UNDERSTANDING THE SPECIATION AND MIGRATION OF METAL POLLUTANTS IN THE MINING-IMPACTED KLIP RIVER WETLAND, JOHANNESBURG

By

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Declaration

The data described in this thesis were collected between March 2017 and June 2018. Experimental work was carried out while registered at the School of Chemistry, University of the Witwatersrand, Johannesburg, under the supervision of Doctor Letitia Pillay and Professor Marc S. Humphries.

This thesis, submitted for the degree of Doctor of Philosophy in the Faculty of Science, University of the Witwatersrand, Johannesburg, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University.

Where use has been made of the work of others, it is duly acknowledged in the text.

Shaeen Chetty
June 2021

I certify that the above statement is correct and as the candidate's supervisor/co-supervisor, I have approved this thesis for submission.

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Abstract

The Witwatersrand Basin in Gauteng, South Africa, is the world's largest gold resource, having yielded more than one third of all the gold ever produced. The majority of these mines are located in Johannesburg and the surrounding areas. Waste from gold mines is considered to constitute the largest single source of waste pollution in South Africa and contributes significantly to acid mine drainage (AMD), which critically affects water resources. One of these major resources, severely affected by these activities is the Klip River wetland, which is the major catchment in the region. The sequestration of metals within the wetland is considered vitally important for water purification as water flowing through the wetland discharges into the Vaal Dam, which supplies potable water to major municipalities.

To establish the sources, transport and sequestration processes of pollutant metals and how wetland attenuation is influenced by AMD contaminants, we identified specific toxic pollutants present in the Klip River wetland and mapped their movement within the wetland. The main sources of contaminants to the wetland were identified using Pb isotope ratios and metal enrichment. Finally, the speciation of As, a major by-product of gold mining, was investigated to assess the attenuation processes.

Analyses reveal that the majority of pollutants are associated with contaminant plumes that emanate from mine dumps and enter the wetland *via* groundwater recharge. This water carries highly enriched concentrations of Co, Ni, Zn, U and rare earth elements, naturally sequestered within the wetland through precipitation and adsorption. While surface runoff from mine dumps severely contaminate watercourses within the upper catchment, surface inputs are considered relatively minor contributors to the overall pollutant load entering the Klip River wetland, although aerosol fallout is the primary source of Pb.

Lead concentrations and isotope compositions were measured ($^{208}Pb/^{207}Pb$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$) to identify major sources contributing to Pb contamination in the wetland. Isotopic ratios of $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ in the wetland ranged between 1.13 – 1.32 and 2.34 – 2.43, respectively. Source identification using Pb isotopes indicated mixing within the system of alkyl lead, coal and Witwatersrand pyrite. Isolating the wetland samples from the rest of the

system, reveals predominant coal and alkyl lead (petroleum) signatures, with minor contributions of the Witwatersrand geology.

To understand the attenuation processes of As in the wetland, its concentration, bioavailability and speciation in samples from several tailing storage facilities (TSFs), sediments from two river tributaries (the Klip River and Klipspruit) feeding into the wetland, and surface and core samples in the wetland were measured. Total As concentrations in TSFs ranged between 77 - 106 mg kg^{-1}, 10 – 90 mg kg^{-1} in the tributaries and an average of 34 ± 25 mg kg^{-1} in the wetland. Arsenic enters the wetland through groundwater inflow. Speciation analysis identified As(III), As(v) and monomethylarsonic acid (MMA) present with concentrations ranging between 0.028 – 1.18 mg kg^{-1}, 0.076 – 7.36 mg kg^{-1}, and 0.012 – 0.22 mg kg^{-1}, respectively. There also appears to be an As-sulfur species present closely linked to the S_{total} concentrations. The predominant As species in the wetland is As(V), and speciation appears dependant on both redox potential and microbial activity (inferred by S concentrations). These processes limit the availability of bioavailable As in the wetland and play an important role in the sequestration process.

The extensive accumulation of metals and specific sources within the Klip River wetland reflects the contaminant legacy primarily associated with gold mining on the Witwatersrand, less to alkyl lead artefacts and highlights the vital role this natural system has played in trapping vast quantities of toxic pollutants and remediating downstream waters. Contaminant plumes associated with mine dumps will likely persist for decades, and preventing further deterioration of the Klip River wetlands is, thus critical for safeguarding water sources in the region.

Dedication

I dedicate this work to my parents – Pravesh and Nirmala Chetty.

Thank you for giving me a good footing in life and for supporting and understanding me all through this journey. Yours sacrifices and love never go unnoticed and will always be treasured.

"In life, if you ever want to be something, win something, or get something, then always listen to your heart. But if you don't get a signal from your heart, then close your eyes and say your mom and dad's names, then watch, you will achieve every goal, every obstacle will become easy, and the victory will be yours... only yours..." - Kabhi Khushi Kabhie Gham (2001)

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*"**The delicate balance of mentoring someone is not creating** them in your own image, but giving them the opportunity to **create** themselves." — Steven Spielberg.*

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"A good friend is like a four leaf clover; hard to find and lucky to have." - *Irish Proverb*

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Finally, I would like to thank the Creator, Shri Krishna, for all is possible only due to Him.

Table of Content

List of abbreviations and acronyms

AMD	Acid Mine Drainage
As (III)	Arsenite
As (V)	Arsenate
CPI	Comprehensive Pollution Index
DMA	Dimethylarsinous acid (V)
DRD	Durban Roodepoort Deep
EF	Enrichment Factor
ERL	Effects Range Low
ERM	Effects Range Median
ERPM	East Rand Proprietary Mines
HREEs	Heavy Rare Earth Elements
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IC-ICP-MS	Ion Chromatography Inductively Coupled Plasma Mass Spectrometry
KR	Klip River
KS	Klipspruit
MC-ICP-MS	Multi-Collector Inductively Coupled Plasma Mass Spectrometry
LOD	Limit of Detection
LOI	Loss on Ignition
LOQ	Limit of Quantification
LREEs	Light Rare Earth Elements
MC-ICP-MS	Multi-collector Inductively Coupled Plasma Mass Spectrometry
MMA	Monomethylarsonous acid (III)
MREEs	Middle Rare Earth Elements
NASC	North American Shale Composite
NRCC	National Research Council of Canada
PAAS	Post-Archean Australian Shale
QC	Quality Control
REEs	Rare Earth Elements
TSFs	Tailing Storage Facilities
UCC	Upper Continental Crust
XRF	X-Ray Fluorescence

Chapter 1

Introduction

One of the major challenges in combating water scarcity in South Africa is managing the water quality of freshwater resources. Water resource management is becoming increasingly difficult as the need for immediate economic growth through industrial and agricultural development overshadows the impact that these activities have on the environment. Waste by-products from the energy, mining and manufacturing sectors include metals, pesticides, and various synthetic chemical compounds released into the environment (Masindi and Muedi, 2018; Naidoo and Buckley, 2003; Singo, 2013; Wuana and Okieimen, 2011).

Metals pose a threat to natural resources since their release and accumulation into the environment are generally undetected. In South Africa, agricultural activities (Addo-Bediako *et al*., 2018; Awofolu *et al*., 2005; Chetty and Pillay, 2019; Edokpayi *et al*., 2016), industrial activities (Addo-Bediako *et al*., 2018; Binning and Baird, 2001; Chetty and Pillay, 2019; Dikole, 2014; Pheiffer *et al*., 2014; Pillay., 2011), and mining activities (Addo-Bediako *et al*., 2018; Pheiffer *et al*., 2014) have resulted in many of the major rivers and estuaries in the country being classified as in poor health with a decline in habitat and population diversity and poor water quality indices (DWS, 2019). According to the Department of Water and Sanitation (DWS) report (2019), the Crocodile, Vaal, Olifants, Berg, Mzimvubu-Tsitsikamma and Pongola-Mtamvuna Rivers are highly impaired and are exposed to modified flows, habitat alteration and pollution. The Vaal River source originates in industrial and mining areas, thus the source is not in a better condition (DWS, 2019). Metals contribute significantly to this problem and widespread contamination of ground and surface water resources have been documented with metals such as Fe (Jackson *et al*., 2009; Roychoudhury and Starke, 2006), Cr (Loock *et al*., 2014; Nussey *et al*., 2000; Roychoudhury and Starke, 2006), Zn (Chetty and Pillay, 2019; Fru, 2020; Jackson *et al*., 2009)., Pb (Monna *et al*., 2006; Nussey *et al*., 2000; Roychoudhury and Starke, 2006), Cu (Fru, 2020; Jackson *et al*., 2009; Roychoudhury and Starke, 2006), Mn (Hess *et al*., 2015; Nussey *et al*., 2000), and metalloids such as As (Pillay, 2011; Roychoudhury and Starke, 2006).

The South African economy and the country's development is driven by the mining sector, which gradually releases heavy metals into the environment from the tailings storage facilities (TSFs), mine voids and tailing dams (Ebenebe *et al*., 2017; Singo, 2013). There is a large sector of the South African population living in rural and informal societies, which depend on untreated surface and groundwater to provide sustenance. The continued consumption of polluted water and biota may have significant health implications. Metabolic and physiological pathways are adversely affected by the accumulation of metals in both humans and wildlife (Pheiffer *et al*., 2014). Despite having potential toxicity to humans, metals may be required in trace quantities for structural, physiological, catalytic or hormonal functions (Tchounwou *et al*., 2012). At higher concentrations some are more toxic than others and, thus pose a significant problem as metals do not biodegrade and can cause major damage to internal tissue (Masindi and Muedi, 2018). Carcinogenesis is the core effect of metals, including Pb, Cu, Zn, Cr, and Ni, which are toxic (Ebenebe *et al*., 2017; Pheiffer *et al*., 2014).

1.1. Pollutant Sources

Increased population requires a continuous supply of resources, including land for agricultural purposes, industrial and commercial development. Technological advances, automation and scientific developments have led to a significant increase in materials and energy requirements. The processing and extraction of materials used for these activities has increased waste by-product generation, introducing a wider, more concentrated range of pollutants into the environment. Pollution is typically produced by anthropogenic activity and is divided be into two classes; point source and non-point source. Point sources of pollution can be identified due to localized contamination such as mining and power stations, while non-point sources are not localized and are distributed over a large area (Schweitzer and Noblet, 2018). The most significant environmental impact is experienced from the mining sector (Akinlabi *et al*., 2019; Hester and Harrison, 1994). In South Africa, mining of coal and precious metals are the primary resources from this activity.

Mining of coal and precious metals has resulted in numerous exposed mine dumps within the Witwatersrand Basin containing unrefined rock waste that contains metal contaminants. Metal contaminants may be released into soil and water or the atmosphere as dust. Coal occurs

predominantly in the north-east provinces of South Africa and is the major energy source for the country. Coal production, over the last 15 years, has been between 250 -260 million tonnes (Burton *et al*., 2018). Coal combustion not only contributes to global greenhouse gas emission (Perera, 2018) but also introduces heavy metals into the atmosphere, soil and water (Seshadri *et al*., 2010). South African coal contains metals, including Fe, Mn, Cr, Pb, Zn, Cu, As, Co and Hg (Kalenga *et al*., 2011). Cities in the vicinity of coal power stations have been reported to have poor air quality with particulate matter containing metals and excess amounts of nitrogen and sulfur oxides continually dispersed from coal stacks (Munnik *et al*., 2010). They may also be deposited in sediment and water and eventually bioaccumulate in plants (Okedeyi *et al*., 2014) and animals (Masindi and Muedi, 2018).

Gold has been mined in the Witwatersrand Basin since 1886 and since then, six billion tonnes of mine waste has been stored in about 270 TSFs across the region (Anglo Gold Ashanti, 2004; Humphries *et al*., 2017; Oelofse *et al*., 2007). These TSFs cover 400 – 500 km^2 (Oelofse *et al*., 2007) and contain approximately 30 million tonnes of sulfur and 430 000 tonnes of uranium due to outdated methods of Au extraction (Weiersbye and Witkowski, 1998). Gold is found in conglomerate reefs along the Witwatersrand Basin together with trace element phases and other minerals, including pyrite. Trace elements, including As (1394 mg kg^{-1}), Co (1006 mg kg^{-1}), Cu (346 mg kg^{-1}), Cr (33 mg kg^{-1}), Ni (1930 mg kg^{-1}), Mn (16 mg kg^{-1}), Sr (3 mg kg^{-1}), Ti (98 mg kg^{-1}), Pb (844 mg kg^{-1}), and Zn (90 mg kg^{-1}), have been found in pyrite from the Witwatersrand Basin (Barton and Hallbauer, 1996; Guy, 2012; Saager, 1976). Metal concentrations from TSFs in the West rand indicated high concentrations of As (79 mg kg^{-1}), Cu (43 mg kg^{-1}), Cr (279 mg kg^{-1}), and Ni (112 mg kg^{-1}).

The largest environmental impacts from mining are acid mine drainage (AMD) (Barton and Hallbauer, 1996; Guy, 2012), contaminant leaching, erosion of soil and mine waste into surface water, water table and groundwater contamination, reduced air quality, and habitat loss (Durand, 2012; McCarthy, 2011). These impacts are historical within the Witwatersrand Basin although, recent secondary mining further escalates the problem (Tutu *et al*., 2008). Abandoned mines begin to flood creating mine voids that are highly acidic, further promoting the development of AMD and widespread contamination of fresh water sources (DWA, 2013). Contamination seeps through rock fractures and migrates into freshwater systems at least 10 km from the contamination source. Aquatic life is decimated, while the water quality is

constantly being affected adversely by AMD carrying dissolved heavy metals (Gheorghe *et al*., 2017; Mudenda, 2018).

1.1.1. Acid Mine Drainage Formation and Chemistry

Gold mined on the Witwatersrand has up to 3% pyrite (FeS_2) associated with the ores. The by-products of mining activities have resulted in the accumulation of mine void water in abandoned mine shafts and stockpiling of fine-grained waste ore into dry-stacked TSFs (Brend, 2007; Fashola *et al*., 2016; Singo, 2013; Tayebi-Khorami *et al*., 2019). TSFs are the largest contributor of pollutants to most mining operations. Exposure to the elements results in wind-borne transport of mining dust and the influx of water into TSFs. The latter promotes acid mine drainage (AMD), which is formed when sulfide minerals, primarily FeS_2, react with oxygenated water (McCarthy, 2011). Pyrite undergoes various oxidation reactions to generate acid.

Reaction 1: $2FeS_2 + 2H_2O + 7O_2 \rightarrow 2FeSO_4 + 2H_2SO_4$
Reaction 2: $4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O$
Reaction 3: $7Fe_2(SO_4)_3 + 8H_2O + FeS_2 \rightarrow 15FeSO_4 + 8H_2SO_4$

Initially, pyrite reacts with water and oxygen to form ferrous ions (Fe^{2+}) and sulfuric acid (Reaction 1). The ferrous ions and sulfuric acid further react with oxygen to produce ferric sulfate and water (reaction 2). These reactions occur under circumneutral pH (Warren, 2011). The pH continues to decrease and below pH = 4, the ferric sulfate reacts with water and pyrite to produce ferrous sulfate and more sulfuric acid (reaction 3). Ferric driven pyrite oxidation yields more sulfuric acid than oxygen driven pyrite oxidation.

Under natural weathering processes, the rate of H_2SO_4 formation is significantly slower allowing for water, calcite or dolomite to buffer/neutralise the acid produced (McCarthy, 2011). However, mining activities fragment rock increasing the surface area and therefore rate of AMD reactions creating an excess of acid. Acidic conditions cause the dissolution of various metals contained in the waste ore (Fe, Al, As, Cu, Zn, Mn, Cd, Co, Ni , Hg, U) and rare earth elements (REEs) comprising of the 15 lanthanides, yttrium and scandium resulting in a highly acidic solution with a high metal and sulfate load (McCarthy, 2011; Yeager, 2004).

1.1.2. Effects of AMD

The most prominent impact of AMD is the contamination of surface water and groundwater. The pH of water is lowered by AMD, solubilizing minerals and increasing metal concentrations rendering the resource unusable (Fadiran *et al*., 2014). Metals enter aquatic systems through leaching and run-off from TSFs and by groundwater seepage from mine voids. AMD polluted sediment and water pose a risk to food security either rendering the area unsuitable for crop growth or facilitating the growth of plants that accumulate toxic metals in their edible parts (Yan *et al*., 2020). Dust from mineral fines in TSFs also contains toxic metals (Mpanza *et al*., 2020; Nakajima and Aryal, 2018). Their windborne distribution and deposition becomes a source of air pollution, and may become a source of respiratory illnesses to communities surrounding mining regions (Mpanza *et al*., 2020). Another significant impact of AMD is the effect of total dissolved solids (TDS) in water. Water quality is affected by TDS, which directly affects ecosystems by disrupting the water-sediment equilibrium, increasing salinity and affecting solubility. Increased TDS concentrations in water are as a result of mineral substrate, including gypsum ($CaCO_3$). Gypsum is predominantly used to neutralise AMD, in mining activities, and contributes to 80-85 % of the TDS in mine water (CSIR, 2013). Primary constituents of TDS, include calcium, magnesium, sodium, and potassium cations and chloride, nitrate, carbonate, bicarbonate, and sulfate anions, which are also constituents of AMD. In natural systems, dominated by dolomites, dolomitic waters neutralise AMD and increases the TDS is the system (Vermaak, 2009). Discharged mine water, high in TDS, is introduced into waterways, which affect the water quality. The adverse effects of metals on the environment are well documented (Obasi and Akudinobi, 2020; Wuana and Okieimen, 2011a). They have far-reaching implications on reproduction, biodiversity and species population (Ali *et al*., 2019; Tovar-Sánchez *et al*., 2018). Bioaccumulation and biomagnification of metals in aquatic plants and animals ultimately affect the human population (Gheorghe *et al*., 2017; Mann *et al*., 2011).

The Vaal River is one of the largest water resources in the country and receives inflow from the Klip River, Blesbokspruit and Grootvlei (McCarthy *et al*., 2007; McKay *et al*., 2018; Pilson *et al*., 2000). Anthropogenic inputs into these rivers are due to primarily AMD and mining activities and to a lesser extent industrial and communal waste. AMD has had a critical impact on water quality resulting in high content of dissolved particulate matter and heavy metals (Coetzee *et al*., 2006a). Controlled contamination by decanting the mine void water into canals and waterways impacts the aquatic ecosystems by negatively affecting biota (Coetzee *et al*.,

2006a). Prior to 2000, the Grootvlei mine, discharged approximately 130 ML/day, which had elevated concentrations of TDS, Mg and sulfate, 4156 mg L^{-1}, 117 mg L^{-1}, and 2085 mg L^{-1}, respectively (Pilson *et al*., 2000). While in 2000, the Grootvlei discharged approximately 26% of the salt load entering the Vaal Barrage (Pilson *et al*., 2000). The salinity of the Vaal River has almost doubled with inputs from the Klip River and Blesbokspruit (McCarthy, 2011). These conditions have improved with the implementation of treatment plants but the salinity of the Vaal River has not improved. The Crocodile River, one of the major rivers in Mpumalanga, has also experienced adverse effects of mining activities. The water quality has indicated elevated heavy metals concentrations above recommended guideline values for South Africa (Nde and Mathuthu, 2018). Like many ecosystems, these rivers have wetlands that direct water into them. The Klip River and Blesbokspruit wetlands discharge into the Vaal River while the Zaalklapspruit wetland discharges into the Crocodile River. These wetlands have been affected by mining activities (Humphries *et al*., 2017; Lourenco and Curtis, 2021; McCarthy, 2010; McKay *et al*., 2018; Oberholster *et al*., 2016), agricultural runoff, stream and bank modifications (Oberholster *et al*., 2016). Elevated metal concentrations in these wetlands have been linked to predominantly linked to mining activities (De Klerk *et al*., 2013; Humphries *et al*., 2017; McCarthy *et al*., 2007). Aquatic biodiversity is diminished and the open water areas reduced due to increased total dissolved solids (TDS) and water flow, which further affects nutrient cycling and microbial and ecological processes (Lourenco and Curtis, 2021; McKay *et al*., 2018; Starke, 2004).

1.1.3. Geochemistry of Attenuation Processes

Attenuation processes occur naturally in all ecosystems reducing the effect of metals released both by weathering of geological material and their introduction through anthropogenic processes. (Povar and Spinu, 2015). These processes are dependent on physical and chemical parameters of the ecosystem, including Eh, pH and speciation of compounds. Attenuation processes include neutralization, oxidation and hydrolysis, precipitation, co-precipitation and sorption, and biological processes (Blowes *et al*., 2005; Lee *et al*., 2002; Sheoran and Sheoran, 2006).

i) Neutralization

Carbonate ore minerals, including calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), ankerite ($Ca(Fe,Mg)(CO_3)_2$), and siderite ($FeCO_3$), are found in sediment and mine waste (Blowes *et al*.,

2005). When they interact with strong acid, they neutralise the acid, dissolving to form bicarbonate, carbonic acid, water, and carbon dioxide (Reactions 4-9) (Webb and Sasowsky, 1994).

Reaction 4: $HCO_3^- + H^+ \rightleftharpoons CO_2 + H_2O$

Reaction 5: $CO_3^{2-} + 2H^+ \rightleftharpoons CO_2 + H_2O$

Reaction 6: $OH^- + H^+ \rightleftharpoons H_2O$

Reaction 7: $KAlSi_3O_8 + 4H^+ + 4H_2O \leftrightharpoons K^+ + Al^{+3} + 3H_4SiO_4$

Reaction 8: $KAl_3Si_3O_{10}(OH)_2 + 10H^+ \leftrightharpoons K^+ + 3Al^{+3} + 3H_4SiO_4$

Reaction 9: $KFe_3(SO_4)_2(OH)_6 + 6H^+ \leftrightharpoons K^+ + 3Fe^{+3} + 6H_2O + 2SO_4^{-2}$

The reaction chemistry is dependent on the pH of the porewater and carbonic acid reactions (reactions 4 & 5) tend to predominate in AMD waters (Fourie, 2007). Depletion of calcium carbonate minerals promotes the dissolution of other carbonate, hydroxide ($FeCO_3$, $Al(OH)_3$ and FeOOH) and silicate minerals to affect the neutralisation process. Continued depletion of these minerals ultimately compromises the underlying geology by changing the porosity, structure and groundwater flow (Moncur, 2006). Neutralisation is one of the most effective treatments of AMD contaminated environments and has been shown to work effectively in several field and lab studies (McCarthy, 2011; Pozo-Antonio *et al*., 2014; Roychowdhury *et al*., 2015).

ii) Oxidation, Hydrolysis, Precipitation, Co-precipitation and Sorption

Labile metal ions may be adsorbed or absorbed by sediment or particulate matter in aquatic systems depending on pH and redox, the main parameters controlling their behaviour in soil (Caporale and Violante, 2016; Lee *et al*., 2002; Namieśnik and Rabajczyk, 2010; Wuana and Okieimen, 2011; Zhang *et al*., 2018). Fe is one of the most abundant metals present in most soils and is often involved in attenuation of metals (Masindi and Muedi, 2018; Wuana and Okieimen, 2011). Its sorption, precipitation and co-precipitation, dissolution is controlled by pH and redox (Figure 1.1.). In mining regions, mineral pyrite (FeS_2) is a common by-product. When pyrite undergoes oxidation to produce acid, the process also produces Fe and S compounds. Pyrite is stable under both oxidizing and reducing conditions over a wide pH range (Figure 1.1.). Under oxidizing (Eh < 0.78 V) and reducing (Eh > -0.58 V) conditions (pH < 7) Fe from pyrite exists as Fe^{2+}. This may affect TDS and promotes the precipitation of FeS and FeS_2. Under oxidizing alkaline conditions the Fe exists as $Fe(OH)_3$, a precipitate, is generally

found in oxic polluted environments (Kacabağ, 1983; Namieśnik and Rabajczyk, 2010). Both ferric and ferrous ions begin to co-precipitate with Si to form mineral, which impacts the underlying geology (Chen *et al*., 2020; Sracek *et al*., 2011). The formation of iron precipitates promotes sorption of organic compounds and other metals.

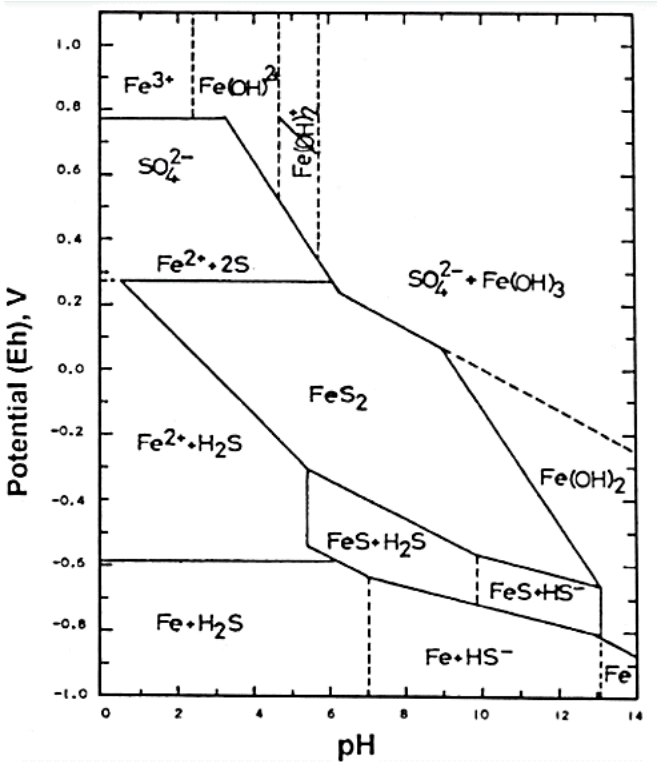

Figure 1.1.: Eh–pH diagram for the pyrite-water system (25°C, 10^{-5} M dissolved species) (Kacabağ, 1983).

The adsorption of metals onto sediment occurs by physisorption or chemisorption. Cation exchange processes occur between sediment and highly anionic surfaces based on weak Van der Waal's attractions, representing a physisorption mechanism (Józefaciuk, 2011; Lee *et al*., 2002). These electrostatic interactions often result in metal co-precipitation with Fe-Mn hydroxides and oxyhydroxides. Iron ions adsorb onto clay and create a charged surface for physisorption to occur with other metals or metal complexes. Iron hydroxides have the same ability to physisorb cations under alkaline conditions (Figure 1.1). Chemisorption processes involve the bonding of pollutants at surface specific sites on the substrate (Lee *et al*., 2002). The substrate is usually clay or organic matter (Lee *et al*., 2002; Sheoran and Sheoran, 2006). Organic matter shows a higher affinity and ability to sequester metals due to the production of humic and fulvic acids, which facilitate the sorption of metal pollutants (Morita, 1980; Tang *et al*., 2014). These organic acids produce various sites for metal interactions and further

degradation of the plant matter may allow for chelation and complexation of the metals (Marchand *et al*., 2010; Sobolewski, 1999). Precipitation and co-precipitation can occur with carbonates, sulfides, hydroxides, and phosphates.

iii) Biological processes

Biological processes use biota to accumulate metals or transform the metal into a different species (Ali *et al*., 2019). Wetlands, natural and constructed may have plants, which are able to accumulate and store metals from water and sediment, thus attenuating the effects of high metal content. One of the most common of these is *Phragmites australis* (common reed), which has been reported to accumulate Cu, Ni and Zn (Rampedi, 2016; Vermaak, 2009).While these processes are more environmentally friendly, they are slow processes occurring over extended periods time.

