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THE EVALUATION OF THE MACROPHYTE SPECIES IN THE ACCUMULATION OF SELECTED ELEMENTS FROM THE VARKENSLAAGTE DRAINAGE LINE IN THE WEST WITS, JOHANNESBURG SOUTH AFRICA

School of Animal, Plants and Environmental Sciences

Master of Science in Environmental Science

A Research Report to be submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science.

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STUDENT No.: 704314

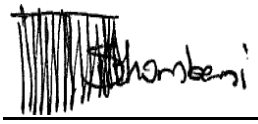
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Declaration

I declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Mthombeni Tinyiko Salome (Signature of candidate)  day of

09TH December 20 15 in Hoedspruit.

ABSTRACT

Mining and associated anthropogenic activities have improved the livelihoods and economy of many countries but negatively impacted the environment and caused detrimental effects on fresh and ground water systems through the generation of acid mine drainage (AMD). The study evaluated three macrophyte species of *P. communis, S.corymbosus* and *T. capensis* for uptake of Mg, P, S, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo, and Pb in acid mine drainage polluted water. The concentration of elements was also determined in sediments and water so as to calculate the bioconcentration and translocation factors in various parts of the macrophtes. The homogenised plant samples were milled using a Fritsch Pulverisette 6 Mill into pulverized powder and element analysis was done using Spectroscout Geo+ XRF Analyzer Pro. Sediment samples were digested with microwave assisted extraction and analysed by inductively coupled plasma optical emission spectrometry (ICP-OES).

Water samples were analysed directly using ICP-OES after filteration with ICP-OES. The concentration levels of these elements in water were compared in all the sites to determine which section of the site (inflow, midflow and outflow) have high amount of the selected elements. The results indicated that elements distribution varied in all the points where the water samples were collected. The concentration level of sediment was compared to the concentration levels of elements in the roots, rhizomes and leaves to determine the translocation and bio concentration factor (TC and BCF). Drinking water quality standards by international organisations were also used as a guideline to compare the concentration levels of elements found in water. Iron (Fe), Nickel (Ni), Manganese (Mn) and Copper (Cu) to determine whether their concentrations in the water were above or below the acceptable levels. the concentrations of Fe, Ni, Mn and Cu were found to be above the international water quality standards for drinking water and their average concentrations was 2230, 282, 5950 and 14080 µg/l respectively.

The study found out that in autumn, Mg, S, P, and Mo were highly accumulated by leaves of *T. capensis, S. Corymbosus* leaves and rhizomes as well as the *P. communis leaves and the highest concentrations were 6.61, 72900, 2.00* µg/g respectively. In autumn, Co was the only element highly accumulated by the roots of *T. capensis* with the highest concentration of 342.80 µg/g. On the other hand, Cr and Fe, was highly accumulated by *S. corymbosus* roots with the highest concentration of 279.20 and 10.03 µg/g in summer. In summer, Cr, Mn, Ni, Cu, Zn and Pb were highly accumulated by the roots of *P. communis* and the concentrations were 279.20, 39390, 204.10, 299.50, 813.80 and 47.5 µg/g respectively.

The results show that although the plant species accumulated the elements in various concentrations, there was no plant species that accumulated all the selected elements in higher concentrations than the other plant species. They

all accumulated a variety of elements in varying amounts and stored them in their different parts. Finally, in all the three plant species analysed, the leaves were the best accumulator of Mg, S, and Mo, whilst the roots were the best accumulators of Cr, Fe, Co, Ni, Cu, Zn and Pb. Since the translocation and bioconcentration factors showed that the macrophyte species accumulated higher concentrations of elements than water and sediments, they can be regarded as hyperaccumulators. Macrophytes species can uptake and accumulate in their different parts various elements and they have the potential to clean the heavy metal polluted sites due to their phytostabilisation and phytoextraction abilities.

List of abbreviations, acronyms and chemical formulae

Acronym	Description	Acronym	Description
AMD	Acid Mine Drainage	**$CaSO_4.2H_2O$**	Gypsum
Cm	Centimetre	**CER**	Centre for Environmental Rights
CGS	Council of Geo Sciences	**CoJ**	City of Johannesburg
DMR	Department of Minerals and Resources	**DO**	Dissolved Oxygen
DWAF	Department of Water and Forestry	**EC**	Electrical Conductivity
ESKOM	Electricity Supply Commission	**GDP**	Gross Domestic Product
FeS_2	Iron pyrite	**Ha**	Hectare
g/cm^3	Gram per cubic centimetre	**JSE**	Johannesburg Stock Exchange
IMC	Inter-Ministerial Committee	**ml**	millilitre
Km	Kilometre	**MPRDA**	Minerals and Petroleum Resources Act
Mm	Millimetre	**PMG**	Parliamentary Monitoring Group
mS/cm	Milli-Siemens per centimetre	**SA**	South Africa
PVC	Polyvinylchloride	**SO_2**	Sulphur Dioxide
T	Tonne	**WW**	West Wits Mining Operations (as division of AngloGold Ashanti Ltd)
WHO	World Health Organisation		

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I would like to express my sincere gratitude in the following manner:

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Chapter 1

1. Introduction

1.1 The History of mining in South Africa

In South Africa, mining began in 1886, whilst in 1867, diamond had already been discovered in the Orange River. In the late 1800s, gold was discovered in Pilgrim's Rest and in Barberton, Mpumalanga Province (MiningSA, Undated). In addition, gold was also discovered at the rocky hills of the Witwatersrand, Gauteng Province. The Witwatersrand basin is estimated to have been the area that has produced approximately 40% of the gold around the world. Other precious minerals that are mined together with gold in the country include platinum, chrome, vanadium, manganese, vermiculite, zirconium, ilmenite, rutile and palladium (AngloGold, 2005). Since the discovery of gold in South Africa, mining has played a significant contribution in the economic, political and social status of the country. Since mining is a pillar sector in the South African economy, mining received a privileged status from the government because of its profitable nature and diversity of minerals that enables it to earn profits locally and from foreign exchange (Adler & Roscher, 2007).

