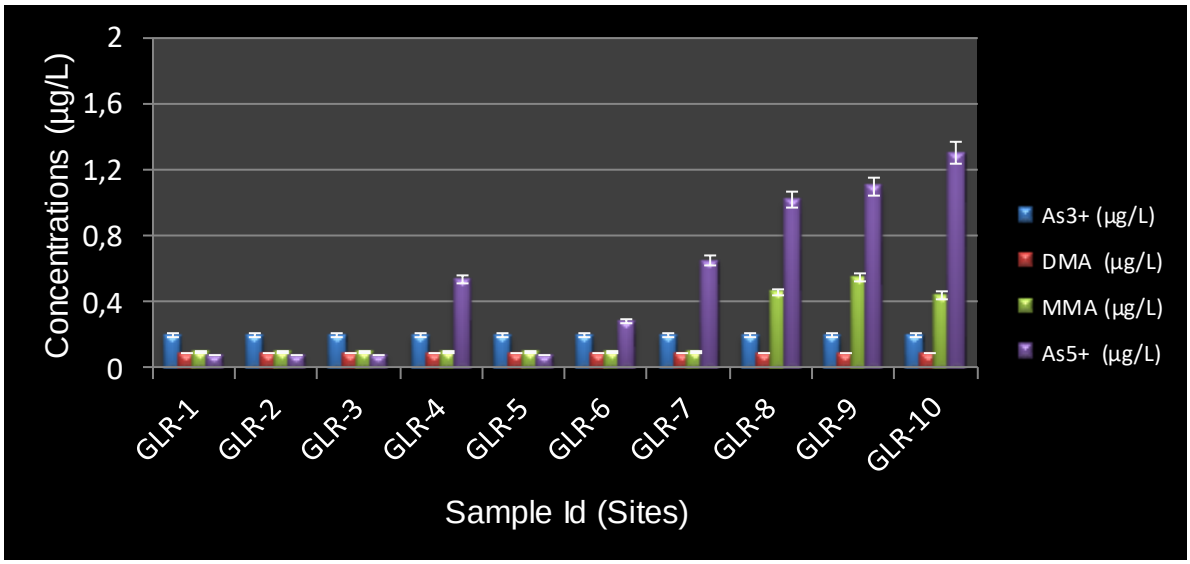

Figure 4.8: Graphical representation of concentrations of As species in water samples of Great Letaba River

The As species concentrations in Great Letaba River increase from upstream to downstream. Although Great Letaba River is characterised by the rocky bed which may elevate As species levels during weathering processes, mining activities and steel operating industries dominate in the vicinity of the upstream.

The graphical representation of As species concentrations of Mokolo River during high and low flow sampling seasons are indicated in figure 4.9 and 4.10, respectively.

The graphical presentation of As species of Mokolo River shows that high flow sampling season (Figure 4.9) have low concentrations whereas low flow sampling season (Figure 4.10) have high concentrations. High species concentrations were observed towards downstream during high flow sampling season, whereas lowest concentrations were also observed in the downstream during low flow sampling season. Mokolo River flow towards site 1 and high flow condition is characterised by the running water which may neutralise contaminants possibly decontaminating river water. The accumulated contaminants tend to mask natural behaviour of aquatic ecosystem (DWAF, 1996).

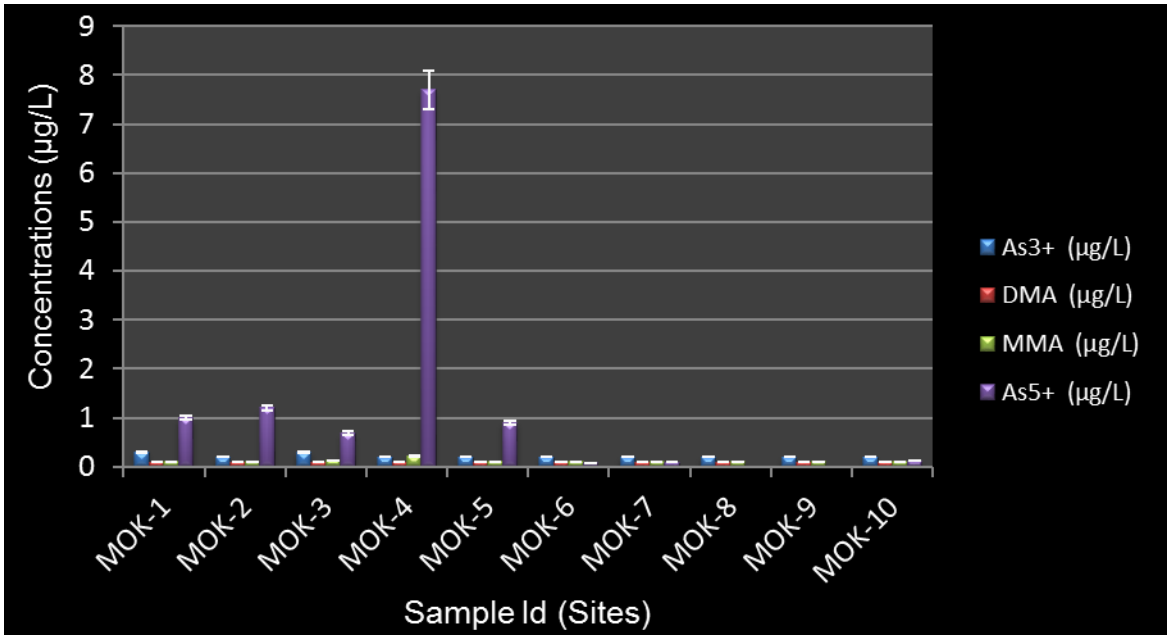

Figure 4.9: Graphical representation of concentrations of As species of Mokolo River water samples during high flow sampling season

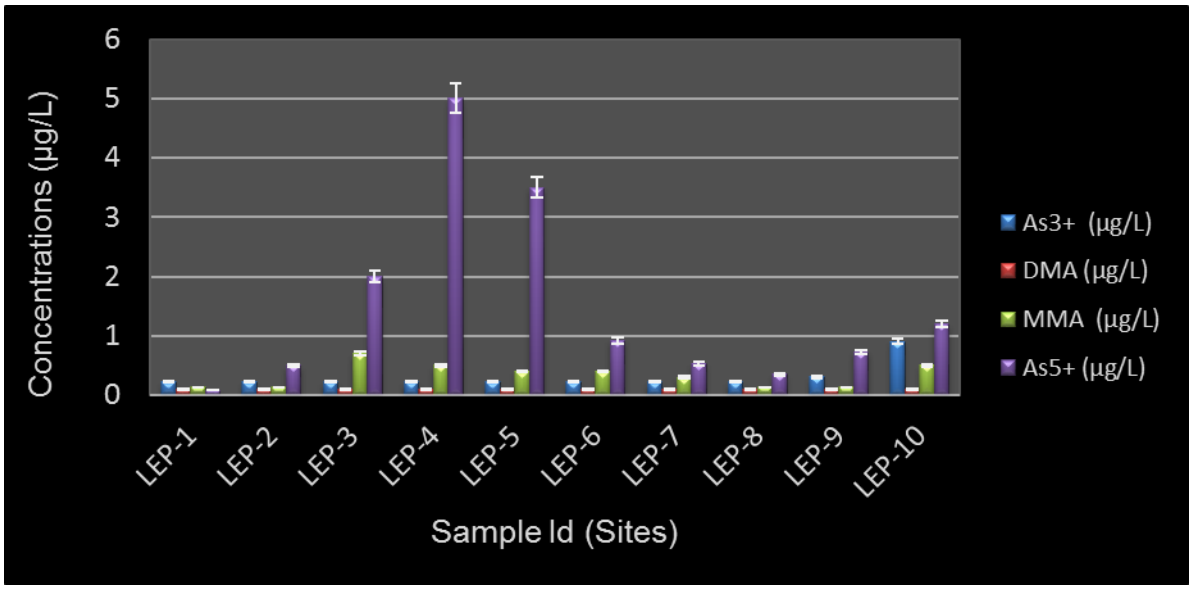

Figure 4.10: Graphical representation of concentrations of As species in water samples of Mokolo River during low flow sampling season

The dominant species in water samples of all river systems is As^{5+}. Chen *et al*. (2006) reported concentrations of 6.14 ± 0.15 µg/L for As^{3+} and 59.8 ± 1.2 µg/L for

As^{5+}, with DMA and MMA not detected in groundwater using IC-ICP-MS. The authors reported that the dominance of As^{5+} was due to As^{3+} oxidation during storage. The results reported by Chen *et al*. (2006) from Bangladesh are higher than the results obtained in this study. Sathrugnan and Hirata (2004) obtained As^{3+} and As^{5+} concentrations in well water samples from Malaysia within the range of 0.19 – 0.87 µg/L and 0.64 – 1.87 µg/L, respectively. The As species were separated and detected using HPLC-ICP-MS. However, MMA and DMA were also not detected in their study. A study conducted by Ronkart *et al*. (2007) reported 0.23 ± 0.03 µg/L and 0.11 ± 0.01 µg/L for As^{3+}, 1.79 ± 0.21 and 0.24 ± 0.03 µg/L for As^{5+}, MMA and DMA were not detected in surface water samples from Belgium using HPLC-ICP-MS. The source of As species in the surface water may have attributed to the contaminants of the organic matter originated from agricultural and municipal wastes (Ronkart *et al*. 2007). Although the analytical results reported by Sathrugnan and Hirata (2004) and Ronkart *et al*. (2007) are from different countries, they are comparable to the As species concentrations obtained in this study. The difference in As species concentrations varies per geological area and are affected by varied factors. The geographical area, water chemistry and varied anthropogenic activities differently affect the contribution of As species in the environment.

The levels of As species in Mokolo River obtained in both sampling seasons were evaluated and figure 4.11 shows the highest levels of species observed per sampling season.

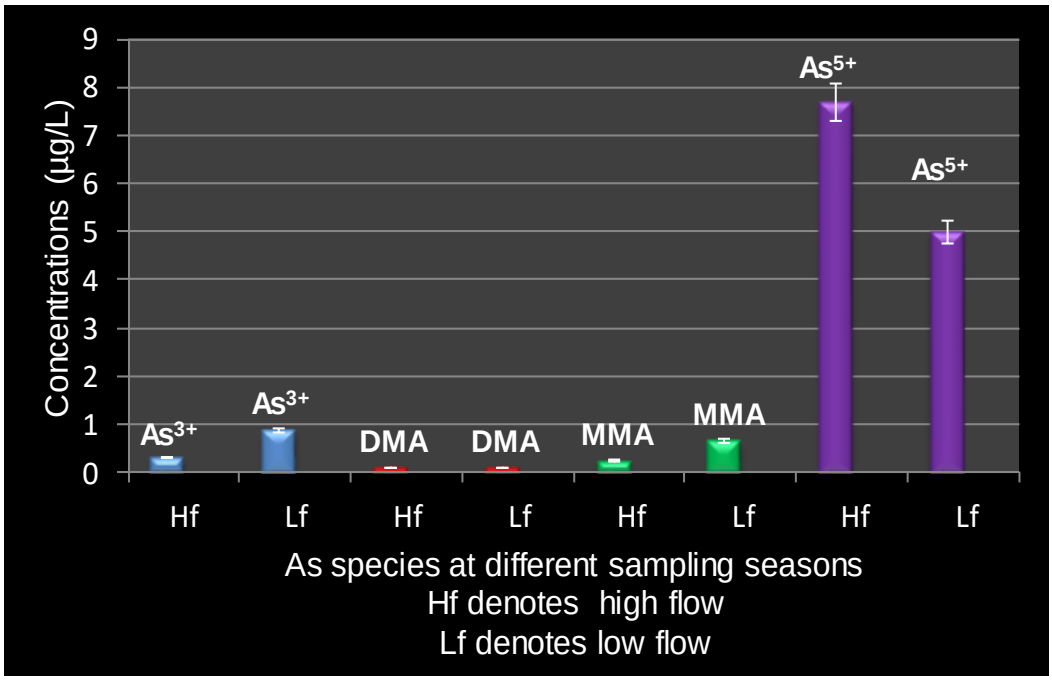

Figure 4.11: Graphical representation of highest levels of As species obtained during high and low flow sampling seasons

The highest concentration of As^{3+} in water samples was in the low flow sampling season analysis results. The DMA was not detected in both sampling seasons whereas highest MMA concentration was found in the low flow sampling season. The highest level of As^{5+} was found in the high flow sampling season results. Currently, MPLs of As species in drinking and irrigation water have not yet been established. Generally, no comparison can be made whether the measured levels of As species in Great Letaba and Mokolo Rivers are in acceptable level or not.

4.5.3 Speciation of arsenic in sediment samples

The As species determination in sediment samples was conducted first by investigating a relevant extraction reagents which are incapable of inducing species interconversion. This was because the integrity of the species should be maintained throughout the analytical procedure in order to avoid the incorrect results of the condition of the river systems.

4.5.3.1 Assessment of extraction conditions for speciation analysis

Different analytical procedures were investigated to adopt the suitable procedure in which the reagents used and microwave extraction condition favours the preservation of the integrity of As species. The extraction of As species in sediment samples was attempted using 1.0 M H_3PO_4 and 0.1 M $C_6H_8O_6$ extraction reagents reported by Garcia-Manyes *et al*. (2002). The extraction of As species using phosphate reagent is through ligand exchange, which involves desorption of As species by phosphate. The extracted As species were separated and detected by employing gradient mode of elution using 20 mM and 60 mM NH_4NO_3 at pH 8.7 mobile phase. The chromatogram of As species in sediment samples obtained using the above procedure is indicated in figure 4.12.