Fungi and algae have also been observed to mitigate the effects of heavy metals in aquatic systems by transforming and accumulating the metals (Kapahi and Sachdeva, 2019). Microorganisms convert toxic metals into less toxic forms by using enzymes. Microbial-mediated redox reactions have been noted to immobilize metals, including Fe and Mn (Lloyd, 2003). These reactions may occur in either oxic or anoxic conditions. Bioremediation is also more economical and produces less toxic by-products but is a less researched attenuation alternative (Heerden *et al*., 2013).

1.2. Wetlands

Wetlands are natural, self-sustaining ecosystems with complex interactions between the hydrology, biology and sediment. These interactions create an environment that is controlled by chemical reactions and physical characteristics. Wetlands are responsible for:

- water purification by removing suspended and dissolved solids, nutrients and contaminants from the surface and ground water and converting them into other forms.
- Floodwater reduction allows wetlands to control the amount of water that is released and store excess water from flood events.
- Groundwater recharge and discharge is influenced by the rate that wetlands move water between the ground water and surface water.
- The physical processes in a wetland control erosion control.

- Chemical and physical processes within the wetland control the biogeochemical cycling of metals and nutrients in the wetland.
- Biodiversity conservation in wetlands allows for the diversity of fauna and flora in the wetland to thrive in a well maintained habitat. (Cantonati *et al*., 2020; Keighley, 2017; Venter and Mitchell, 2015).

1.2.1. Wetland functioning

Wetlands are able to filter out dissolved and suspended nutrients, organic matter, sediment and pollutants from surface or groundwater providing pollutants and other suspended material trapping them in sediment or vegetation (Puckett *et al*., 1993; Reddy *et al*., 2010; Zhang *et al*., 2014). Increases in human population and industrial activities create significant changes to the natural functioning of a wetland by reducing water flow, altering vegetation, biogeochemical cycles, increasing eutrophication and pollution resulting in wetland degradation (Cantonati *et al*., 2020; Galatowitsch, 2018; Oberholster *et al*., 2016). This has important consequences for the ability of wetlands to act as important sinks in the environment, which assists in the attenuation of contaminants and the natural purification of water. An increase in metal contamination due to an increase in anthropogenic activities has resulted in constructed wetlands. Constructed wetlands are designed and carefully controlled to provide selected attenuation of pollutants. Foreign substances that enter any wetland are classified as a pollutant. These include sediment, nutrients, trace metals, organic pollutants, and microorganisms.

i) Sediment

Hydrophytes within the wetland reduce the flow rate of water, thus allowing for the settling of sediment in the wetland. Suspended sediment in water carry adsorbed pollutants (Sheoran and Sheoran, 2006), which may be ingested and cause health issues. High sedimentation within a wetland reduces the capacity of the wetland to actively remove pollutants from water (Puckett *et al*., 1993). Along with the sediment, the pollutants are attenuated in the wetland *via* a sedimentation mechanism (Sobolewski, 1999). This mechanism was seen in the Nylsvely wetland in Limpopo where the comparison of surface sediment and water entering and exiting the wetland reported elevated metal concentrations in the sediment (Dalu *et al*., 2020). This indicates that the wetland is effectively sequestering metals.

ii) Nutrients

Nitrogen and phosphorus are the two most common nutrients in plants that promote root and leaf growth. In excess amounts, these elements become pollutants that contribute to eutrophication, promoting algal blooms and growth of invasive species of hydrophytes, including water hyacinth (*Eichhornia crassipes*). Algal bloom causes a reduction in oxygen to benthic organisms, thus collapsing the ecosystem. Subsequent death of algae releases toxins into the water further degrading water quality (Durand, 2012). In severe cases, the ecosystem become dystrophic. Wetlands generally receive nutrients from run-off resulting in the over-fertilization of crops. They remove nutrients by bioaccumulation in biota, sedimentation and nitrogen fixation. The latter releases nitrogen into the atmosphere. Bioaccumulation and sedimentation are temporary sinks. As soon as the biota die or sediment physical conditions change, the nutrients are re-mobilized. The precipitation of Fe, Al, and Ca in the form of phosphates (Pozo-Antonio *et al*., 2014) may also immobilize the phosphorus. The formation of the precipitate assists in the attenuation of the metals (Wuana and Okieimen, 2011). Nutrient removal efficiency is often more effective in constructed wetlands due to the controlled nature of wetland dynamics. In some instances, constructed wetlands in South Africa, including the Mpophomeni, Letlhabile and Ladybrand wetlands, which have been constructed in decommissioned mining sites, have all shown more than 90% removal efficiency of nutrients from water (Wood and Steffen, 1999).

iii) Metals

Metals are primarily removed from solution through adsorption onto suspended and settled particles either sediment or organic (Wuana and Okieimen, 2011). Other processes that remove trace metals from water include precipitation, complexation/chelation to organic matter, and bioaccumulation. The higher the organic matter content, the greater the efficiency of the wetland to remove metals from water. Loss of the peat (organic matter) diminishes the ability to sequester the trace metals. The precipitation of metals as oxides, hydroxides, carbonates, phosphates, and sulfides are promoted by aerobic conditions (Wuana and Okieimen, 2011). These can be re-mobilized under the influence of physical parameter changes. Healthy wetlands can easily attenuate Cu, Zn and Fe (Pat-Espadas *et al*., 2018). Constructed wetlands are utilised where wetland degradation and the need for metal removal from the effluent is required. One of the key parameters is the

control of pH, which has shown effectiveness in removing Fe from effluent waste (Sheridan *et al*., 2013).

iv) Organic pollutants

Organic pollutants (*e.g*., pesticides, pharmaceuticals and sewage) generally adsorb onto sediment and undergo degradation. The process is promoted under anaerobic conditions and include photo-degradation and degradation by microorganisms. Pesticides are one of the most abundant organic pollutants in wetlands. Metabolites from the degradation of organic pollutants assist in the complexation of metals, thus removing the metals by forming colloids or complexes (Zhang *et al*., 2008). Research in removing organic pollutants in natural wetlands is scarce however, constructed wetlands show greater efficiency in removing these pollutants (Almasi *et al*., 2016; Kaur *et al*., 2020; Matamoros *et al*., 2008).

v) Microorganisms

Effluent from industrial, agricultural and urban run-off introduce substantial quantities of microorganisms into the wetland, including *Salmonella* and *Enterococci*, which are hazardous to humans (Müller *et al*., 2020). These organisms are eliminated *via* various mechanisms, including solar radiation, adsorption to sediment, physical conditions (pH), and the presence of toxic substances. Some of these microorganisms have developed mechanisms to accumulate and utilize trace elements in their functioning, thus attenuating the metal (Sheoran and Sheoran, 2006; Stanton *et al*., 2007). Constructed wetlands have been shown to remove Faecal Coliforms from wastewater (Arias *et al*., 2003; Ramprasad *et al*., 2017) and promote microbial growth, which assist in removing pollutants (Srivastava *et al*., 2017). Constructed wetlands have shown positive results in promoting bacteria that biodegrade cyanobacteria, which promote eutrophication (Bavithra *et al*., 2020; Lemes *et al*., 2015).

1.3. Pollutant mobility and transport in wetlands

The mobility of metal pollutants in the wetland is related to the species of the pollutant and is dependent on the physical conditions, redox potential and pH, and on the concentration of other chemical species (Caporale and Violante, 2016; Namieśnik and Rabajczyk, 2010). Metal transport however, occurs *via* dissolved or particulate matter (Masindi and Muedi, 2018; Wuana and Okieimen, 2011). Lateral or surface transportation of metals occurs *via* the movement of sediment or organic matter that have adsorbed metals whereas vertical transport of metals is facilitated by the porewater concentration gradients (Boano *et al*., 2014; Hasegawa *et al*., 2016). During sedimentation processes, water is trapped in sediment, forming porewater, where it is considered to be in equilibrium with the solid phase sediment (Batley and Giles, 1979; Ouddane *et al*., 2004). As metals are oxidized in sediment, they transverse into the sediment porewater, thus increasing the metal concentration (De Jonge *et al*., 2012; Ouddane *et al*., 2004). Generally, metal concentrations in porewater decrease with depth due to more compact sediment, changes in physical parameters and hydrology (Liu *et al*., 2017; Ouddane *et al*., 2004; Rezanezhad *et al*., 2016). Pollutants are then attenuated at lower depths due to the attenuation mechanisms that predominate at those depths. Surface attenuation is predominated by organic matter complexation or adsorption whereas clay content at lower depths facilitate adsorption due to the larger surface area and high electrostatic interactions (Carrillo-González *et al*., 2006). Mechanisms employed in the wetland are intended to immobilize pollutants, thus attenuating the pollutants. The chemical speciation of certain metals such as As, Hg and Cr, change with pH and redox potential, rendering them more (or less) toxic, bioavailable and mobile (Olaniran *et al*., 2013; Wuana and Okieimen, 2011).

1.3.1. Wetland conditions that facilitate the removal of pollutants in water

Wetlands are permanently or seasonally water-logged either by catchment run-off or groundwater discharge and are dense with vegetation (Morita, 1980). The air spaces between the soil particles are filled with water and this reduces the diffusion of oxygen into the soil. Hydrophytes and microorganisms contribute to depletion of oxygen from the water-logged soil, which leads to an anaerobic system. The anaerobic environment promotes reducing conditions, which leach Fe^{2+} and Mn^{2+} ions from sediment. These ions are mobile and leave the sediment particles exposed as grey particles. The ions that leach from the soil enter biota due to their mobility in ecosystems and cause ecotoxicological problems. Hydrophytes that grow in

wetlands bioaccumulate metals, which assists in the purification of water (Tangahu *et al*., 2011). When hydrophytes die, their plant matter forms a new layer on the sub-waterlogged surface, classified as peat. Peat is a complex material predominantly composed of f partially decomposed plant biomass and soil that is unique to wetlands. It is a highly porous material with large and small pores, which facilitates the diffusion of solutes between a mobile region and immobile region within the peat (Rezanezhad *et al*., 2016). Peat has a range of organic functional groups with oxygen, nitrogen and sulfur as the heteroatom providing a polar surface to which metals adsorb. The hydrophytes within a wetland reduce the flow of water, allowing for the settling of sediment particles in the wetland. The biota within wetland bioaccumulates inorganic pollutants, which assist in the removal of these pollutants from water. Information from natural wetlands, including sequestration mechanisms and bioaccumulating hydrophytes has led to the design of constructed wetlands.

Constructed wetlands are more effective and have been used to treat AMD passively. They follow the same chemical processes that exist in natural wetlands but in a controlled and carefully adjusted environment. South Africa has more than 70 constructed wetlands that have been utilized in mining and industrial sites (Calheiros *et al*., 2007; Khan *et al*., 2009), stormwater and urban catchment management (Birch *et al*., 2004; Walaszek *et al*., 2018), riverine rehabilitation and protection, groundwater recharge and development of urban nature reserves and ecological sites (Wood and Steffen, 1999). The design of constructed wetlands is dependent on operational parameters, heavy metal uptake mechanisms by plants, microorganisms, and support material (Pat-Espadas *et al*., 2018). Surface water and sub-surface flow wetlands are the two main constructed wetlands. Surface wetland flow allows pollutants to be exposed to the atmosphere whereas the sub-surface flow wetlands allow for maximum interactions with plant roots and substrate for maximum retention (Pat-Espadas *et al*., 2018). With sub-surface flow wetlands, heavy metals are exposed to an anaerobic environment that leads to metal removal by adsorption and precipitation (Pat-Espadas *et al*., 2018).

1.3.2. Chemical Speciation

The distribution, mobility, toxicity and availability of metals in the environment is dependent on the chemical speciation, which refers to the stable distribution of an element in a system based on their oxidation state, ligands and complexes (Reis and Gonçalves, 2015; Wasik and Namiesnik, 2001). Metal pollutants entering soil may undergo species transformation enabling

their uptake into plants, solubilisation into water, or precipitation onto the soil surface. The specifics of their fate is directed by various chemical and physical parameters, including pH, redox , salinity, presence of ligands, temperature and nutrient concentrations.

Metals are often mobilized and transported between environmental compartments, which are directly related to their speciation (McLean and Bledsoe, 1992; Wuana and Okieimen, 2011). Additionally, speciation studies provide bioavailability and toxicological information (Adelana *et al*., 2016; Benamer, 2014; Wang *et al*., 2019). The species available for biological uptake is referred to as being bioavailable and is absorbed and translocated to the various or specific organelle. Metals may bioaccumulate posing adverse health effects to biota.

Metals in mining waste include Fe, Al, As, Cu, Zn, Mn, Cd, Co, Ni, Hg, U and REEs (Kamunda *et al*., 2016; Marchand and Plumb, 2005; Peng *et al*., 2009). Some of these metals have a range of oxidation states, and speciation stability, which are dependent on physical parameters. Metals, including Fe, Al, and Mn form insoluble precipitates whereas metals, including As, Cu and Hg become mobile and are transported in the presence of organic matter (Namieśnik and Rabajczyk, 2010). Initially, these metals are in insoluble mineral form and upon leaching by AMD, become soluble ions. These ions undergo various chemical processes in solution, thus changing their species. Metal species, including mercury, lead and plutonium become insoluble as they form complexes or precipitates while other species become bioavailable and are accumulated in biota (Singh *et al*., 2011).

Arsenic is often found naturally in ores, including arsenopyrite (FeAsS), arsenical pyrite (FeS_2-As), realgar (As_2S_2), orpiment (As_2S_3), and cobalite (CoAsS) as well as in coal (O'Day, 2006). The common species of As include As(III), As(V), monomethylarsonous acid (MMA) and dimethylarsinous acid (DMA) (Cordos *et al*., 2006). In AMD affected soil and water, As exists as predominantly as arsenite (As(III)) or arsenate (As(V)) bound to Fe precipitates (Paikaray, 2015). They can transform to methylated species in the prescence of S or S-bacteria. They may also precipitate depending on the levels of Fe.

1.3.3. Lead Isotopes

Complex environmental systems, including wetlands and estuaries that have been affected by anthropogenic activity requires a better understanding of the behaviour and impact of specific metals, and the source identification of these metals. Various stable isotopes have been successfully used for a range of applications, including environmental geochemistry, biological analysis, clinical and metabolic studies, pollution source tracing, forensics, and geographical provenance tracing (Degryse *et al*., 2010; Liu *et al*., 2014). Isotopic variations in the environment can be due to natural processes, including biogeochemical processes and biological mediated processes (Pallavicini, 2016), and anthropogenic activity, including agriculture, sanitation, industrial discharge waste disposal, traffic, and mining (Anaman, 2013; Pallavicini, 2016). Anthropogenic activities alter the natural isotopic signatures therefore enabling the tracing and identification of pollution sources (Abiye, 2013). Metals may undergo isotopic fractionation and the resultant isotopic composition is unique, thus allowing for the identification of sources (Bullen, 2012).

Isotopic studies have become a powerful tool in environmental studies, with multiple isotopes being used to trace sources, authenticate isotopes and provenance, especially with advances in modern mass spectrometry (Bartelink and Chesson, 2019; Irrgeher and Prohaska, 2016). Stable isotopes do not change over time and can be quantified using mass spectrometry while radiogenic isotopes provide information on the origin and age of minerals (Bartelink and Chesson, 2019). Stable isotopes do not change over time, whereas radiogenic isotopes decay in time. This forms the premise for the use of radiogenic and stable isotopes in environmental studies.

Radiogenic isotopes, which are the daughter products of the decay of a radioactive parent, can be used to identify the age and origin of minerals and artefacts (Miller, 2019). Radiogenic isotopes make ideal environmental tracers due to their precise and accurate determination, wide range of isotopic abundance applications, and minimal fractionation. One such isotope is Pb. Lead isotopes have been used extensively in the characterization of natural and anthropogenic sources in various environments and sample types (Cheng and Hu, 2010; Komárek *et al*., 2008; Miller, 2019; Wiederhold, 2015). Major sources of Pb in the atmosphere are from the combustion of fossil fuels, mining activities, non-ferrous metal refining and waste incineration (Graedel, 2012). Lead transport can be short- or long-range and is often associated with other

metal emissions (Bollhöfer and Rosman, 2000). Lead has four stable isotopes, ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb. The ^{206}Pb, ^{207}Pb and ^{208}Pb isotopes are classified as radiogenic and are products of the radioactive decay of ^{238}U, ^{235}U and ^{232}Th. The fourth isotope of Pb, ^{204}Pb, is a cosmogenic isotope and isotopic ratios are often reported as radiogenic/cosmogenic isotopes. These ratios are compared to known Pb isotope ratios to determine the source of pollution or to trace the migrations of pollutants (Saint-Laurent *et al*., 2010). Environmental isotope tracing presents several challenges, including poorly or undefined anthropogenic source isotopic compositions, alteration of isotopic ratios by intermediate processes, overlapping of potential sources, and natural background isotopic composition (Wiederhold, 2015).

1.4. Study Area

The Gauteng region has approximately 15 million of the South African population within provincial borders and this number is expected to rise to 18 million by 2030 (National Planning Commission, 2013). This population growth places stress on resources, including land and water. Currently, the world faces a freshwater crisis and South Africa is no exception (Machethe, 2011). The need for purification and identification of clean and safe freshwater has become imperative.

The Witwatersrand Basin holds the world's largest gold reserves (Frimmel, 2019), comprised of siliciclastic sedimentary rocks divided into lower Westrand and upper Central Rand (Viljoen, 2009). Tectonic activity, deposition of Au and U-rich conglomerates and sandstone compressed the lower sediments of the Witwatersrand Basin (Viljoen, 2009). The Central Rand Group is divided into Johannesburg and Turffontein subgroups, where Main Reef, South Reef, the Bird Reef, Kimberley Reef and Elsburg Reef were the zones of mineralisation. Run-off from the Central Rand Goldfield discharges into rivers and wetlands, the most prominent of these being the Klip River wetland (McCarthy *et al*., 2007; Vermaak, 2009). The Klip River catchment drains the southern Witwatersrand before flowing into the wetland, located southwest of Johannesburg, South Africa (Figure 1.2). The Klip River wetland is predominantly dolomite underlain with basaltic volcanic rocks. The underlying dolomite forms aquifers, which sustain the wetland by recharging and discharging groundwater (McCarthy *et al*., 2007; Vermaak, 2009; Winde and Erasmus, 2011).

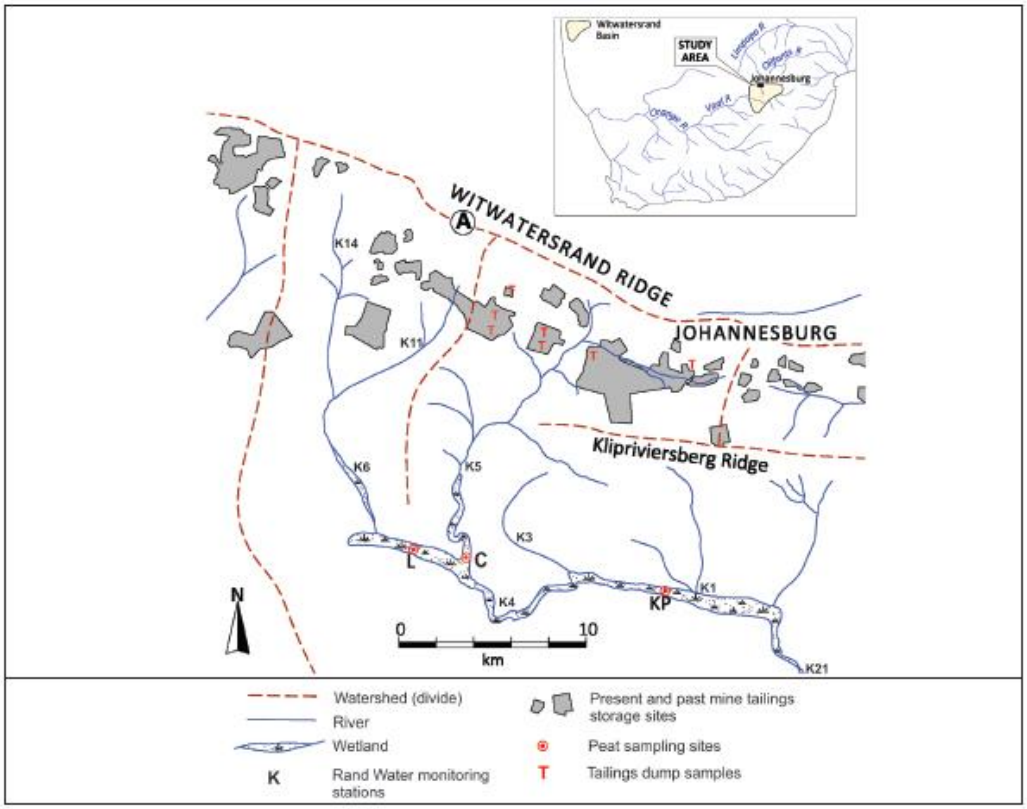

Figure 1.2: Map of the Klip River wetland study area (Humphries *et al*., 2017).

The Klipspruit River and the Natalspruit River both drain into the Klip River, which in turn flows into the Vaal River. The Vaal River supplies water to three provinces in the country and is a drainage system for southern Gauteng. The Upper Klip catchment is characterised by formal and informal settlements, mines, and tailing dumps. Both these communities extract groundwater from the underlying aquifers for domestic and agricultural purposes. Consumers of the water are seemingly unaware of the toxic effects of some of the elements in the water. These elements are bioaccumulated in fresh produce grown by these settlements and enter the human body, thus creating adverse health effects. Industries within the region are mainly based near waterways and often discharge effluent into the waterways. These industries have been identified as mining and mining-related industries. The Klip River wetland has been severely impacted by mining activities and numerous studies involving biota, heavy metal contamination and wetland degradation have been reported (Humphries *et al*., 2017; McCarthy, 2011; Vermaak, 2009).

Research into the Klip River system has encompassed geomorphological investigations (Vermaak, 2009), biodiversity counts (Durgapersad, 2005), water quality assessments

(Humphries *et al*., 2017; Marara and Palamuleni, 2020), the measurement of heavy metal contamination (Humphries *et al*., 2017; Marara and Palamuleni, 2020; McCarthy and Venter, 2006; McCarthy, 2011) and wetland degradation (McCarthy *et al*., 2007). A significant portion of the research has focused on the issue of AMD and the associated contaminants and risks to the region (Humphries *et al*., 2017; McCarthy, 2011). Geomorphological surveys show that peat mining and canalisation have severely degraded wetland function in the lower reaches of the wetland, changing vegetation and water flow dynamics (McCarthy *et al*., 2007). The influx of water discharge from sewerage plants has exacerbated the process leading to eutrophication and failure of the wetland to sequester nutrients. The increase in informal settlements and industrial activities has decreased the natural size of the wetland (Tooth, 2018; Tooth *et al*., 2009).

Several water quality studies have been undertaken over the years. Most reflect high metal concentrations (Humphries *et al*., 2017), increased bacterial counts (Durgapersad, 2005), diminished invertebrate diversity (Wepener *et al*., 2015) and degradation of ecological integrity (Kotze, 2005; Wepener *et al*., 2011). Metal pollutants in biota, water and sediments from various tributaries and in the wetland have shown bioaccumulation of metals in macroinvertebrates and fish (Mahlangu, 2013), elevated Pb, Cu, Co, Zn and Ni concentrations in sediments (Humphries *et al*., 2017) and the presence of U in both water and sediment as a result of AMD (Tutu *et al*., 2008). Recently, the Klip river catchment was classified as critically polluted for domestic and aquatic use according to the Comprehensive Pollution Index (CPI) measurement (Marara and Palamuleni, 2020). This index utilizes the ratio of pollutant concentration to background concentrations, divided by the number of sampling sites. It indicates pollution if water quality guidelines are available. When comparing the 2015/2016 seasonal data to the 2016/2017 seasonal data for As, Al, Fe, U, V and Zn, the concentrations have increased (Marara and Palamuleni, 2020).

The spatial distribution of metal contaminants using surface and sediment core data provides potential scenarios for metal entry into the wetland and attenuation. Fe-Mn oxy-hydroxides are the predominant phase for precipitation of Ni, Pb and Zn in the wetland. At depth, sulfides and organic matter precipitate Ca, Mg, Cu and Co (Humphries *et al*., 2017). Metal contaminants appear to enter the wetland at the surface (Cu and Pb) and groundwater (Ni, Co and Zn). An assessment of uranium, a common by-product of mining showed that AMD from TSFs mobilised and transported oxidised U in the water of the upper Klipspruit (Tutu *et al*., 2008).

Geochemical modelling of U in TSFs indicated that subsequent precipitation would be dependent on Fe, the redox conditions. The summary of the research carried out, thus far indicates a system that is highly contaminated, with an unknown remaining attenuation capacity and limited understanding of the processes driving metal mobility and the continued impact of industrial activity in the region.

1.5. Aims and structure of the thesis

The aim of this study was to establish the sources of metal pollutants into the Klip River wetland and investigate the mechanisms by which they are transported and sequestered to better understand the impact of AMD contaminants on wetland attenuation and functioning.

Objectives:

1. To investigate the transport and accumulation processes of various metal contaminants affecting the Klip River wetland and selected tributaries.
2. To identify the specific sources contributing to Pb pollution in using isotope ratio measurements.
3. To analyse the arsenic geochemistry of AMD and its effects in the wetland using speciation analysis.

These objectives will be addressed in chapters 2, 3 and 4, written and formatted as journal articles. The focus of each paper is distinct although there is minor repetition between chapters. The overall structure of the thesis is summarised below:

Chapter 2:
This chapter examines the concentration and distribution of trace metals and REEs in the wetland by examining water, porewater and sediment concentrations. The extent of pollution, migration pathways, and variations in accumulation and sequestration processes of specific metals are addressed. This chapter represents a manuscript submitted to the South African Journal of Science (SAJS) (accepted on 11 February 2020, published on 29 July 2021; manuscript number: 8668).

Chapter 3:

The potential sources (both natural and anthropogenic) of Pb in the Klip River wetland are examined using isotope analysis. Source apportionment is assessed using a basic mixing model and the primary contributors of Pb identified. Isotopic Pb signatures revealed that the major sources of Pb in the wetland are due to coal and alkyl lead (fuel). The article is intended for submission to Environmental Science and Technology (ACS Publication).

Chapter 4:

In this chapter, arsenic speciation, using hyphenated techniques, is examined to elucidate the specific species changes given the dynamic nature of the wetland. The predominant As species was As(V), with speciation dependent on redox and microbial activity. The predominance of As(V) indicates that the wetland remains successful in attenuating this metal. The article is intended for submission to Mine Water and the Environment (Springer).

Chapter 5:

This chapter integrates the overall findings from all the papers and implications to the wetland system. Future studies that may enhance the understanding of the Klip River wetland and pollution are recommended. Results from this study indicate that better management of AMD related pollution is required to mitigate the effects on natural ecosystems, particularly those beneficial systems such as wetlands. The pollution in the region can be attributed to AMD and mining activities increased urbanisation and industrialization in the region. Therefore, a deeper understanding of how pollutants impact wetland dynamics are impacted by pollutants is crucial to ensure the longevity and health of the wetland to ensure the proper functioning and minimal adverse effects to the broader environment.

1.6. Author contributions

Paper 1:

Shaeen Chetty (SC), Letitia Pillay (LP) and Marc S Humphries (MSH) carried out fieldwork and sample collection SC conducted the sample analysis and data extraction. SC carried out data interpretation under the supervision of LP and MSH. SC drafted the original manuscript, which was edited by LP and MSH. The study was conceptualised by LP and MSH.