The discovery of gold led to the start of Witwatersrand Goldfields, which was known as the largest stock market in Africa, and it was then renamed Johannesburg Stock Exchanges (JSE) Limited. It started in 1887 and it was established mainly to fund the mining sector. Among other parastatals in South Africa, Eskom, Spoornet and Rand Water came into existence after the gold and diamond mining sectors. The mining sector played a role in the development of the City of Johannesburg and is regarded as the economic hub of South Africa, contributing 47% to the Provincial economy (CoJ, 2012). Johannesburg is also known as 'Egoli', which when translated means a place of gold (AngloGold, 2005). Approximately 40% of the world's total gold reserves found in the Witwatersrand area of South Africa. The development of the mining sector in SA has led to the development of industries that have placed SA in a position at top of the industrialised countries in the Sub-Saharan Africa. Mining has also led to the development of the major infrastructures such as roads, water, electricity, railways and manufacturing industries and other major services (AngloGold, 2005). According to the records of the mining sector that were first collected in 1884, this sector has produced a maximum of 55, 055 t of gold in 2004 which contributed approximately 33% of the total gold when compared to the estimated gold production that was mined around the world. The remaining gold reserves are estimated to contain some 40 000 t of gold, about to 1000 to 8000 t are thought to be recoverable economically as this will depend on the price of gold (AngloGold, 2005).

For the past 120 years, South Africa has been the main contestant in the mining sector globally and also experienced a peak production of 1000 t in 1970 which is approximately 67 % of mine supply when compared with other countries globally. Since 2004, the industry has grown in terms of production and has reached the mature stage where

production has started to decline to 342 t per year (Mining SA, undated). To date, this sector remains the largest employer and significantly contributes towards 2 % of the gross domestic product (GDP) and 10 % of the foreign exchange (AngloGold, 2005).

Although SA was the biggest producer of gold globally and despite the fact that this industry is experiencing the drawbacks of closure of older mines and shafts, it is also experiencing a decline in the production of minerals as well as the generation of acid-mine drainage (AMD) from the mines and tailing storage facilities, it is also experiencing the exhaustion of gold reserves, affected by low gold price globally, high energy requirements for deep level mining, high labour wage demands and social unrests. These setbacks have made the economic position of mining in South Africa to decline. There are detrimental impacts that arise from the cessation of the large-scale mining operations (Inter-ministerial committee, 2010). In order to access gold resources far underground, mining operations resorted to dewatering activities, in order to keep the groundwater level away from the mining operations. Once mining ceased, the voids began to flood with rainwater and groundwater as well as with surface water resulting in acidic water.

The acidic sulphur rich wastewater or effluent from the mining and industrial environments has greater consequences of AMD in both actively operating and abandoned mines (Johnson, 2004). The occurrence of minerals containing sulphide resulted in the generation of AMD in the abandoned and active mines. During the mining process, the extraction of the gold bearing conglomerate layer is crushed and the gold extracted (Sutton, 2012). After the gold has been extracted, the crushed rock is deposited on heaps known as slimes or tailing dumps. Generally, gold bearing conglomerates contain about 3% of pyrite which gets deposited in slimes and tailing dumps (Akcil and Koldas, 2004, Johnson, 2004, IMC, 2010) resulting in AMD.

Acid Mine Drainage occurs in regions with historic and current mining activities throughout the world. AMD is defined as a natural process (more correctly termed “acid rock drainage”, or ARD) that occurs when sulphur containing minerals become exposed to water and oxygen, in the presence of bacteria known as the *Acidithiobacillus* and *Ferrooxidans* (Akcil and Koldas, 2004). Sulphides in pyrite rock (Fool’s gold) then react with oxygen and water and leading to the production of sulphuric acid (McCarthy, 2011). Dissolution of iron sulphide lead to the production of acid and in can be described by the equation below.

$$4FeS_2\,(s) + 15O_2 + 14\,H_2O \longrightarrow 16H^+\,(aq) + 8\,SO_4^{2-}\,(aq) + 4Fe\,(OH)_3\,(s)$$

The sulphuric acid percolates through the slimes dam and dissolves some of the heavy metals. The resultant acidic, net acidic and saline plume enters the surrounding soils, and eventually enters the groundwater and surface water bodies (McCarthy, 2011 and Johnson *et al,* 2005). AMD is a slow process characterized by low pH and high salinity levels with higher concentrations sulphate, iron, aluminium and manganese and possibility of radionucleids

(Oeloefse, 2008). The dark, reddish-brown and low pH water (often lower than 2.5) is difficult to rectify (Akcil and Koldas, 2004).

The most important salts and heavy metals associated with AMD that pose serious contamination threats to environment and human health are metals such as Fe, Mn, Al and other heavy metals as well as metalloids such as arsenic (Johnson *et al*., 2005 and Hu, 2002). Heavy metals such as mercury and metalloid such as arsenic can become toxic and pose additional risk to the environment even when they are introduced to the water system in minute amounts (Johnson *et al*., 2005 and Mohiuddin *et al*. 2011).

The introduction of excess concentrations of metals into fresh water bodies does not only impact on water quality, it also has a negative effect on the food chain and health status of the aquatic systems. In addition, it affects other life forms that depend on the fresh water bodies and sediments for survival (Cheng, 2003). When elements are being transported in a water body, their chemical properties may change due to the processes of dissolution, precipitation, sorption and complexation. These processes also affect the bioavailability and behaviour of metals in the water and sediments (Akcay *et al*., 2003).

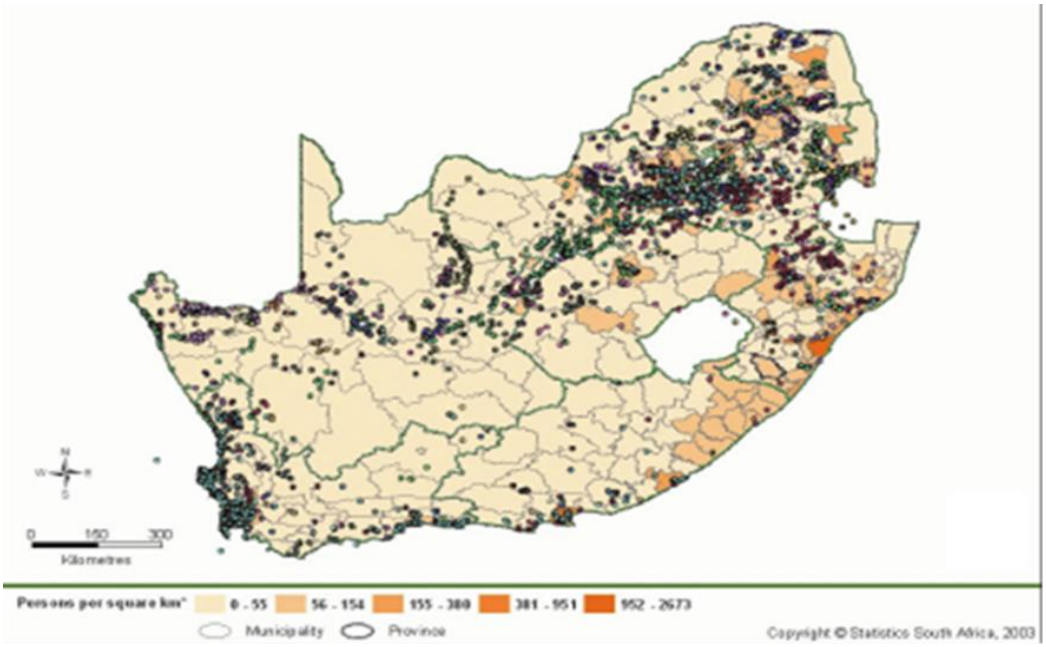
In order to gain an understanding of AMD, one has to understand its characteristics. According to McCarthy, (2011) the negative impacts of AMD depends upon other factors such as the geomorphology, climate and the area covered by the deposits significantly contribute towards the AMD generation. The Vaal River catchment is located in the area that most of the gold and coal operations take place. The upper catchments of the Vaal and the Olifants rivers in particular are extensively underlain by coal deposits. The coal and gold-rich regions receive the highest rainfall in the area. As a result, most of the seepage from tailings from these deposits has the possibility of finding its way downstream from the catchment. The mining process enhances the formation of AMD through the exposure of rocks containing pyrite to the surface with water and oxygen (McCarthy, 2011).