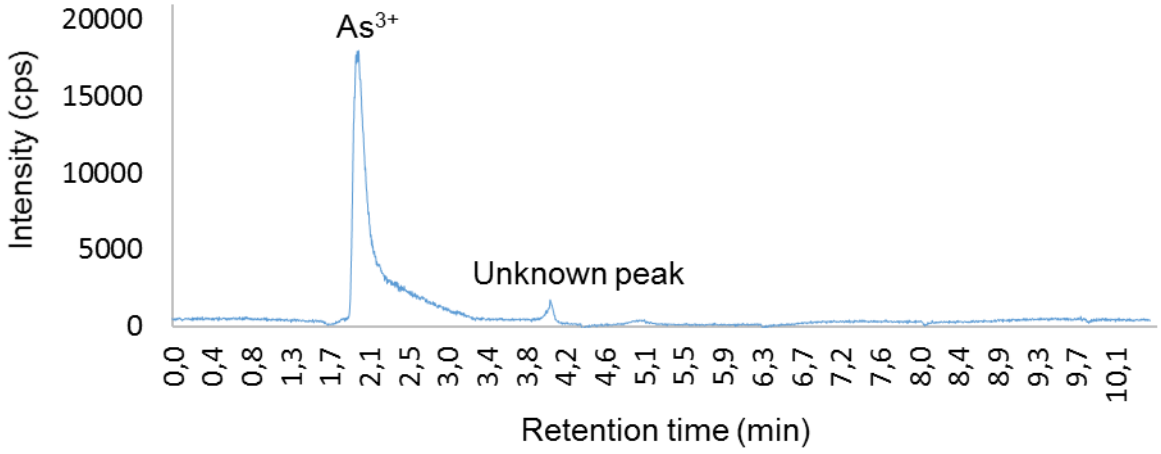

Figure 4.12: Chromatogram of As species for sediment samples obtained using 1.0 M H_3PO_4 and 0.1 M $C_6H_8O_6$

The peaks on the chromatogram were identified as As^{3+} and the unknown peak. The As species obtained using the suggested method show the possibility of reduction of As^{5+} to As^{3+} which may have induced by the use of reducing agent $C_6H_8O_6$. In the study by Garcia-Manyes *et al*. (2002), As^{3+} peak was also observed with no peak corresponding to As^{5+} in the chromatogram. The authors further reported that the extraction efficiency was affected by Fe, Mn and Al which have high affinity for retaining As species. Moreover, extraction efficiency depends on the matrices

composition of the materials. These trace elements are usually co-extracted with As species forming complexes such as Fe-As, Mn-As, Al-As species which are adsorbed by the stationary phase of the column (Pillay and Kindness, 2016).

The extraction reagents were further optimised with the use of EDTA instead of $C_6H_8O_6$ at pH 7 by employing the similar chromatographic conditions as when $C_6H_8O_6$ was used. The EDTA was recommended because of the ability to stabilise As^{3+} and also to chelate Fe, Al and Mn by the formation of complexes such as Fe-EDTA and Mn-EDTA hoping to preserve the integrity of native species (Pillay and Kindness, 2016). The chromatogram of the blank solution obtained using the optimised extraction reagents of 1.0 M H_3PO_4 and 50 mM M EDTA at pH 7 is indicated in figure 4.13.

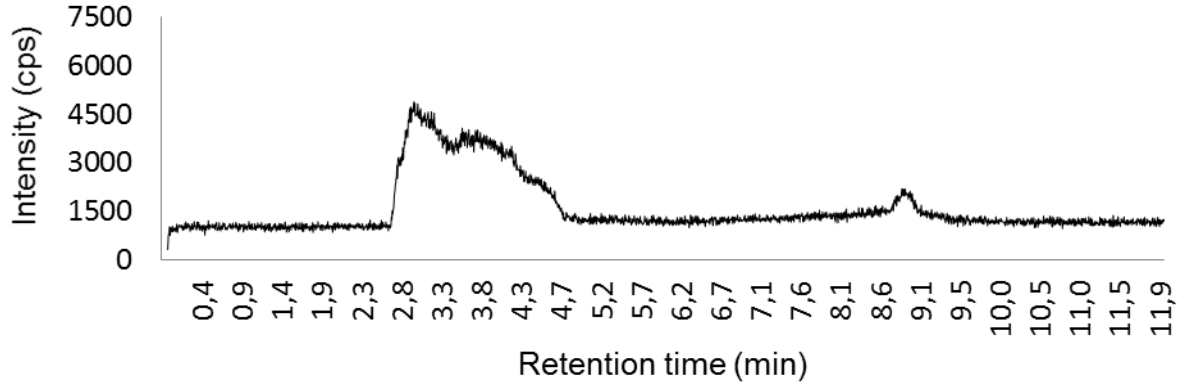

Figure 4.13: Chromatogram of the extraction reagents constituted of 1.0 M H_3PO_4 and 50 mM M EDTA at pH 7

The chromatogram of the proposed method show unknown peaks which may have attributed to contaminated mobile phase or interferences. However, observation of these unknown peaks led to investigation of constituents of H_3PO_4 and was found out that H_3PO_4 was prepared with As containing reagent in the parts per million (ppm) range of less than 1 mg/L. The use of H_3PO_4 for extraction of As species could produce unreliable analytical data because of the observed interferences. Chen *et al*. (2006) conducted speciation of As in an aerobic soil using H_3PO_4 an extraction reagent. The authors were able to detect and quantify As^{5+} since there was no other peaks corresponding to any species. They concluded their observation by stating

that H_3PO_4 and aerobic soil used favoured the oxidation of As^{3+} and stabilisation of As^{5+} in the samples. The ultra-pure acid phosphate reagents were further investigated in this study for extraction of As species in sediment samples.

4.5.3.2 Suitable reagents for species extraction in sediments

The $(NH_4)_2HPO_4$ acid and EDTA were investigated for the suitability of extraction of As species in sediment samples. The preliminary analysis of the extracts showed that the capability of the extractant reagents to be used without species interconversion is expected. The $(NH_4)_2HPO_4$ was assayed at different concentrations of 0.1, 0.3 and 1.0 M $(NH_4)_2HPO_4$ with 50 mM EDTA adjusted to neutral pH conditions which is similar to the environmental conditions. The extracted species were separated and detected by employing gradient mode of elution using optimised 10 mM NH_4NO_3 and 60 mM NH_4NO_3 at pH 8.9 adjusted with NH_3 solution. The extraction reagents of 0.3 M $(NH_4)_2HPO_4$ with 50 mM EDTA yielded excellent results which were further investigated for analytical figures of merit and method validation for testing the applicability of the proposed analytical procedure for monitoring of As species in sediments samples. The chromatogram of mostly extracted As species in sediment samples using the developed analytical procedure is indicated in figure 4.14.

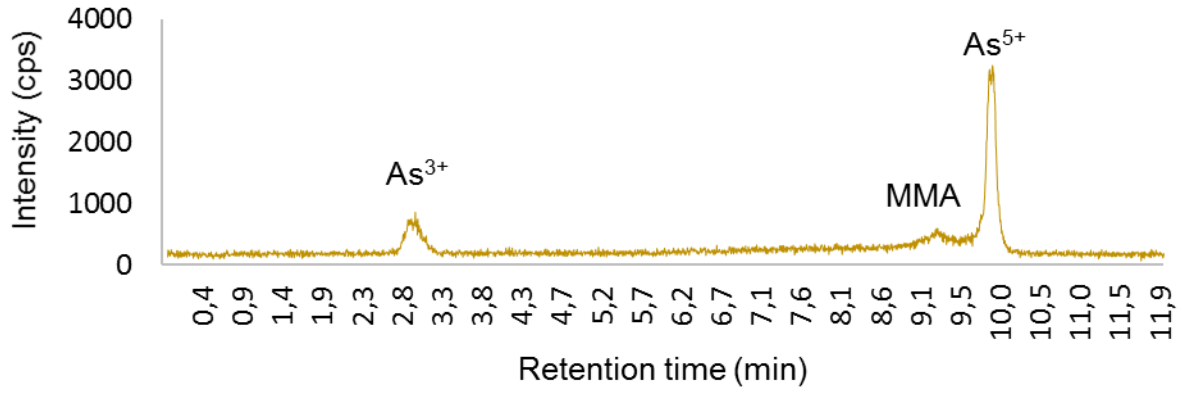

Figure 4.14: Chromatogram of As species for sediment samples

4.5.3.3 Arsenic species determination in sediments

The As species concentrations in sediments of the river systems were extracted and monitored with a suitable procedure. The species concentrations in sediment samples are indicated in tables 4.14 – 4.16.

Table 4.14: Concentrations of As species in sediment samples of Great Letaba River

Sample Id	As^{3+} (ng/g)	DMA (ng/g)	MMA (ng/g)	As^{5+} (ng/g)
GLR-1	74.0 ± 2.0	< 0.07	97 ± 5.5	457 ± 27
GLR-2	337 ± 1.7	< 0.07	401 ± 1.1	881 ± 42
GLR-3	1190 ± 16	< 0.07	449 ± 6.3	1150 ± 49
GLR-4	630 ± 14	< 0.07	318 ± 28	903 ± 43
GLR-5	482 ± 6.1	< 0.07	261 ± 2.1	601 ± 16
GLR-6	340 ± 2.1	< 0.07	159 ± 14	312 ± 39
GLR-7	< 0.11	< 0.07	132 ± 3.0	291 ± 9.2
GLR-8	< 0.11	< 0.07	< 0.09	152 ± 12
GLR-9	< 0.11	< 0.07	< 0.09	< 0.03
GLR-10	< 0.11	< 0.07	311 ± 5.4	189 ± 5.4

The detected As species in Great Letaba River sediments were in the range of 74 – 1190 ng/g for As^{3+}, 97 – 449 ng/g for MMA and 152 – 1150 ng/g for As^{5+}. The DMA was not detected in all sites. The results were precise with RSDs of less than 10%. The highest concentration was observed at site 3 and may have been attributed to the weathering processes of rocks. Various types of rocks were dominated in site 3 and as such, As containing rocks may be converted to inorganic As species which is then dissolved in water and further adsorbed into river sediments (Winkel *et al*., 2008). The activities of micro-organisms in sediments methylate the inorganic species to organic species (O'Reilly *et al*., 2001). Therefore, MMA concentrations were resulted by the effects of metabolic activities of micro-organisms. The As species concentrations in sediment samples are higher than in water samples. The underlying sediments protect water quality of the river system through adsorption of water contaminant (Terlecka, 2005). Thus, higher As species concentration were observed in sediment samples.

The As species concentrations of Mokolo River samples obtained during different seasons are indicated in table 4.15 and 4.16.

Table 4.15: Concentrations of As species in sediment samples of Mokolo during high flow sampling season

Sample Id	As^{3+} (ng/g)	DMA (ng/g)	MMA (ng/g)	As^{5+} (ng/g)
MOK-1	4350 ± 450	< 0.07	1600 ± 90	13000 ± 92
MOK-2	2560 ± 74	< 0.07	2280 ± 74	13100 ± 160
MOK-3	7950 ± 500	< 0.07	< 0.09	12800 ± 1200
MOK-4	3760 ± 160	< 0.07	< 0.09	15600 ± 85
MOK-5	2580 ± 140	< 0.07	935 ± 87	2580 ± 140
MOK-6	3920 ± 140	< 0.07	2060 ± 120	6160 ± 590
MOK-7	3930 ± 220	< 0.07	1870 ± 53	4120 ± 120
MOK-8	2890 ± 59	< 0.07	1710 ± 180	4110 ± 230
MOK-9	3140 ± 99	< 0.07	1850 ± 280	4060 ± 200
MOK-10	3350 ± 100	< 0.07	1610 ± 62	4420 ± 190

The concentration of As^{3+}, MMA and As^{5+} in sediment samples found during different flow sampling seasons are considerably higher than those reported by Ellwood and Maher (2003). During high flow sampling season, highest concentration of 7950 ng/g As^{3+} was found in site 3, 2280 ng/g for MMA was observed in site 2 and 15600 ng/g As^{5+} was observed in site 4. During low flow sampling season (Table 4.16), the highest concentration of 8870 ng/g As^{3+} was found in site 3, 1860 ng/g for MMA was found in site 9 and 9630 ng/g As^{5+} was observed in site 4. The precision of the results were monitored in all the cases and RSDs of less than 10% obtained show good precision. The DMA was not detected in both sampling season.