Paper 2:

SC, LP and MSH carried out fieldwork and sample collection. SC conducted the sample preparation, analysis. SC, LP and MSH performed data analysis and SC drafted the original manuscript. The study was conceptualised by MSH and LP.

Paper 3:

SC, LP and MSH conducted the sample collection. SC prepared the samples while Katharina Blümlein (KB) conducted the sample analysis. SC, KB and LP carried out data interpretation. SC drafted the first version of this chapter, which was edited by LP and MSH. The study was conceptualised by LP and MSH.

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Chapter 2

Gold mining's toxic legacy: pollutant transport and accumulation in the Klip River catchment, Johannesburg

Abstract

Waste from gold mines is considered the largest single source of waste pollution in South Africa and contributes significantly to acid mine drainage, which remains one of the country's most serious environmental and socio-economic issues. Run-off from the Central Rand Goldfield discharges into wetlands along the Klip River, known to be important sinks for toxic pollutants. The aim of this study was to examine the transport, migration and sequestration of metal pollutants in the upper Klip River catchment in further detail. Analyses reveal that the majority of pollutants are associated with contaminant plumes that emanate from mine dumps and enter the wetland *via* groundwater recharge. This water carries highly elevated concentrations of Co, Ni, Zn, U and rare earth elements, which are naturally sequestered within the wetland, largely through precipitation and adsorption. While surface run-off from mine dumps severely contaminates watercourses within the upper catchment, surface inputs are considered relatively minor contributors to the overall pollutant load entering the Klip River wetland, although aerosol fallout is an important source of Pb. The extensive accumulation of metals within the Klip River wetland reflects the contaminant legacy associated with gold mining on the Witwatersrand and highlights the vital role this natural system has played in trapping vast quantities of toxic pollutants and remediating downstream waters. Contaminant plumes associated with mine dumps will likely persist for decades, and preventing further deterioration of the Klip River wetlands is, thus critical for safeguarding water sources in the region.

2.1. Introduction

The Witwatersrand Basin is the world's largest gold resource, having yielded more than one third of all the gold ever produced (Tucker *et al*., 2016). Although gold mining gave birth to the city of Johannesburg and formed the foundation of the country's economy for over a century, today it presents one of the largest threats to South African water resources and human health (Hobbs and Cobbing, 2007; McCarthy, 2010). Gold mining is associated with numerous environmental and health issues, although acid mine drainage (AMD), which emanates both from underground workings and waste disposal sites, is undoubtedly the most serious of these (McCarthy, 2011).

Acid mine drainage arises from the oxidation of pyrite, ubiquitous in the Witwatersrand gold-bearing conglomerates, to form sulfuric acid. The acid produced mobilizes constituents in the waste material and underground mine workings, releasing toxic metals such as cobalt, nickel, zinc and uranium. The major source of AMD on the Witwatersrand gold mines is associated with seepage from mine waste dumps, known as tailings storage facilities (TSFs). Waste from gold mines is considered the largest single source of waste pollution in South Africa (Oelofse *et al*., 2007). There are more than 270 TSFs in the Witwatersrand Basin alone, most of which are unlined and pose serious threats to both groundwater and surface water quality. Although some TSFs have been partially or completely reclaimed, their chemical footprint continues to contaminate groundwater even after the waste material has been removed for reprocessing (Rösner and Van Schalkwyk, 2000). AMD also arises from the discharge of contaminated water from flooded defunct gold mines and discharge of partially treated water pumped from producing mines has also contributed to the problem in recent years (Durand, 2012; Hobbs and Cobbing, 2007). Many gold mines on the Central and West Rand are situated beneath karst aquifers and must be constantly dewatered to prevent effluent rising to the surface and decanting from flooded mine voids.

The Central Rand, just south of Johannesburg, has been a prolific producer of gold for more than 100 years and is severely affected by the impact of mine tailings. Run-off from the Central Rand Goldfield discharges into wetlands along the Klip River, the principal drainage of the southern portion of Johannesburg. As a result, the Klip River wetland has been accumulating pollution since the founding of Johannesburg and previous studies have highlighted the extensive pollutant load sequestered within the wetland sediments (Humphries *et al*., 2017;

McCarthy and Venter, 2006). While the lower reaches of the wetland are severely degraded, the upper reaches, which receive polluted water from old gold mines, are still reasonably intact and have a beneficial impact on the quality of water entering the Vaal River (McCarthy *et al*., 2007). Given the economic and environmental value of the Klip River wetland, this study was undertaken to examine the transport, migration and sequestration of metal pollutants in the upper Klip River catchment in further detail. Firstly, we assess the significance of surface runoff and groundwater seepage as potential sources of pollutants by examining metal concentrations in sediment and water samples collected from the upper Klip River catchment. Secondly, we examine the ingress of metal contaminants and variability in their sequestration across the Klip River wetland through the detailed geochemical analysis of a series of sediment cores.

2.2. Study area

2.2.1. Regional setting

The Klip River basin is a sub-basin of the Vaal-Orange River system and represents the most significant drainage system for the southern Witwatersrand region (Figure 2.1A) (Vermaak, 2009). The Klip River and Klipspruit are the primary tributaries draining the Witwatersrand mining-industrial complex and discharge into the extensive wetland area further downstream.

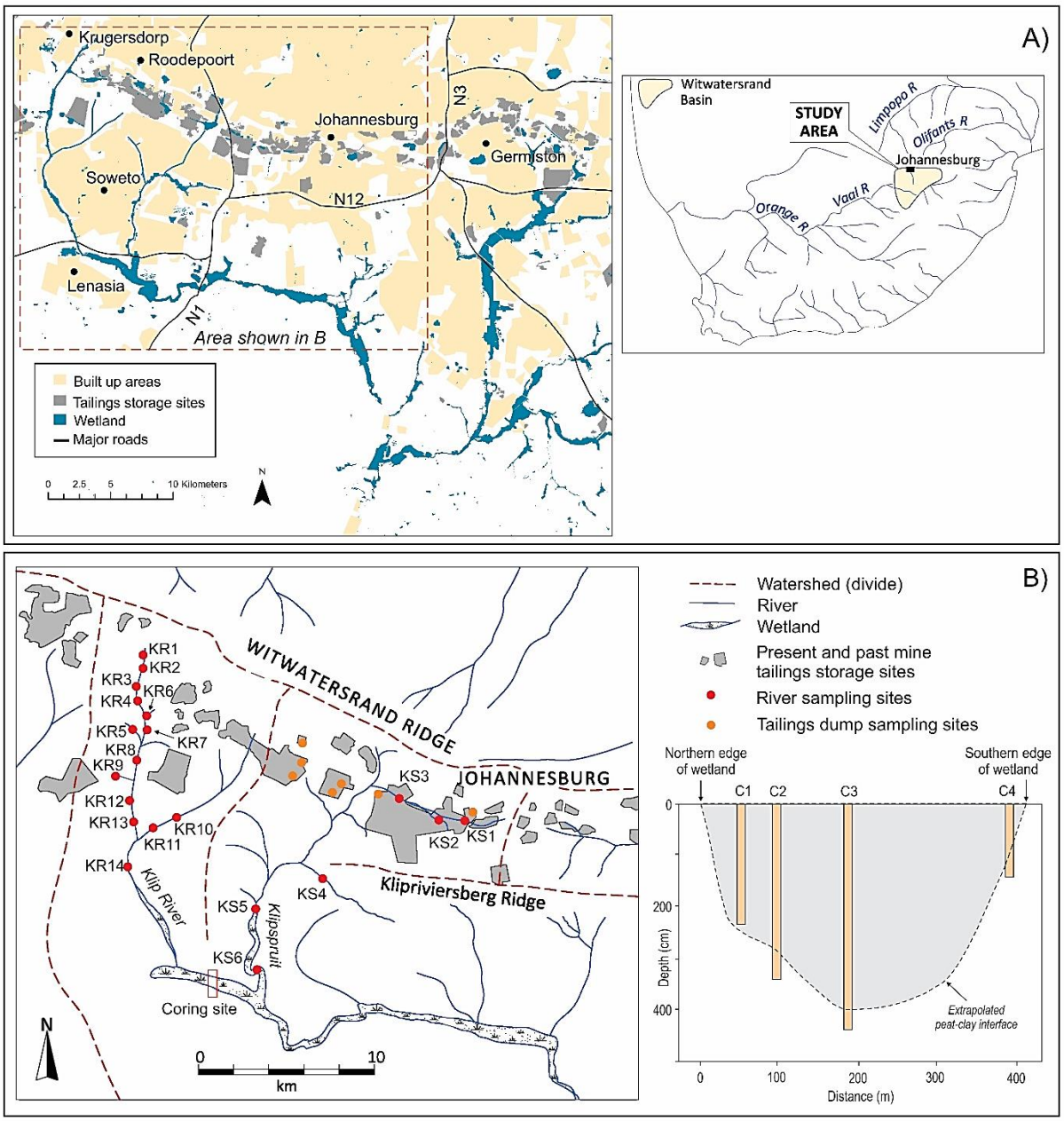

Figure 2.1: A) Map of the study area showing the drainage network of the upper Klip River catchment in relation to mining activities and major urban centres in the Johannesburg area. The location of water and sediment sampling sites along the Klip River (KR1 – 14) and Klipspruit (KS1 – 6) also indicated. B) Cross-sectional transect across the Klip River wetland showing the position of the four cores (C1 – 4) collected.

2.2.2. Geology and geohydrology

The bedrock geology of the region is summarised in Figure 2.2. Gold is found in quartz pebble conglomerates, hosted in a sequence of siliceous quartzites of the Witwatersrand Supergroup. The Witwatersrand strata are conformably overlain by basaltic volcanic rocks of the Ventersdorp Supergroup, which in turn are overlain by dolomitic rocks of the Transvaal Supergroup. These rocks are karstic and host the Klip River wetlands. The wetlands are sustained predominantly by groundwater recharge from the underlying dolomite. During summer, this discharge is augmented by surface run-off from rainfall, with the Klip River and Klipspruit being the dominant fluvial inputs. The Klip River wetland drains into the Vaal River, which supplies approximately 23% of the South African population with potable drinking water (Chinyama *et al*., 2016). Groundwater from underlying aquifers is also extracted for domestic consumption and informal settlements use untreated river water for domestic and agricultural purposes (Abiye *et al*., 2011a).

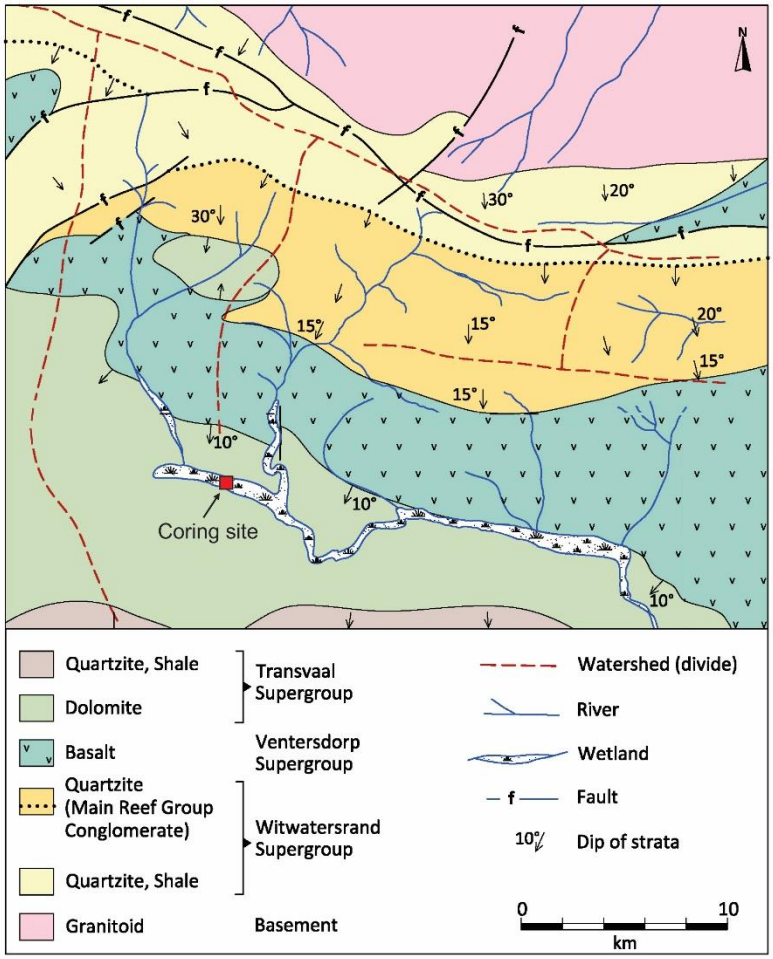

Figure 2.2: Simplified geology of the study area (Mellor, 1917).

2.2.3. Pollutant sources

TSFs represent important sources of pollution within the Witwatersrand Basin and are typically associated with extensive acid plumes that contaminate the local groundwater. The formation of acid plumes is caused by the infiltration of rainfall through the TSFs, resulting in the discharge of contaminated water into nearby streams and rivers (Naicker *et al*., 2003). The Klip River wetland also receives runoff from several large informal settlements and industrial regions, as well as discharge from three sewerage treatment plants.

2.3. Methods

2.3.1. Sampling

Water (n = 20) and surface sediment (n = 15) samples were collected from the upper Klip River (KR1 – 14) and Klipspruit (KS1 – 6) tributaries during March 2017 (Figure 2.1). Sampling sites were established based on accessibility and in an attempt to capture downstream variations. Surface samples (0-10 cm) were collected from the riverbed by hand. The pH and electrical conductivity (EC) of samples were measured in field using a portable combination meter (Thermo Scientific Orion Star) calibrated against appropriate standard solutions. Water samples were immediately passed through 0.45 µm filters, acidified with 1% HNO_3 and then refrigerated. Sediment samples were placed in bags and stored at 4 °C. Surface material from several mine TSFs in the upper catchment was also sampled (Figure 2.1) in order to characterise the chemical composition of gold mine tailings.

A series of four sediment cores (C1 – 4) were collected along a 350 m north-south orientated transect established in the upstream, western section of the Klip River wetland (Figure 2.1). This section of the wetland is characterised by an intact reed-covered swamp dominated by *Phragmites australis* and is considered to have remained relatively undisturbed for the last ~80 years based on examination of historical aerial photography and maps (McCarthy *et al*., 2007). Samples were collected to refusal depth using a Russian peat corer. Sub-samples were taken at 10 cm intervals and sealed in plastic bags for subsequent analysis.

2.3.2. Chemical analysis

Tributary and wetland representative sediment samples were air-dried, milled and combusted at 500 °C for 4 hours to determine loss on ignition (LOI). Combusted sample powders (~25 mg) were microwave digested in 9 mL HF:HNO_3:HCl (6:3:1) using an Anton Paar Multiwave Go system to ensure REEs and complete metal dissolution (Balaram and Rao, 2003). Solutions were evaporated to dryness following the addition of perchloric acid (0.5 mL), before finally being diluted with 1% HNO_3 and spiked with Rh as an internal standard. Metal and REEs (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) concentrations were measured against external matrix-matched standards using an Agilent 7700x ICP-MS. Chemical interferences were eliminated with the use of the KED-He mode. Procedural blanks were analysed with each digestion batch and used to correct final sample concentrations. Sediment standards PACS-2 and CGL-111 were used to assess the accuracy and reproducibility of the method, with recoveries ranging between 88 and 110% (n = 6). Internal precision was typically <3% for all elements. Major elements from the ashed samples were analysed by powder X-ray fluorescence (XRF) using a Bruker Ranger S2 standard sediment method, following calibration against a range of local and USGS rock standards.

Metal and REEs concentrations in water samples were measured by ICP-MS as reported above. Where possible, porewater was extracted from wetland core samples using direct centrifuge drainage (Di Bonito *et al*., 2008). The pH and metal content of porewater samples was determined using the procedures described above.

2.3.3. Enrichment factors

To account for variations in sample composition and mineralogy, metal concentrations are expressed as enrichment factors (EF). While the calculation of EF values is subject to several assumptions (see for example Reimann and Caritat, 2005), we apply this normalisation in examining the spatial distribution in metal concentrations within the study area. Enrichment factors were calculated relative to upper continental crust (UCC) values (Wedepohl, 1995) using Li as the normalising element (Equation 1). Lithium behaves conservatively in the environment and is not associated with mine waste.

$$EF = \frac{\left[\frac{C_{Metal}}{C_{Li}}\right]_{Sediment}}{\left[\frac{C_{Metal}}{C_{Li}}\right]_{UCC}} \qquad (1)$$

Calculated EF values were assessed and classified into seven categories: (1) <1: no enrichment; (2) 1-3: minor enrichment; (3) 3-5: moderate enrichment; (4) 5-10: moderately severe enrichment; (5) 10-25: severe enrichment; (6) 25-50: very severe enrichment; (7) > 50: extremely severe enrichment (Harikumar and Jisha, 2010; Rudnick *et al*., 2003). Relationships between metal concentrations were examined using analysis of variance (ANOVA), with significance set at $p < 0.05$.

2.4. Results

2.4.1. Surface sediment and water chemistry

Water samples collected from the upper Klip River catchment and Klipspruit reveal a high degree of chemical variability (Table 2.1). The highest trace metal concentrations were generally measured in water samples from the Klipspruit, which were typically characterised by high EC and elevated levels of Fe and Al. Several other metals were detected in significant concentration in the Klipspruit, notably Co, Ni and Zn. The most contaminated sample was collected in the vicinity of TSFs located in the upper Klipspruit catchment (site KS3) and contained highly elevated concentrations of Fe (330 mg/L), Co (2.1 mg/L), Ni (2.7 mg/L), Zn (2.8 mg/L) and U (0.35 mg/L).

Table 2.1: Chemistry data for surface water catchment samples and porewater samples from the Klip River wetland

	pH	EC (mS/cm)	Al	Fe	Co	Ni	Zn	Cu	Pb	U
			mg/L							
Klip River (n = 14)										
Max.	7.6	95	0.84	2.5	0.032	0.20	0.48	0.4	0.012	0.10
Min.	4.3	15	*<DL*	*<DL*	0.0002	0.0012	*<DL*	*<DL*	*<DL*	*<DL*
Mean	6.7	52	0.091	0.30	0.0082	0.075	0.15	0.086	0.0028	0.020
SD	0.8	33	0.23	0.68	0.0088	0.064	0.12	0.10	0.0044	0.028
Klipspruit (n = 6)										
Max.	7.8	379	60	330	2.1	2.7	2.8	0.35	0.0076	0.34
Min.	6.5	15	*<DL*	0.012	0.0002	0.0044	0.073	*<DL*	*<DL*	*<DL*
Mean	7.1	112	8.9	47	0.33	0.46	0.66	0.10	0.0019	0.055
SD	0.6	150	22	124	0.77	1.0	0.99	0.14	0.0029	0.13
Wetland porewater (n = 94)										
Max.	7.9	60	1000	804	8.9	47	30	14	1.4	8.1
Min.	5.8	0.9	*<DL*	*<DL*	*<DL*	0.006	0.36	0.026	0.004	*<DL*
Mean	6.9	26	66	55	0.75	3.9	5.5	1.2	0.15	0.36
SD	0.4	5.6	155	118	1.4	7.3	6.6	1.9	0.27	1.1
DWAF (Holmes, 1995)	6.5-8.4	<70	0.15	0.1	0.5	0.15	5	1	0.02	0.02
Central Basin void mine water (n =12) (DWA, 2013)	3.0	397	120	40	4.7	10.6	9.1	0.33	0.028	0.61

<DL = Below detection limit, DWAF = Department of Water and Forestry, DWA = Department of Water Affairs

Metal concentrations measured in porewater samples from the Klip River wetland were extremely variable. Average concentrations exceeded those measured in both tributaries, with several porewater samples characterised by exceptionally high levels of Al, Fe, Co, Ni and Zn and lower concentrations of Cu, Pb and U were detected. Maximum measured metal porewater concentrations exceeded those typically found in mine void water from the Central Witwatersrand Basin.

Most trace metals were present in highly elevated concentrations in sediments collected from the Klipspruit and upper Klip River catchment (Table 2.2). Sediments from the Klipspruit were visually characterised by considerably higher proportions of clay, while those from the Klip River tended to be more organic-rich. Normalised metal concentrations show Klip River sediments to be substantially more enriched compared to the Klipspruit. Most metals were present in Klip River sediments at severe enrichment levels, while minor to moderate enrichment characterised sediments from the Klipspruit. Surface material from TSFs comprised predominantly (~95%) SiO_2 and Al_2O_3, but showed enrichment with respect to some trace metals, notably Pb and U. Cobalt and Cu exhibited minor enrichment, while Ni and Zn were not detected in any of the TSF samples analysed.

Table 2.2: Mean concentrations (mg kg^{-1}) and enrichment factors (EF) for metals in tributary surface sediments and material from tailings storage facilities (TSFs).

	Klip River (n = 10)		**Klipspruit (n = 5)**		**TSFs (n = 7)**	
	[Mean] ± SD	**EF**	**[Mean] ± SD**	**EF**	**[Mean] ± SD**	**EF**
C_{org} (%)	18 ± 14	-	12 ± 12	-	0	-
Li	17 ± 9.6	-	108 ± 95	-	7.6 ± 3.9	-
Al (%)	3.6 ± 1.5	0.50	6.6 ± 3.3	0.20	3.4 ± 0.89	1.0
Fe (%)	5.4 ± 3.0	1.2	4.6 ± 3.2	0.50	2.9 ± 1.1	1.7
Co	104 ± 73	13	198 ± 304	2.8	13 ± 13	2.1
Ni	312 ± 234	11	328 ± 483	1.8	*<DL*	-
Zn	512 ± 445	13	700 ± 298	2.6	*<DL*	-
Cu	219 ± 218	13	114 ± 66	1.3	25 ± 27	2.8
Pb	79 ± 56	8.5	62 ± 54	1.8	33 ± 17	7.2
U	20 ± 21	11	23 ± 25	4.6	12 ± 12	13
ΣREEs	125 ± 54	-	304 ± 175	-	100 ± 19	-
$\Sigma REEs_{PAAS}$	8.7 ± 3.9	-	15 ± 5.4	-	5.5 ± 1.3	-

<DL = below detection limit, PAAS = Post-Australian Archean Shale

2.4.2. Core characteristics and major chemical composition

The cores retrieved from the Klip River wetland ranged in length from 140 to 440 cm. An underlying clayey sand layer was intercepted at all sites, except site C1, and marked the depth at which point of refusal was reached. The upper sections of all cores were generally composed of organic-rich sediment (peat), with LOI varying between 65 and 85% (Figure 2.3A). Little variation was observed in pH, both with depth and between individual coring sites.

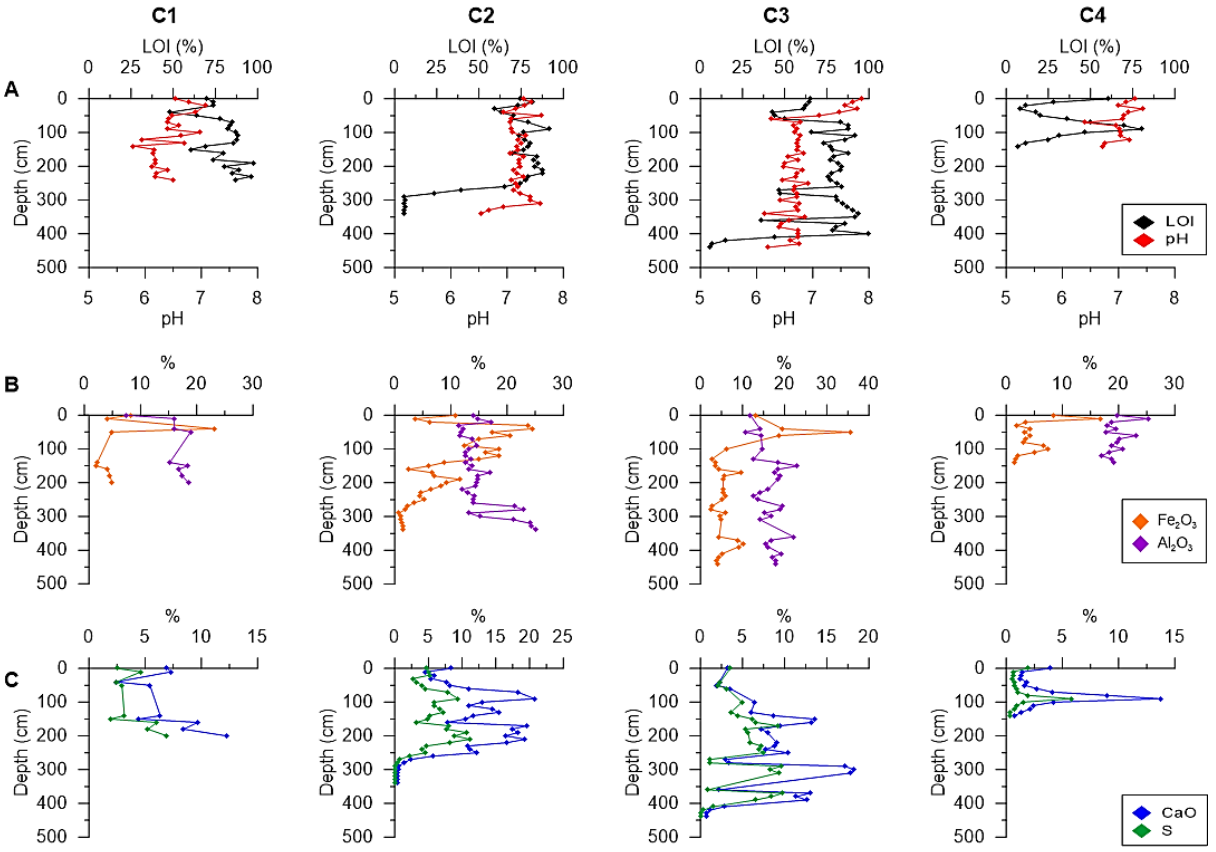

Figure 2.3: Downcore profiles showing variations in (A) sediment pH and loss on ignition (LOI), (B) Fe_2O_3 and Al_2O_3 (ashed samples) concentrations , and (C) CaO and S concentrations at coring sites C1 – 4.

The inorganic fraction of the peat was dominated by fine-grained material composed largely of SiO_2 (59 ± 6.9 %) and Al_2O_3 (16 ± 3.5 %), with cores closer to the edge of the wetland (C1 and C4) characterised by slightly higher SiO_2 and Al_2O_3 contents. Marked variations in Fe_2O_3 and CaO abundances were observed both downcore and across the wetland (Figure 2.3B, C). Fe_2O_3 was typically enriched in the upper peat layers, with enrichment most prevalent in C2. Peat CaO concentrations ranged between 0.3 and 21%, with cores C2 and C3 characterised by particularly high concentrations. In contrast to Fe_2O_3, CaO concentrations were typically highest within the middle and lower sections of the peat profiles and were closely matched by variations in total sulfur, which ranged between 0.1 and 11%.

2.4.3. Trace metal profiles

Metal profiles show substantial downcore variations, although enrichments occur in different sections of the peat profiles. Cobalt and Ni show similar downcore trends, with highest enrichment typically found near the base of the peat sequences (Figure 2.4A, B). Overall, Ni is significantly more enriched compared to Co, with EF values varying between 0.2 – 68 and 0.2 – 87, respectively. However, both metals show similar lateral trends, with enrichment in the peat generally decreasing from north (C1) to south (C4). Cobalt and Ni concentrations are approximately 15 and 6 times lower in C4 compared to C1. Zinc was concentrated in the middle and lower sections of the peat profiles, where it was present at highly enriched levels (Figure 2.4C). Enrichment in Zn tended to increase towards the centre of the wetland, with EF values of up to 300 present in C3. The lowest Zn concentrations were measured in C4 (EF <10).