Acid mine drainage pollution has been reported in the catchments around the Witwatersrand Basin (IMC, 2010). In addition, decanting polluted mine water from the abandoned mines of the Western, Central and Eastern basins is on-going (DWAF, 2012). The Central basin is situated to the South and South East of Johannesburg. Since its dewatering pump was switched off in 2008, it was expected to begin decanting of underground mine-water in 2012 (Roche-Kelly & Van Dyk, 2011). According to the Inter Ministerial Committee, (2010), the first flooding was experienced in the western basin of the Witwatersrand gold fields, which occurred in the closed shaft in September 2002. To date, the Western Basin is still a problem. The water levels in the Central and Eastern Basins are also growing (DWAF, 2012). The outflow of the polluted water from the abandoned and closed mines ran into rivers and then into farmland through irrigation (PMG, 2011).

1.2 The history of abandoned mines in South Africa

South Africa has a well-developed mining sector and operates in the large-scale commercial mining. The abundance of mineral reserves in South Africa has on the other hand led to the abandoning of mines in South Africa. According to a report by the Council of Geoscience (CGS), (2008) there are approximately 5906 mines that are officially listed as abandoned mines in South Africa, prior to the year 2008. The majority of these mines closed down prior to 2002, when the Minerals, Petroleum Resources Development Act (MPRDA) No. 28 of 2002 was promulgated. Under this Act, an owner of the mine will by law remain accountable for the liabilities that are associated with the mine and the associated mining activities, until a legal certificate of closure had been issued by the Department of Mineral Resources. Sections 41 and 43 of the said Act entail that an owner to make a considerable provision for all the environmental liabilities incurred by the mine (Centre for Environmental Rights, 2009).

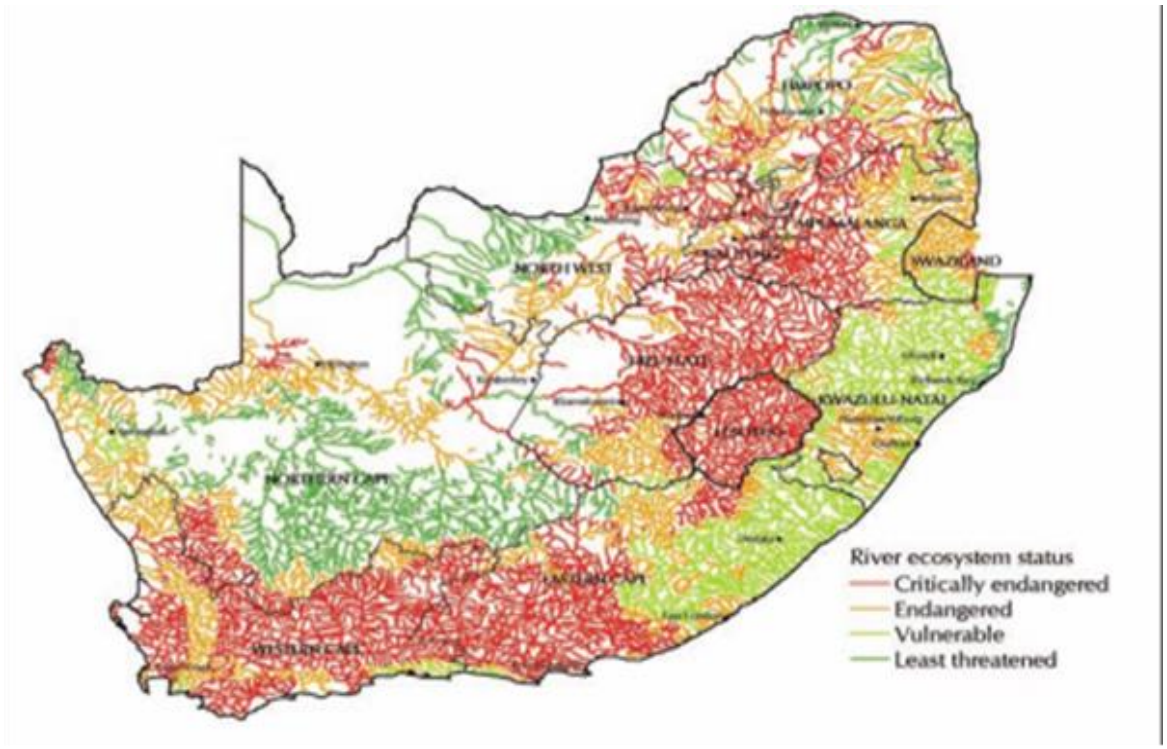
The Department of Minerals and Resources (DMR) has identified the key issues that result because of the abandoned mines. These risks include air pollution due to dust generation from mine dumps, contamination of surface and groundwater resources with acid, salts and heavy metals, combustion products from burning mine workings, and physical hazards posed by sites with open shafts and unstable slopes (CER, 2009 and Schachtschneider, 2011). Thus abandoned mines do not only pose huge health threats but also negatively impact the fresh surface and underground water resources. Figure 1 shows the areas where the abandoned mines occur throughout South Africa. This indicates that the health of the general public is under a great risk. In addition, the surface and groundwater resources as well as the ecosystem are under severe pressure.



Adopted from: Auditor General Report, South Africa, 2009.