Table 4.16: Concentrations of As species in sediment samples of Mokolo River during low flow sampling season

Sample Id	As^{3+} (ng/g)	DMA (ng/g)	MMA (ng/g)	As^{5+} (ng/g)
LEP-1	311 ± 9.9	< 0.07	1320 ± 22	9260 ± 18
LEP-2	3920 ± 480	< 0.07	1030 ± 110	8170 ± 640
LEP-3	8870 ± 510	< 0.07	1370 ± 160	7730 ± 200
LEP-4	6610 ± 130	< 0.07	1400 ± 93	9630 ± 100
LEP-5	6780 ± 360	< 0.07	832 ± 95	7900 ± 290
LEP-6	6440 ± 31	< 0.07	959 ± 100	6590 ± 340
LEP-7	7020 ± 340	< 0.07	1600 ± 11	1490 ± 170
LEP-8	3380 ± 180	< 0.07	728 ± 94	1750 ± 78
LEP-9	3350 ± 140	< 0.07	1860 ± 56	1570 ± 110
LEP-10	3640 ± 330	< 0.07	1810 ± 110	1410 ± 48

The concentration of As^{3+} was higher during low flow sampling season whereas As^{5+} was higher during high flow sampling season. This may have been attributed by redox processes and geochemical nature of sediments (O'Reilly *et al*., 2001). The anthropogenic and microbial activities also affect the levels of As species. Mokolo River during high flow sampling season was subjected to the reducing conditions which favours the dissolution of sediment bound to metal-oxides in particular of Fe, Mn or Al (O'Reilly *et al*., 2001); hence, more of As^{5+} was released. The low flow sampling season is linked to oxidising condition of Mokolo River because sediments were exposed to air and increased dissolved O_2 concentration (O'Reilly *et al*., 2001). The oxidative dissolution of species such as Fe, Mn or Al led to less As^{5+} in the river system (O'Reilly *et al*., 2001).

The metabolic activities of micro-organisms in sediments methylate inorganic As species to MMA and DMA. Hence levels of MMA were observed in sediments of both sampling seasons. The highest concentration of particular inorganic species in water and sediment samples were predominating the site 4 of the river in both sampling seasons. The heavy rainfall season may distribute and elevate levels of As species from site 4 throughout the entire river system. This, leads to adverse impacts on water quality and aquatic ecosystem.

Mokolo River has higher concentration of As than Great Letaba River. Ellwood and Maher (2003) reported the range of As^{3+} and As^{5+} concentrations of 230 – 2430 ng/g and 110 – 7930 ng/g, respectively in marine sediments obtained from Lake Macquari, Australia using HPLC-ICP-MS. The concentrations of As^{3+} and As^{5+} in sediments reported by Ellwood and Maher (2003) are lower than data obtained from Mokolo River in both sampling seasons. However, their concentrations values were comparable with data obtained from Great Letaba River. The authors reported that the concentrations of As species obtained depend on the extraction procedure. The main anthropogenic sources of As are from use of arsenical pesticides, fertilisers, irrigation, ash from the burning of fossil fuels, and leachates from industrial waste such as mine tailings. Chen *et al*. (2006) detected 72.9 ± 1.4 µg/g for As^{5+} in soil sample using IC-ICP-MS which is higher than As^{5+} concentrations reported in this study.

4.6 Total concentration of trace elements in water and sediment samples

4.6.1 Quantification of trace elements in water samples

Hudson-Edwards *et al*. (2004) have indicated that the pathways of As from soil and sediment to water, plants and animals depend on the solid-phase partitioning of the As. Therefore, with the total concentration of Fe, Mn, and Al determined; the distribution of As in sediments and its pathways to water, plants and animals could be properly estimated. The total concentrations of As, Fe, Mn and Al in water samples were quantified using the validated analytical procedures. The quantified total concentrations in water samples of the river systems are presented in tables 4.17 – 4.19.

Table 4.17: Concentrations of trace elements in water samples of Great Letaba River

Sample Id	As[(a)] (µg/L)	Fe[(b)] (µg/L)	Mn[(b)] (µg/L)	Al[(b)] (µg/L)
GLR-1	< 0.0441	572 ± 29	656 ± 24	192 ± 17
GLR -2	< 0.0441	368 ± 30	228 ± 24	185 ± 7.8
GLR 3	< 0.0441	239 ± 18	99.2 ± 4.0	131 ± 2.6
GLR -4	0.0961 ± 0.0053	178 ± 11	86.6 ± 9.0	63.6 ± 5.5
GLR -5	0.447 ± 0.0014	274 ± 24	131 ± 8.0	95.9 ± 6.3
GLR -6	0.286 ± 0.029	179 ± 6.6	82.9 ± 5.6	5.89 ± 0.42
GLR -7	0.669 ± 0.063	158 ± 11	96.9 ± 5.7	32.9 ± 3.6
GLR -8	1.456 ± 0.077	151 ± 12	99.6 ± 8.3	168 ± 0.71
GLR -9	1.596 ± 0.077	88 ± 7.1	99.6 ± 8.3	156 ± 4.2
GLR -10	1.75 ± 0.063	178 ± 2.1	129 ± 7.3	177 ± 2.8

[(a)] denotes determination using ICP-MS
[(b)] denotes determination using ICP-OES

Water samples from Great Letaba River show that high concentrations of Fe were dominating as compared to concentrations of Mn, Al and As. The concentrations of As were high at downstream of the river with the maximum concentration of 1.75 µg/L. The Mn and Al concentrations were found within range of 82.9 – 656 µg/L and 5.89 – 192 µg/L, respectively. The trace elements concentrations observed in this study were compared with other studies. Ali (2010) found As concentrations in groundwater collected from Gravellote, Leeupoort and Kwagga in South Africa within 14 – 18 µg/L, 12.0 – 21.8 µg/L and 0.10 – 0.40 µg/L, respectively. The levels of Fe,

Mn and Al were not measured. The As concentrations obtained for Gravellote and Leeupoort samples are higher than the concentrations reported in this study. However, As concentrations obtained from water samples of Kwagga are comparable to concentrations observed in this study. Ogola *et al*. (2011) reported higher As concentrations of 1.0 mg/L as compared to this study in water around Ebenezer Dam from Limpopo province, South Africa. Rahman *et al*. (2015) obtained As, Fe and Mn concentrations of 262 µg/L, 3.90 mg/L and 809 µg/L, respectively in groundwater samples of Bangladesh and West Bengal. The concentrations of trace elements reported by Rahman *et al*. (2015) are higher than the data obtained in this study. Abdulhamid *et al*. (2013) reported Fe and Mn concentrations in the range of 0.09 – 0.26 mg/L and 0.03 – 0.05 mg/L, respectively. The study was conducted in groundwater samples of Kalambaina community and Al and As were not measured. The levels measured were less than Fe and Mn concentrations obtained in this study. The different concentrations of As, Fe and Mn in water samples vary from region to region based on regional geology and varied anthropogenic activities.

The water quality assessment study was also conducted for Great Letaba River by the development of reconciliation for the Levhubu and Letaba water supply systems (DWAF 2012). The assessment study was prompted by the effects of eutrophication in the river system, affecting nutrients in water to toxic levels. The nutrients such as phosphate, nitrate and ammonia were reported to be above MPLs of nutrients standard guidelines of water (EEA, 2005). The report revealed that gradual degradation of water quality is affected by return flows of agricultural runoff from intensively cultivated lands of banana and citrus which are impacted by fertilisers, salts, nutrients and pesticides (DWAF, 2012).

The concentrations of trace elements in water samples of Mokolo River during high and low flow sampling seasons are presented in tables 4.18 and 4.19.

Table 4.18: Concentrations of trace elements in water samples of Mokolo River during high flow sampling season

Sample Id	As[(a)] (µg/L)	Fe[(b)] (µg/L)	Mn[(b)] (µg/L)	Al[(b)] (µg/L)
MOK 1	0.956 ± 0.0057	< 0.0862	< 0.0574	57.9 ± 5.1
MOK 2	1.38 ± 0.048	130 ± 11	5.4 ± 0.51	161 ± 21
MOK 3	0.905 ± 0.047	121 ± 11	122 ± 16	49.9 ± 4.1
MOK 4	7.92 ± 0.2	< 0.0862	969 ± 101	< 0.0218
MOK 5	< 0.0441	204 ± 4.4	< 0.0574	94.9 ± 9.6
MOK 6	< 0.0441	214 ± 35	179 ± 14	130 ± 9.9
MOK 7	< 0.0441	264 ± 15	187 ± 8.5	189 ± 12
MOK 8	< 0.0441	336 ± 34	118 ± 2.1	164 ± 16
MOK 9	< 0.0441	266 ± 9.1	< 0.0574	101 ± 9.5
MOK 10	< 0.0441	252 ± 4.9	139 ± 7.1	195 ± 12

[(a)] denotes determination using ICP-MS
[(b)] denotes determination using ICP-OES

Mokolo River in high flow sampling season was observed having highest concentration of 7.92 µg/L for As, 336 µg/L for Fe, 969 µg/L for Mn and 195 µg/L for Al based on the analytical results. The As concentration were not detected in the upstream site 5 to site 10 of the river. This may be due to flowing water distributing As from upstream to the downstream. The low flow sampling season results of Mokolo River (Table 4.19) show elevated levels of trace elements. The As concentration was detected in all sites with the measured concentrations of 0.0456 – 5.66 µg/L. The Fe concentrations were found within 249 – 382 µg/L whereas Mn and Al were detected within 151 – 2760 µg/L and 93.2 – 249 µg/L, respectively.

The trace elements levels in water samples obtained from Mokolo River in both sampling seasons were comparable with other studies reported by Ali (2010), Abdulhamid *et al*. (2013) and Rahman *et al*. (2015). The As concentrations in water samples of Mokolo River were lower than concentrations reported by Ali (2010); particularly of the samples obtained from Gravellote and Leeupoort. Moreover, As levels of Kwagga samples also reported by Ali (2010) were comparable with the concentrations reported in this study. Rahman *et al*. (2015) reported As and Fe levels quite higher than that of Mokolo River. The site 4 of high flow sampling season and site 1 and 3 of low flow sampling season were having Mn concentrations higher than levels reported by Rahman *et al*. (2015). The levels of Fe and Mn reported by

Abdulhamid *et al*. (2013) are generally lower than that reported in this study. The Al was not reported hence the levels of Al were not compared to any other study.

Table 4.19: Concentrations of trace elements in water samples of Mokolo River during low flow sampling season

Sample Id	As[(a)] (µg/L)	Fe[(b)] (µg/L)	Mn[(b)] (µg/L)	Al[(b)] (µg/L)
LEP-1	0.0532 ± 0.0055	285 ± 28	2760 ± 38	178 ± 16
LEP-2	0.081 ± 0.0061	382 ± 33	431 ± 28	198 ± 12
LEP-3	2.63 ± 0.12	317 ± 24	2250 ± 24	239 ± 24
LEP-4	5.66 ± 0.3	251 ± 24	541 ± 45	93.2 ± 3.2
LEP-5	3.52 ± 0.1	258 ± 21	155 ± 15	101 ± 9.5
LEP-6	1.16 ± 0.15	249 ± 19	165 ± 19	183 ± 5.0
LEP-7	0.934 ± 0.035	421 ± 35	723 ± 33	210 ± 12
LEP-8	0.0456 ± 0.0065	333 ± 32	151 ± 11	249 ± 20
LEP-9	0.837± 0.11	253 ± 16	680 ± 12	172 ± 4.9
LEP-10	2.26 ± 0.12	300 ± 13	584 ± 56	183 ± 5.0

[(a)] denotes determination using ICP-MS
[(b)] denotes determination using ICP-OES

The Council of Scientific and Industrial Research (CSIR) investigated water quality of Mokolo River (Sibisi, 2009). The CSIR study was based on the development of ecological indicators which provided estimation of the ecological status of Mokolo river and wetland ecosystem (Sibisi, 2009). The study succeeded in formulating the methods for predicting toxic blue-green algae (eutrophication) known to be posing significant health threats in the aquatic ecosystem. The filamentous green algal species in water indicated the negative impacts of agricultural activities on the aquatic ecosystem of Mokolo River (Sibisi, 2009).

4.6.1.1 Comparison with maximum permissible levels in drinking water and irrigation water

Total concentrations of trace elements quantified in water samples collected from Great Letaba and Mokolo River systems were assessed for safe drinking and irrigation with reference to the standard guidelines. The Food and Agriculture Organisation (FAO), WHO and USEPA have established MPLs of trace elements to set mandatory water quality standards for drinking water and irrigation (FAO, 1991;

WHO, 2011). The guideline values for trace elements provide water quality targets which could be used to verify the level of contamination of trace elements in water (FAO, 1991; WHO, 2011). The MPLs for trace elements in drinking and irrigation water recommended by the WHO and FAO organisation are indicated in table 4.20.

Table 4.20: Standard guidelines for trace elements in drinking and irrigation water

Element	WHO MPLs (µg/L)¤	FAO MPLs (µg/L)#
As	10	100
Fe	300	5000
Mn	100	200
Al	200	5000

¤ denotes guidelines for drinking water
# denotes guidelines for irrigation water

The assessment of water quality was prompted by observed collection of water for household purposes and water abstraction systems installed in the rivers for irrigation purpose during the sampling campaigns. The As concentrations in Great Letaba River samples were below the MPLs guidelines for drinking water and irrigation water (FAO, 1991; WHO, 2011). The Fe concentrations exceeded MPL guideline for drinking water at site 1 and 2 whereas Mn concentrations exceeded the guideline at site 1, site 2, site 5 and site 10 (WHO, 2011). The Al concentrations were below MPLs guidelines at all sites. The As, Fe and Al levels in Great Letaba River water samples were below MPLs recommendations for irrigation water. However, Mn concentrations exceeded MPLs recommended for drinking irrigation water at site 1 and 2 (FAO, 1991; WHO, 2011).