In contrast to the subsurface enrichments displayed by Co, Ni and Zn, Pb was found at highest concentrations within the upper (< 100 cm) sections of the peat sequences (Fig. 2.4D). At the surface, Pb enrichment typically ranged between 6 and 8, showing little lateral variation across the wetland. Copper and U exhibited similar downcore distributions, with highest enrichments typically confined to the upper 100 cm of the peat profiles (Fig. 2.4E, F). Both metals were significantly enriched in C1, with pronounced decreases in concentrations towards the centre of the wetland.

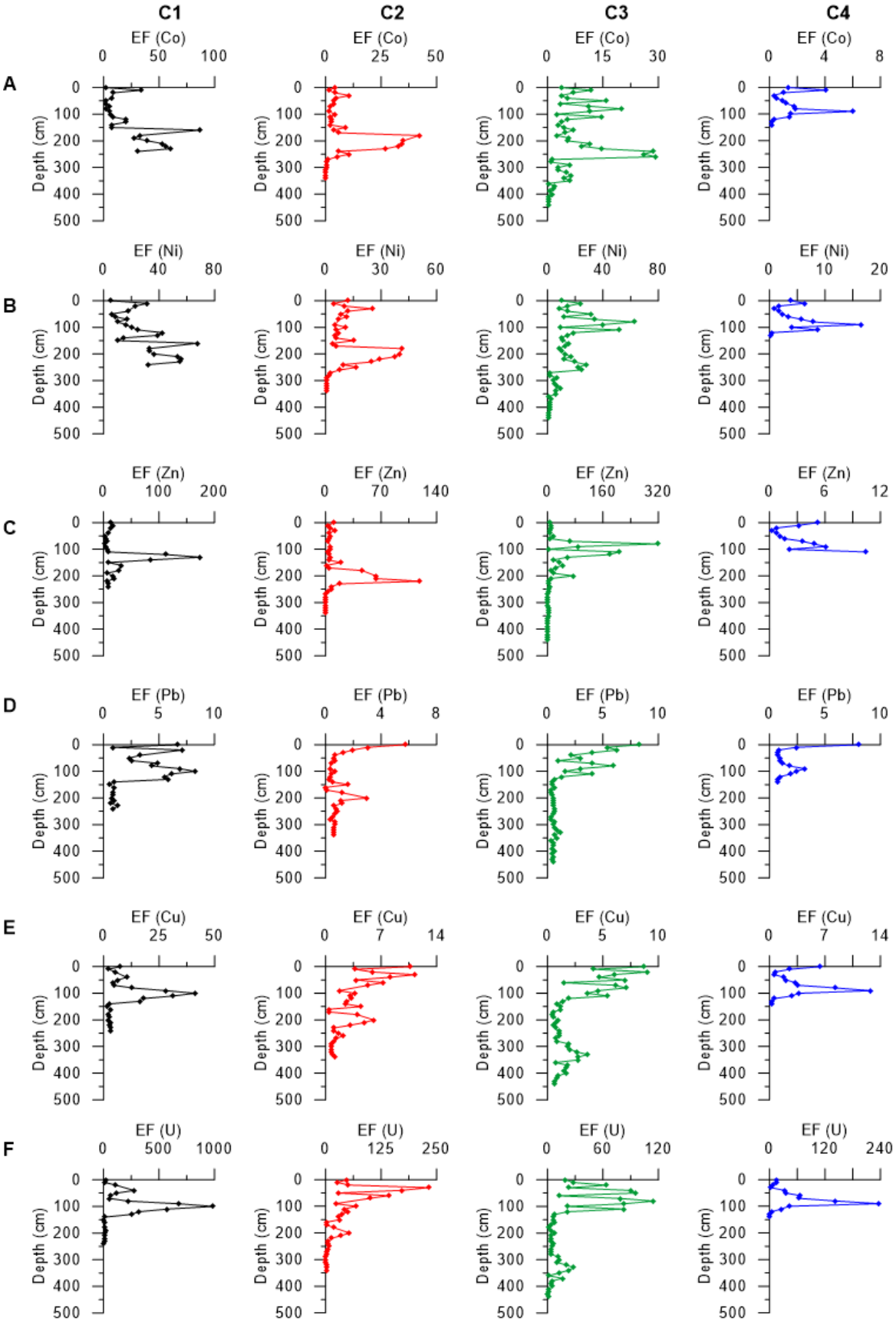

Figure 2.4: Downcore metal enrichment profiles for sites C1 – 4. Enrichment factor (EF) values calculated relative to upper continental crust (Wedepohl, 1995).

2.4.4. Rare earth element patterns

The downcore distributions in ∑REEs concentration were remarkably similar across all four profiles (Figure 2.5A). Highest concentrations were typically confined to a pronounced zone of enrichment between 50 and 150 cm. There were modest variations in ∑REEs concentration across the wetland, although highest concentrations (~6000 mg kg^{-1}) were found in C1. Post-Archaean Australian Shale (PAAS)-normalised patterns indicate that REEs in the upper section of C1 are enriched 3-fold relative to the other cores (Figure 2.5B). Enrichment in the middle REEs (MREEs) relative to both the light (LREEs) and heavy REEs (HREEs) is a characteristic across all coring sites.

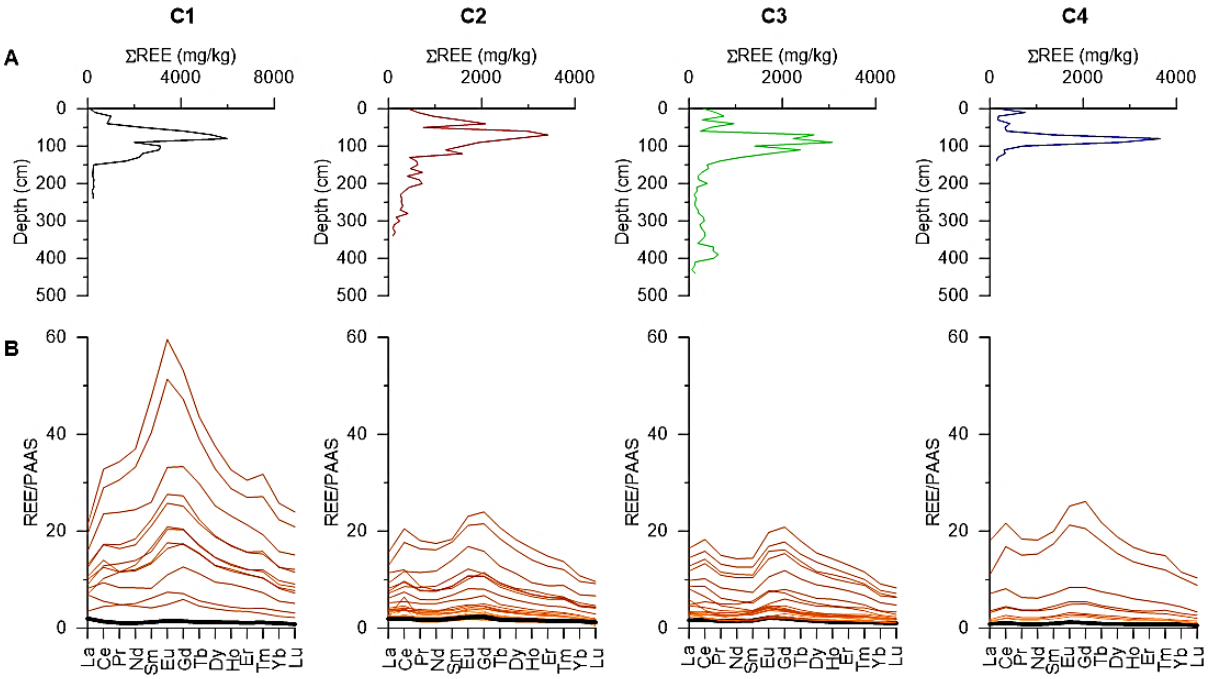

Figure 2.5: A) Downcore total rare earth element (∑REEs) concentration profiles for sites C1 – 4, and B) Post-Archaean Australian Shale (PAAS)-normalised REEs patterns. REEs enriched samples (REEs/PAAS >1) are shown in orange, while the average of non-enriched samples is indicated in black.

2.5. Discussion

2.5.1. Pollution extent, sources and pathways

The Central Basin extends approximately 55 km, from Durban Roodepoort Deep (DRD) in the west, where the Main Reef terminates against the Roodepoort Fault, to East Rand Proprietary Mines (ERPM) in the east (Figure 2.6). Mining operations commenced in 1886, and although the mines started as separate entities, the underground workings eventually merged over time forming a continuous mine void extending from the East Rand to the West Rand (Durand, 2012). Large-scale mining began to curtail in the 1950s, with the closure of DRD in 1999 and ERPM in 2008 bringing to an end deep-level operations in the basin. A long history of mining operations, together with the progressive closure of mines, has resulted in the wide-scale discharge of contaminants into the streams and watercourses draining the Central Basin (Humphries *et al*., 2017; McCarthy *et al*., 2007; Naicker *et al*., 2003).

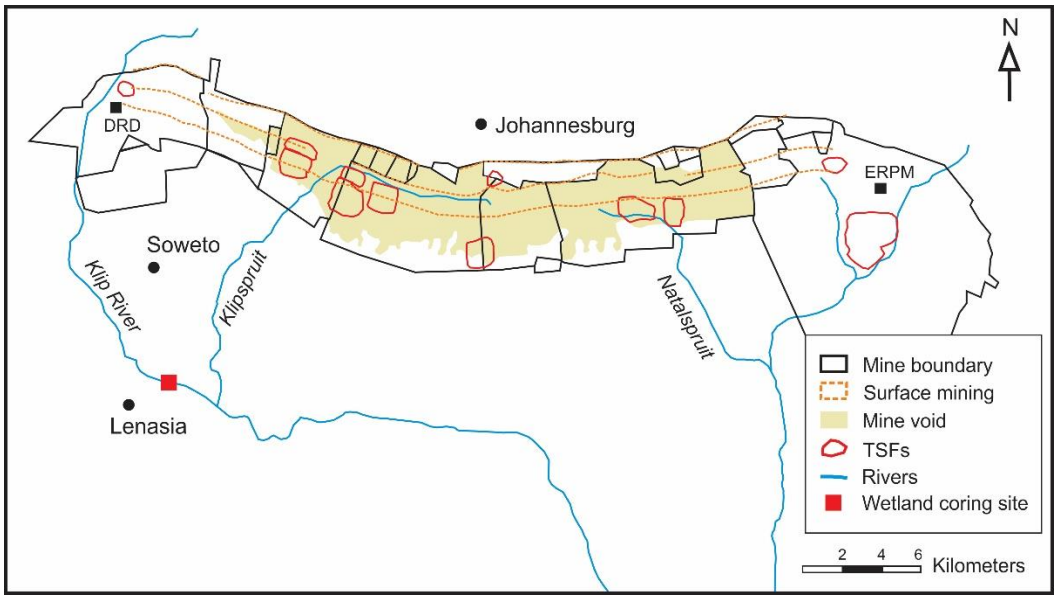

Figure 2.6: Map showing the extent of mining activities and areas of major water ingress into the Central Basin mine void (DWA, 2013).

Acid mine drainage issuing from gold mines on the Witwatersrand is characterised by high concentrations of Ni, Co, Zn and U (DWA, 2013). These metal contaminants enter the river systems through run-off from TSFs as well as through decant of effluent from slimes dams and

mine voids. The two streams sampled in this study, the Klip River and Klipspruit, drain the western portion of the Central Basin, where several large mining operations were located, including DRD and Crown Mines (Figure 2.6). Samples collected from the Klip River and Klipspruit revealed widespread contamination of watercourses in the region. Metal concentrations often exceeded accepted maximum levels, particularly for Al, Fe, Ni and U. Higher concentrations measured in water samples from the Klipspruit compared to the Klip River likely reflect the degree to which the upper reaches of each tributary have been affected by mining. The Klipspruit drains a portion of the Central Basin that has been intensively mined out and which is characterised by the presence of several large TSFs. Interestingly, sediments from the Klip River revealed higher levels of metal enrichment compared to the Klipspruit. To some extent, this appears to reflect differences in the nature of the material analysed, with sediment from the Klip River characterised by substantially lower aluminium concentrations (reflecting a lower clay content) and higher amounts of organic carbon (averaging ~20%) compared to the Klipspruit. Effluent from industrial and residential sources, particularly around Roodepoort and Soweto, may also be a significant contributor to pollutant loads in the Klip River.

Contaminants associated with AMD may also enter the groundwater *via* seepage through TSFs or directly from flooded mine voids. TSFs on the Witwatersrand are known to be significant contributors to AMD (Naicker *et al*., 2003; Tutu *et al*., 2008) and often constitute the single largest source of mining-related water pollution (Coetzee *et al*., 2006). Tailings material, which is dominated by unconsolidated silicate minerals, is prone to leaching and percolation of rainwater through the dumps results in the formation of polluted groundwater plumes. The geochemical processes characterising Witwatersrand TSFs have been fairly well studied (e.g., Coetzee *et al*., 2006; Hansen, 2015), with the mobility of metals within dumps largely controlled by a combination of pH and redox parameters. Impoundments are generally considered to consist of two key zones; an outer oxidation zone characterised by the presence of sulfate minerals (especially jarosite and gypsum) and secondary oxy-hydroxides, and an inner reduction zone (Hansen, 2015). Pyrite on the Witwatersrand is associated with several trace metals found in relatively high concentration, notably Co (176 mg kg^{-1}), Ni (477 mg kg^{-1}) and Zn (90 mg kg^{-1}) (Barton and Hallbauer, 1996). These metals are released during the oxidation process, which also results in the formation of sulfuric acid. Most metals,

including Fe, Al, Ni and U are highly soluble under acidic reducing conditions and are leached from TSFs into the groundwater.

Samples collected from several TSFs located in the Central Basin show that tailings surface material is characterised by several trace metals that are present in elevated concentrations, notably U and Pb (Figure 2.7B). Elevated concentrations of U (up to 5.8%) are a prominent feature of Witwatersrand gold-bearing conglomerates (Coetzee *et al*., 2006b). Although originally mined as a by-product of the gold industry, most of the U extracted as part of the gold mining process was dumped in slimes dams on the Witwatersrand (Rösner and Van Schalkwyk, 2000). Uranium is found in the mineral form uraninite (UO_2), which reacts in acidic solutions to produce soluble U^{4+} ions. Mine dumps, thus contain and leach considerable quantities of U, which accumulates in wetlands and river sediments in the region (Coetzee *et al*., 2006b; Winde and Sandham, 2004). Lead is often present in the Witwatersrand conglomerates as galena (PbS), but it also found in association with pyrite (Barton and Hallbauer, 1996). Although galena may be oxidised under acidic conditions, Pb is not a notable feature of AMD on the Witwatersrand and has been reported in relatively low concentration (<1 mg/L) within shallow acidic groundwaters (Naicker *et al*., 2003). This is consistent with Pb enrichments measured in tailings material, suggesting that Pb is present in insoluble oxide and sulfate forms, or absorbed to Fe-(oxy)hydroxides or onto clay minerals, limiting the degree to which it is leached from TSFs. However, several other important metals were either not detected in tailings material (e.g., Ni and Zn) or showed only moderate enrichment (e.g., Al, Fe, Co and Cu). These metals are leached from the tailings under acidic conditions and form the major constituents in seepage from mine dumps on the Witwatersrand.

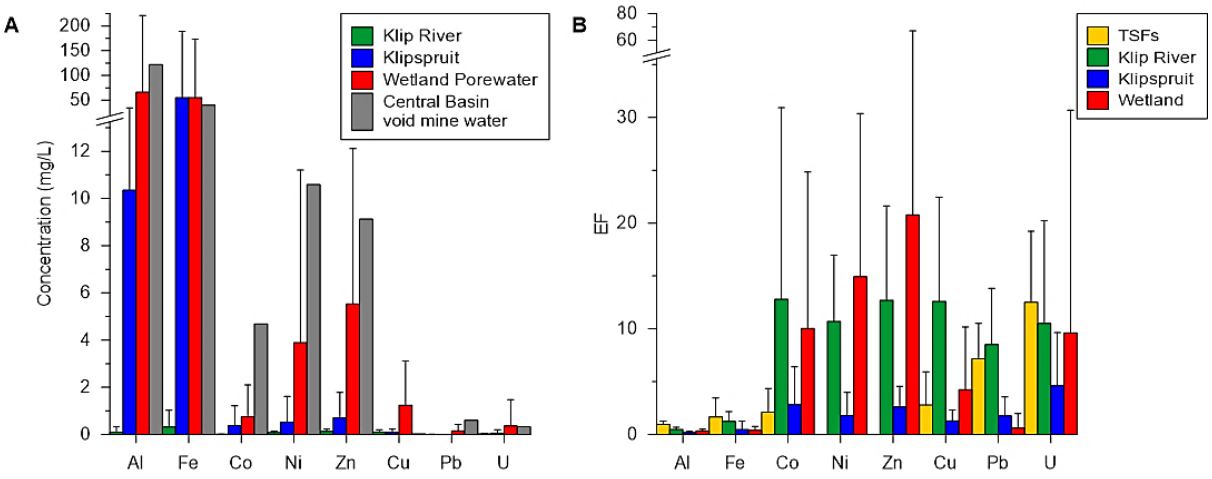

Figure 2.7: A) Comparison between average metal concentrations measured in water from the Klip River, Klipspruit, Klip River wetland (this study) and the Central Basin mine void (DWA, 2013) , and B) average metal enrichment factors (EF) measured in material collected from TSFs, Klip River, Klipspruit and wetland. Error bars indicate standard deviation not analytical errors.

Acidic seepage from TSFs drains directly into underlying aquifers. Because the Witwatersrand Supergroup lies adjacent to the karstified dolomites of the Malmani Subgroup, seepage from TSFs results in widespread groundwater contamination in the area. Many tailings dumps and slimes dams along the Witwatersrand were purposefully positioned on dolomite to facilitate water drainage, resulting in drier, more stable impoundments (Durand, 2012). Seepage of AMD directly into the karst system below not only results in the contamination of deeper groundwater but also the dissolution of the dolomite.

Neutralisation is likely an important process for metal sequestration and is a common process employed in wetlands constructed for AMD treatment (Mathuthu *et al*., 2018; Pat-Espadas *et al*., 2018). The high concentrations of Ca (up to 20%) measured in peat from the Klip River wetland indicate the important role the underlying dolomite substrate likely plays in sequestering metals from groundwater flowing into the wetland. Sulfate salts would be expected to form when sulfuric acid reacts with the alkaline dolomite and the strong relationship between Ca and S abundances suggests the widespread precipitation of gypsum within the wetland. The high metal load carried in the acidic groundwater plumes oxidise and precipitate within the wetland under higher pH (>6) conditions, resulting in substantial peat enrichments, particularly with respect to Ni, Zn, Co and U (Figure 2.7B).

2.5.2. Variations in pollutant accumulation and sequestration processes

The contamination of both surface water and groundwater discharging from the Central Basin causes pollutants to enter and accumulate in different parts of the Klip River wetland. Contour plots show that metals strongly associated with AMD (Co, Ni and Zn; Figures 2.8A-C) enter the wetland along the northern edge *via* groundwater flow and accumulate predominantly within the deeper peat (typically below 1 m). The ingress of these contaminants into the wetland is associated with acidic groundwater plumes, which originate from the numerous TSFs that dominate the Central Rand. Although these contaminants enter the wetland *via* the same transport processes, their migration and sequestration within the peat is influenced by various physico-chemical factors. Cobalt and Ni show similar distribution patterns (R^2 = 0.73), suggesting that these metals are sequestered *via* a common mechanism. Humphries *et al*. (2017) assessed the partitioning of metals within Klip River sediments and found that Co and Ni were preferentially associated with the reducible (Fe-hydroxides) and oxidisable (organic matter/sulfides) fractions. Zinc, which shows no correlation with Co and Ni, is instead predominantly (>50%) associated with Fe-hydroxides (Humphries *et al*., 2017), which precipitate readily under wetland conditions (pH = 6-8). Uranium and Cu also exhibit similar distributions (R^2 = 0.76), and although entering the wetland *via* the same groundwater process, these metals tend to accumulate predominantly within the upper sediments of the wetland (Figures 2.8D, F). Copper is primarily associated with the oxidisable fraction (Humphries *et al*., 2017) and it is likely that Cu and U are sequestered as sulfides or bound to organic matter. REEs appear to remain fairly mobile within the peat and migrate laterally across the wetland (Figure 2.8E). Their sequestration predominantly within the upper 1.5 m is likely associated with Fe-hydroxide adsorption.

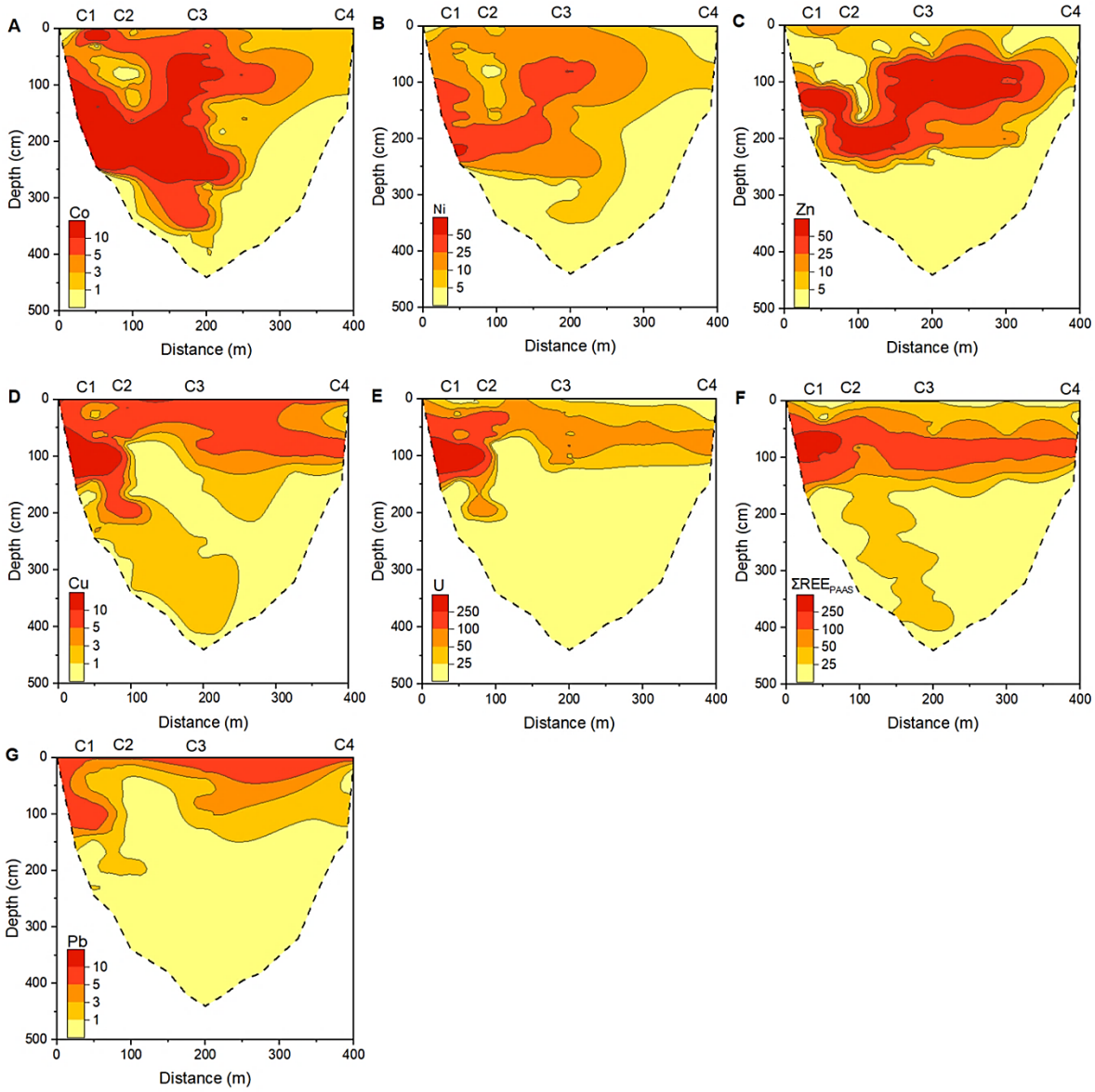

Figure 2.8: Contour maps illustrating the ingress of pollutants using the EF in the wetland

Distribution patterns suggest that Pb (Figure 2.8G) is predominantly unrelated to the ingress of AMD-contaminated groundwater. Instead, localised surface enrichment within the wetland suggests that Pb contamination most likely originates from surface runoff and/or atmospheric fallout. Lead aerosol deposition may be related to historical combustion of Pb-gasoline or dust originating from TSFs (Mathuthu *et al*., 2018) and other industrial activities.

2.6. Summary and conclusions

The results of this study reveal the Klip River wetland to be an important sink for pollutants originating from mining activities on Witwatersrand. Pollutants enter and accumulate within the wetland *via* different processes, summarised in Figure 2.9. The majority of pollutants are likely associated with AMD plumes that emanate from TSFs and enter the wetland *via* groundwater recharge. This water transports highly elevated concentrations of Co, Ni, Zn and U, which are sequestered within the wetland sediment through a variety of precipitation and adsorption processes. While surface runoff from TSFs severely contaminates watercourses within the upper catchment of the Klip River wetland, surface inputs are considered relatively minor contributors to the overall pollutant load entering the Klip River wetland. Aerosol deposition is, however, likely an important source of Pb.

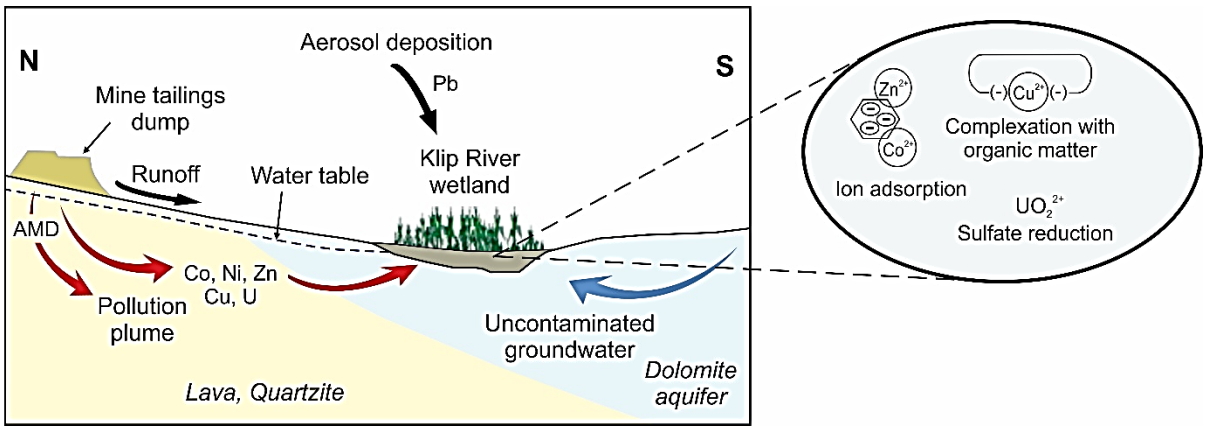

Figure 2.9: Conceptual hydrogeological model showing the main transport pathways of metal contaminants to the Klip River wetland (diagram not to scale).