Figure 1: Map of South Africa showing abandoned mines

The abandoned mines are the major threats to the river systems of South Africa. Figure 2 below shows the main tributaries that are impacted by the abandoned mines because of the surface and underground drainage in South Africa. The main river systems that play a major role in supplying fresh water are under a severe pressure from AMD from the abandoned mines. Amongst the other major rivers in South Africa, the Vaal river and the Olifants river systems are the main systems that most of its water are used in the mining, domestic, agricultural, industrial, commercial, and other sectors in South Africa which are severely impacted by AMD from the abandoned mines in the Witwatersrand basin and coal mines in Mpumalanga (DWAF, 2012). South Africa is regarded as a semi-arid country and it obtains most of its water from the Lesotho Highlands. This makes it a necessity for the abandoned mines to be rehabilitated by the DMR although this will require the input of huge capital into the abandoned mines for rehabilitation purposes, as this will reduce the severe stress that the surface water and groundwater resources are exposed to as well as the vulnerability of biodiversity and ecosystem that are threatened by the legacy of abandoned mines (McCarthy, 2011, DWAF, 2013, IMC, 2010). In addition, most of the critically endangered rivers occur in areas where most of the abandoned mines are located. Many of these affected catchments are found on the interior parts where most of the gold and coal and other abandoned mines are found on the Western towards the southern parts of the South Africa.



Source: SA Environment Outlook: A report on the state of the environment, DEAT

Figure 2: Ecosystem status of the rivers in South Africa.

1.3 Land uses in the Witwatersrand basin and associated catchment

An overview of the land uses in the Witwatersrand basin includes a variety of highly urbanised areas. From the perspective of water quality, the most crucial land uses include mining, power generation, and steel industry, waste water treatment works that are linked with the urban and municipal areas. Mining, waste water treatments, urban and municipal areas are dependent on the water from the Vaal river, and this have greater impact on the water quality and the water quantity of this river and other streams that are connected in these system, (DWAF, 2012).

Gold mining has been dependent on water from the Vaal River and Elsburgspruit rivers for mining activities such as cooling and mineral washing (AngloGold, 2008). The Kraalkop Nature Reserve is situated within the vicinity of the West Wits Operations, and it is a game farm that provides recreation to individuals and around the mining area. The main activities that take place within the game reserve include ecotourism and conservation education. The recreational activity that is allowed in the dam within the game farm is fishing (AngloGold, 2009). Other facilities around the West Wits operations include property and residences, commercial facilities and training as well as exploration (AngloGold, 2009).

1.4 AMD in the Witwatersrand basin and the West Wits operations

Contamination of the environment by heavy metals results from the anthropogenic activities such as mining, and processing plants, where waste conveying huge amounts of heavy metals is channelled, percolate or leach into freshwater or ground water bodies. The accumulation of heavy metals by fresh water bodies have negative impact on the biodiversity that depends on the fresh water for survival. Heavy metal contamination also has various impacts of the different aspects of water use and result in the depletion of oxygen in the fresh water. Eutrophication is another typical example that results from the mass loading of elements such as Nitrogen (N) and Phosphorus (P) into the fresh water systems, and it can also result in the mortality of aquatic fauna due to the rapid depletion of oxygen content. Accumulation of heavy metals in the bodies of plants and animals is another serious impact that can be observed as a result of AMD. The exposure of aquatic plants and animals to AMD contaminated bodies impacts negatively on the growth, development as well as reproduction of organisms in the water.

When polluted water enters and surfaces in the streams that drain around the dumps, the streams are often become characterised by high toxic conditions with relatively low pH conditions, high sulphate and heavy metal concentrations (Oelofse, 2008, Akcil and Koldas, 2004, and McCarthy, 2011). The most crucial negative impact associated with AMD is the availability of metals that cover the soil and rocks and such impact of metal toxicity have the ability to hamper and destroy the function and structure of the ecosystem (Briden, 2004).

1.5 Acute biodiversity impacts of AMD

As stated earlier, mining causes a range of negative environmental impacts from land disturbances, habitat fragmentation to air pollution as well as soil and water pollution (Genthe *et al.,* 2013). Consequently, the living standards of people are affected negatively. Mining effluent from seepage, tailings dam and waste rock impoundments also pose threats to fresh water resources and its associated aquatic life forms in ecosystems that receive the effluent water from the tailing dams (Düzgün and Demirel, 2011).

AMD is associated with low pH, high sulphates and the accumulation of heavy metals, which lead to the contamination of surface and ground waters, as well as soils (Adler and Rascher, 2007). AMD can reduce the growth and development of various organisms across the trophic level which then negatively affects the food chain that supports various ecosystems (Gavin *et al.,* 2009). Acid conditions result in the leaching of nutrients from the soil, thus reducing nutrient availability to plants, by blocking the intake of crucial elements such as Calcium (Ca), N and P which are required by the plants for growth and development. This often takes place when the soil pH drops below 4.5. The low pH conditions caused by AMD also favour the growth and development of invasive plants, which thrive at the expense of indigenous plants (Briden, 2004).

The low pH associated with AMD causes the release of high concentrations of heavy and trace metals into the environments, thus making metals more bioavailable (Jennings *et al.,* 2008). In waters that receive AMD directly, the fish become directly exposed to the metals and H+ ions (Jennings *et al.,* 2008). The metals get into the body of the fish through the gills. Once the metals entered the fish gills, the fish become susceptible to respiration failures as a result of acute toxicity. Fish and other organisms in the AMD contaminated water that feed on sediments and clays are vulnerable to increased metal levels in the aquatic system. This occurs mostly during the process of ingestion and it is therefore passed on into the food chain (Jennings *et al.,* 2008).

During the contamination of water bodies with AMD, Iron hydroxide is formed, also known as a red coloured precipitate that covers the bottom of streams (Jennings *et al.,* 2008). Iron hydroxide covers all surfaces in streams, making it impossible for aquatic organisms to feed on their preferred vegetation. This consequence of AMD poses a serious threat to the physical, chemical and biological processes that underpin food chains. In a study conducted by Oberholster *et al.,* (2011), there is evidence of Iron (Fe) coating the intestines and fat of the aquatic invertebrates that are found in areas affected by AMD.

Macrophyte aquatic plant species have different capacities of metal uptake into the tissues of their roots and also in their aboveground parts (Aksoy, 2005, Duman and Sezen, 2011). The degree at which metals are taken up by the plant and distributed in the various parts of the plant is influenced by the residence time of the plants. Metal uptake

and distribution within the plants have been investigated in numerous studies including Larsen and Schierup (1981), Koorimannil *et al.,* (2010), Phukan, *et al.,* 2015, Cardwell *et al.,* (2002) and Ye *et al.,* (1997). Hyperaccumulators are plants species that can uptake metals and translocate in the organs in various concentrations. According to Lasat (2000) some plant species of hyperaccumulators can uptake metals to concentrations that are 100 times greater than the eventual amounts that can be accumulated by other plant species. Some of the hyperaccumulators can take up to 10 ppm Hg, 100 ppm Cd, 1 000 ppm Co, Cr, Cu and Pb, 1000 ppm Ni and Zn.