The As levels of Mokolo River in both sampling seasons were below MPLs guidelines for drinking and irrigation waters. However, site 4 has an astonishing level of As which may exceed MPL guideline for drinking water provided sediments undergoes reducing environmental conditions. The reducing conditions usually favours the leaching of As from sediments into river water, resulting in an elevated levels of As. The As is not an essential plant nutrient at any concentration. Although it may stimulate plant growth, however depression of crop production may result due to increased accumulation levels (DWAF, 1996). The main effect of As in plants appear to be destruction of chlorophyll in the foliage, as a consequence of inhibition

of reductase enzymes (DWAF, 1996). Furthermore, bioaccumulation of As in edible plants may lead to As exposure through consumption of As contaminated crops.

The Fe level at site 8 in high flow sampling season exceeded MPL for drinking water. In low flow sampling season, Fe concentrations were higher than MPL recommended for drinking water at site 2, 3, 7 and 8 samples. In both sampling seasons, Fe levels were below MPL guideline for irrigation water.

The Mn concentrations in high flow sampling season of Mokolo River were below MPL recommended for drinking water at site 1, 2, 5 and 9 whereas the levels exceeded MPL at all sites in low flow sampling season. The Mn levels were discovered higher than MPL guideline for irrigation water at site 4 only of high flow sampling season and below the guideline at site 4, 5, 6 and 8 in low flow sampling season. The crops irrigated with higher levels of Mn may suffer from productivity (DWAF, 1996). The Al levels in low flow sampling season exceeded MPLs recommended for drinking water at site 3, 7 and 8 and were discovered below the guideline at all sites in high flow sampling season. In both sampling seasons, Al levels were below the MPL recommendation for irrigation water. The Al is not a plant nutrient and it may cause non-productivity in acid soils when irrigated with concentration above guideline value for irrigation water (DWAF, 1996).

Great Letaba and Mokolo Rivers water should not be recommended for safe drinking and suitability for irrigation unless other studies such as microbial, physical and chemical parameters for water quality assessment has been done and evaluated.

4.6.2 Quantification of trace elements in sediment samples

Garcia-Manyes *et al*. (2002) outlined the relationship between As and elemental oxides of Fe, Mn and Al in complex matrices like sediments. The major species of As are sorbed onto sediments or soil minerals as well as hydrous Fe, Mn and Al oxides. The induced affinity between these trace elements and As affect extractability of As because the adsorption is enhanced by cations of Fe, Al and Mn acting as bridging complexes for As (Manning and Goldberg, 1997). Therefore, total concentration determination of As, Fe, Mn and Al was conducted in sediment samples to further investigate the correlation between major species with the trace elements.

4.6.2.1 The concentrations of As, Fe, Mn and Al in sediments of Great Letaba and Mokolo Rivers

The total concentration determination was conducted using the validated analytical procedure. The quantified total concentrations of As, Fe, Mn and Al in sediment samples of the river systems are presented in tables 4.21 – 4.23.

Table 4.21: Concentrations of trace elements in sediment samples of Great Letaba River

Sample Id	As[(a)] (µg/g)	Fe[(b)] (µg/g)	Mn[(b)] (µg/g)
GLR-1	0.614 ± 0.025	1970 ± 201	482 ± 35
GLR -2	2.30 ± 0.025	7040 ± 622	285 ± 18
GLR -3	3.31 ± 0.24	905 ± 28	395 ± 39
GLR -4	2.21 ± 0.21	2990 ± 254	688 ± 53
GLR -5	1.74 ± 0.076	2910 ± 125	404 ± 15
GLR -6	1.00 ± 0.037	967 ± 114	275 ± 62
GLR -7	0.335 ± 0.037	587 ± 65	201 ± 14
GLR -8	0.146 ± 0.0042	385 ± 47	137 ± 4.8
GLR -9	0.187 ± 0.016	478 ± 62	208 ± 64
GLR -10	0.607 ± 0.016	1820 ± 87	353 ± 12

[(a)] denotes determination using ICP-MS
[(b)] denotes determination using ICP-OES

The concentrations of As in sediment samples from Great Letaba River were within the range of 0.146 – 3.31 µg/g. The mostly high As concentrations were observed in the upstream of the river. The Fe concentrations were found with the highest concentrations of 385 – 2990 µg/g as compared to As and Mn. The Al was not measured in sediment samples of Great Letaba River. The levels of trace elements could have been influenced by the regional geology, natural processes and anthropogenic activities.

Mols (2016) reported total concentrations of 605 µg/g for As, 2700 µg/g for Fe, 1260 µg/g for Mn and 34280 µg/g for Al in sediments samples in a study for As speciation and sorption in soils, sediments and tailings from the Chenzhou region in China. The As and Mn levels reported by Mols (2016) are higher than the ones reported in this study. The Fe concentrations at site 2, 4 and 5 observed to be higher than the levels reported by Mols (2016). Lou *et al*. (2008) reported As concentrations in the surface

soil in the range 5.70 – 23.0 µg/g which are higher than concentrations obtained in sediments of Great Letaba River. The Fe, Mn and Al levels in a study by Lou *et al*. (2008) were not measured. Olivares-Rieumont *et al*. (2005) reported total concentrations in the range 26000 – 45000 µg/g for Fe, 460 – 1630 µg/g for Mn and 18000 – 68000 µg/g for Al in the surface sediments of Almendares and San Francisco Rivers.

The concentrations of trace elements in sediment samples of Mokolo River during high and low flow sampling seasons are presented in tables 4.22 and 4.23.

Table 4.22: Concentrations of trace elements in sediment samples of Mokolo River during high flow sampling season

Sample Id	As[(a)] (µg/g)	Fe[(b)] (µg/g)	Mn[(b)] (µg/g)	Al[(b)] (µg/g)
MOK 1	21.9 ± 1.8	512 ± 18	14.2 ± 0.14	6530 ± 316
MOK 2	23.5 ± 0.014	700 ± 38	30.8 ± 1.2	8370 ± 362
MOK 3	24.6 ± 2.3	653 ± 73	31.0 ± 3.9	6790 ± 359
MOK 4	21.3 ± 0.27	358 ± 16	9.1 ± 1.1	3760 ± 483
MOK 5	7.15 ± 0.48	347 ± 19	28.5 ± 0.15	4250 ± 538
MOK 6	14.5 ± 1.4	282 ± 8.8	16.7 ± 0.38	7210 ± 926
MOK 7	11.0 ± 0.054	417 ± 31	50.8 ± 1.3	6780 ± 487
MOK 8	10.9 ± 1.1	412 ± 11	23.3 ± 1.6	7300 ± 561
MOK 9	13.3 ± 0.55	952 ± 21	27.9 ± 1.3	8032 ± 425
MOK 10	12.2 ± 0.082	368 ± 44	105 ± 3.1	9080 ± 232

[(a)] denotes determination using ICP-MS
[(b)] denotes determination using ICP-OES

The high flow sampling season sediments results of trace elements show the concentrations of As in the range of 7.15 – 23.5 µg/g. The Fe, Mn and Al were obtained in the range 282 – 952 µg/g, 9.1 – 105 µg/g and 3760 – 9080 µg/g, respectively. The low flow sampling season results (Table 4.23) were observed in the range 12.3 – 24.3 µg/g for As, 548 – 1630 µg/g for Fe, 113 – 1290 µg/g for Mn and 14520 – 19540 µg/g for Al.

Table 4.23: Concentrations of trace elements in sediment samples of Mokolo River during low flow sampling season

Sample Id	As[(a)] (µg/g)	Fe[(b)] (µg/g)	Mn[(b)] (µg/g)	Al[(b)] (µg/g)
LEP-1	12.3 ± 0.85	1040 ± 83	544 ± 43	15050 ± 1565
LEP-2	14.9 ± 1.5	933 ± 58	272 ± 9.5	16050 ± 871
LEP-3	19.7 ± 0.18	1590 ± 253	1040 ± 130	18830 ± 2035
LEP-4	19.1 ± 0.49	1630 ± 159	1290 ± 131	15300 ± 1483
LEP-5	15.0 ± 0.29	1090 ± 131	273 ± 48	14520 ± 1374
LEP-6	15.5 ± 1.3	1000 ± 44	300 ± 9.9	14920 ± 1113
LEP-7	24.3 ± 1.4	1270 ± 27	1260 ± 58	17540 ± 1879
LEP-8	22.4 ± 0.38	852 ± 54	261 ± 17	15210 ± 791
LEP-9	22.0 ± 0.43	971 ± 46	171 ± 12	15630 ± 1238
LEP-10	21.7 ± 2.4	548 ± 42	113 ± 9.9	19540 ± 614

[(a)] denotes determination using ICP-MS
[(b)] denotes determination using ICP-OES

The As, Fe, Mn and Al concentrations obtained in both sampling seasons were lower than the levels reported by Mols (2016). Lou *et al*. (2008) reported As levels which are comparable to the concentrations obtained in this study in both sampling seasons. The Fe and Al levels obtained in a study by Olivares-Rieumont *et al*. (2005) are higher than the concentrations found in both sampling season of Mokolo River. The Mn concentrations obtained in high flow sampling season are comparable with the levels reported in a study by Olivares-Rieumont *et al*. (2005), whereas Mn concentrations in site 1, 3, 4 and 7 in low flow sampling season, were found to be in the range of the similar study.

4.6.2.2 Comparison of levels of trace elements in Mokolo River sediments for two sampling seasons

The levels of trace elements for each sampling site were compared between high flow (HF) and low flow (LF) sampling seasons. The graphical representations of the levels of As, Fe, Mn and Al in both sampling seasons are presented in figure 4.15 – 4.18, respectively.

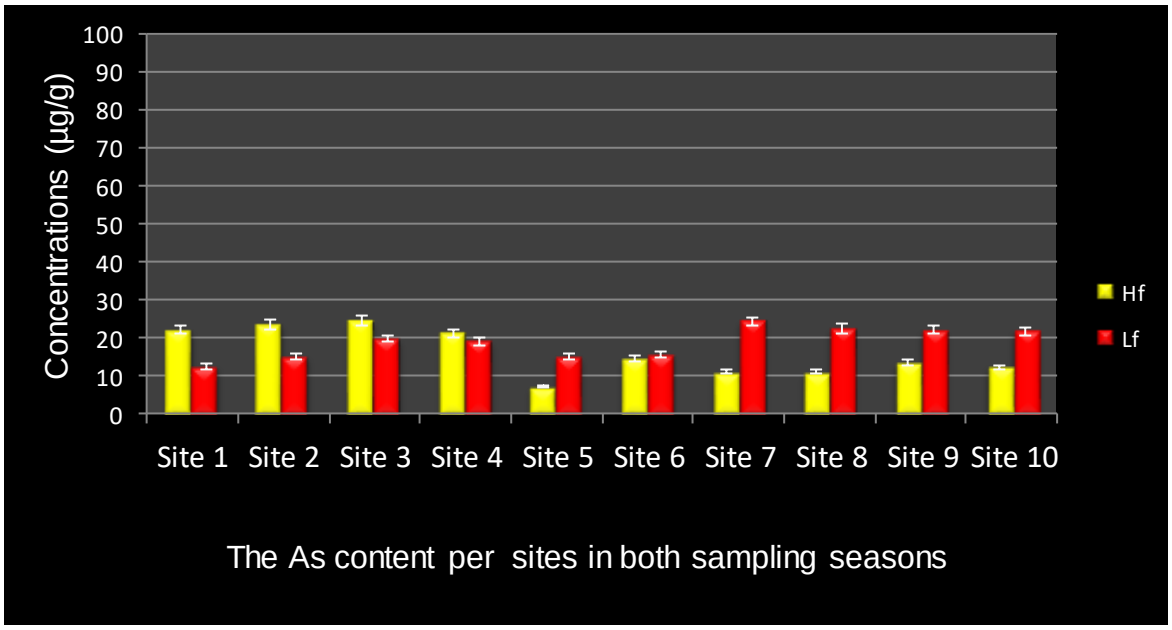

Figure 4.15: Graphical representation of As levels of Mokolo River in both sampling seasons

The graphical representation of As levels in sediment samples collected from Mokolo River (Figure 4.15) show the elevated levels of As at different sites in different sampling seasons. During high flow sampling season, As levels are high towards downstream of the river with the lowest level found at site 5. In low flow sampling season, As levels were discovered elevated in the upstream of the river with highest concentration found in site 7.

The levels of Fe in sediments obtained in both sampling seasons are presented graphically (Figure 4.16) to observe which sampling season was dominated by Fe levels.