The extensive accumulation of metals within the Klip River wetland reflects the contaminant legacy associated with gold mining on the Witwatersrand that has spanned over a century. While the efflux of AMD is a particularly difficult and costly problem to control, this study highlights the effectiveness of natural wetlands in trapping vast quantities of toxic pollutants and remediating downstream waters. From this perspective, the Klip River wetlands are undoubtedly some of the most valuable natural assets in Johannesburg. However, wetlands have the potential to release trapped pollutants, particularly if the natural biogeochemical conditions favouring metal sequestration are disrupted. Preventing erosion and further deterioration of the

Klip River wetlands is, thus critical for the protection of freshwater resources in the region, particularly as the contaminant plumes associated with TSFs will likely persist for decades. Despite 130 years of mining, the Witwatersrand goldfield is still estimated to contain in the order of 30 000 tonnes of gold, representing almost 45% of the world's known gold deposit (Viljoen, 2009). Although much of this gold is found at depth and in lower-grade narrow reefs, mining and metallurgical advancements could revitalise operations in the future. Under such circumstances, the role of the Klip River wetland in trapping toxic metals emanating from the Central Basin will be critical.

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Chapter 3

Tracing sources of lead pollution in the Klip River catchment, Johannesburg

Abstract

South Africa is a water-scarce, high-energy-demand country with mining as one of its' most serious environmental issues. Stable Pb isotopes are increasingly used to differentiate between natural and anthropogenic sources of Pb, one of the most common pollutants in South Africa. Lead concentrations and isotope compositions were measured from the Klip River wetland system, a highly contaminated system, Johannesburg, South Africa, impacted by urban and industrial pollution. Isotopic ratios ($^{208}Pb/^{207}Pb$, $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$) were used to identify major sources contributing to Pb contamination in the wetland with potential sources, including alkyl lead (fuel), coal, geological material, paint, industrial input and urban incineration. The isotopic composition of $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ in the wetland ranged between 1.13 – 1.32 and 2.34 – 2.43, respectively. These ratios were similar to those in the tributaries (1.14 – 1.31 and 2.35 – 2.41) but lower than the tailing storage facilities (TSFs) (2.51 - 3.06 and 1.78 – 1.95). This was compared to Pb isotope ratios for South African fuel, coal, and natural geological material. The predominant end members for source apportionment were alkyl lead ($^{206}Pb/^{207}Pb$ 1.06 – 2.34 and $^{208}Pb/^{207}Pb$ 1.07 – 2.44), coal ($^{206}Pb/^{207}Pb$ 1.21 – 1.23 and $^{208}Pb/^{207}Pb$ 2.44 – 2.49) and Witwatersrand geologic ($^{206}Pb/^{207}Pb$ 0.82 – 2.92 and $^{208}Pb/^{207}Pb$ 1.61 – 2.30). Isolating the wetland samples which contained the highest concentrations of Pb in the system (2.74 – 283 mg kg^{-1}) from the rest, reveals predominant coal and alkyl lead (fuel) signatures, with minor contributions of the Witwatersrand geology.

***Key words**: lead, isotopes, source tracing, pollution, sediment*

3.1. Introduction

Lead emissions in South Africa, result predominantly from mining and coal burning activities, as well as the historical use of alkyl-leaded fuels (Witt *et al*., 2006). It may also be a by-product of manufacturing and smelting (Félix *et al*., 2015; Martin *et al*., 2014). Lead in the environment is a toxic, persistent pollutant (Cheng and Hu, 2010; Komárek *et al*., 2008; Tong *et al*., 2000) that bioaccumulates in organisms (Wani *et al*., 2015). Understanding sources, movement and fate of Pb in the environment is therefore crucial in understanding the long-term effects and mitigation of its impacts. Stable Pb isotopes (^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb) have been successfully used to trace source contamination, migration and transport pathways, and release timings in different environmental matrices (Cheng and Hu, 2010; Vecchia *et al*., 2017). As Pb isotopic signatures are not significantly affected by physical or chemical fractionation, they are particularly useful in tracking pollution sources (Cheng and Hu, 2010; Vecchia *et al*., 2017). Broad isotopic ratio ranges can be used to distinguish between natural and anthropogenic sources, while specific ratios have been successfully used in numerous studies to classify and identify pollutant sources (Cheng and Hu, 2010; Díaz-Somoano *et al*., 2009; Kong *et al*., 2018; Monna *et al*., 2006). In South Africa, isotopic Pb ratios have been derived for coal (Díaz-Somoano *et al*., 2009), petroleum (Monna *et al*., 2006), geological material (Barton and Hallbauer, 1996; Coetzee *et al*., 2004; Koppel and Saager, 1974; Louw, 1954), blood (Hess *et al*., 2013), and paint (Díaz-Somoano *et al*., 2009). They have been successfully used to pinpoint the dispersion of coal in the atmosphere and tracing contaminated water from uranium mining processes (Mathuthu and Khumalo, 2018; Vecchia *et al*., 2017). This research has provided the reference and background ratios used for comparison in our study.

In this study, potential sources contributing to Pb contamination in the Klip River wetland system were investigated. The Klip River wetland has previously been identified as being heavily impacted by mining and industrial pollution (Humphries *et al*., 2017; McCarthy *et al*., 2007; Vermaak, 2009). Wetland sediments accumulate high quantities of Co, Ni, Zn, and U, transported in acid mine drainage (AMD) plumes and infiltrate the wetland through groundwater (Chapter 2). High concentrations of Pb (2.74 – 283 mg kg^{-1}) also accumulate in the wetland, although spatial variations in accumulation indicate that Pb is not predominately associated with AMD (Chapter 2). The aim of this study was to use isotopic compositions to

determine the origin of Pb pollution in the Klip River wetland and thereby identify important sources of Pb pollution impacting South Africa's most populous city.

3.2. Methods

3.2.1. Study site

The Witwatersrand Basin is host to one of the largest goldfields in the world (Frimmel, 2019). Witwatersrand gold-bearing conglomerates have been mined for over a century leaving the natural topography of the region modified by mine dumps (Durand, 2012; McCarthy, 2010).Pollution sources arising from the Witwatersrand basin are predominantly by-products of anthropogenic activities and urban waste. Vehicular emissions in the region are elevated due to the dense population and the expansion of the city. Combustion of coal is used as a primary source of energy generation for both industries and residential areas. Informal settlements are located along tributaries and rivers in the province with direct input of waste and waste products into the waterways. The development and urbanisation of the area have also introduced and increased anthropogenic pollution load on the system.

The city of Johannesburg was developed central to mining activity with mining operations split into the West Rand, the Central Rand and East Rand. The Central Rand mining activity can be found south of the city of Johannesburg. Run-off from the Central Rand Goldfield discharges into wetlands along the Klip River, the principal drainage of the southern portion of Johannesburg. The Klip River (Figure 3.1A) drains into the Vaal River, and is one of the most economically important wetlands in South Africa as it reduces costs of water purification especially run-off from mining activities (McCarthy *et al*., 2007).

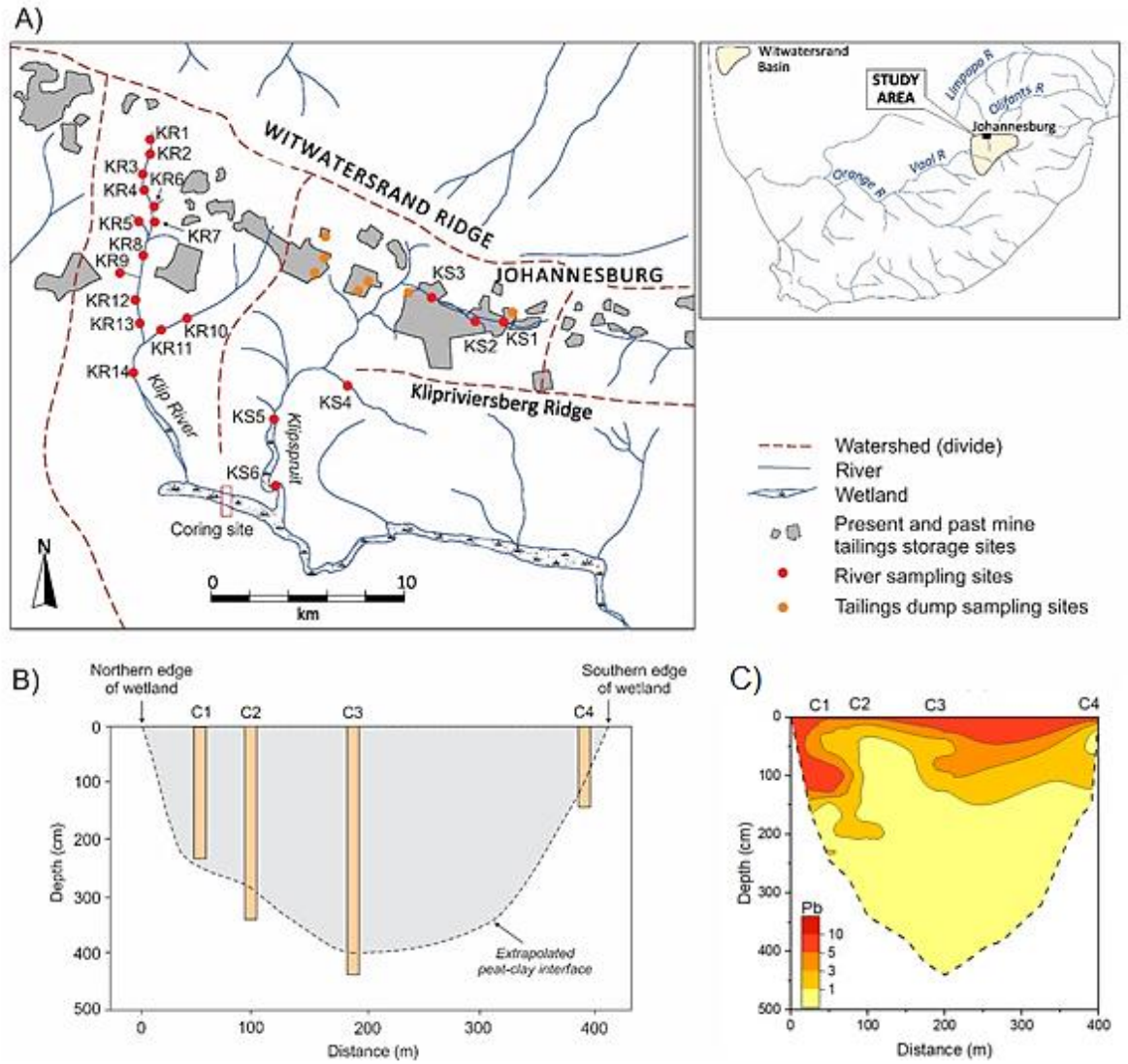

Figure 3.1: A) Map of the study area showing the drainage network of the upper Klip River catchment in the Johannesburg area. The location of water and sediment sampling sites along the Klip River (KR1 – 14) and Klipspruit (KS1 – 6) are also indicated. B) Cross-sectional transect across the Klip River wetland showing the position of the four cores (C1 – 4) collected. C) Distribution in Pb enrichment across a section of the Klip River wetland.

3.2.2. Sample collection

Surface sediment samples were collected from the upper Klip River (KR1-14) and Klipspruit (KS1-6) tributaries during March 2017 (Figure 3.1A). Sampling sites were established based on accessibility and in an attempt to capture downstream variations. Surface material from several mine TSFs in the upper catchment was also sampled. Of these, three TSFs samples, two Klipspruit samples and three Klip River samples were subsequently selected for Pb isotope analysis.

A series of four sediment cores (C1 – 4) were collected along a 350 m north-south orientated transect established in the upstream, western section of the Klip River wetland (Figure 3.1B). This section of the wetland is characterised by intact reed-covered swamp dominated by *Phragmites australis* and is considered to have remained relatively undisturbed for the last ~80 years based on examination of historical aerial photography and maps (McCarthy *et al*., 2007). Samples were collected to refusal depth using a Russian peat corer. Sub-samples were taken at 10 cm intervals and sealed in polyethylene bags for subsequent analysis. Pb isotope analysis was conducted on selected samples from C1 (0 cm and 20 cm), C3 (0 cm, 70 cm, 140 cm, 220 cm, 330 cm, and 440 cm) and C4 (0 cm) to characterise the Pb isotope composition of wetland sediments. These cores were selected to determine the Pb isotopes across the wetland.

3.2.3. Enrichment Factors

Variations in sample composition and mineralogy, metal concentration was expressed as an enrichment factor (EF). Enrichment factors were calculated relative to upper continental crust (UCC) values (Wedepohl, 1995) using Li as the normalising element (Equation 1). Lithium behaves conservatively in the environment and is not associated with mine waste.

$$\mathrm{EF} = \frac{\left[\frac{C_{Pb}}{C_{Li}}\right]_{Sediment}}{\left[\frac{C_{Pb}}{C_{Li}}\right]_{UCC}} \qquad \text{(Equation 1)}$$

Calculated EF values were assessed and classified into seven categories: (1) <1: no enrichment; (2) 1-3: minor enrichment; (3) 3-5: moderate enrichment; (4) 5-10: moderately severe enrichment; (5) 10-25: severe enrichment; (6) 25-50: very severe enrichment;

(7) > 50: extremely severe enrichment (Barbieri, 2016; dos Santos *et al*., 2017). Analysis of variance (ANOVA, $p < 0.05$) was used to examine the relationships between metal concentrations.

3.2.4. Pb isotope analysis

All laboratory treatments were conducted in a clean-room environment at the University of the Witwatersrand Isotope Geosciences Laboratory (WIGL) following standard analytical protocols. Briefly, 250 mg powdered samples, obtained by milling air-dried samples, were digested in 3 mL distilled HF in sealed Teflon® vessels on a hot-plate at 100°C for two days Concentrated HNO_3 was added to the vessels before evaporation to dryness. This process was repeated three times (Smet *et al.*, 2010; Varga *et al.*, 2009). Samples were reconstituted in 0.5 mL HBr and lead was separated from the matrix in HBr media using AG® 1-X8 200-400 mesh resin (Bio-Rad). The Pb isotope fraction was collected using 0.6 M HCl and subsequently evaporated to dryness and diluted with 2% HNO_3. Due to high Pb concentrations in the samples, spiking with known Pb isotopic standards was not done. All chemicals were double distilled in the WIGL facility before use while the water was ultrapure. Blanks (n = 3) and the standards NIST SRM981 and USGS BCR2, were processed following the same sample preparation.

Pb isotopes were measured using a Nu Plasma II MC-ICP-MS (Nu Instruments) at the University of Johannesburg (Table 3.1). Samples and standards were introduced in a 2% HNO_3 solution using wet plasma. The nebuliser was coupled with a Peltier cooled quartz (Glass Expansion) Twister spray chamber at 7°C. Thallium (62.5 µg L^{-1}) was used as an internal standard to correct for signal drift and instrument instability. Isobaric interferences of $^{204}Hg^{2+}$ with $^{204}Pb^{2+}$ arising from sample preparation were monitored. Blank Pb concentrations (n = 3) were below 1% indicating no residual carry-over of analyte between sample sets. Standard bracketing data reduction technique was used to correct the Pb data every five samples using SRM981. The isotopic Pb ratios were compared to the certified values of the certified reference material to obtain a correction factor. The correction factors ranged from 0.973 – 0.992. The correction factors were then applied to the samples to obtain the correct isotopic ratios. Precision and accuracy for Pb isotopic ratios were within the accepted range (Table 3.2).

Table 3.1: Instrumental operating conditions for the Nu Plasma II MC-ICP-MS

Instrument	Nu Plasma III MC-ICP-MS
RF Power	1300 W
Reflected Power	1 W
Accelerating voltage	6000 V
Analyser pressure	~3×10^{-8} mbar
Cones	Nickel
Nebuliser	Glass Expansion MicroMist U-series
Sample uptake rate	200 µL min^{-1}
Faraday cups	16
Ion counters	5
Scans	40

Table 3.2: Certified and determined Pb isotope ratios with correction factors for SRM981 and BCR2 standards

	SRM981 (n = 7)			**BCR2 (n = 2)**		
	Pb^{206}/Pb^{207}	Pb^{208}/Pb^{207}	Pb^{208}/Pb^{206}	Pb^{206}/Pb^{207}	Pb^{208}/Pb^{207}	Pb^{208}/Pb^{206}
Certified	1.0933	2.3704	2.1681	1.2008	2.4778	2.0635
Measured	1.1239	2.3696	2.1682	1.2005	2.4789	2.0640
±2σ	0.0006	0.0016	0.0005	0.0035	0.0032	0.0034
Correction factor	1.0068	0.9932	0.9866	1.0067	0.9934	0.9869

3.3.Results

The TSFs indicate minor to moderately severe enrichment to the system showing the impact of anthropogenic input to the environment (Table 3.3)The Pb isotopic composition of tributary samples collected from the Klip River and Klipspruit varied between 1.14 and 1.30 ($^{206}Pb/^{207}Pb$) and between 0.47 and 0.56 ($^{208}Pb/^{206}Pb$) (Table 3.3). Wetland surface samples were similarly characterised by a narrow range in isotopic composition, ranging from 1.128 – 1.154 ($^{206}Pb/^{207}Pb$) and 0.474 – 0.480 ($^{208}Pb/^{206}Pb$). Considerably higher $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ ratios were measured in material collected from TSFs compared to wetland and tributary samples, with average ratios of 2.81 ± 0.28 ($^{206}Pb/^{207}Pb$) and 1.51 ± 0.22 ($^{208}Pb/^{206}Pb$).

Overall, tributary and wetland samples were isotopically similar but distinct from TSF material (Figure 3.2A). Natural or background Pb isotope signatures in U-rich geology typically have $^{206}Pb/^{207}Pb$ ratios > 1.2, while anthropogenic sources are typically characterised by lower values (Kong *et al*., 2018; Mathuthu and Khumalo, 2018, Zheng *et al*., 2007). The wetland and tributary samples indicate a more anthropogenic input while the TSF samples have a distinct natural background (Figure 3.2B).

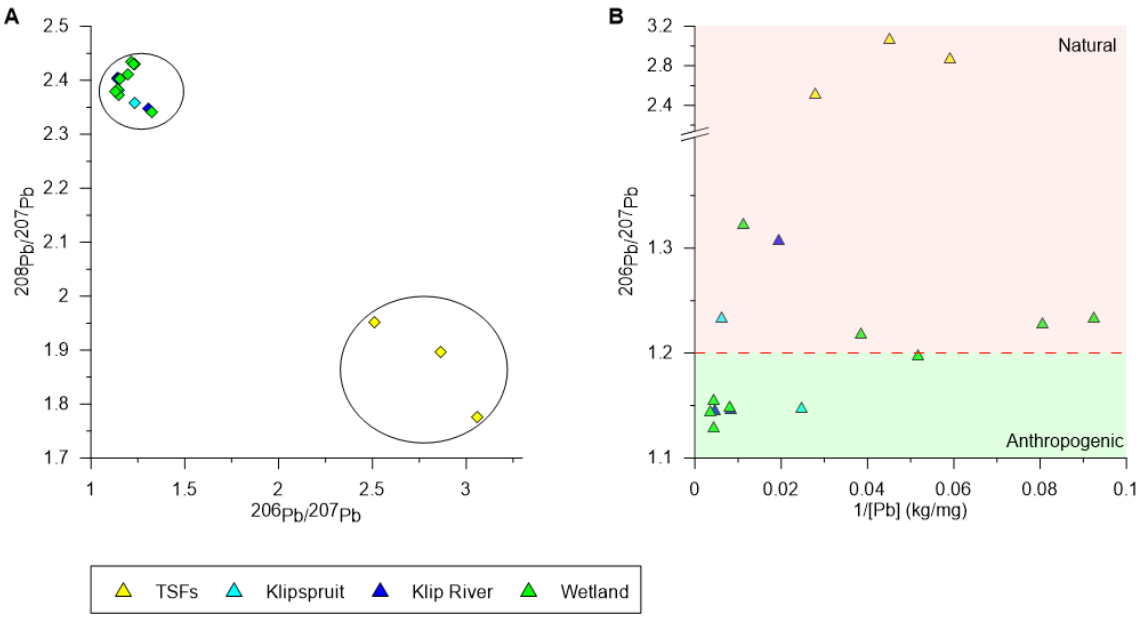

Figure 3.2: (A) $^{208}Pb/^{207}Pb$ ratio plotted against the $^{206}Pb/^{207}Pb$ ratio for samples from the TSFs, Klip River, Klipspruit and the wetland cores; (B) Natural and anthropogenic source discrimination of samples using $^{206}Pb/^{207}Pb$ against 1/[Pb] as indicated by the $^{206}Pb/^{207}Pb$ ratio of 1.2 (Red line) (Zheng *et al*., 2007).

Table 3.3: Lead total concentrations and enrichment factors (EF) and isotopic composition of surface and wetland sediment samples

Site / Sample Depth	[Pb] (mg kg^{-1})	EF	$^{208}Pb/^{207}Pb$ ($\pm 2\sigma \times 10^{-5}$)	$^{206}Pb/^{207}Pb$ ($\pm 2\sigma \times 10^{-5}$)	$^{208}Pb/^{206}Pb$ ($\pm 2\sigma \times 10^{-5}$)
TSFs					
T1	22.2	1.8	1.776 (± 6)	3.058 (± 10)	1.722 (± 6)
T4	16.9	6.2	1.897 (± 9)	2.867 (± 13)	1.511 (± 7)
T7	35.8	2.7	1.952 (± 7)	2.509 (± 8)	1.285 (± 5)
Klipspruit					
KS1	160	2.8	2.358 (± 9)	1.232 (± 5)	0.522 (± 2)
KS4	40.5	0.16	2.381 (± 13)	1.147 (± 6)	0.481 (± 3)
Klip River					
KR1	212	10.2	2.405 (± 12)	1.144 (± 5)	0.476 (± 2)
KR8	122	8.7	2.402 (± 8)	1.145 (± 3)	0.477 (± 2)
KR10	51.7	1.4	2.347 (± 14)	1.306 (± 7)	0.557 (± 3)
C1					
0 cm	123	4.6	2.403 (± 10)	1.154 (± 5)	0.480 (± 2)
20 cm	262	6.9	2.374 (± 13)	1.148 (± 6)	0.484 (± 3)
C3					
0 cm	283	8.1	2.384 (± 9)	1.143 (± 4)	0.479 (± 2)
70 cm	88.8	3.9	2.341 (± 8)	1.322 (± 4)	0.564 (± 2)
140 cm	10.8	0.40	2.429 (± 10)	1.232 (± 5)	0.507 (± 2)
220 cm	26.1	0.54	2.434 (± 11)	1.218 (± 5)	0.500 (± 2)
330 cm	19.3	1.1	2.410 (± 12)	1.197 (± 6)	0.497 (± 3)
440 cm	12.4	0.47	2.430 (± 10)	1.227 (± 5)	0.505 (± 2)
C4					
0 cm	225	7.81	2.380 (± 11)	1.128 (± 5)	0.474 (± 2)

TSFs = Tailing Storage facilities

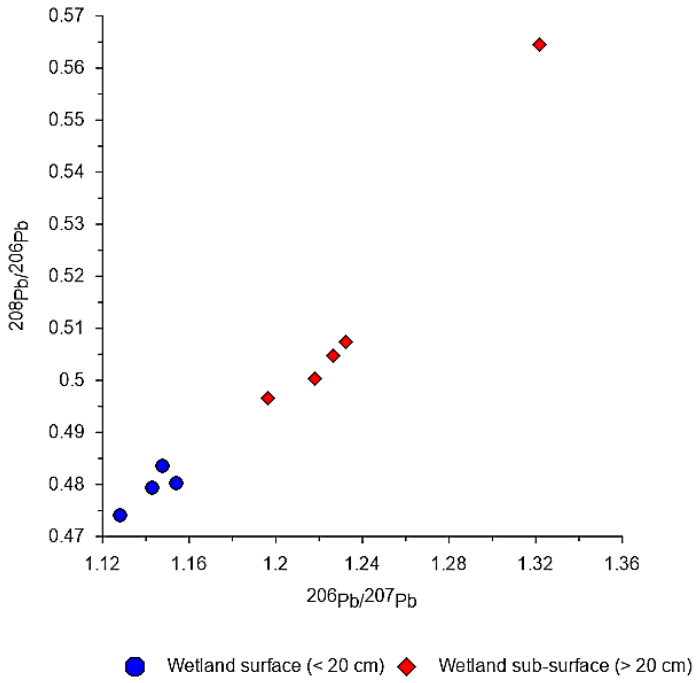

Figure 3.3: Relationship between $^{208}Pb/^{206}Pb$ and $^{206}Pb/^{207}Pb$ indicating a linear trend between the wetland surface (< 20 cm) and wetland sub-surface (> 20 cm) sediment samples.

Downcore variations in Pb isotope composition reveal that wetland surface sediments are typically characterised by lower $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}Pb$ ratios relative to the underlying material (Figure 3.3). A linear trend is observed ($R^2 = 0.9391$) between the core surface and core sub-surface samples.

3.4. Discussion

3.4.1. Surface concentrations and enrichment

The Benguela Current Large Marine Ecosystem (BCLME), in 2006, which encompasses Angola, South Africa and Namibia investigated the different sediment quality guidelines in an attempt to define sediment quality guidelines for the African coast and it was proposed that South Africa used the values stipulated by the NOAA (Taljaard, 2006). These guideline values fall between three ranges by two threshold limits; the "effects range low" (ERL) and the "effects range median" (ERM). Below the ERL value, organisms exhibit minimal toxic effects and

values above the ERM indicate toxic effects on biological organisms (Long *et al.* 1995). Pb surface concentrations in the Klip River wetland are elevated and well above the South African ERM guidelines (218 mg kg^{-1}) for sediment (Long *et al*., 1995). Enrichment factors indicate anthropogenic input however, the difference in its entry (*via* surface input) into the wetland compared to other metals (groundwater) suggests multiple sources of Pb contaminants (Muzerengi, 2017). Additionally, the decreasing EF values downstream from the TSFs compared to the values for the wetland support this.

3.4.2. Potential sources of Pb pollution

There is a multitude of sources of Pb from industrial applications. However, Johannesburg has no urban incinerators, smelters, Pb ore exploitation, or heavy metal industries that use Pb or produce it as a waste by-product (Monna *et al*., 2006), thus eliminating them as potential sources. The major sources considered were natural mineralogy, coal- and petroleum-based applications.