Reeves and Baker (1999) have identified up to 400 plant species that are well known for their capability to accumulate higher amounts of metals in them. In order for the plants to grow and develop through their life cycle, both the macronutrients and the micronutrients have to be taken up by the plants. Plants that uptake metals perform a crucial role of acting as biological indicators for monitoring of heavy metals on polluted sites. As a result, they can be used in the process of phytoremediation (Aksoy *et al*., 2005 & Wislocka, 2006). The utilisation of various plant species for cleaning of metal contaminated sites is a widely used low cost technology in both developed and developing countries around the world. There are many plant species that are used for this role of metal cleaning. Both the terrestrial an aquatic plants are globally used for this process (Aksoy *et al*., 2005).

1.6 Justification of the study

Although mining, industrial and agricultural activities are amongst the important activities in the economy for the improvement of livelihoods in the world, they also have a consequence of environmental pollution and Acid Mine Drainage which is one of the pressing issues because of its serious environmental and human health threats. Effluent discharged from mining have pollutants that are discharged either in dissolved or suspended forms or suspended in materials in aquatic bodies and this decrease the water quality and increase the ecological risk of human and animals (Harguinteguy *et al.,* 2013). Heavy metals and metalloids released from mining and other industrial effluent have been and are currently of great environmental concern, and they also have a great negative impact on biodiversity. Some elements when released to the environment, they are taken up by the plants for physiological purpose whilst some become toxic and cause harm to aquatic fauna and flora as well as changing the chemical, biological and physical forms and functioning of the environment. Although there are a variety of costly technologies worldwide that are used to remediate contaminated soils from mining, agricultural, industrial and settlement related contamination, phytoremediation is still regarded as the most cost-effective, aesthetic and long-lasting solution to the remediation of the contaminated sites (Yoon *et al.,* 2006).

There are different types of macrophyte species that hyperaccumulate many toxic elements from the soil and water bodies and improve the quality of soils and water as well as to protect and ensure a healthy environment that promote a basic human right according to the constitution of South Africa (Act No. 108 of 1996) as cited by Weiersbye *et al.,* (2006). In this study three macrophyte species were investigated to quantify the quantities of elements accumulated and stored in the leaves, rhizomes and roots as well as to compare the quantified amounts of elements accumulated by the plants in autumn and summer seasons. The levels of elements analysed in the water samples collected were compared with the international standards for drinking water to determine whether the quantities of elements in water were above or below the acceptable benchmark of the drinking water standards.

1.7 Aim of the study

The aim of this study was to quantify the removal of sulphur and metals from acid mine drainage by plantings of the macrophyte species *P. communis, T. capensis* and *S. corymbosus* in the Varkenslaagte stream, situated at the West Wits Mining Operations area on the Highveld of South Africa.

Specific objectives include:

1. To determine the kinds and levels of metal accumulations in water, sediments and plants in the Varkenslaagte Drainage line of the WW operations.
2. To quantify the concentrations and amounts of carbon, nitrogen, sulphur and metals accumulated by *T. capensis*, *S. corymbosus* and *P. communis,* from the wetland plants located along the canal drainage line for the parts of the plant below and above the ground as well as their relative allocation to each other, the sites and the plants parts.

1.8 Outline of the study

This study consists of 5 chapters. The first chapter highlight the introduction, the history of abandoned mines in South Africa, economic significance of mining in the Witwatersrand basin, land uses in the West Wits basin and associated catchment, AMD in the WW basin and West Wits operations, acute biodiversity impacts of AMD, aims of the study as well as the research questions. Chapter 2 focuses on the literature review of the study which explores the three macrophyte species in the Varkenslaagte drainage line and the elements which were analysed in the study. It further highlights the process of phytoremediation of the elements pollution by the plant species *S. corymbosus*, *P. communis* and *T. capensis*. The third chapter is the research methodology and looks at the site description, description of sampling sites, site core sampling and sediments sample collection, plants sampling and sample preparation, water sampling and water analysis and total nitrogen analysis as well as data presentation. Chapter 4

give brief discussions of the research findings and results of the study. In this chapter, the trends of selected elements analysed in the study from different data collection tasks were briefly discussed and presented in the form of appropriate graphs and tables. The last chapter gives the summary, conclusion and recommendations of the study. These are followed by the references that are included throughout the body of the report, and the Appendices of statistics and raw data of the variables analysed in the study.

CHAPTER 2 Literature Review

2.1 Introduction

Western basin of the Witwatersrand is a mining intensive area and it is known that the water bodies found close to the mines also receive harmful or toxic elements from the mine dumps that end up in the fresh water bodies through seepage, runoff and percolation. In this regard, fresh water bodies in a gold mining area such as the West Wits are likely to contain, in either the water or in the sediments, detectable or elevated concentrations of potentially toxic metals such as magnesium (Mg), phosphorus (P), sulphur (S), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), molybdenum (Mo) and lead (Pb). Some or all of these metals may be adsorbed on, or absorbed by, organs (roots, rhizomes, stems and leaves) of wetland plant species that grow in these environments. Examples relevant to this study are the wetland macrophytes *S. corymbosus, P. communis* and *T. capensis spp* that are common to both the natural drainage line and a series of shallowly excavated wetlands in the Varkenslaagte canal which drains the West Wits Mining Operations northern tailings facilities (Weiersbye et al.,2006).

Due to the known risks of AMD, metals and radionuclides entering the fresh water bodies, an extensive mechanical operation was conducted to remove all spilled tailings from this canal, in order to rehabilitate the polluted effluents and stream sediments using various aquatic macrophyte species. Many aquatic macrophytes are classified as heavy metal accumulators (Kärenlampi *inter alia*, 2000) and they are known to accumulate metals to various degrees, and store them in below-ground tissues (rhizomes, roots) or above-ground tissues (leaves, stems). In some cases, aquatic macrophytes have been found to absorb higher concentrations of metals than are found in the water (Tilahun and Ashagre, 2011).

Macrophytes have unique distinguishing characteristics. They have the ability to uptake elements from various polluted sites and translocate these elements in the tissues for various purposes. They are also known to be tolerant to the various concentrations of metals that they accumulate such as Iron, Al and other elements. Macrophyte species have high biomass production rates. Moreover, macrophyte species are generally known to accumulate more than one element from the soil and store elements in their roots, rhizomes and stems as well as in their leaves (Kärenlampi *et al.,* 2000). Although some growth and development of plants in general is impacted by the high availability of toxic metals in the water, sediment and soil substrate, there are also some macrophytes that are able to survive and reproduce in the metal contaminated environment, which is they are tolerant to such conditions. These are known as metallophytes (Baker, 1981).