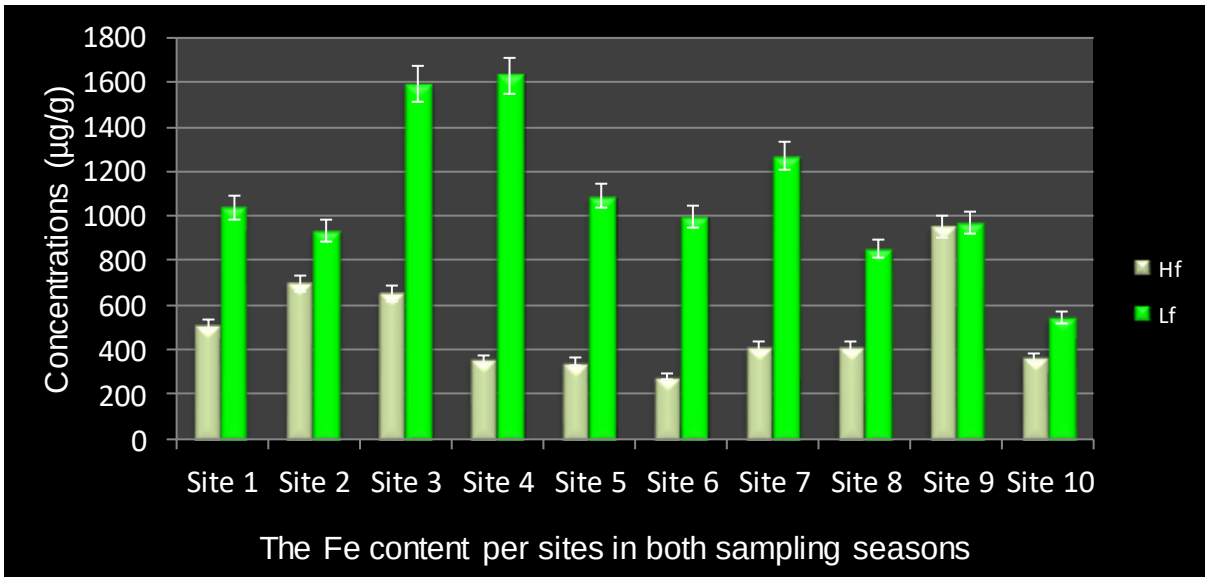

Figure 4.16: Graphical representation of Fe levels of Mokolo River in both sampling seasons

The distribution of Fe during high flow sampling season was relatively low as compared to the levels found in high flow sampling seasons. The seasonal variation of Fe levels may have attributed to the redox condition, microbial activities, physical and chemical nature of the river sediments. For instance, during the reductive dissolution process which is likely to occur as a consequence of flooding or submersion of sediments may influence elevated levels of Fe (O'Reilly *et al*., 2001). Furthermore, Fe is an essential micronutrient. However, toxicity effect may arise due to higher Fe contents which are likely to be contributed by Fe dust from coal mining and possibilities of sewage effluents surrounding Mokolo River (Manning and Goldberg, 1997). In addition, Fe containing agricultural herbicide and pesticides may contribute to elevated levels of Fe in river sediments since the agricultural farmers were found in the vicinity of Mokolo River.

The graphical representation of the distribution of Mn content in sediments of Mokolo in high and low flow sampling season are indicated in figure 4.17.

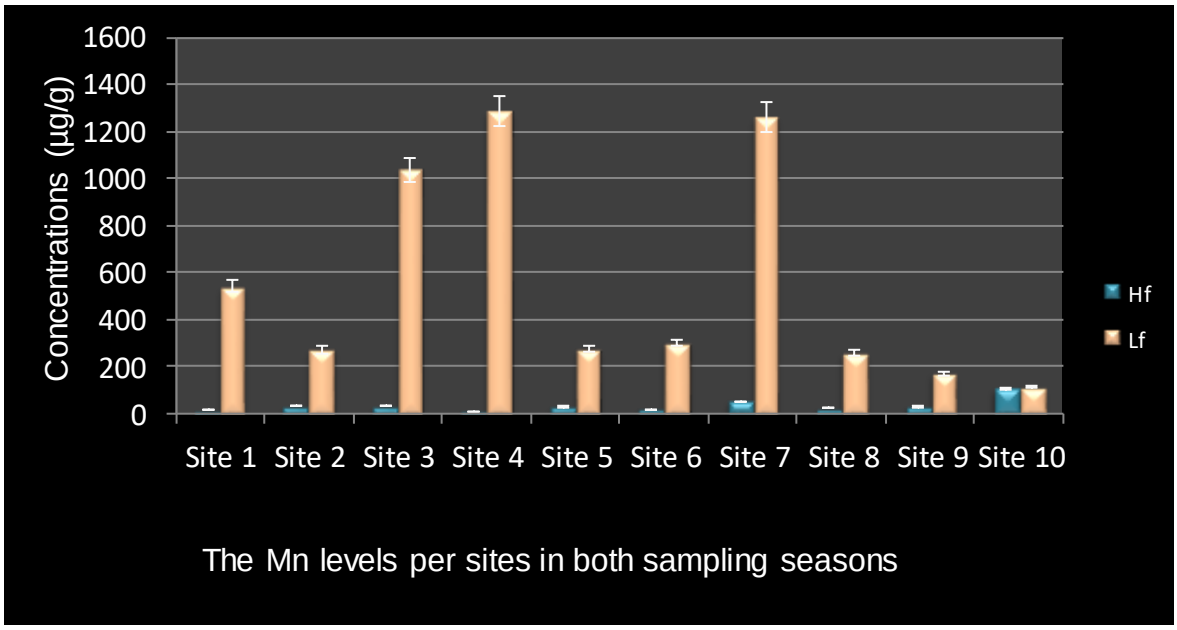

Figure 4.17: Graphical representation of Mn levels of Mokolo River in both sampling seasons

High levels of Mn are discovered in low flow sampling season (Figure 4.17). However, site 10 was found to have the same Mn levels in both sampling season. The Mn is a naturally occurring element that is found in all environmental matrices. The lower Mn levels obtained during high flow may have been influenced by the dilution effect during rainy seasons.

The graphical representation of the distribution of Al content in sediments of Mokolo in high and low sampling season are indicated in figure 4.18.

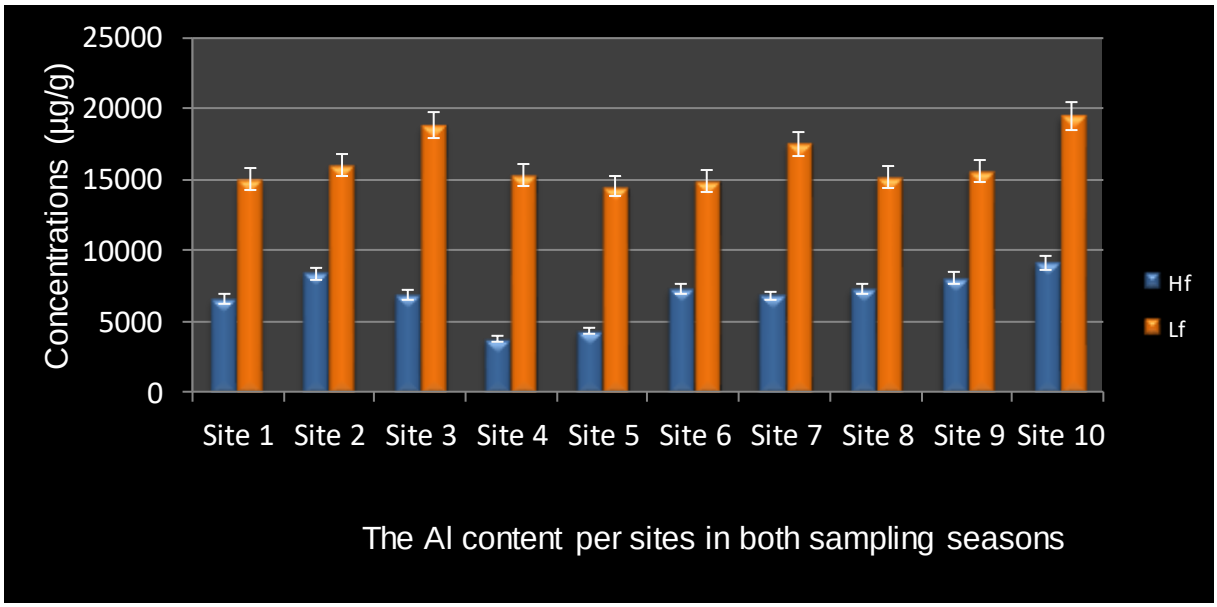

Figure 4.18: Graphical representation of Al levels of Mokolo River in both sampling seasons

The higher Al levels were found during low flow sampling season as compared to high flow sampling seasons. The Al is the most abundant metal and the third most abundant element in the earth's crust (O'Reilly *et al*., 2001). Due to its prominence, natural weathering processes contribute to its release to the environment through the weathering of rocks and minerals than the anthropogenic sources (O'Reilly *et al*., 2001). In exceptional to natural weathering processes, levels of Al in sediments may have influenced by the atmospheric deposition of Al containing dust during coal mining, municipal waste water effluents as well as solid wastes primarily associated with industrial processes occurring near Mokolo River in Lephalale area.

4.6.2.3 Comparison with sediment quality guidelines

The sediment quality guidelines are a set of standards guidelines developed for the protection of aquatic biological resources. The guideline values for each trace element indicate the lowest effect levels (LEL) of sediment contamination at which the majority of aquatic organisms are unaffected (OMEE, 1993; CCME, 2001). The severe effect levels (SEL) of trace elements in sediments may pronounce disturbance of the sediments dwelling community which would be detrimental to the

majority of aquatic organism (CCME, 2001; OMEE 1993). The levels of trace elements in sediments of the river systems were assessed for quality with reference to sediments quality guidelines recommended by Canadian Council of Ministers of Environment (CCME) and Ontario Ministry of Environment and Energy organisations (OMEE) (OMEE, 1993; CCME, 2001). The CCME and OMEE recommendations for LEL and SEL values are indicated in table 4.24.

Table 4.24: Standard guidelines for sediments quality

Trace element	LEL (µg/g)	SEL (µg/g)
As	6.00	33.0
Fe	21200	43766
Mn	460	1100
Al	NR*	NR*

*NR denote not recommended

The standard quality guidelines to assess the level of contamination for As species in the environmental samples are not yet established. However, contamination levels of As species in river sediments were assessed through the evaluation of total concentration determination with reference to quality guidelines. The As species concentrations in sediments of Great Letaba which contribute to total concentrations were found to be less than the recommended LEL value. Moreover, the highest concentrations of Fe and Mn were found exceeding the LEL values but less than SEL values. The analytical results show that sediments of Great Letaba River could maintain sediment dwelling aquatic organisms without detrimental effects.

The As concentrations in sediments of Mokolo in both sampling seasons have exceeded the recommended LEL values but below SEL values with highest concentration of 24.0 µg/g. The observed As levels in sediments of Mokolo River pose a threat to sediments dwelling and aquatic ecosystem because As is not an essential nutrient for living organisms at any concentration (DWAF, 1996). Although, As concentrations in Mokolo River sediments are below recommended SEL value, nonetheless sediments dwelling community are at risk of As exposure due deteriorating quality of sediments.

The highest levels measured for Fe in both sampling seasons were below LEL value. The Mn concentrations were below LEL in high flow sampling season and exceeded LEL and SEL recommendations in low flow sampling season. The Fe and Mn levels obtained may be beneficial to sediments dwelling aquatic organisms since Fe and Mn are essential micronutrients. Moreover, no information was reported on toxicity of Mn and Fe to aquatic biota (OMEE, 1993; CCME, 2001). The CCME and OMEE organisations have not established the LEL and SEL guidelines values for the assessment of quality of Al in sediments.

4.7 Correlation between inorganic arsenic species and trace elements in sediments

The levels of Fe, Mn and Al in sediment samples were investigated to monitor the effects of these trace elements on extraction efficiency of inorganic As species in sediment samples. This investigation was prompted by interaction between inorganic species with trace elements. The As^{3+} and As^{5+} are known to be sorbed onto common soil or sediments minerals including clays, hydrous Al, Fe and Mn oxides (Hudson-Edwards *et al*., 2004). The interactions between total As and trace elements were evaluated in this study. No correlation was observed between total As levels with any levels of these trace elements. Ellwood and Maher (2003) observed weak correlation between the extracted concentrations of As and Fe levels with R^2 value of 0.30. The R^2 value of 0.49 was observed between extracted As with Mn (Ellwood and Maher, 2003). The correlation investigation between inorganic As species with Fe, Mn and Al are presented and error bars represent ± standard deviations.

4.7.1 The correlation between Fe, As^{3+} and As^{5+}

The relationship of Fe with As^{3+} and As^{5+} is indicated in figure 4.19 and appendix 1. The correlation between Fe and As^{3+} is stronger than the one with As^{5+} in terms of R^2 values of 0.4067 and 0.2754, respectively. However, levels of Fe and As^{5+} indicated in appendix 1, shows interaction by grouping between concentrations from 2 µg/g As^{5+} to 1270 µg/g Fe and from 6 µg/g As^{5+} to 1630 µg/g Fe. The correlation between

Fe and As^{3+} by grouping of concentrations is also found by clustered levels of Fe and As^{3+} at different concentration as compared to interaction of Fe and As^{5+}. The difference in the interactions of Fe with As^{3+} and As^{5+} is influenced by the pH of the surrounding (O'Reilly *et al*., 2001). The stronger correlation between Fe with As^{3+} is attributed to the neutral pH (6.5 – 8.5) of the river condition which favour the adsorption of As^{3+} onto Fe-oxide surface of the sediments (Jablonska-Czapla, 2014). The adsorption of As^{5+} onto Fe-oxide surface is likely to occur at pH 4 of the environmental condition (Jablonska-Czapla, 2014). Therefore, weak correlation between Fe and As^{5+}, in terms of R^2 value is an indication of weak phase association between Fe and As^{5+} affected by the pH.