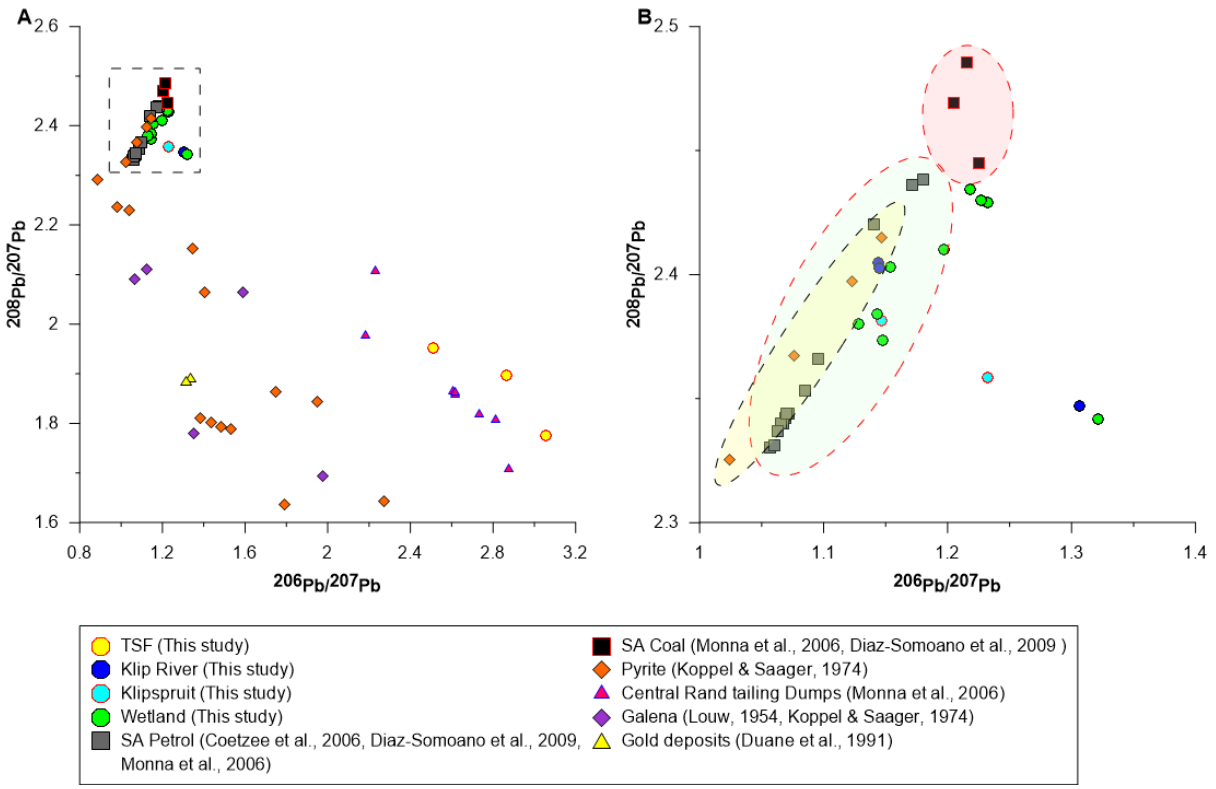

Figure 3.4: (A) Pb source apportionment using a plot of $^{208}Pb/^{207}Pb$ ratio against the $^{206}Pb/^{207}Pb$ ratio for the Klip River system, and (B) expanded area of the wetland samples as indicated by the area encompassed by the block in (A). Field lines of major sources (petroleum (blue), coal (red) and pyrite (yellow)) are indicated.

3.4.2.1. Natural sources

Natural sources of Pb are derived from the geology of the region and are composed of a combination of minerals (galena and pyrite), rocks (quartzite, shale, dolomite, and basalt) and gold deposits (Humphries *et al*., 2017; McCarthy and Venter, 2006) and have isotopic signatures specific to this region. Other contributions arise from the gold mining process, including quartz pebble conglomerates, uraninite and pyrite (Mineeva, 2005). TSFs are a composite of these minerals generated from mining waste (Vriens *et al*., 2020). The isotopic signature for the TSFs is significantly different from the other samples (Figure 3.4A) and reflects values closer to the natural geology of the Witwatersrand Basin. These samples generally have higher radiogenic Pb compositions ($^{206}Pb/^{207}Pb$) due to the U parent material, thus making their Pb isotopic composition unique (Coetzee *et al*., 2006). The TSFs samples have a close correlation with the Central Rand tailing dumps (Monna *et al*., 2006). Samples from the Klip River (KR1 and KR8), which are in close proximity to the TSFs have similar ratios to pyrite (Figure 3.5B). Galena, pyrite and gold deposits were the key natural sources of Pb in the system.

3.4.2.2. Anthropogenic sources

The potential sources of Pb in the Klip River system are coal, alkyl Pb and pyrite (Figure 3.4B). Field lines were used to identify the potential regions of influence for the coal (red) and alkyl Pb (blue) sources. Samples with isotopic $^{206}Pb/^{207}Pb$ ratios between 1.232 and 1.322 represent samples that are appreciably influenced by natural sources in addition to coal and petroleum.

Wetland samples cluster around the South African coal, petroleum and the Witwatersrand pyrite signatures (Figure 3.4B). These sources exhibit a collinear relationship ($R^2 = 0.9629$). Although alkyl lead-based fuel (petroleum) was completely phased out in 2006 in South Africa, it is the major source of Pb to wetland samples. Coal is the minor signature for wetland samples. It has been reported as a contributor to Pb in the atmosphere around the country (Monna *et al*., 2006).

Alkyl lead may be persistent in the environment for decades (Filella and Bonet, 2017). It degrades in the atmosphere and water to inorganic Pb and may remain airborne for up to 10 days before wet or dry deposition (Filella and Bonet, 2017) implying long range transport and contamination. In soils, alkyl lead may be present at low concentrations, which are eventually either transformed or undergo microbial degradation to inorganic Pb (Filella and Bonet, 2017). Very rarely, alkylation of Pb may be mediated by microbes under anaerobic conditions (Smith, 1995), thus introducing them into the environment. Rapid industrialisation and population growth in South Africa has increased the demand for energy based resources and coal, further promoting Pb release into the environment. The mobility of Pb is greater in air and water, allowing for dispersion over longer ranges. In soil, it is generally precipitated with oxyhydroxides (Martín *et al*., 2014).

3.4.3. Implications of Pb pollution

The two anthropogenic sources of Pb, coal and petroleum, and the natural source, geological material, contribute to the Pb present in the Klip River wetland system. The mobility of Pb is greater in air and water compared to soil, which immobilizes Pb. The migration of Pb from the wetland *via* surface water is of concern due to the solubility of Pb and the toxicity of the metal. South Africa continues to rely on coal as an energy source, thus the introduction of Pb into the atmosphere is inevitable. An increase in the population would require more resources, thus a greater input of Pb into the atmosphere. The constant cycling and further introduction of Pb into the wetland would increase its potential for water solubility, thus ingestion and toxicity. The transformation of Pb species introduces a multitude of environmental consequences, thus further research would be required to determine the transformation and movement of Pb species.

3.5. Conclusions

Lead contamination can arise from various sources, which can be traced using Pb isotope ratios. While South Africa does not have a large lead isotope database, this study was able to successfully utilize stable Pb isotope ratios to differentiate between natural and anthropogenic sources and further discriminate between individual pollutant groups. The isotopic signatures were related to the composite geological parent material, coal and alkyl lead based fuel; the latter sources predominant on the wetland surface. South Africa should see a gradual decline in the alkyl Pb-based signature; however, there are several isotopic signatures, which would add value to this research, including Pb-based signatures from vehicular emissions, waste incineration and domestic and industrial wastewater.

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Chapter 4

An investigation into arsenic speciation in a wetland impacted by acid mine drainage

Abstract

The Klip River wetland system has been exposed to acid mine drainage (AMD) resulting from extensive mining activities within the Witwatersrand Basin, Johannesburg, South Africa. Well-documented sources of AMD include metal leachate from tailing storage facilities (TSFs) and flooded mine voids. The resultant metal accumulation, together with AMD has contributed to the degradation of this wetland, altering the water chemistry, and influencing the biodiversity. Arsenic, a common by-product of AMD-chemistry, is a well-documented toxic metal with severe impacts to both humans and animals. To understand the fate of As in the wetland, we investigated its concentration, bioavailability and speciation in samples from several TSFs, sediments from two river tributaries (the Klip River and Klipspruit) feeding into the wetland and surface and core samples within the wetland. Arsenic enters the wetland through groundwater inflow. Total As concentrations in TSFs ranged between 77 - 106 mg kg^{-1}, 10 – 90 mg kg^{-1} in the tributaries and an average of 34 ± 25 mg kg^{-1} in the wetland. Despite high concentrations present in the TSFs, As bioavailability in the system was fairly low (3 – 17 %) except in the Klip River sediment (5 – 74%). The As species (As(III), As(V), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)) in surface and core sediment from tributaries and wetland were measured using Ion Chromatography Inductively Coupled Plasma Mass Spectrometry (IC-ICP-MS). Identified species present in the wetland were As(III), As(V) and MMA with concentrations ranging between 0.028 – 1.18 mg kg^{-1}, 0.076 – 7.36 mg kg^{-1}, and 0.012 – 0.22 mg kg^{-1}, respectively. One unidentified peak (postulated to be an As-sulfur species) was present with As likely complexed to a sulfide or sulfate. The predominant As species in the wetland is As(V), and speciation appears dependant on both redox potential and microbial activity (inferred by S concentrations). These processes limit the bioavailability of As in the wetland and play an important role in the sequestration process.

***Key words**: arsenic, speciation, acid mine drainage, arsenic geochemistry, sediment*

4.1. Introduction

Elevated levels of arsenic are commonly associated with gold-bearing ores and co-exist with heavy metals due to its affinity for sulfides and iron hydroxides (Sami and Druzynski, 2003). Mining activities accelerate the natural weathering process of these minerals and the consequent formation of acid mine drainage (AMD) results in the oxidative dissolution of sulfide-bearing minerals, releasing As into the environment (Paikaray, 2015). The speciation of As has a significant influence on its fate and toxicity in the environment. There are a range of oxidation states in which As exists (-3, 0, +3, +5) with arsenite and arsenate (+3 and +5) being the predominant states (Smedley and Kinniburgh, 2001). The trivalent oxidation state is generally considered to be more toxic than the pentavalent state, and the inorganic forms significantly more toxic than the organic forms (monomethyl arsenic (MMA), dimethyl arsenic (DMA), arsines, arsenosugars, trimethylarsine oxide (TMAO), tetramethylarsonium ion (Ma_4As^+) arsenocholine (AsC) and arsenobetaine (AsB)) (Nguyen *et al*., 2018; Paikaray, 2015). The wide-ranging impact of AMD on sediment geochemistry, particularly changes in acidity, and Fe-Mn oxide precipitation, together with the redox sensitive nature of As makes understanding the behaviour of this element complex (Pillay and Kindness, 2016; Yano *et al*., 2020; Zhang *et al*., 2020).

Mobility of As is controlled by a variety of processes, including redox reactions; adsorption and desorption; ion exchange; precipitation and dissolution; and biological activity (Smedley and Kinniburgh, 2001). These processes are often interlinked and several may be occurring simultaneously. Adsorption and precipitation of arsenic is also dependent on the amount of clay minerals present and the soil pH (Fendorf *et al*., 2010; Neil *et al*., 2014; Pillay and Kindness, 2016). These parameters dictate the speciation and/or dissolution of As minerals (Barral-Fraga *et al*., 2020; Pillay and Kindness, 2016). Under anoxic conditions, the more toxic As(III) species form the predominant arsenic compounds while As(V) is more stable under oxic conditions (Smedley and Kinniburgh, 2001). Under reducing conditions, sulfides present, together with Fe and As may form As-S complexes (thioarsenites) which precipitate from solution (Pillay and Kindness, 2016; Saunders *et al*., 2018; Wilkin *et al*., 2003). Arsenous acid ($As(OH)_3$) undergoes substitution reactions with thiol groups (SH^-) to produce various thiol-As complexes (Reactions 1-6). Chain substitution reactions (Reactions 1-4) with additional thiol groups forms

sulfarsenite (AsS_3^{3-}) (Reaction 5). This can either terminate the chain reaction or continue to react and form a complete thioarsenate ($As(SH)_4^-$) (Reaction 6).

Reaction 1: $As(OH)_3^0 + HS^- + H^+ \rightarrow As(OH)_2(SH)^0 + H_2O$
Reaction 2: $As(OH)_3^0 + HS^- \rightarrow As(OH)_2S^- + H_2O$
Reaction 3: $As(OH)_2S^- + HS^- \rightarrow As(OH)S_2^{2-} + H_2O$
Reaction 4: $As(OH)S_2^{2-} + HS^- + H^+ \rightarrow AsS_3H^{2-} + H_2O$
Reaction 5: $As(OH)S_2^{2-} + HS^- \rightarrow AsS_3^{3-} + H_2O$
Reaction 6: $AsS_3H^{2-} + HS^- + 2H^+ \rightarrow As(SH)_4^-$

Inorganic arsenic tends to adsorb to metal oxides in sediments removing the As from solution (Smedley and Kinniburgh, 2001). However, As and sulfides may also form, which then has an impact on the solubility and mobility of As in the environment. Microbial activity may also result in the oxidation, reduction or methylation of arsenic species (Zhang *et al*., 2015). Bacteria, which thrive in acid rich environments may reduce or oxidise Fe, S, and As changing the speciation and in some instances producing volatile methylated compounds.

Microorganisms within the wetland rapidly adapt to the contamination of the system and develop coping mechanisms to survive and thrive. Adaptions of these microorganisms, include genetic, intrinsic biochemical, structural, and physiological properties (Aktan *et al.*, 2013). Metal resistance mechanisms, include exclusion of toxic heavy metal ions from the cell by the alteration of membrane transport systems involved in initial cellular accumulation, extra- and intra-cellular sequestration of metal binding components similar to metallothioneins. cation/anion efflux systems that are encoded by resistance genes, and enzymatic detoxification of heavy metals from toxic to less toxic forms (Hynninen *et al*., 2009).

The Witwatersrand Basin, located in the Gauteng province, is the largest gold reserve in the world and has been extensively mined (Frimmel, 2019). Water resources and wetlands in the region have been severely affected by AMD (McCarthy and Venter, 2006). One of the most prominent of these is the Klip River wetland system, which has been reported to be in critical condition due to the accumulation of pollutants and deterioration of the site by anthropogenic activities, including dumping and development (Humphries *et al*., 2017; McCarthy and Venter, 2006). Elevated concentrations of metals and organic pesticides have been reported. Our earlier

work (Chapter 2) has shown that metals (Cu, Co, Ni, Pb, U and Zn) are significantly enriched in the river tributaries and wetland, transported *via* groundwater plumes and sequestered in the sediment.

The objective of this study was to better understand the behavior, fate and mobility of As in a wetland heavily affected by anthropogenic activities. Specifically, the changes in As speciation due to AMD and (related to biogeochemical processes) were investigated in sediment.

4.2. Methods

4.2.1. Study Area

The Klip River catchment is a sub-basin of the Vaal-Orange River system and represents the most significant drainage system for the southern Witwatersrand region (Figure 4.1A) (Vermaak, 2009). The Klip River and Klipspruit are the primary tributaries draining the Central Witwatersrand mining-industrial complex and discharge into the extensive wetland area downstream. The Klip River wetland drains into the Vaal River. The river is a key water resource and supplies approximately 23% of the South African population with potable drinking water (Chinyama *et al*., 2016).

TSFs represent important sources of pollution within the Witwatersrand Basin and are typically associated with extensive acid plumes that contaminate the local groundwater (Govender, 2011; McCarthy, 2011). Mining and industrial activities are located, predominantly to the north of the wetland and in close proximity to river tributaries (McCarthy *et al*., 2007). The waterways contain leached metals from TSFs in surface and groundwater, which ultimately enter the Klip River wetland (Humphries *et al*., 2017). Groundwater from underlying aquifers is also extracted for domestic consumption and informal settlements use untreated river water for domestic and agricultural purposes (Abiye *et al*., 2011a). Additionally, the Klip River wetland receives runoff from several large informal settlements and industrial regions, as well as discharge from three sewerage treatment plants.

Sewage plants and direct communal discharge into the rivers promote the occurrence of microorganisms. The presence and identification of bacteria have reported in the Klip River wetland with the identification of 15 different species (Chihomvu *et al.*, 2014). Three of these 15 bacteria have been identified as microorganisms capable or responsible for arsenite oxidation, which include *Acinetobactor species* (Chihomvu *et al.*, 2014, Nguyen *et al*., 2017) and *Pseudomonas species* (Chihomvu *et al.*, 2014, Rehman *et al.*, 2010).

4.2.2. Sampling

Surface sediment (n = 7) samples were collected from the upper Klip River (KR1, KR2, KR3, KR4) and Klipspruit (KS1, KS2, KS3) tributaries during March 2017 (Figure 4.1B). Sediment samples were placed in bags and stored at 4 °C. Surface material from three TSFs (T1, T2 and T3) in the upper catchment were also sampled (Figure 4.1B). A series of four sediment cores (C1 – 4) were collected along a 350 m north-south orientated transect established in the upstream, western section of the Klip River wetland (Figure 4.1B). Samples were collected to refusal depth using a Russian peat corer. Sub-samples were taken at 10 cm intervals and sealed in plastic bags for subsequent analysis.

Porewater was extracted by centrifuging the sediment sub-samples at 1500 rpm for 15 minutes and preserved by storing below 4°C (Steiner *et al*., 2018). The pH of sediment, core sub-samples and (where possible) the redox potential of porewater samples were measured using a portable combination meter (Thermo Scientific Orion Star) calibrated against appropriate standard solutions.

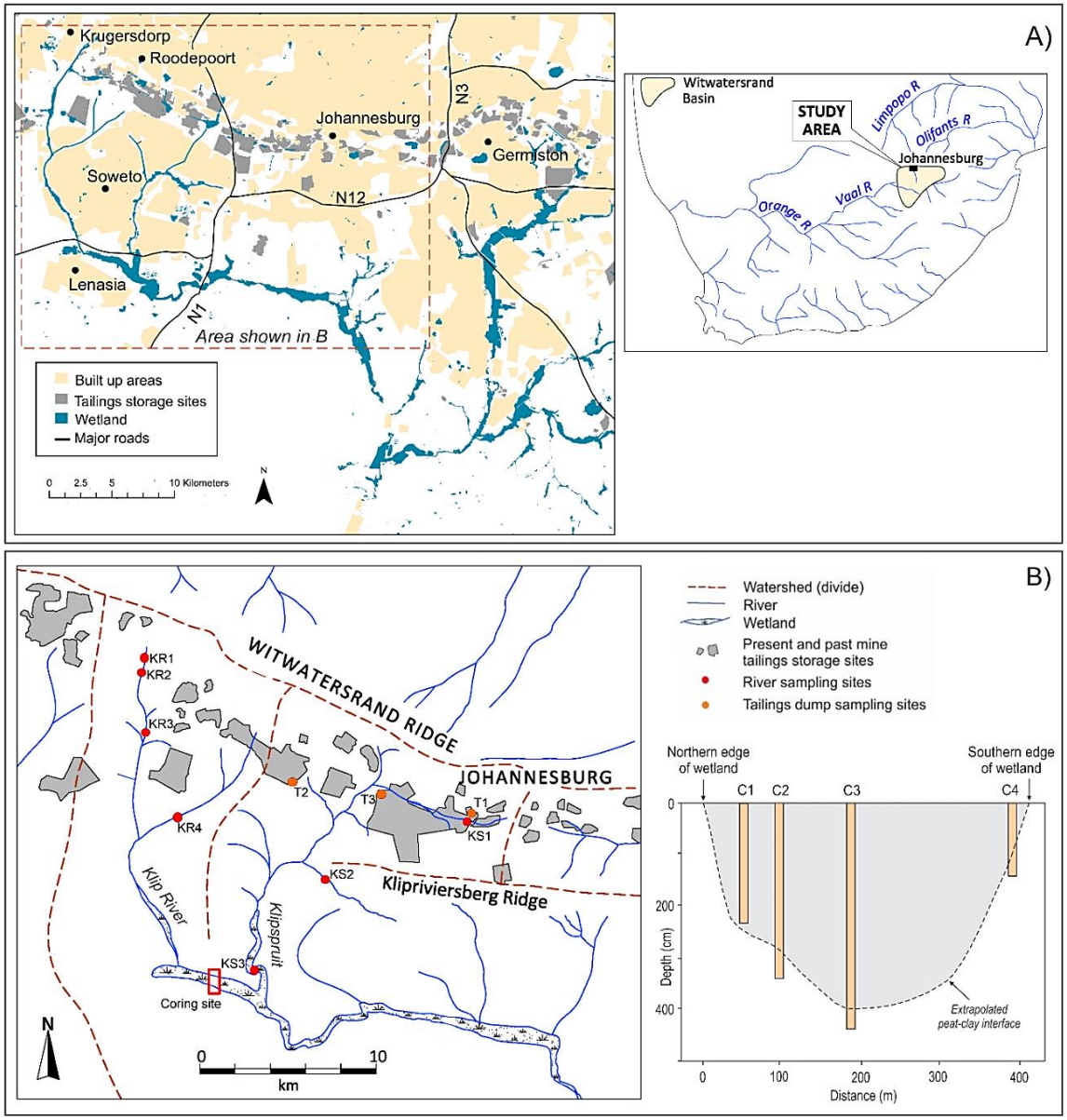

Figure 4.1: (A) Map of the Witwatersrand Basin showing the urbanised areas, wetland, tailing storage facilities and national roads, (B) Map of the study area showing the drainage network of the upper Klip River catchment in relation to mining activities in the Johannesburg area. The location of water and sediment sampling sites along the Klip River (KR1 – KR4) and Klipspruit (KS1 – KS3) are also indicated. Cross-sectional transect across the Klip River wetland showing the position of the four cores (C1 – 4) collected.

4.2.3. Chemical analysis

4.2.3.1. Total As concentrations

Tributary and wetland sediment samples were air-dried, milled and combusted at 500 °C for 4 hours to determine loss on ignition (LOI). Combusted sample powders (~25 mg) were microwave digested in 9 mL HF:HNO_3:HCl (6:3:1) using an Anton Paar Multiwave Go system (Balaram and Rao, 2003b). Solutions were evaporated to dryness following the addition of perchloric acid (0.5 mL), before finally being diluted with 1% HNO_3 and spiked with Rh as internal standard. Metal concentrations (As, Co, Cu, Ni, Pb, U, and Zn) were measured against external matrix-matched standards using an Agilent 7700x ICP-MS. Procedural blanks were analysed with each digestion batch and used to correct final sample concentrations. The certified reference material PACS-2 from NRCC was used to assess the accuracy and reproducibility of the method, with recoveries ranging between 88 % and 106 % (n = 6). Internal precision was typically <3% for all elements. Major elements (Al_2O_3, Fe_2O_3, and CaO) were analysed by powder X-ray fluorescence (XRF) using a Bruker Ranger S2, following calibration against a range of local and USGS rock standards. Metal concentrations in porewater samples were measured by ICP-MS as reported above.

4.2.3.2. Arsenic speciation

Standard solutions of As species (As(III), As(V), MMA and DMA) were made using sodium salts (> 98%, Sigma-Aldrich). Arsenic standard stock solutions were prepared by dissolving appropriate amounts of the individual As standards in deionized distilled water. Appropriate dilutions of stock solutions were made from working standards. The phosphate stock solution was prepared by dissolving appropriate amounts of K_2HPO_4 and KH_2PO_4 in distilled water resulting in a solution of 50 mM K_2HPO_4 and 50 mM KH_2PO_4. All chemicals were used without further purification. Carbonate mobile phase solutions ($(NH_4)_2CO_3$ and NH_4HCO_3) for ion chromatography (IC) analysis was prepared by dissolving appropriate amounts of $(NH_4)_2CO_3$ and NH_4HCO_3 in ultrapure water, purified with a Synergy UV device, to make 200 mM and 2 mM solutions, respectively.

A set of 60 representative sediment samples and 26 porewater samples for speciation analysis were selected on the basis of their total As concentrations. Data for the C2 core is presented for porewater analysis as higher porewater volumes were collected for this core. The sample preparation technique was adapted from Moriarty *et al*. (2014) and Rattanachongkiat *et al.* (2004). Arsenic species were considered relatively stable in air during sample preparation as the rate of oxidation is relatively slow both in situ and with material prepared in the laboratory. Stability of the species has been reported to range from a period of days to weeks (Smedley and Kinniburgh, 2001). Chemical extractions were carried out using 100 ± 50 mg of ground, air-dried sediment. The extraction mixture consisted of 5 mL 50 mM KH_2PO_4 and 5 mL 50 mM K_2HPO_4. Samples were vortexed before being placed in an ice cooled ultra-sonic bath for 30 minutes. Samples were centrifuged at 1500 g and the supernatant extracted and stored at 4 °C before analysis. Samples were diluted (1:5) with 0.1 M HCl for speciation analysis (Georgiadis *et al*., 2006; Yuan *et al*., 2007). The determination of As species concentrations in the extracts was carried out by IC-ICP-MS. Chromatographic separation was performed by elution with a 0.5 mL min^{-1} flow rate through an anion-exchange Dionex Ion Pac AS7 analytical column (2 x 250 mm) and a ThermoScientific Dionex ICS-5000+ IC system with a ThermoFischer iCAP TQ detector. A gradient elution program was used consisting of 200 mM $(NH_4)_2CO_3$ (eluent A) and 20 mM NH_4HCO_3 (eluent B). Basically, 0-2 min 100% eluent B, 2-10 min 50% eluent B, and 10 min 100% eluent B. Reagent blanks were included in sample sequences and quantification was done against the As(V) species. In order to exclude possible interference from $^{40}Ar^{35}Cl^{+}$, the determination of ^{75}As (dwell time = 100 ms) was carried out in the SQ-KED mode using He as the collision gas.

Calibration of each As species was performed on six points over a range of 0.05 to 5.0 µg L^{-1}. Calibration curves with good correlation coefficients ($R^2 > 0.999$) were obtained. The limits of detection (LOD) and limits of quantification (LOQ) were based on repeated measurements of the blank (n = 41) and monitoring As(V). Three quality control (QC) standards were used (0.075 µg L^{-1}, 0.50 µg L^{-1} and 0.90 µg L^{-1}) with percent accuracy ranging from 77 % (As (III)) to 117 % (As(V)). One-way analysis of variance (ANOVA) was done using Grapher Graphing Software (V 8.4.969) from Golden Software Inc. The data indicated statistical significance ($p < 0.05$) for the total and As species.

4.2.3.3. Chromatographic separation

Ion-exchange chromatography hyphenated to ICP-MS was used to separate and identify the four different arsenic species in a 0.5 µg L^{-1} combined species standard (Figure 4.2A). All targeted arsenic species were adequately baseline separated. The formation of a shoulder was evident at low concentrations for DMA but did not affect the analysis and subsequent quantification. Peak areas of As(V) standards produced linear calibration curves (R^2 = 0.999), which were used to quantify analytes present in sediment samples.