Some metallophyte species that grow and develop well in metal contaminated sites are thought to grow and reproduce faster because they do not have competitors. They are thus effective in covering the polluted soil, and they play an important role of colonising the disturbed sites which are characterised by physicochemical characteristics which does not support the growth of many plants (Mehes-Smith inter alia (*et al)*, 2013). Although such plants survive in the slimes dams and polluted substrates, depressed seed production and seedling abnormalities is common in plants because of the high levels of inorganic contents (Weiersbye *et al*., 2006).

In order for the plants to survive, they must adapt to the chemical and physical characteristics of the soil, water, atmosphere and climate. Plants that grow and survive in metal contaminated environments (metallophytes) have the distinct characteristic of tolerance. Plants also may be categorised as the metal excluders, indicators, accumulators or even as hyperaccumulators (Baker, 1981). Hyperaccumulators have the ability to translocate metals to their above-ground organs, and thereby extract and accumulate quantities that exceed any other species in the same environment (Salt *et al*., 1998).

In order for a plant to be defined as a hyperaccumulator, the plant must have a root:shoot quotient of >1, i.e. it is able to translocate and accumulate more metals in their shoots than it can accumulate in the roots (Baker, 1981; Kachout *et al*., 2009). Some plants take up a certain metal in relatively higher concentrations than the other metals whilst only a few plants accumulate multiple metals in higher amount. Some plant species of *Thlaspi* and *Alyssum* have been discovered and proven to accumulating some reasonable amounts of elements of Zn, Pb and Cd in the plant parts. Such plants are known to have a ratio of >1 when the total concentration of elements in the shoots divided by the concentration of elements on the roots (Mehes-Smith, 2013).

Plant species differ in their ability and tolerance of metal uptake and accumulation (Aksoy, 2005). Some plant species can accumulate high concentrations of a single metal and translocate it to the roots, rhizomes, stems and/or leaves, while others can accumulate more than one element in different parts. Another category of plant are known as the excluders, and have the ability to tolerate metal-rich environments by reducing the amount of elements translocated from the below ground parts to the aboveground parts. This mechanism in the plants is said to achieve the lowest possible concentration of metals in their shoots. These plants store most of the metals they uptake from the metal containing sediments and water in the roots rather than in the shoots. Baker and Walker (1990) have noted a wide range of unrelated excluder plant species such as *Oenothera biennis, Commelima communis, Silene maritime, Agrostis stolonifera* L.*, and Pinus radiate,* representing annual and perennial herbs, grasses and trees.

Baker and Walker (1990) categorise the third tolerance strategy of plant species in the metal rich sites as resulting in so-called “Indicator species”, which also accumulate their metals in the shoots, but only to levels in equilibrium with the substrate. The concentration of the metals in the leaves is considered to be a reflection of the concentrations of metals in the substrate environment in which they grow.

2.2 Phytoremediation of metal pollution

Although it is difficult to rectify the exacerbating effects of AMD on metal availability, it is essential to consider feasible solutions, including those of low cost technology. This can include the use of aquatic macrophyte species for phytoremediation, i.e. to stabilise, adsorb, absorb or even accumulate elements from AMD polluted sites. Phytoremediation is defined as the use of higher plants to immobilise or extract elements from the contaminated soil, water and or sediments (Chaney *et al*., 1997). Phytoremediation can be applied to both organic and inorganic pollutants present in the soil, water and air (*Salt et al*., 1998). Phytoremediation is the use of green plants and their associated micro-organisms, soil amendments and agronomic techniques to remove contain or render harmless environmental pollutants (Cunningham and Berti, 1993). Hence the technology is suitable for AMD contaminated areas with elevated metal concentrations.

Plant species exhibit varying degrees of tolerance, and respond differently to contaminated environments. This response may include accumulating different amounts of metals and concentrating them in the roots, shoots and stems. Plants that can accumulate elements to levels that exceed soil levels are classified as hyper accumulators (Salt et, al., 1998). According to Lasat (2000), citing Baumann (1885), plant species of *Thlaspi caerulescens* and *Viola calaminaria* were the first recorded plants with high levels of metals in their leaves in the 19th century. They have the ability to tolerate and accumulate metals such as Zn, Co, Cu and Mn in their shoots (Lasat, 2000). The ability of these plants to tolerate such heavy metal concentrations then prompted further investigations into the physiology and mechanisms that plants develop in order to accumulate high amounts of heavy metals (Baker and Brookes, 1980).

Table 1: Some herbaceous plants used in mine sited for phytoremediation

Botanical name	Common Name	Process	Reference
Amorphia fructicosa	Indigo bush	Accumulation of Cu, Pb and Zn.	Seo *et al.,* 2008
Achillea millefolium	Yarrow	Accumulation of Pb, Cd and Cu	Radulescu *et al.,* 2012
Azolla pinnata	Water velvet	Biosorption and bioaccumulation of metals of Cu, Pb, Cr, Cd and Zn	Shafi *et al,* 2015
Bacopa monnieri	Water hyssop	Accumulation of Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Zn	Koorimannil *et al.,* 2010
Hydrilla verticillata	Hydrilla	Hyperaccumulation of Cr and Cd	Phukan, *et al.,* 2015
Myriophyllum aquaticum	Parrot feather	Translocation and degradation of metals	Cardwell *et al.,* 2002
Phragmites australis	Common reed	Reed bed treatment systems for accumulation of Zn, Cu and Se. Accumulation of Zn, Pd & Cd	Ye *et al.,* 1997
Brassica juncea	Indian mustard	Hyperaccumulation of As, Cd, Mo and Cr	Reisinger *et al.,* 2008
Allium Schoenoprasum	Chives	Hyperaccumulation of Pb, Zn, Ni, Cu, Co and Cd	Soudek *et al*., 2009
Thlaspi caerulescens	Alpine pennycress	Hyperaccumulation of Zn, Cu, Pb and Cd.	Basic *et al.,* 2003
Pteris vittata	Brake fern	Hyperaccumulation of As	Caille *et al.,* 2003

2.3 Habitat types and plant communities of *S. corymbosus, P. communis* and *T. capensis*

Schoenoplectus corymbosus L., Phragmites communis L. and *Typha capensis L.* are the three common aquatic macrophytes that are widespread in the West Wits wetlands, and therefore have been planted or allowed to colonise the artificial wetlands excavated along the canal. They are known to often form monospecific stands which exclude other plant species in the habitat they occupy by fast growth, persistent shading and extensive utilisation of space and by spreading their roots (Aksoy *et al*., 2005). All three species occur in areas of anthropogenic wetlands that receive acid mine drainages from gold mine tailings dams in South Africa (Weiersbye *et al*., 2006), and favour clayey and seasonally or perennially saturated soils, where they form dense stands (Filmalter, 2010).