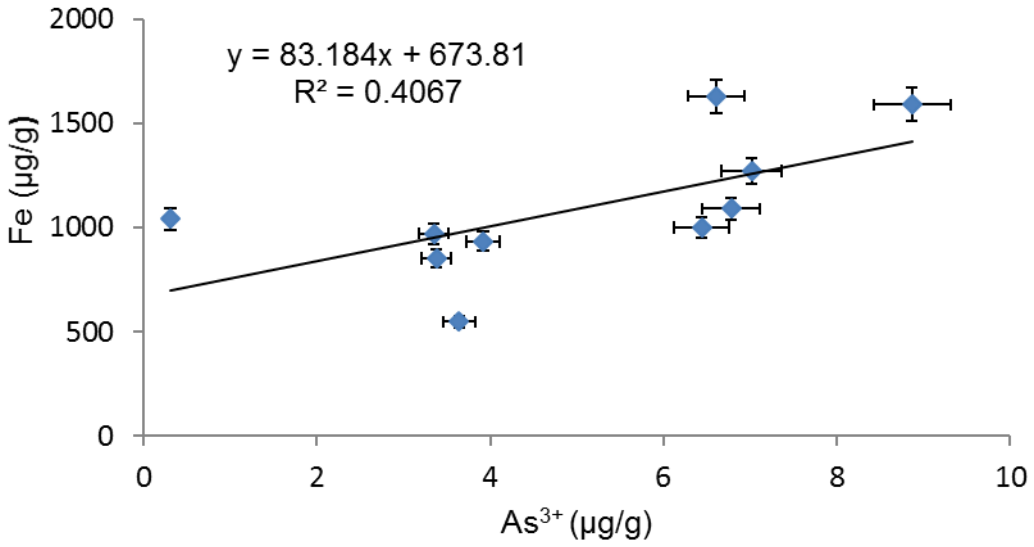

Figure 4.19: The correlation between Fe and As^{3+}

4.7.2 The correlation between Mn, As^{3+} and As^{5+}

The relationship of Mn with As^{3+} and As^{5+} is indicated in appendix 2 and appendix 3, respectively. The Mn exhibit a fair correlation with As^{5+} with the observed R^2 value of 0.5879. The levels of Mn and As^{5+} are clustered at different concentrations showing the phase association between Mn and As^{5+}. The R^2 value of 0.1953 and scattered concentrations of Mn and As^{3+} in the plot area is an indication of weak interaction. The As^{+5} is likely to be deposited into sediments surface by Mn oxides during the

environmental conditions which favours the persistence of As^{5+} (Johnson, 2008). This result with the strong interaction observed between Mn and As^{5+} than the one with As^{3+}.

4.7.3 The correlation between Al, As^{3+} and As^{5+}

The relationship of Mn with As^{3+} and As^{5+} is indicated in appendix 4 and appendix 5. The R^2 value of Al with As^{3+} exhibit no correlation. Buschmann *et al*. (2007) demonstrated the existence of the interaction between Al and As^{3+} over pH range of 6 – 9.5. However, interaction within stipulated pH was not observed in this study. Manning and Goldberg (1997) demonstrated that Al hydroxide may retain appreciable concentrations of As^{5+}, which may be indicated by grouped interaction at different concentrations. The R^2 value of Al with As^{5+} exhibit poor correlation nonetheless, grouped concentrations show phase association between Al with As^{5+}.

4.8 Solid phase extraction using multi walled carbon nanotubes with branched polyethyleneimine

The SPE technique was adopted for this study due to faster operation, pre-concentration efficiency, merit of satisfactory recovery and easier compatibility with analytical instruments (Chen *et al*., 2014). The SPE technique was used to pre-concentrate As^{5+} in water samples by employing MWCNTs-BPEI as the adsorbent materials.

4.8.1 Characterisation of adsorbent material

The control MWCNTs, oxidised MWCNTs and MWCNTs-BPEI were characterised using XRD, FTIR and TGA to verify the successful modification of the nanocomposite before use as an adsorbent.

4.8.1.1 Characterisation using X-ray diffraction technique

The characterisation using XRD was employed for phase identification and crystallinity through the modification process of MWCNTs with BPEI polymer. The XRD spectrum of control MWCNTs, oxidised MWCNTs and MWCNTs-BPEI are indicated in figure 4.20.

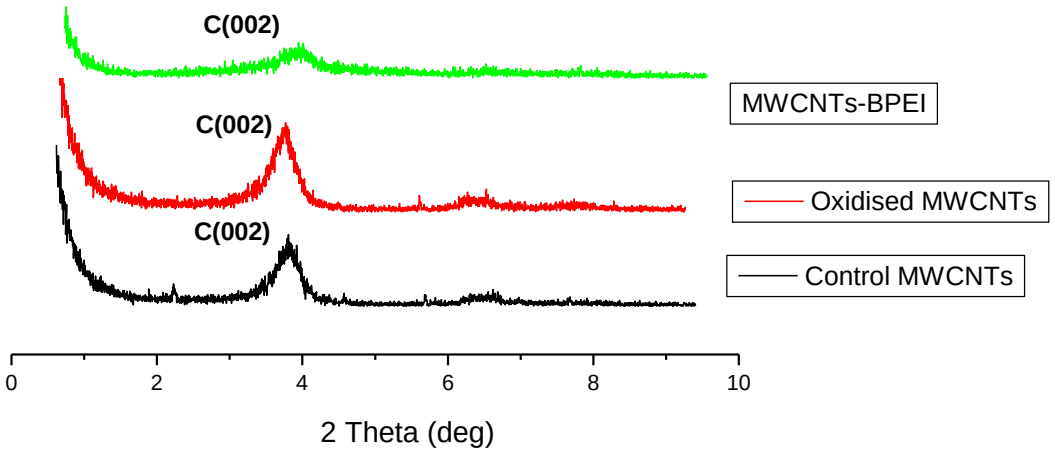

Figure 4.20: The XRD patterns for control MWCNTs, oxidised MWCNTs and MWCNTs-BPEI

The XRD profile shows a peak in the control MWCNTs which is identified as graphitic carbon indexed to C(002) plane reflections of hexagonal graphite (Mkhondo and Magadzu, 2014). The intensified graphitic peak of the oxidised-MWCNTs is attributed to formation of sp^3 hybridised carbon (C-H) due to effects of acid treatment as compared to sp^2 hybridised carbon (C=C) of the untreated or control MWCNTs (Malikov *et al*., 2014). The enhanced peak on the oxidised MWCNTs further indicates a formation of more ordered structure of MWCNTs with the walls remaining intact without significant damage. The impurities present in the control MWCNTs are due to thin layer of amorphous carbon on the nanotube surface and the encapsulated iron catalyst residue (Stobinskia *et al*., 2010). A decrease of graphitic carbon indexed to C(002) plane reflection of the MWCNTs-BPEI shows the successful interaction of MWCNTs with BPEI polymer.

A decrease of graphitic carbon indexed to C(002) plane reflection of the spectrum of MWCNTs-BPEI shows the successful interaction of MWCNTs with BPEI polymer. The XRD pattern of BPEI polymer (Figure 4.21) was investigated for identification of BPEI peaks on the XRD spectrum of MWCNTs-BPEI. The attenuation of the graphitic peak intensity of MWCNTs-BPEI observed on the XRD pattern occurred as a result of physisorption of a thin layer of amorphous BPEI coating on the surface of oxidised MWCNTs (Malikov *et al*., 2014).

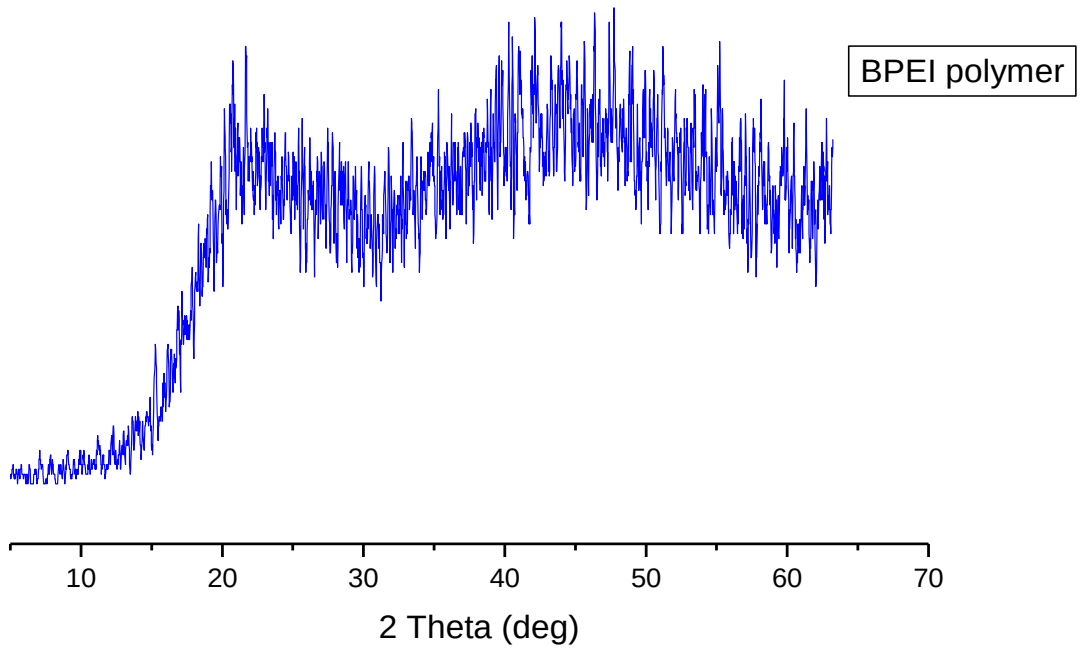

Figure 4.21: The XRD pattern for BPEI polymer

4.8.1.2 Characterisation using Fourier transform infrared spectroscopy

The FTIR technique was employed to investigate the functional groups introduced on the surface of MWCNTs during the acid modification processes.

The FTIR spectrum of control MWCNTs is indicated in figure 4.22 showing the absence of C-O, –CH and –OH functional groups. The absorption peak at 1536 cm^{-1} is assigned as C=C stretching vibrations is due to aromatic ring of MWCNTs (Stobinski *et al*., 2010; Malikov *et al*., 2014).

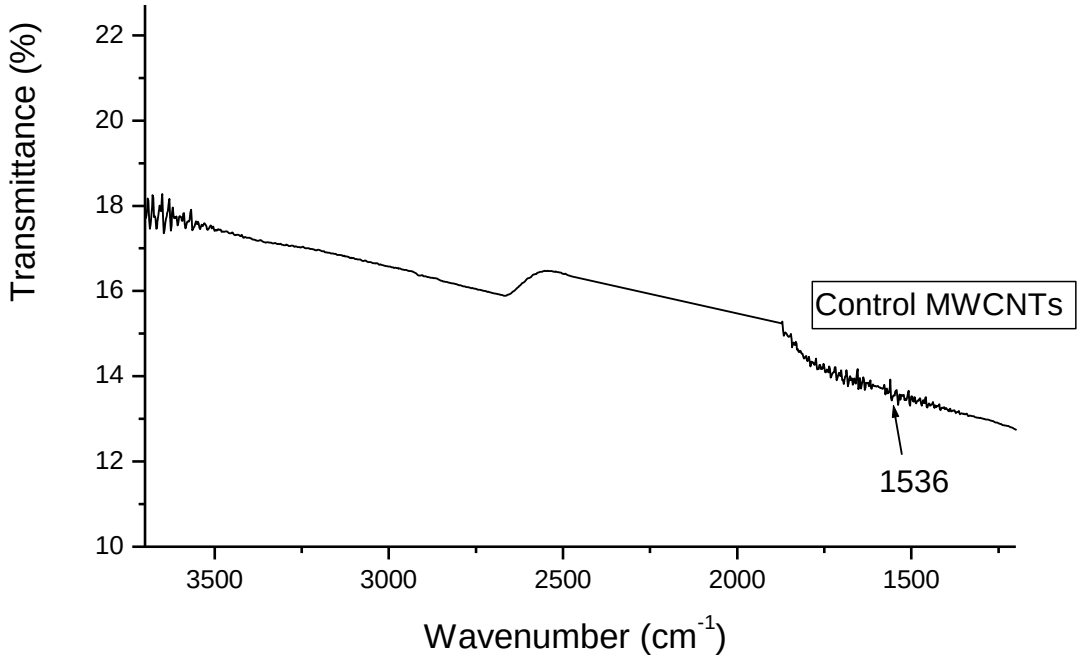

Figure 4.22: The FTIR spectrum of control MWCNTs

The FTIR spectrum of MWCNTs-BPEI is indicated in figure 4.23. The absorption peaks at 3383 cm^{-1} and 2893 cm^{-1} in the FTIR spectrum of MWCNTs-BPEI may have been attributed to stretching vibrations of –NH and –CH, respectively on the surface of nanocomposite. The peak at 1740 cm^{-1} corresponds to the stretching vibrations of –CN (Geng *et al*., 2015). These results verify the successful noncovalent modification of MWCNTs with BPEI.