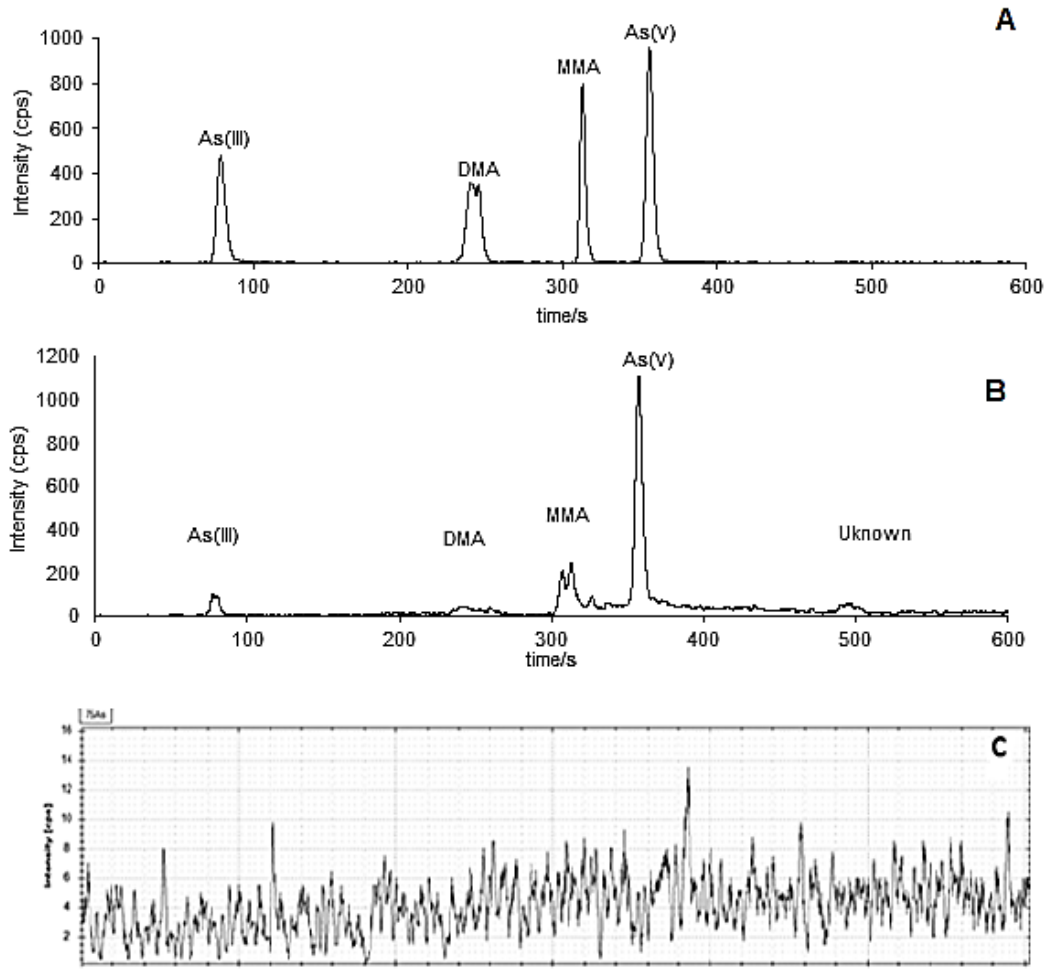

Figure 4.2: (A) Anion-exchange IC-ICP-MS chromatogram of a 0.5 µg L^{-1} mixed As standard on a Dionex Ion Pac AS7 column, elution with $(NH_4)_2CO_3$ buffers. (B) Chromatogram of extracted sediment sample (C3, 280 cm). (C) Chromatogram of buffer solutions for analysis of blanks and LOD and LOQ calculations.

The QC standards analyzed during the course of each analysis batch, bracketing the samples, indicated stability (80-90% recovery) for DMA, MMA and As(V) while the phosphate buffer mobile phase/standard matrix introduced some variability in the As(III) quantification. A correction factor (1.3 – 1.4) was applied to the data accordingly. Limits of detection (0.04 ng L^{-1}) and quantification (0.12 ng L^{-1}) were calculated using a series of phosphate buffer injections for blank measurements (Figure 4.2C). Sediment samples were extracted with a phosphate buffer, diluted and appropriately, pH adjusted. Samples had an unknown As peak (Figure 4.2B) at approximately 500 s with As(V) being the major species in the samples.

4.3. Results

4.3.1. Total Arsenic

Surface samples from TSFs, and the two tributaries Klip River (KR) and Klipspruit (KS) show highly variable As concentrations (Table 4.1). It is evident that proximity to TSFs influences As concentrations with higher As concentrations in the Klipspruit. Material from TSFs have significantly ($p > 0.05$) higher concentrations, ranging between 77 and 106 mg kg^{-1} compared to both sediments from the Klip River (10 – 33 mg kg^{-1}) and Klipspruit (17 – 90 mg kg^{-1}). The sum of extracted (available) As species provides an indicator for As mobility and bioavailability. Despite having lowest total As concentrations, As was relatively bioavailable in the Klip River (28.4 ± 30.7%), with KR1 having 73.6% of total As in bioavailable form. Sites closer to the wetland (KS3 and KR4) tended to have higher total and bioavailable arsenic (32.6 – 89.5 mg kg^{-1} and 9.18 - 15%, respectively).

Average As values remain elevated (51.1 ± 31.1 mg kg^{-1}) across the surface of the wetland. A contour plot (Figure 4.3) shows that As enters the wetland along the northern edge predominantly *via* groundwater flow and diffuses throughout the wetland along a decreasing concentration gradient. Higher concentrations of As move towards the surface compared to the sub-surface. Bioavailability of As in wetland sediment was low with the surface ranging from 2 - 12% in the upper 130 cm and decreasing to between 1 – 5% at depths below 130 cm (Appendix B).

Table 4.1: Total and extracted As in the surface sediment samples from the TSFs, Klip River and Klipspruit.

Sample	Total As	Extracted As	Available As*
	mg kg^{-1}		%
T1	77.4	4.32	5.58
T2	99.6	16.7	16.8
T3	106	3.49	3.31
KS1	70.3	2.91	4.14
KS2	17.5	0.45	2.59
KS3	89.5	8.22	9.18
KR1	10.1	7.40	73.6
KR2	30.1	1.60	5.32
KR3	20.5	4.01	19.6
KR4	32.6	4.89	15.0

** %Available As was calculated as the fraction of the extracted As from the total*

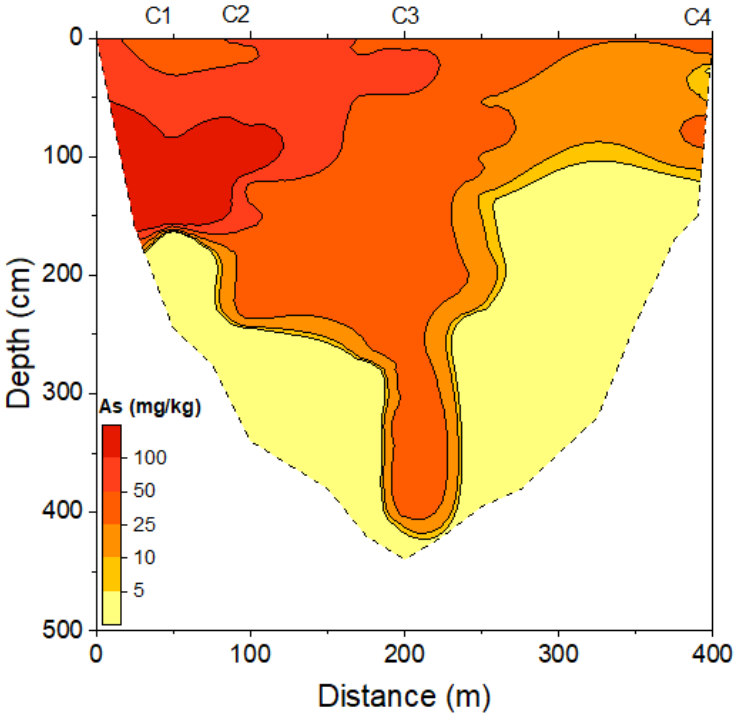

Figure 4.3: Distribution of As in the Klip River wetland.

Arsenic geochemistry is commonly linked with Fe-Mn oxyhydroxides, organic matter and S (Herath *et al*., 2016; Wuana and Okieimen, 2011b; Zhang *et al*., 2014). Correlation plots between As and minerals indicate weak but likely associations. (Figure 4.4). For the wetland samples, the strongest relationship was observed between Fe and As content (R^2 = 0.4905). Poor correlations (R^2 < 0.25) were observed between both LOI and Mn with As. Additionally, the expected significant correlation between As and S was not evident. This suggests a combination of parameters likely influence the distribution of As in wetland sediments.

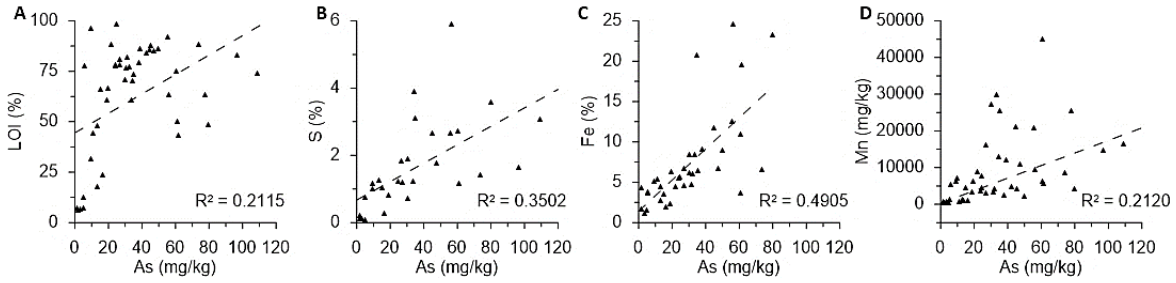

Figure 4.4: Correlation plots of (A) LOI, (B) S, (C) %Fe and (D) Mn with As in sediment

4.3.2. Arsenic porewater concentrations

The pH of the sediment core C2 is relatively stable (pH 7.08 – 7.33) until 320 cm where the pH starts to decrease. There are no noticeable trends from the surface downcore for Eh, $As_{porewater}$ and $As_{sediment}$ concentrations (Figure 4.5). The $As_{porewater}$ tends to be inversely related to the $As_{sediment}$ concentrations and directly proportional to Eh indicating an exchange process dependant on redox potential. The exchange between the sediment and interstitial water is closely linked to Eh. Surface and core profiles indicate that the wetland sediments are anaerobic.

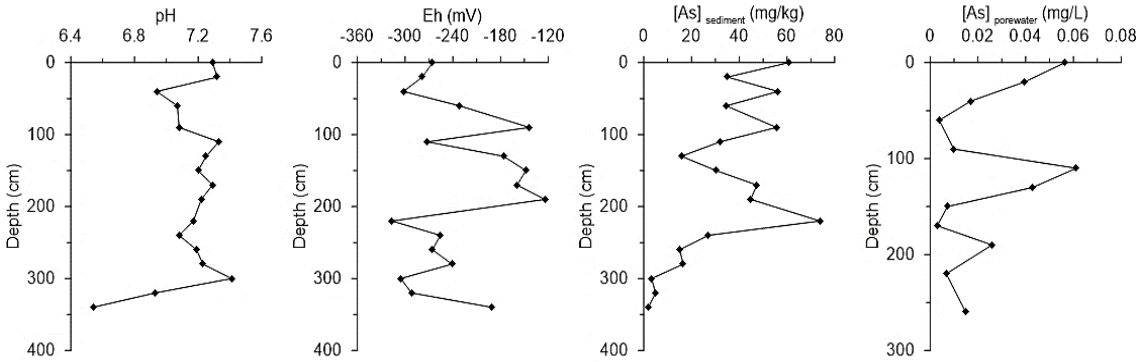

Figure 4.5: Vertical profiles of pH, Eh, As concentrations in sediment and porewater of the C2 core.

4.3.3. Arsenic Speciation

Phosphate buffer extractions yielded As species for all of the sediment samples analysed. The predominant species was As(V), followed by As(III), MMA and an unidentified As species (Table 4.2, Figures 4.6 and 4.7). As(V) was the predominant species in the TSFs (Table 4.2 and Figure 4.6) and tributary river samples. This is probable since arsenic minerals exposed to air undergo oxidation and their speciation is generally heavily skewed towards As(V) (Fendorf *et al*., 2010; Smedley and Kinniburgh, 2001). Concentration depth profiles through the wetland reveal variability in As speciation at the surface (Figure 4.7A). As(III) and As(V) follow a similar trend with elevated concentrations (0.04 - 1.17 mg kg^{-1} and 0.35 – 7.36 mg kg^{-1}, respectively) in the upper 100 cm followed by a general decline in concentration. Despite significantly lower concentrations of MMA in the cores, the concentrations of MMA generally decreases across the wetland (0.055 – 0.035 mg kg^{-1}) and with increasing depth (0.012 – 0.202 mg kg^{-1}) (Figure 4.7B). There appears to be an inverse relationship between MMA and S with depth (Figure 4.7B). The methylation of As species has commonly been linked with arsenic sulfide minerals and microbial methylation processes (Cullen, 2014).

Table 4.2: Total and extracted As in surface sediment samples from TSFs, Klip River and Klipspruit.

Sample	As(III)	DMA	MMA	As(V)	Unknown
			mg kg^{-1}		
KS1	0.98	BLQ	BLQ	1.8	0.14
KS2	0.17	BLQ	BLQ	0.29	BLQ
KS3	1.8	0.03	0.11	6.2	0.11
KR1	1.2	ND	0.033	5.9	0.25
KR2	0.68	ND	0.056	0.81	0.049
KR3	0.63	ND	BLQ	3.4	ND
KR4	1.6	ND	0.035	3.1	0.15
T1	BLQ	BLQ	ND	4.32	ND
T2	ND	0.064	BLQ	16.6	ND
T3	BLQ	BLQ	ND	3.49	ND

BLQ – Below Limit of Quantification; ND – Not Detected

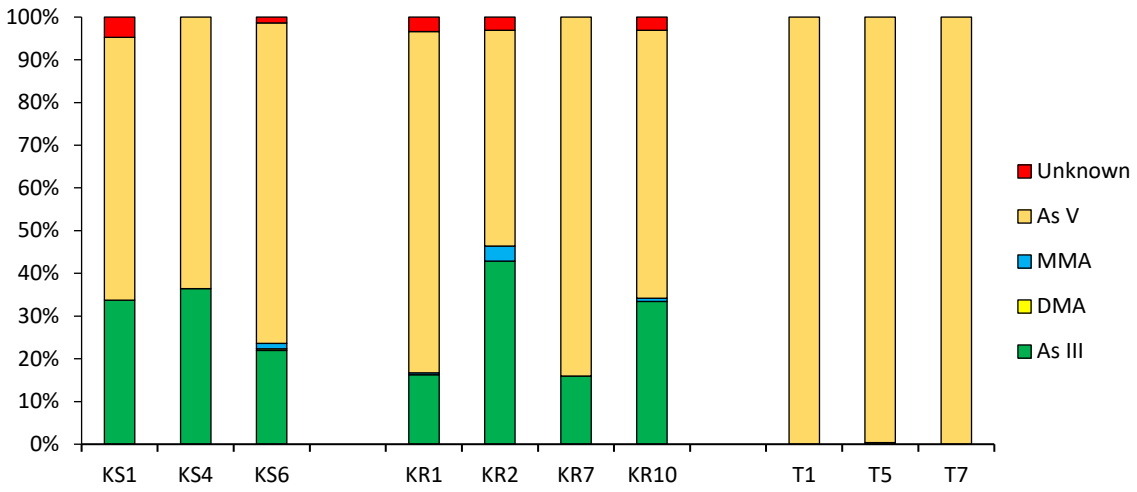

Figure 4.6: As speciation present in the Klip River, Klipspruit and TSFs

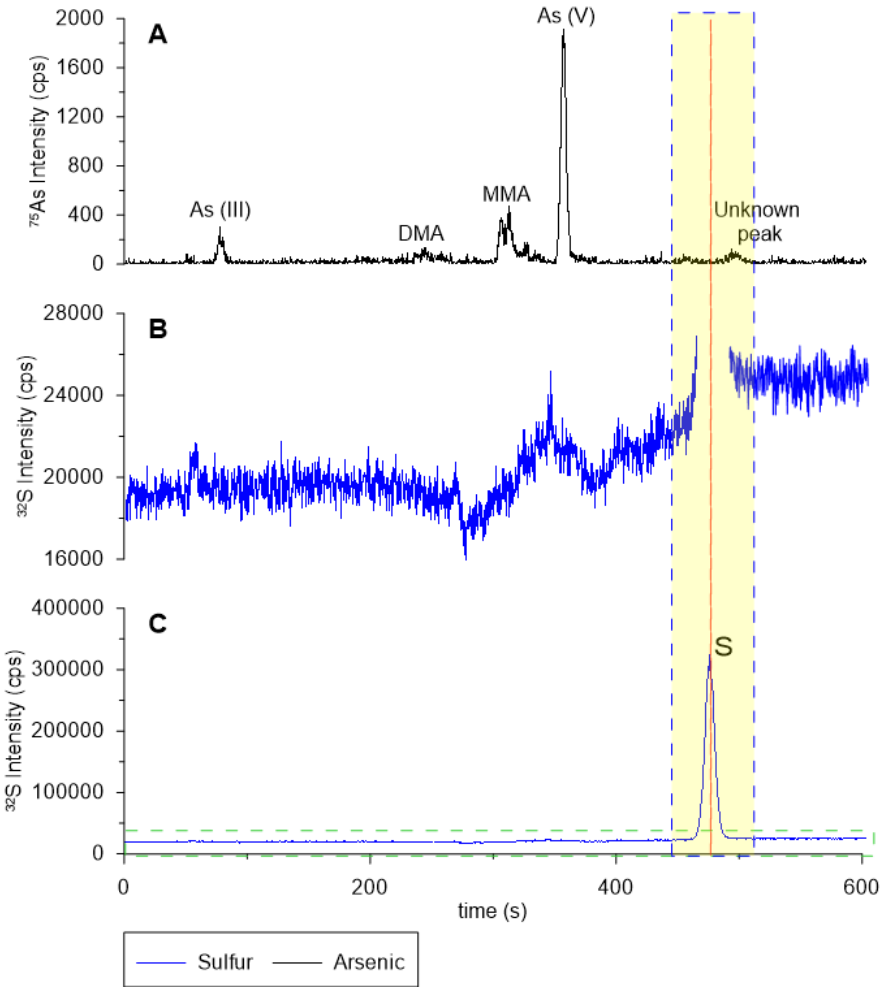

Figure 4.7: Chromatograms indicating (A) a sample containing all As species, (B) expanded chromatogram of the area indicated by the green, and (C) single S standard. The shaded area indicates the plausible region for S-species.

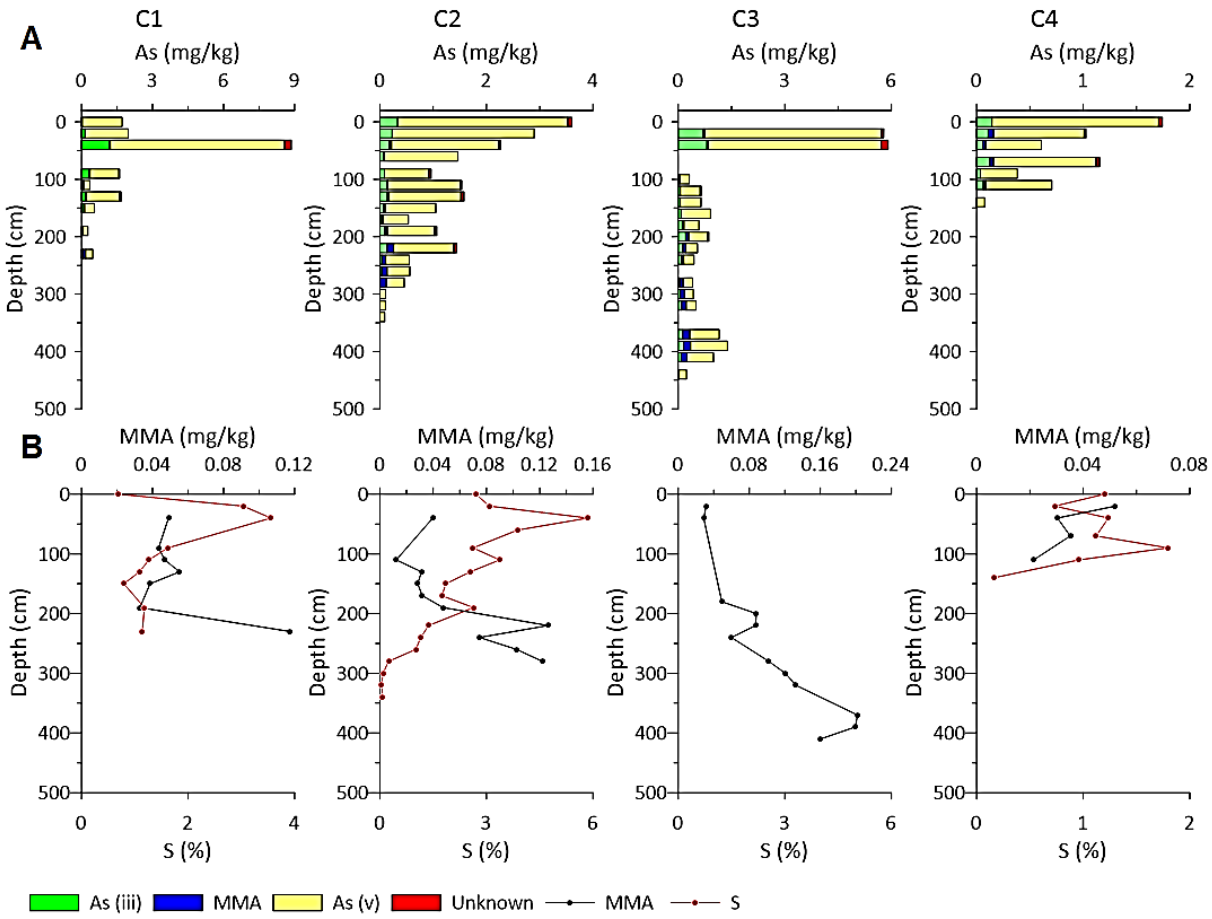

Figure 4.8: (A) Vertical profile of As species in sediment across the wetland, and (B) verticles profiles of sulfur and MMA (S in C3 not determined).

Given the nature of As speciation in wetlands affected by AMD, it is plausible that the unidentified species is an As-sulfur species (Figure 4.7). While slight shifts in retention times of samples containing As and S have been reported with the cause being degradation or reaction of the sulfur species (Kalenga, 2011; Vu *et al.*, 2019), the distinct differences in retention time and the physico-chemical parameters of the wetland suggests that the As may be complexed to a sulfate or sulfide species (Couture *et al.*, 2013a). This postulated As-S species (Unknown) is only present where elemental S concentrations are > 0.07% (Figure 4.8) and it is therefore likely that As and S are related.

4.4. Discussion

4.4.1. Transport and accumulation of As in the wetland

Arsenic concentrations in the Klip River wetland system indicate the long term and systemic entry of this pollutant into the environment. Introduction of As into the wetland is predominantly *via* groundwater with lesser contributions from surface inflow (Figure 4.3). Arsenic in heavily mined areas is typically associated with Fe-, Al-, Mn- oxyhydroxides, and arsenic containing minerals (pyrite, realgar, cobaltite) (Kocourková-Víšková *et al*., 2015; Sami and Druzynski, 2003). Arsenic is one of the most common by-products of mining activities. The two primary sources are the residual waste stored in TSFs along with other metals, minerals, organic matter and water (Herath *et al*., 2016), and mine voids that contain highly acidic and sulfidic water, with high concentrations of dissolved metals, including As. Dry TSFs are typically characterised by an outer oxidation zone and an inner reducing region (Hansen, 2015b) and this together with the other biogeochemical processes of desorption, dissolution and microbial-mediated activity (reduction andmethylation) have a significant influence on the behaviour of As in the environment (Huang, 2014). In the outer oxidation zone, As is likely to be stable and precipitated to Fe, Al or Mn oxides. The inner reducing region, where As is most likely mobile, contributes as one of the sources of As entry into the tributaries and wetland. Both aerobic and anaerobic zones contain microbes. Microbial oxidation of sulfur containing ores also promotes the leaching of As from substrates into aqueous media (Manchisi *et al*., 2012; Muravyov and Panyushkina, 2020). Flooding or partial water inflow into abandoned mines of the Witwatersrand basin has resulted in a process of continued production of AMD, and high sulfide concentrations increasing the concentration of dissolved As, which may contaminate aquatic resources (Ramontja *et al*., 2011).

Differences in the concentrations and mobility of As in the two tributaries is likely due to the differences in sedimentological characteristics. The Klipspruit generally has higher average concentrations of metals (Cu, Co, Ni, Pb, U and Zn) than the Klip River. Despite the lower clay content and higher organic matter in the Klip River, the bioavailable concentration in the Klip River is high (1.60 – 7.40 mg kg^{-1}). A ternary plot of the most likely As-associations shows three distinct clusters (Figure 4.9). The first cluster (A) contains the TSFs, which are characterised by high concentrations of Fe+Mn and As and low concentrations of organic

material. The second cluster contains core samples from the bottom of each of the sediment cores. These are samples that were collected from the clay layer underlying the wetland. This indicates that with depth, and lower concentrations of As and the influence of Al and Fe comes to the fore. The wetland surface and core sample cluster has a significantly higher organic content, which suggests that the behaviour and speciation of As will be directed by this parameter with likely sorption to natural organic matter and fibrous peat.

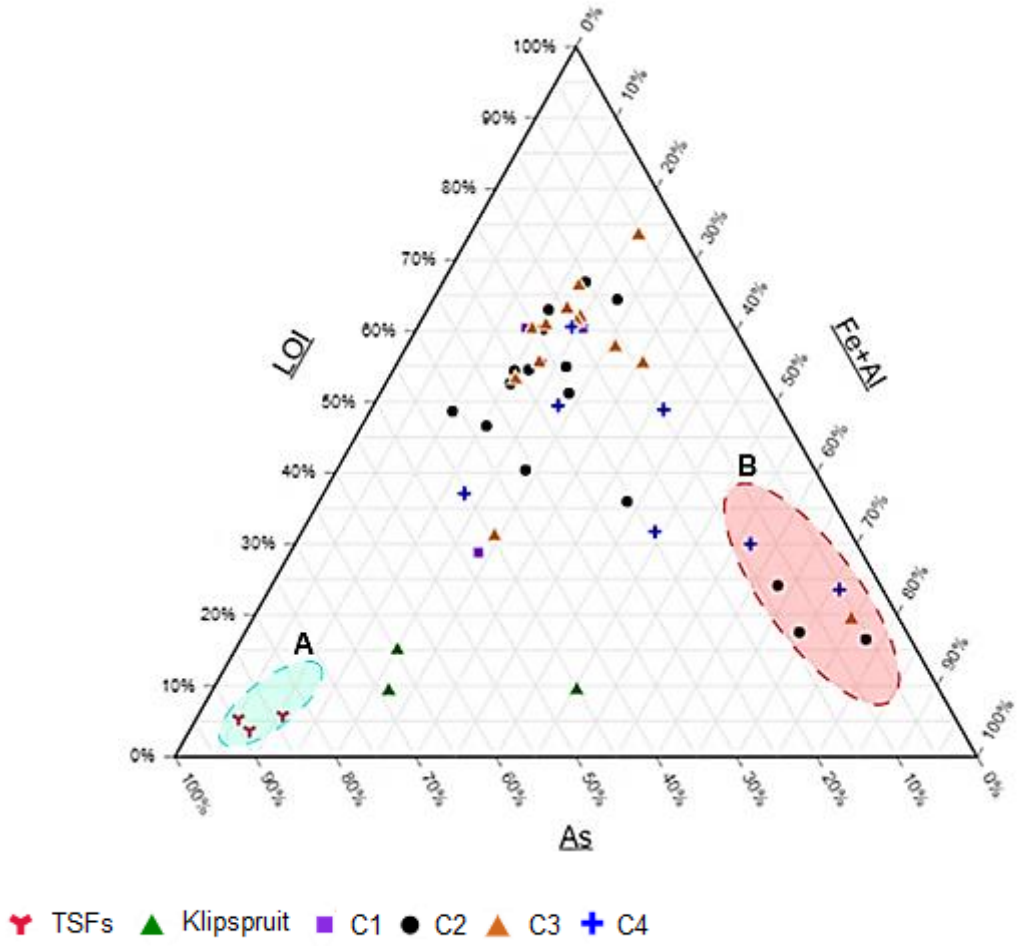

Figure 4.9: Ternary plot indicating the relationship between As, LOI and Al+Fe oxides

4.4.2. Speciation

Arsenate and arsenite are both present in the system with arsenate the predominant form given the oxidising conditions under which AMD processes occur (Paikaray, 2015). The prevalence of arsenate in AMD polluted environments is well documented (Cassone *et al*., 2018; Kocourková-Víšková *et al*., 2015; Meharg and Zhao, 2012) and the noted distribution is due to

the affinity for surface sorption to metal oxides (Fe-, Mn-oxides) and bacteria-catalysed oxidation (Bruneel *et al*., 2003; Shi *et al*., 2020; Wakao *et al*., 1988). Bacteria facilitated oxidation forming arsenate results in increased rates of surface adsorption and removal of arsenic from porewater (Fendorf *et al*., 2010). The arsenite present may undergo a relatively slow oxidation to form arsenate species, which may be precipitated by sulfur, or remain in solution (Paikaray, 2015). Within the wetland, As(V) remains the predominant species even at depth and this strongly indicates a process that is driven by microbial activity and organic matter. Its persistence at anoxic depths may be due to the elevated Fe-Mn oxyhydroxide concentrations (Kuhn and Sigg, 1993). The entry mechanism into the wetland does not seem to affect the predominant species. This is further supported by the appearance and dependence of MMA on sulfur. Sulfur is often utilised as the electron donor in the methylation processes of As within bacteria (Chen *et al*., 2019). The decrease of S and increase in MMA content (Figure 4.7) infers that microbes are using this element in the methylation mechanism.