P. communis is one of the commonly known aquatic macrophyte species that has the ability to absorb up to four different trace elements of Fe, Mn, Zn and Ni and store them in both the roots and in their shoots (Van der Merwe *et al.,* 1990). In the study conducted by Aksoy et al., (2003), It has been indicated that most of the macrophyte species used for phytoremediation store most of their accumulated elements in the roots, and the leaves whilst the stems and rhizomes are the organs where the least of the element are stored (Aksoy *et al*., 2003). Schierup and Larsen (1981) and Peverly (1995) have reported that there are some trace elements such as zinc which are found in high concentrations in the aboveground shoots whereas some of the metals were discovered on the belowground zone especially in the roots. The concentration of heavy metals in reeds is generally low. These species accumulate most of the heavy metals in stems but the only metals that are concentrated in high amounts in the leaves is Zn, and the accumulation of high amounts of Zn in the leaves is assumed to be due to the role that zinc play in the biosynthesis and its requirements for hormonal growth (van der Merwe *et al*., 1990).

P. communis inhibit the uptake of metals to the shoots and this is believed to be the tolerance mechanism of most wetland plants (McCabe *et al.,* 2001, Windham *et al.,* 2003 and Weis & Weis, 2004). Wetland plant species protect themselves against metal toxicity in different ways. Some plants release a small portion of metals through the tissues of the leaves during the growing period (Burke, 2000). In metal polluted habitats, *P. communis* do not transform the uptake and distribution of metal as a way of coping with stress resulting from the environment (Ye *et al,* 1997). Furthermore, the study conducted by Schierup and Larsen (1981a and b), titled “Heavy metal uptake by reeds in a polluted and non-polluted site”, indicates that heavy metal uptake from non-polluted sites were relatively higher than those that were found in the polluted site.

Factors such as pH, redox potential, soil type have a greater impact on the metals uptake than on the total levels of heavy metals available in the sediments and surface waters. The types of soil have significant effect on the uptake of

metals. In addition, clay soil has a higher sorption capacity which in turn reduces the availability of the metals. clay soil with high organic matter enhance the conditions that favour highly reduced conditions and successive precipitation of sulphide and this has the ability to decrease the available metals at the lower depths. On the other hand, soils with less clay and organic matter content tend to release metals from the sediments and improve the metal uptake by the plants. Salinity also improves the rates of metal uptake, especially Cr, Ni and Zn (Du Liang, 2006).

Another important factor affecting metal uptake in *P. communis* is the water levels. The seedlings of *P. communis* grown under flooded conditions accumulate more heavy metals in the biomass than seedlings grown in dry conditions (Ye *et al,* 1998). *P. communis* indeed obtains its nutrients from sediments. The stems of the reeds are the main potential parts to observe the factors that affect metal uptake, since the levels of metals analysed on the roots and rhizomes may indicate only the metals that are taken up by the roots (Weis & Weis, 2004).

T. capensis is widely known as cattail or bulrush. It is a monoecious plant also known as a perennial aquatic weed. *T. capensis* is a member of the family *Typhaceae*. It has a fast growing rate and it can grow up to a reasonable length of 2 m or more in height. It is distinguished from other aquatic plants from its twisted and spongy leaves that are like strap. The bluish-grey to green leaves with parallel veins can grow up to a length of 0.5 to 1.5 m or even more. It is well known for spreading through its rhizomes and colonising stream banks, ponds, canals and marshes and in dry areas in many parts of Africa (Voigt & Porter, 2007).

According to Van Der Merwe *et al.,* (1990) variations in water pH affect the ability of metals in water to be soluble. Furthermore, this also impacts the deposition ability of metals in the sediments as well as in the flowing water. The concentrations of Zn, Mn and Ni in sediment have a direct bearing on the increased uptake of elements by plants (Mortimer, 1985). This result from the fact that some of the metals such as Zn, Mn and Ni are also taken up by the plants for metabolic activities. Furthermore, there are a number of extraneous factors that affect the intake of these metals by plants such as season, competition and water flow. Changes in seasonal growth rates and patterns of the plants, physical age of the plant and metal ion involved in the absorption process are the most crucial factors that play a role in plants metal uptake. According to the study conducted by van Der Merwe, it has been indicated that there have been some successful observations made about the absorption of Ni and Mn on *T. capensis.* This macrophyte species is distinguished from other aquatic plants due to its tolerating mechanisms on both the acidic and alkaline conditions of various metals from polluted sites with different sources of contaminants. *T. capensis* can

also accumulate higher amounts of element such as Cu, Fe and Ni Fe and they accumulate in the aboveground shoots (Van der Merwe, *et al.,* 1990).

The pattern of copper absorption is in a descending order. Most of the accumulated copper is stored in the white part of the stems, then into the green part of stems and then into the green leaves. The most important storage organ of elements in the plant is the roots. Whilst, according to observations made by Ying Ma (2005), Cr was found to be the least absorbed element throughout the body tissues of *T. capensis. T. capensis* has the capacity to concentrate various elements such as Fe, Mn, Ni and Cr in the aboveground stems and leaves as well as in the below ground parts of the plants such as the roots (Ying Ma, 2005). Although the amount of elements accumulated can be relatively higher and at varying degree, but it can be treated as an aquatic macrophytes suitable for bioaccumulation.

According to Schachtschneider *et al.,* (2012) *S. corymbosus* is a hyperaccumulator of metals Al, Fe and Mn. Its metal uptake by the shoots far exceeds the metal uptake of the *P. communis*. As a result, *S. corymbosus* can be regarded as an effective plant to be utilised for phytoremediation processes for some trace metals. *S. corymbosus* can withstand various salt stresses that result from various activities such as mining, agricultural land uses, wastewater treatments which have various pH levels (Ying, Ma, 2005).

Chapter 3

3.1 Materials and methods

The following sub-sections briefly discuss the methods by which water, sediments and plants samples were collected. This section also highlights each sample type as well as the methods of sample analysis. The sample size (n=15) and it makes statistical comparisons easier as it is not limited by the sample size.

3.2 Site description

The study area is found in the West Wits and it is situated along the 15 kilometres (km) Varkenslaagte drainage line, which is also known as the old canal of the West Wits Operations (AngloGold Ashanti). The area is divided by a rocky ridge called Gatsrand. It covers approximately 3785.5 ha and approximately 38.58% which equates to 14604 ha is under deep mining (Mponeng, Tautona and Savuka mine). The rest of the area is occupied by mining related infrastructures such as tailing dams, mining plants, shafts, related operations, residential as well as excavations. The Northern part of the mining area has been converted into agricultural land and mining activities while only a small portion of the area is still in its natural state.