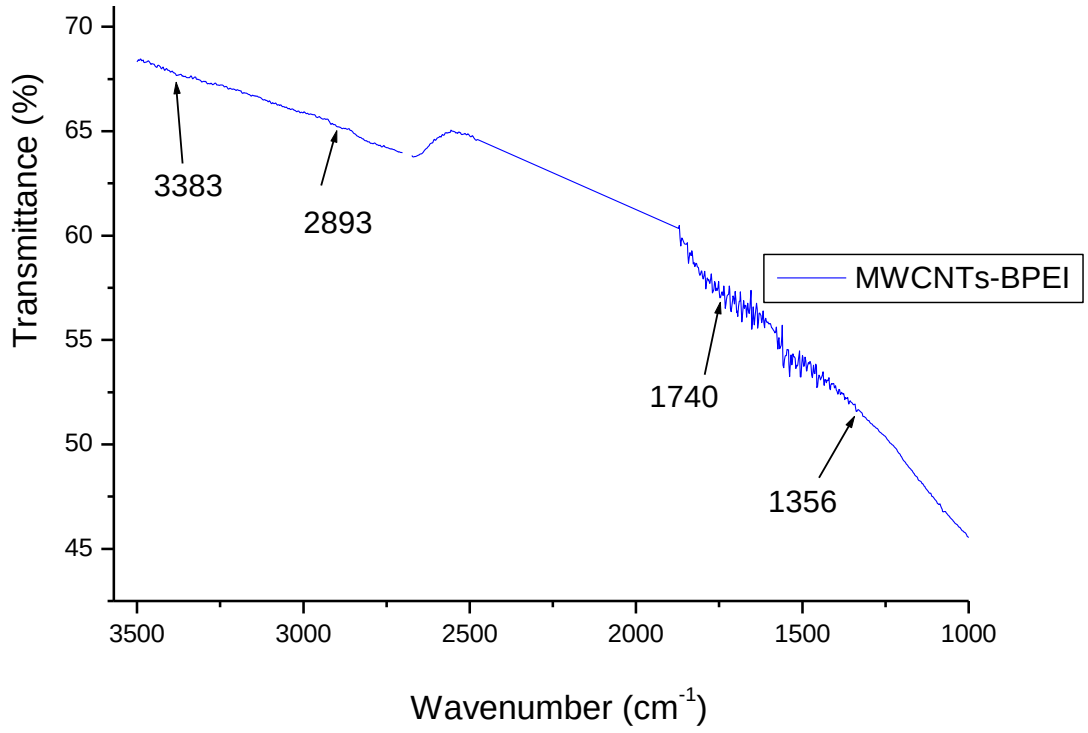

Figure 4.23: The FTIR spectrum of MWCNTs-BPEI

4.8.1.3 Characterisation using TGA technique

The characterisation of modified MWCNTs was explored to investigate thermal stability, purity and oxidative temperature. The TGA profile of the modification of MWCNTs is indicated in figure 4.24.

The TGA profiles of the modified MWCNTs reveal difference in the initiation temperatures where materials started to decompose and at the oxidative temperatures defined as the point of maximum weight loss (Bom *et al*., 2002). The thermal stability of the control MWCNTs is directly attributed to the aromatic bonding within the MWCNT structure. However, stability could be influenced by the number of walls, the presence and composition of catalyst, defects within the tubes and the presence of other materials within the structure such as amorphous carbon and graphitic particles. The oxidative temperature of control MWCNTs was observed between 280 – 400 ^{o}C (Yu *et al*., 2007). The catalytic impurities of amorphous carbon have lower oxidation temperatures, i.e., between 200 – 350 ^{o}C (Bom *et al*., 2002); hence the observed weight losses are likely to be due to oxidative temperature of amorphous carbon. A further weight loss observed at 400 ^{o}C may

have attributed to residual mass of metal catalyst in particular Fe used to manufacture the nanotubes as well as the oxidation products of the catalyst (Bom *et al*., 2002). The oxidised MWCNTs show an improved stability due to high purity of the material without defects. The oxidative temperature of MWCNTs-BPEI was observed approximately at 280 ^{o}C and may be attributed to functional groups added to oxidised MWCNTs through electrostatic interaction with BPEI (Bom *et al*., 2002). The BPEI polymer is less stable as compared to oxidised MWCNTs and TGA profile shows successful modification of MWCNTs-BPEI because of observed higher stability. Although stability is lost at higher temperature, the application of MWCNTs-BPEI as adsorbent material was not affected since experiment was performed at room temperature.

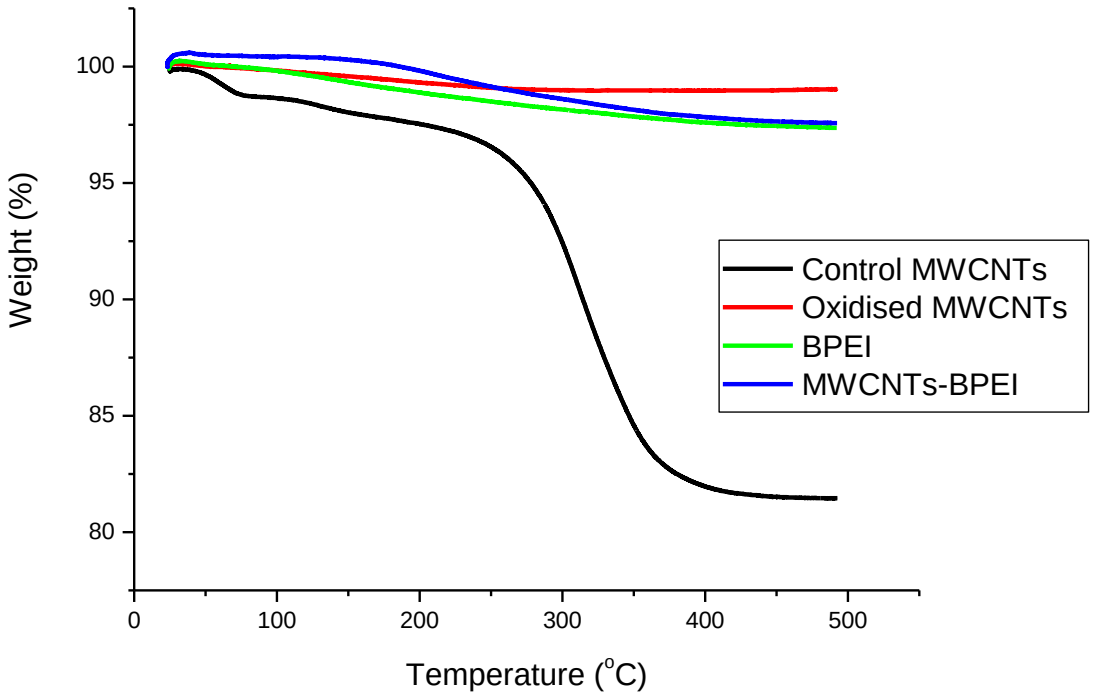

Figure 4.24: The TGA profiles for control MWCNTs, oxidised MWCNTs, MWCNTs-BPEI

4.8.2 Concentrations of As^{5+} in water samples obtained using solid phase extraction

The MWCNTs functionalised with BPEI exhibit excellent sorbent material for the selective retention of As^{5+} in the presence As^{3+} in the aqueous samples. The

successful modification of MWCNTs-BPEI led to validation of SPE analytical procedure and the pre-concentration factor of 23.3, with adequate percentage RSDs achieved using off-line mode. The results for SPE procedure are comparable with HPLC-ICP-MS as shown in Tables 4.25 – 4.27.

Table 4.25: Concentrations of As^{5+} in water samples of Great Letaba River

Sample Id	As^{5+} [(a)] (µg/L)	As^{5+} [(b)] (µg/L)
GRL-1	< 0.0537	< 0.078
GRL-2	< 0.0537	< 0.078
GRL-3	< 0.0537	< 0.078
GRL-4	< 0.0537	0.537 ± 0.0099
GRL-5	< 0.0537	< 0.078
GRL-6	0.204 ± 0.021	0.274 ± 0.013
GRL-7	0.597 ± 0.025	0.653 ± 0.023
GRL-8	0.774 ± 0.050	1.02 ± 0.057
GRL-9	0.888 ± 0.105	1.05 ± 0.0014
GRL-10	0.855 0.066	1.271 ± 0.012

[(a)] denotes analysis using ICP-MS after separation by SPE
[(b)] denotes analysis using HPLC-ICP-MS

The results obtained by SPE procedure are in good agreement with the HPLC-ICP-MS results. This agreement show that MWCNTs-BPEI composites exhibit a favourable adsorption efficiency for As^{5+} in water samples. The highest concentration of As^{+5} in water samples of Great Letaba River obtained using SPE procedure was found at site 9 whereas highest concentration obtained using HPLC-ICP-MS was found at site 10. The difference in the application of the modes of sample analysis between the SPE and HPLC-ICP-MS may have led to the difference of the concentrations of As^{5+} obtained. The SPE procedure was performed through off-line mode while HPLC-ICP-MS used on-line mode of analysis. For instance, the advantageous use of HPLC-ICP-MS is that the random errors which may be induced during sample injection, separation of the analytes and detection are minimal. The SPE has the capability to provide the samples which are concentrated enough for detection. Due to the interaction between the adsorbent material, analyte of the interest and eluent, the SPE procedure is reliable to resolve the interferences of polyatomic and matrices (Zwir-Ferenc and Biziuk, 2006).

The optimised SPE procedure was also employed to monitor the inorganic species of the water samples collected from Mokolo River. The concentrations of As^{5+} obtained during high and low flow sampling seasons are presented in tables 4.26 – 4.27.

Table 4.26: Concentrations of As^{5+} in water samples of Mokolo River during high flow sampling season

Sample Id	As^{5+} (a) (µg/L)	As^{5+} (b) (µg/L)
MOK-1	0.712 ± 0.023	0.951 ± 0.029
MOK-2	1.03 ± 0.034	1.55 ± 0.21
MOK-3	0.432 ± 0.038	0.681 ± 0.024
MOK-4	7.52 ± 1.50	7.70 ± 0.24
MOK-5	< 0.0537	< 0.078
MOK-6	< 0.0537	< 0.078
MOK-7	0.0737 ± 0.0014	0.0895 ± 0.0036
MOK-8	< 0.0537	< 0.078
MOK-9	< 0.0537	< 0.078
MOK-10	0.107 ± 0.009	0.118 ± 0.016

(a) denotes analysis using ICP-MS after separation by SPE
(b) denotes analysis using HPLC-ICP-MS

The SPE results of Mokolo River obtained during high and low flow sampling seasons are also in good agreement with HPLC-ICP-MS data. The SPE procedure showed potential for the routine speciation analysis of As^{5+} in aqueous matrices. This was attributed to MWCNTs-BPEI composites for providing the potential approach of monitoring As^{5+} in water samples. The accuracy and precision of the results show the efficiency of SPE procedure for sample pre-treatment.

Table 4.27: Concentrations of As^{5+} in water samples of Mokolo River during low flow sampling season

Sample Id	As^{5+} [(a)] (µg/L)	As^{5+} [(b)] (µg/L)
LEP-1	0.0612 ± 0.0032	< 0.078
LEP-2	0.307 ± 0.029	0.449 ± 0.005
LEP-3	1.85 ± 0.12	2.03 ± 0.020
LEP-4	3.65 ± 0.22	4.99 ± 0.027
LEP-5	2.27 ± 0.14	3.24 ± 0.044
LEP-6	0.702 ± 0.04	0.920 ± 0.013
LEP-7	0.367 ± 0.038	0.529 ± 0.010
LEP-8	0.153 ± 0.043	0.324 ± 0.027
LEP-9	0.493 ± 0.067	0.733 ± 0.031
LEP-10	0.762 0.087	1.15 ± 0.0013

[(a)] denotes analysis using ICP-MS after separation by SPE
[(b)] denotes analysis using HPLC-ICP-MS

The study conducted by Chen *et al*. (2013) reported As^{5+} concentrations of 1.52 ± 0.08 µg/L in snow water and 2.04 ± 0.39 µg/L in rain water with the pre-concentration factor of 16.3 when using AFS detection system after the SPE procedure. The results obtained in a study by Chen *et al*. (2013) are comparable with the results obtained in this study. However, concentration of As^{5+} in water samples differ with respect to the potential sources of As in water sample. The chemistry and pH of water also play a crucial role on the influence of the existence of As species in water.

4.8.3 The effects of pH on adsorption behaviour of As^{5+} and As^{3+}

In SPE, the pH is crucial in the adsorption of As^{5+} because it determines the ionic characteristics of both As^{3+} and As^{5+} as well as the surface properties of the MWCNTs-BPEI composite (Zwir-Ferenc and Biziuk, 2006; Chen *et al*., 2013). The polymer adopted contains primary and secondary amines in its backbone structure thus making the surface of MWCNTs-BPEI composite positively charged. The pKa values for As^{5+} falls within wide range of pH 2 – 9 where it exist as an anionic species whereas As^{3+} exist as an uncharged species at pH less than 9 since the pKa values are active at pH between 9.2 – 13.4 (Famah, 2012; Smedley and Kinniburgh, 2012; Chen *et al*., 2013). The pH of the river systems during sampling seasons were found in the range of 7.1 – 8.0. Therefore, under such pH conditions As^{5+} is retained

by MWCNTs-BPEI through electrostatic interaction whereas the adsorption of As^{3+} is negligible. At the pH less than 2, both As^{3+} and As^{5+} exist as an uncharged species and no adsorption of either As^{3+} or As^{5+} is anticipated because in this case both species are neutral molecules while the surface of MWCNTs-BPEI is positively charged (Chen *et al*., 2013). This is an indication that the applicability of SPE is mainly determined by the adsorbent material used in the extraction column (Zwir-Ferenc and Biziuk, 2006).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

This chapter concludes the entire project; with recommendations given for further future work in the speciation analysis of As.

5.1 General conclusions based on the analysis report

- In this study, various analytical approaches were explored to precisely identify and quantify different species of As in water and sediment samples collected from Great Letaba and Mokolo Rivers. The analytical procedures for total concentration determination, speciation analysis and offline separation of arsenic by SPE were developed, optimised, validated and successfully applied to water and sediment samples from the two rivers.

- The optimised method for speciation analysis of As in water samples requires a mere filtration of samples using membrane with 0.22 µm pore size followed by a direct injection into HPLC-ICP-MS without further treatment.