4.4.3. Microbial mediated biotransformation

Arsenic biotransformation has a significant influence on As speciation. It may result in a change of oxidation state As(III) to As(V), a change in between the inorganic and organic forms (i.e. production of methylated species) and a resultant influence on the mobility and toxicity of the produced species. *Acinetobactor* and *Pseudomonas species* identified as present in the Klip River wetland (Chihomvu *et al.*, 2014) may be involved in the biotransformation of As.

Biotransformation may occur in one of four pathways;

a) oxidation of As(III) to As(V) by heterotrophic or autotrophic bacteria (Muravyov and Panyushkina, 2020; Zhang *et al*., 2015). These species are able to oxidise As through either a natural detoxification mechanism or by using As(III) as an electron donor for respiration (Kudo *et al*., 2013).
b) methylation of As rendering it less toxic. This occurs through S-adenosylmethionine (amino acid product) or methyl cobalamin (vitamin produced by bacteria) and can act as a methyl donor producing MMA through a catalysed process (Bentley and Chasteen, 2002). Sulfate reducing bacteria may also play a role in methylation (Chen *et al*., 2019).
c) oxidation of Fe(II) to Fe(III) by iron-oxidising bacteria result in the formation of Fe-hydroxides, which acts as a good surface adsorbent for As (Hao *et al*., 2018).

d) microbial SO_4^{2-} reduction. This process, facilitated in substrates with high organic matter, results in the formation of sulfides. Arsenic is then sequestered as thiolated species (Couture *et al*., 2013b).

Our data clearly reflects a methylation process but it is likely that all these processes are actively occurring in the wetland resulting in the dominant As(V) species.

Although the wetland is predominantly reducing in nature, there may be some isolated oxidation zones, which promote the development of specific types of bacteria facilitating and sustaining biotransformation. Monomethylarsenate may be further transformed by the presence of sulfur to thiolated arsenic species, sulfide-containing As(V) metabolites e.g. monomethyl monothioarsonic acid and dimethyl monothioarsinic acid. These species may account for the decrease in S with depth in the sediment cores. It appears that the wetland is able, through biotransformation, to contain the As species, as As(V). This species is readily adsorbed or co-precipitated onto Fe-oxyhydroxides rendering them unlikely to be bioavailable (Drahota *et al*., 2012).

However, the dynamic nature of a wetland and the on-going anthropogenic activity in this area puts the As(V) stability at risk. Disrupting the microbial balance and sequestration mechanisms currently utilised to oxidize As, could result in dissolution of Fe-oxyhydroxide minerals releasing As(V) into the environment. Small changes, including release of mine void water and groundwater plumes, to the influx of AMD, rainfall patterns, peat removal will effectively result in the release of sequestered As (Drahota *et al*., 2021). While As is likely to re-precipitate with Fe and sulfide minerals, reduction of As(V) to the more mobile As(III), the production of volatile As species may also occur and will have a detrimental effect on this environment . The occurrence of volatile As-species poses the threat of short and long range As transport, which contaminates other environments, thus expanding As exposure to the broader region. The reduction of As(V) to the As(III) state will promote the oxidation of metals (Fe and Mn) and dissolved organic matter. The oxidation of organic matter reduces the ability of the wetland to sequestrate metals *via* sorption processes (Wang and Mulligan, 2006), thus collapsing the natural filtration system. Arsenic speciation behaviour in the Klip River wetland system may provide insight to the behaviour of other metalloids, including Sb and Se (Cooksey, 2012), which are found in mined minerals.

4.5. Conclusion

Speciation analysis shows that AMD is responsible for the release and transport of As from the TSFs and mine voids *via* groundwater plumes to the wetland. Bioavailable As concentrations in the Klip River may pose a threat to the environment as As biomagnifies through the food chain. Informal settlements are located along both, the Klip River and Klipspruit, where the water and sediment from the rivers are likely used for consumption and agricultural purposes. The wetland appears to be attenuating As primarily through two mechanisms; oxidation of As(III) to As(V) and the subsequent surface sorption to Al and Fe oxides; and microbial biotransformation of As(III) to As(V) or methylated As species. While microbial remediation of As seems to be ideal in sequestering As, the predominance of these specific types of bacteria may begin to change microbe diversity, their presence changing the natural cycle of that habitat. Bacteria may also volatilise methylated As species, releasing them into the atmosphere. The Klip River wetland should be carefully monitored as small changes to chemical parameters easily affect transformation of As species.

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Chapter 5

Conclusions & Future work

This study was conceived to establish sources of metal pollutants into the Klip River wetland and to investigate the mechanisms by which they are transported and sequestered to better understand the impact of AMD contaminants on wetland attenuation. Key pollutant stressors in this system include input from mining, industrial and agricultural activities.

To address the aim, the three main research objectives were;

1. To identify and quantify toxic metal contaminants affecting the Klip River wetland system and to investigate their movement and sequestration into and through the wetland using a combination of geochemical techniques.
2. To identify the specific sources contributing to Pb pollution using isotope ratio measurements.
3. To assess the behaviour of As species and the influence of AMD on the distribution of the As species.

The study highlighted the significant and sustained increase in pollutant metals specifically As, Co, Cu, Ni, Pb, U and Zn over time. The majority of pollutants (except Pb) are associated with AMD plumes that emanate from TSFs and enter the wetland *via* groundwater recharge. Lead appears to originate from aerosol deposition. Surface runoff is an additional albeit minor contributor. AMD plumes that emanate from TSFs transport high concentrations of Co, Zn, Ni and U into the wetland *via* the tributaries. These are then, sequestered through precipitation and sorption processes. The wetland continues to act as an important sink for pollutants originating from mining activities on Witwatersrand.

Source identification for Pb indicated isotopic signatures related to composite geological parent material, coal and alkyl lead-based fuel, the latter source predominant on the wetland surface. The finding also highlights the long-term environmental challenges created by the Pb-based fuels despite its prohibition from the South African market in 2006.

The concentration and distribution of As confirmed that AMD was responsible for its' release and transport from TSFs and mine voids *via* groundwater plumes into the wetland. Speciation is heavily dependent on pH and Eh with the predominant species being As(V). The wetland appears to be attenuating As primarily through two mechanisms; oxidation of As(III) to As(V) and the subsequent surface sorption to Al and Fe oxides; and microbial biotransformation of As(III) to As(V) or methylated As species. While microbial remediation of As seems to be ideal in sequestering As, the predominance of these specific types of bacteria may begin to change microbe diversity, their presence changing the natural cycle of that habitat. Bacteria may also volatilise methylated As species, releasing them into the atmosphere.

Significant contributions, including the mode of entry of AMD into the wetland, source identification of Pb and biogeochemical cycling of As in the wetland adds towards understanding the processes directing the behaviour of metals in wetlands affected by AMD. The predominant entry pathway for metals into the wetland is *via* groundwater plumes rather than surface inflow. This information raises concerns about discharge transport pathways of contaminated groundwater and the potential health implications for users of untreated water. It may also provide useful for the development of suitable reactive barriers at appropriate intervals should remediation of this wetland become a viable option. AMD pollutants are no doubt highly destructive to this environment and are the primary reason for the wetland degradation; however, the identification of coal and fuel-based pollutants should not be marginalised. These pollutants may be widely dispersed and affect a wider population.

One of the functions of a wetland is to attenuate toxic metals by transforming the metal to less toxic species. One of more useful ways to achieve this type of attenuation in the Klip River wetland was through microbial biotransformation seen with As species. Microbes effectively created an environment, which promoted As precipitation. Presumably, this would also apply to other metals such as Se, Sb, and possibly Hg. This presents a viable option for wetland remediation opportunities.

5.1. Future Research

The findings from this study raised several questions, which deserve further investigation.

These include:

- Porewater and sediment analysis was used in this study to determine the infiltration of AMD pollutants into the wetland. Further studies are required to understand the migration away from the wetland and into residential or agricultural areas. By understanding the migration routes of AMD plumes, permeable reactive barriers could be installed in "hot-spot" areas to attenuate the effects of AMD *via* groundwater. This would ensure that communities that depend on borehole or groundwater are at a lower risk of consuming contaminated water.

- Studies on the bioaccumulation of metals in accumulator plants would allow indigenous plants to be utilised in phytoremediation (Ramla and Sheridan, 2015). This would reduce the metallic load on the sediment and allow the attenuating capacity of the wetland to increase. This strategy has been used in the West Rand Basin where Zn, Cu and Ni in gold mine tailings were accumulated within with vetiver grass (*Chrysopogon zizanioides*) (Melato *et al*., 2016). Other suitable plants commonly found in wetland include *Phragmites communis, Phragmites australis, Carex flacca* and *Typha latifolia* are two common wetland plants that effectively remove metals from polluted water (Nagy *et al*., 2020; Zhang *et al*., 2010).

- Studies using microbes to assess the bioaccumulation and biotransformation of other metals. Some microbes have been found to remove up to 90% of sulfate from AMD along with Cu, Zn, Fe, Ni and Mn (Ayangbenro *et al*., 2018; Nancucheo *et al*., 2017). Bacillaceae, which are aerobic bacteria, were successfully used to partially remediate AMD effluent from a gold mine in Gauteng, removing Cr, As, Pb and Al from the environment (Ijoma *et al*., 2019). Selected microbes would need to be examined for their effectiveness to bioaccumulate and biotransform metals. These can then be introduced into the wetland naturally or in bioreactors to attenuate the effects of AMD pollution.

- Conducting lab studies on peat and plants based on the information contained in this study and applying the results to constructed wetlands across the Witwatersrand Basin to increase attenuation capacity in natural wetlands. Microbes are useful in attenuating As by methylation into a less toxic species while organic matter and Al-Fe oxyhydroxides adsorb metals, including Co, Cu and Ni. Constructed wetlands could be built using this knowledge to channel AMD and attenuate pollutants before the water is released into natural systems.

Natural wetlands, including the Klip River wetland are intricate systems that sustain flora and fauna, while purifying water. The ability of these systems to mitigate and attenuate toxic pollutants is unrivalled. Therefore, these systems should be valued and any endeavour to sustain and ensure the longevity of the wetland should be considered.

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Appendix

Appendix A (Chapter 2)

Table A: Sediment concentration data for the core samples (C1-C4) from the Klip River wetland.

Depth /cm	Co	Ni	Zn	Pb	Cu	U	ΣREEs
	mg kg^{-1}						
Core 1							
0	68.99	497.6	1516	123.2	329.3	37.87	100.6
10	2421	4976	3167	26.02	168.1	34.04	314.3
20	793.7	4508	3319	262.3	473.8	482.2	1001
40	473.6	2357	1545	83.97	641.1	823.6	835.5
50	152.5	1200	351.0	89.68	627.6	550.1	2326
60	145.2	1412	825.8	82.79	326.4	239.2	4142
70	391.3	2856	1328	151.5	359.0	203.6	5310
80	241.2	2373	875.3	197.8	1408	1164	5978
90	256.1	1745	607.6	139.3	1395	1633	2004
100	308.6	2198	814.3	168.1	2070	2389	3127
110	410.2	2828	1084	131.5	1655	1459	3069
120	765.0	3554	11616	85.59	681.9	590.0	2356
130	654.8	2804	15279	78.52	531.3	395.6	2206
140	458.4	2107	15432	27.70	164.9	41.38	1638
150	709.8	1992	2145	20.07	130.1	14.64	260.0
160	2742	4727	2696	13.02	105.6	17.04	235.5
180	1856	4118	4221	19.03	125.9	22.50	215.1
190	1607	4243	953.1	19.26	149.1	53.41	267.1

Depth /cm	Co	Ni	Zn	Pb	Cu	U	ΣREEs
	mg kg^{-1}						
Core 1 continued							
200	2258	4660	2661	18.17	127.4	23.21	244.7
210	2491	5617	2455	17.97	139.2	26.51	269.1
220	2876	6403	1002	13.74	124.5	26.36	189.2
230	3499	7090	1302	31.51	190.2	28.36	237.6
240	1262	2879	990.3	14.32	132.1	10.19	235.1
Depth /cm	**Co**	**Ni**	**Zn**	**Pb**	**Cu**	**U**	**ΣREEs**
	mg kg^{-1}						
Core 2							
0	264.2	1696	1885	151.6	690.0	151.3	431.5
10	180.0	1346	1214	163.9	499.3	167.3	637.9
20	434.7	2380	1848	87.20	647.8	270.1	968.8
30	973.3	5034	2781	46.53	1036	1028	1536
40	642.2	3682	1652	36.93	1158	1171	2081
50	634.8	3314	2823	45.85	727.6	251.4	751.2
60	432.5	2894	1263	31.18	827.0	778.0	2988
70	232.7	1728	1028	16.90	586.9	527.1	3417
90	282.8	2085	2868	22.41	313.1	195.9	1937
100	603.6	3444	2153	41.16	532.4	471.1	1583
110	300.7	1797	1308	25.73	428.3	285.8	1226
120	346.7	2090	1391	19.10	454.5	334.7	1586

Depth /cm	Co	Ni	Zn	Pb	Cu	U	ΣREEs
	mg kg^{-1}						
Core 2 continued							
130	132.7	592.3	989.0	5.685	148.8	102.4	456.3
140	234.4	1306	1252	22.82	278.7	151.2	605.0
150	567.2	2169	3237	43.32	292.8	97.13	627.8
160	987.9	2313	1414	2.740	123.4	17.18	474.6
170	2420	5219	5035	6.862	202.8	35.72	730.0
180	2289	5001	6810	25.91	217.3	49.48	407.0
190	1748	4729	13151	117.7	466.5	122.6	664.2
200	1734	4369	8485	61.40	304.6	129.0	725.1
210	1567	3807	7899	20.09	225.6	78.41	446.7
220	1754	3479	17270	26.33	167.0	35.87	344.6
230	1963	4001	3380	18.27	80.51	14.83	259.1
240	425.1	1507	1380	21.47	74.56	16.51	285.7
250	638.7	2182	1128	20.38	93.59	19.74	279.5
260	389.4	1321	590.2	22.37	171.3	16.33	309.0
270	158.3	804.7	150.4	27.86	187.4	8.769	251.9
280	180.8	1015	132.6	41.04	284.0	15.16	419.6
290	16.05	69.36	13.37	12.93	33.91	2.208	166.1
300	27.91	120.5	31.36	19.11	55.04	3.695	243.7
310	7.491	53.58	4.24	10.54	32.35	2.182	124.9
320	7.425	58.32	11.36	10.43	33.57	3.102	99.74
330	8.331	82.67	21.83	15.90	53.28	4.602	146.6

Depth /cm	Co	Ni	Zn	Pb	Cu	U	ΣREEs
	mg kg^{-1}						
Core 2 continued							
340	6.112	57.14	32.27	9.899	42.92	2.227	95.52
Depth /cm	**Co**	**Ni**	**Zn**	**Pb**	**Cu**	**U**	**ΣREEs**
	mg kg^{-1}						
Core 3							
0	308.4	1862	1516	283.2	729.8	78.17	304.7
10	1239	5527	2629	233.4	436.3	141.3	571.7
20	605.6	2781	2018	224.9	792.4	269.12	748.0
30	297.4	1493	1158	133.8	487.9	89.85	279.2
40	396.8	2457	1307	66.85	358.1	329.6	960.9
50	662.9	2892	1768	51.40	295.1	192.6	471.5
60	227.8	1694	1370	26.51	99.03	40.33	244.6
70	603.9	4011	9430	88.80	334.5	206.1	2680
80	560.1	3857	23959	68.43	200.0	156.5	2231
90	552.0	4253	11644	58.92	224.4	193.7	3072
100	123.7	1050	239.0	34.29	187.5	54.67	1406
110	585.1	4546	22279	65.96	219.5	159.7	2386
120	258.2	1962	22709	25.36	89.79	48.81	1735
130	214.7	1786	8836	13.85	80.84	22.61	1112
140	190.8	1398	2952	10.82	53.66	20.00	664.5
150	446.5	2477	9685	18.41	114.7	29.94	387.4

Depth /cm	Co	Ni	Zn	Pb	Cu	U	ΣREEs
	mg kg^{-1}						
Core 3 continued							
160	570.5	2723	9297	20.13	98.52	30.21	415.2
170	540.7	3192	6827	16.98	65.42	17.60	278.7
180	370.3	2748	3436	17.19	54.32	12.88	192.5
190	657.9	2622	5024	20.83	63.62	17.84	208.7
200	571.2	2900	20858	25.13	105.2	41.42	390.3
210	1130	3722	2308	23.34	74.29	19.64	162.1
220	1044	2915	541.6	26.08	66.51	18.68	172.1
230	1023	2994	415.6	14.43	48.98	12.77	107.8
240	1900	4071	1328	17.81	67.67	20.31	156.8
250	1717	3188	701.9	15.89	71.69	15.34	130.6
260	2026	3779	428.1	16.59	71.59	13.10	130.4
270	172.6	553.6	25.19	17.59	104.6	21.09	186.5
280	128.1	455.2	58.49	17.95	117.6	25.64	207.4
290	504.3	1207	219.1	21.54	159.9	48.75	303.8
300	302.5	897.5	148.89	23.01	182.9	63.96	329.2
310	204.0	735.8	159.15	16.63	141.4	34.09	232.4
320	288.9	879.9	259.34	19.63	148.4	54.89	266.8
330	259.3	838.9	345.13	19.30	151.0	57.57	330.5
340	333.3	1056	548.28	19.18	212.0	82.78	345.9
350	332.2	733.3	290.8	17.61	150.9	31.65	280.1
360	39.44	262.3	58.44	19.02	115.5	8.618	185.5

Depth /cm	Co	Ni	Zn	Pb	Cu	U	ΣREEs
	mg kg^{-1}						
Core 3 continued							
370	192.3	582.5	120.6	22.32	166.9	73.18	521.0
380	145.6	409.5	98.43	20.77	157.8	22.79	514.2
390	81.55	421.6	118.2	22.61	183.4	22.58	625.1
400	158.2	458.3	114.5	28.98	197.4	28.54	529.5
410	21.27	304.1	72.18	15.53	90.87	4.964	134.4
420	15.16	101.3	39.78	11.67	48.07	2.394	116.7
430	7.662	34.23	11.59	6.328	21.33	0.8775	59.57
440	16.54	83.22	19.30	12.43	38.72	2.256	127.8
Depth /cm	**Co**	**Ni**	**Zn**	**Pb**	**Cu**	**U**	**ΣREEs**
	mg kg^{-1}						
Core 4							
0	89.60	575.8	944.4	224.5	436.2	50.01	177.2
10	663.1	2296	1407	162.6	419.0	124.5	754.3
20	83.20	319.7	177.4	27.95	59.50	31.79	183.8
30	22.59	147.8	50.49	22.24	48.34	10.19	169.8
40	48.13	352.9	196.8	30.40	175.4	155.1	427.7
50	61.78	348.0	211.3	26.80	142.6	114.9	324.4
60	60.39	382.8	228.2	19.28	166.6	163.8	357.9
70	197.0	1446	1091	52.60	415.3	351.7	1349
80	240.2	2271	1696	98.30	1106	916.2	3658

Depth /cm	Co	Ni	Zn	Pb	Cu	U	ΣREEs
	mg kg^{-1}						
Core 4 continued							
90	436.1	2646	1195	93.75	937.0	833.8	2800
100	125.2	735.1	473.4	81.46	302.2	169.2	706.1
110	88.10	1168	1712	49.12	173.8	75.11	302.4
120	29.70	97.80	ND	35.17	58.40	25.30	326.3
130	14.79	33.11	ND	26.35	30.59	3.403	169.6
140	14.10	ND	ND	24.36	22.29	3.434	143.0

ND – Not Determined

Appendix B (Chapter 4)

Table B: Total As, As species and extracted As in the core sediment samples.

Sample	$[As]_{Total}$	As(III)	DMA	MMA	As(V)	Unknown	$[As]_{Extracted}$	Available As
	mg kg^{-1}							%
Core 1								
C1-0	30.20	0.04	<*dl*	<*dl*	1.68	<*dl*	1.72	5.68
C1-20	109.0	0.16	<*dl*	<*dl*	1.81	<*dl*	1.97	1.81
C1-40	79.75	1.17	<*dl*	0.05	7.36	0.28	8.86	11.11
C1-90	96.80	0.32	<*dl*	0.04	1.22	0.03	1.61	1.66
C1-110	276.6	0.05	0.03	0.05	0.26	<*dl*	0.39	0.14
C1-130	485.5	0.16	<*dl*	0.06	1.41	0.05	1.67	0.34
C1-150	18.93	0.10	<*dl*	0.04	0.41	<*dl*	0.55	2.89
C1-190	24.70	0.04	<*dl*	0.03	0.19	<*dl*	0.27	1.08
C1-230	9.61	0.06	<*dl*	0.12	0.29	<*dl*	0.48	4.95
Sample	$[As]_{Total}$	As(III)	DMA	MMA	As(V)	Unknown	$[As]_{Extracted}$	Available As
	mg kg^{-1}							%
Core 2								
C2-0	60.61	0.33	<*dl*	<*dl*	3.19	0.08	3.60	5.94
C2-20	35.08	0.23	<*dl*	<*dl*	2.66	<*dl*	2.90	8.25
C2-40	56.18	0.18	<*dl*	0.04	2.01	0.03	2.26	4.02
C2-60	34.51	0.08	<*dl*	<*dl*	1.39	<*dl*	1.46	4.24
C2-90	55.57	0.09	<*dl*	<*dl*	0.83	0.04	0.96	1.73

Sample	$[As]_{Total}$	As(III)	DMA	MMA	As(V)	Unknown	$[As]_{Extracted}$	Available As
	mg kg^{-1}							%
Core 2 continued								
C2-110	31.93	0.14	<*dl*	0.01	1.36	0.03	1.54	4.81
C2-130	15.74	0.14	<*dl*	0.03	1.35	0.06	1.58	10.03
C2-150	30.35	0.07	<*dl*	0.03	0.95	<*dl*	1.05	3.46
C2-170	47.33	0.03	<*dl*	0.03	0.47	<*dl*	0.54	1.13
C2-190	44.78	0.09	<*dl*	0.05	0.88	0.04	1.07	2.38
C2-220	73.81	0.13	0.02	0.13	1.13	0.05	1.47	1.99
C2-240	27.07	0.04	<*dl*	0.07	0.44	<*dl*	0.55	2.05
C2-260	15.01	0.04	<*dl*	0.10	0.42	<*dl*	0.56	3.75
C2-280	16.51	<*dl*	0.02	0.12	0.34	<*dl*	0.48	2.91
C2-300	3.26	<*dl*	<*dl*	<*dl*	0.11	<*dl*	0.11	3.27
C2-320	4.94	<*dl*	<*dl*	<*dl*	0.11	<*dl*	0.11	2.13
C2-340	1.91	<*dl*	<*dl*	<*dl*	0.09	<*dl*	0.09	4.71
Sample	$[As]_{Total}$	As(III)	DMA	MMA	As(V)	Unknown	$[As]_{Extracted}$	Available As
	mg kg^{-1}							%
Core 3								
C3-20	77.82	0.71	<*dl*	0.03	4.98	0.07	5.79	7.44
C3-40	61.56	0.81	<*dl*	0.03	4.88	0.20	5.91	9.60
C3-100	19.67	0.04	<*dl*	<*dl*	0.27	<*dl*	0.31	1.59
C3-120	38.88	0.06	<*dl*	<*dl*	0.56	0.04	0.65	1.68
C3-140	5.74	0.06	<*dl*	<*dl*	0.58	<*dl*	0.64	11.15

Sample	$[As]_{Total}$	As(III)	DMA	MMA	As(V)	Unknown	$[As]_{Extracted}$	Available As
	mg kg^{-1}							%
Core 3 continued								
C3-160	21.77	0.08	<*dl*	<*dl*	0.83	<*dl*	0.91	4.16
C3-180	24.22	0.11	<*dl*	0.05	0.42	<*dl*	0.58	2.41
C3-200	42.50	0.21	<*dl*	0.09	0.53	0.04	0.86	2.02
C3-220	24.12	0.13	<*dl*	0.09	0.32	<*dl*	0.54	2.24
C3-240	32.35	0.10	<*dl*	0.06	0.29	<*dl*	0.44	1.37
C3-280	13.35	0.04	<*dl*	0.10	0.26	<*dl*	0.40	3.03
C3-300	31.23	0.06	<*dl*	0.12	0.24	<*dl*	0.42	1.35
C3-320	45.44	0.09	<*dl*	0.13	0.27	<*dl*	0.50	1.10
C3-370	49.95	0.13	0.03	0.20	0.82	<*dl*	1.19	2.38
C3-390	38.03	0.15	0.04	0.20	1.03	<*dl*	1.42	3.74
C3-410	11.10	0.08	0.03	0.16	0.75	<*dl*	1.02	9.20
C3-440	1.78	0.03	<*dl*	<*dl*	0.20	<*dl*	0.23	13.00
Sample	$[As]_{Total}$	As(III)	DMA	MMA	As(V)	Unknown	$[As]_{Extracted}$	Available As
	mg kg^{-1}							%
Core 4								
C4-0	33.46	0.14	<*dl*	<*dl*	1.56	0.04	1.74	5.21
C4-20	5.21	0.11	<*dl*	0.05	0.85	0.02	1.03	19.67
C4-40	13.17	0.06	<*dl*	0.03	0.52	<*dl*	0.61	4.61
C4-70	60.91	0.12	<*dl*	0.04	0.96	0.03	1.15	1.90
C4-90	26.79	0.04	<*dl*	<*dl*	0.35	<*dl*	0.38	1.43

Sample	$[As]_{Total}$	As(III)	DMA	MMA	As(V)	Unknown	$[As]_{Extracted}$	Available As
	mg kg^{-1}							%
Core 4 continued								
C4-110	9.29	0.06	<*dl*	0.02	0.62	<*dl*	0.71	7.59
C4-140	1.57	<*dl*	<*dl*	<*dl*	0.08	<*dl*	0.08	4.84

<*dl: below detection limit*