- The available species in water samples were detected and quantified using the validated analytical procedure. The predominant species in Great Letaba and Mokolo Rivers was determined to be As^{5+}. Weathering of rocks, agricultural activities and atmospheric deposition of As containing dust released from the nearby mining operations are considered potential sources responsible for the As species in the Great Letaba River. The As species in Mokolo River on the other hand could have been mainly affected by As containing dust released from coal mining, power stations and As containing herbicides used as fertilisers by the agricultural farmers.

- The developed extraction method for assessment of As species in sediments is characterised with adequate efficiency and consistency in terms of

precision and is considered reliable due to its capability to preserve species. The As species in sediments of the two river systems were monitored using HPLC-ICP-MS and it was found that As^{3+} and As^{5+} are distributed evenly in Great Letaba River. In Mokolo River, however As species in sediments varied with sampling season with notably higher values during high flow sampling season. This may be attributed to reductive dissolution process which favours release of As in sediments bound to Fe-oxide. Elevated levels of As species may also be influenced by anthropogenic sources such as municipal wastewater discharges, sewage effluents, mining and combustion of fuels observed in areas surrounding the Mokolo River.

- Total concentrations of As, Fe, Mn and Al in water and sediments were determined to assess the level of contamination relative to MPLs guidelines for trace elements in drinking and irrigation waters. It was found that in Great Letaba River, As and Al levels were below the recommended MPLs for drinking and irrigation water whereas Fe and Mn exceeded MPLs for drinking water at few sites. In Mokolo River, As levels were below MPLs recommendations for drinking and irrigation water in both high and low flow sampling seasons.

- We further investigated SPE procedure using nanocomposites as adsorbent material. Efficiency of the nanocomposite material to execute speciation of As in water has been evaluated through physical characterisation and quantitative measurement of the analytical figures of merit.

- MWCNTs were successfully modified with BPEI and the structure of the composite remained intact as confirmed by XRD, FTIR and TGA.

- The water quality assessment report for the Levhubu and Letaba water supply systems (DWAF 2012) indicates a gradual decline in quality of the Great Letaba River and attributes the observed decline to diffuse pollution including

afforestation in the downstream, agricultural runoff from intensively cultivated lands of banana and citrus which are impacted by fertilisers, salts, nutrients, pesticides (DWAF, 2012). Our findings on the levels of As species, Fe and Mn in water and sediments from Great Letaba River further revealed potential effects of mining activities since mining is the major source of As emission in the environment.

- Mokolo River has been threatened by As contamination based on the levels of As species and trace elements determined during low flow and high flow sampling seasons. In water samples, determined levels of As, Fe, Mn and Al were 7.92, 421, 2760 and 249 µg/L, respectively. Higher levels of trace elements were found in sediment samples. The redox conditions of Fe, Mn and Al usually occurring in sediments, further elevate levels of As species due to observed correlations. The diversity of anthropogenic activities such as coal mining, power stations and agriculture are related to As levels and distribution, which may negatively impact aquatic ecosystem of Mokolo River.

5.2 Recommendations

- Efficient methods, which have been developed and validated for successful quantification of As species in water and sediments using HPLC-ICP-MS can be applied for similar studies in future.

- The continuous monitoring of As species in Great Letaba and Mokolo Rivers is strongly suggested so that the accurate identification of the potential sources could be achieved to mitigate further As inputs into the river systems.

- Water from Great Letaba and Mokolo Rivers should not be recommended for drinking and irrigation unless their fitness confirmed by additional studies on microbial, physical and chemical parameters for water quality assessment.

- The optimised and validated offline mode of SPE procedure coupled with ICP-MS, for separation and quantification of As^{5+} in water is recommended for successful speciation analysis of inorganic As species.

- In general, outcome of this study predicts the ongoing gradual changes in the aquatic ecosystems of Great Letaba and Mokolo Rivers. We suggest that our findings will provide baseline management guidelines to policy makers to stimulate direct management actions aimed at remediation for As contamination and other long term sustainable use of aquatic ecosystems. Therefore, we strongly recommend continuous assessment of As contamination in Great Letaba and Mokolo Rivers as the methods for assessment have been developed.

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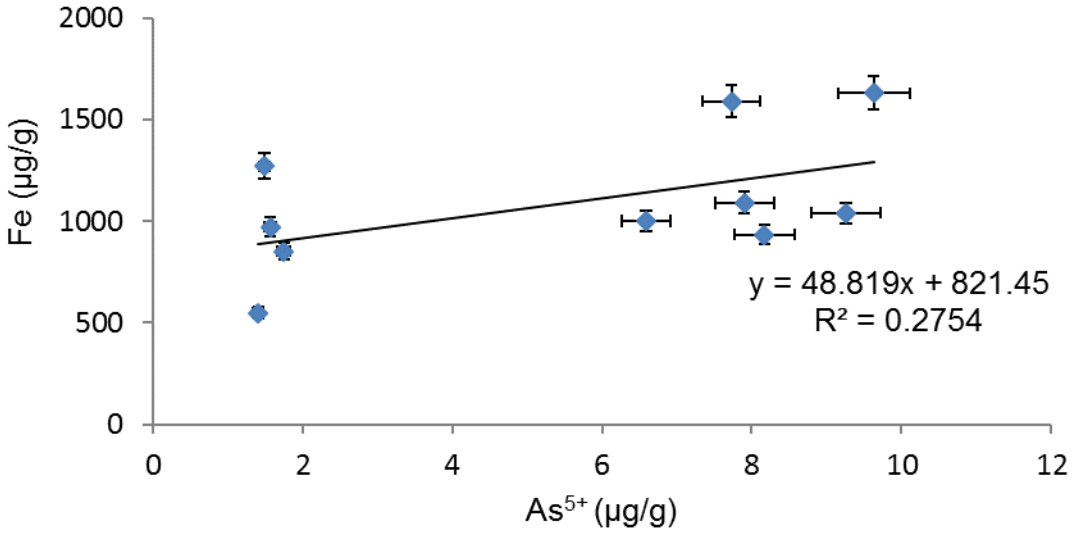
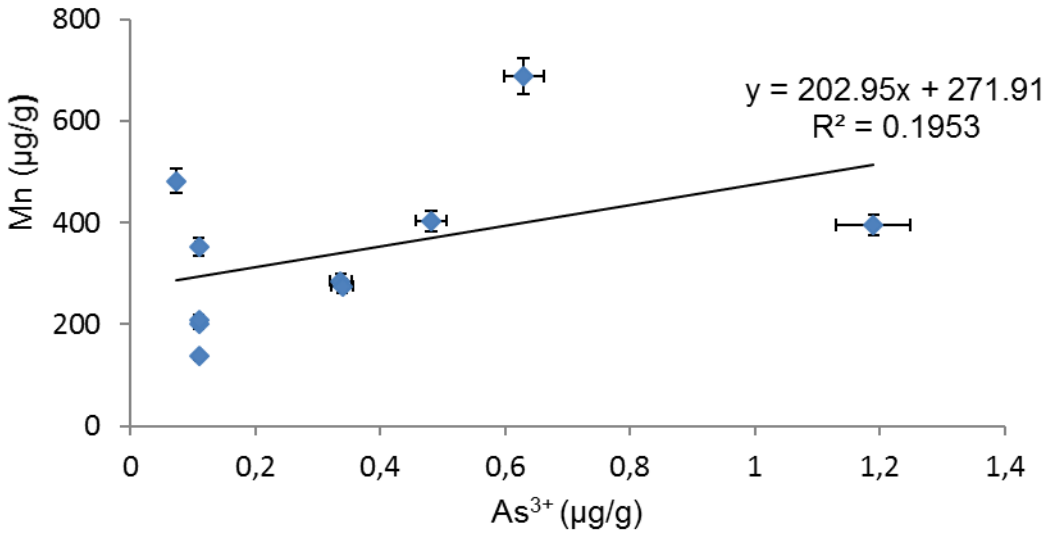
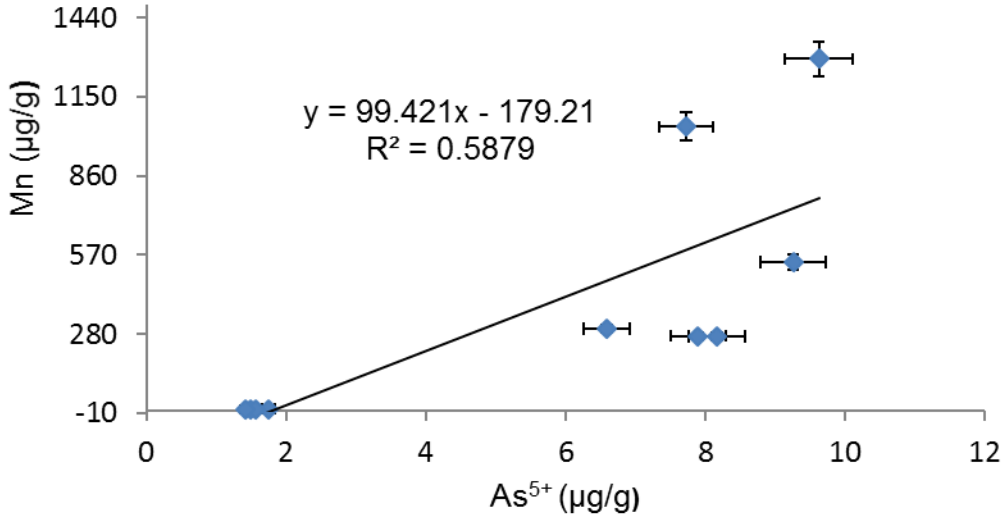
APPENDICES



Appendix 1: The correlation between Fe and As^{5+}



Appendix 2: The correlation between Mn and As^{3+}



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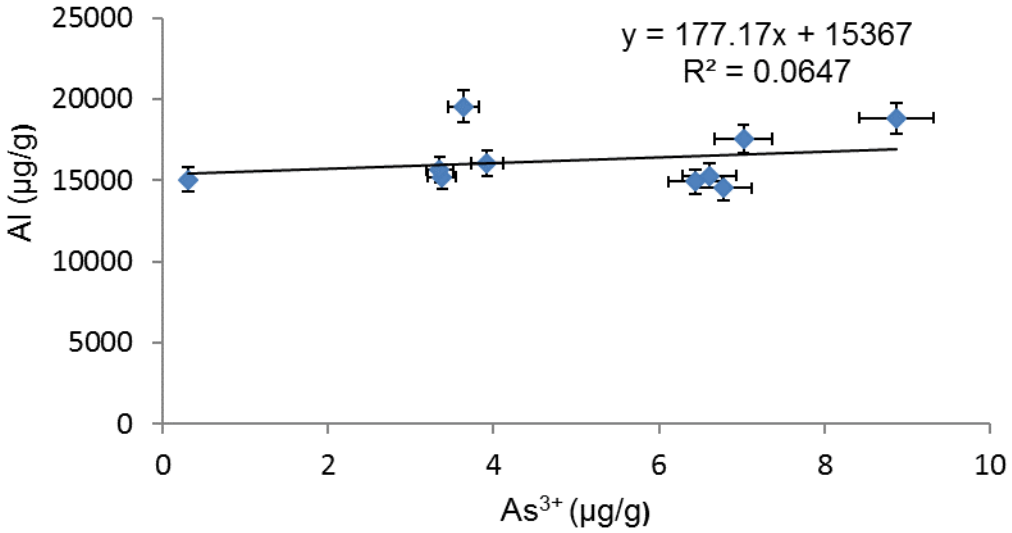
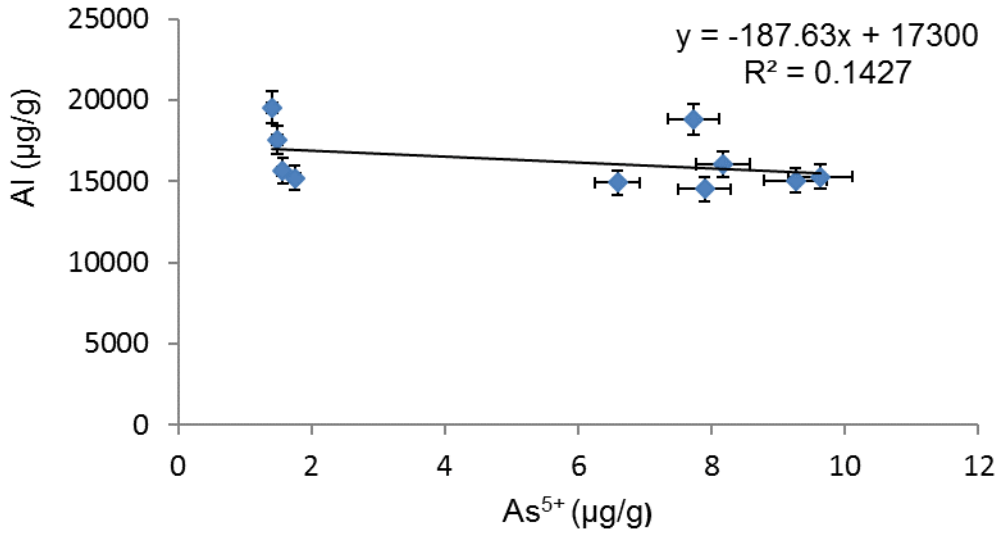


Appendix 4: The correlation between Al and As^{3+}



Appendix 5: The correlation between Al and As^{5+}