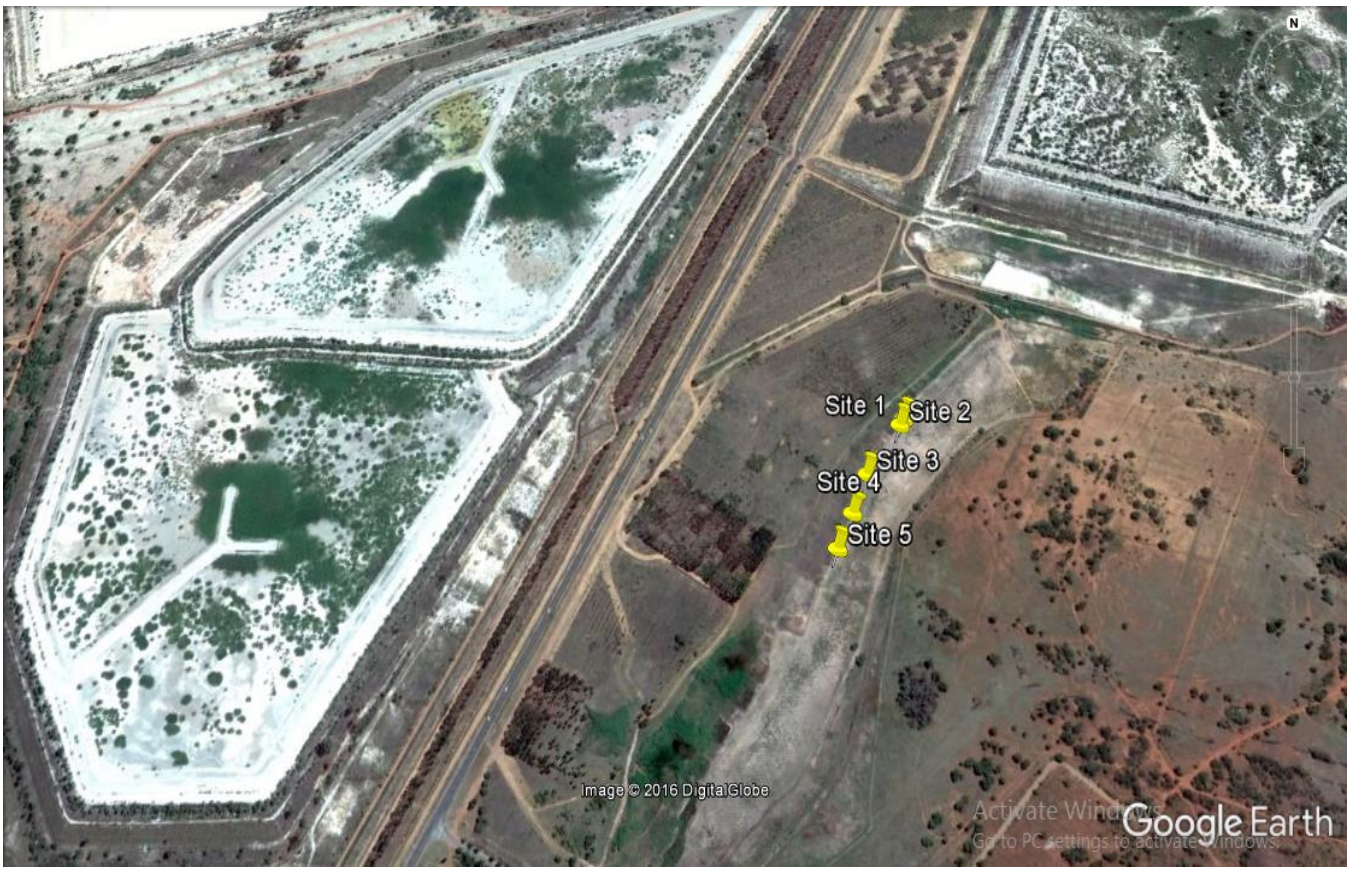


Figure 3: Map of West Wits showing the sampling sites

The West Wits operations are situated approximately 75 km from Johannesburg within the Gauteng Province and about 7 km South of Carletonville.

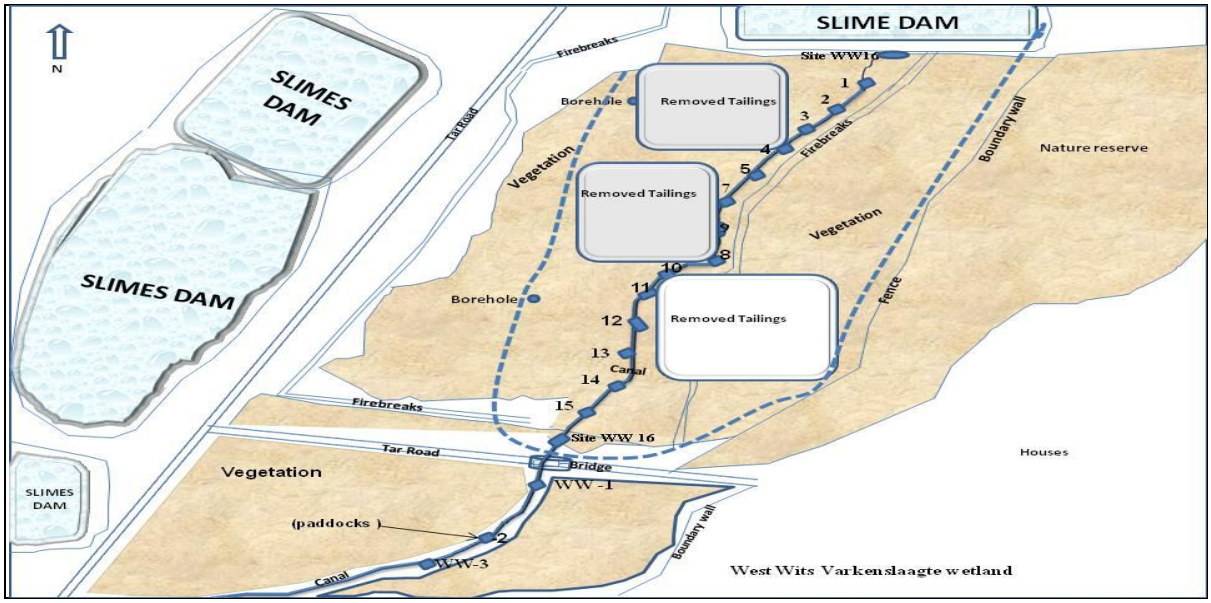


Figure 4: Map of the West Wits showing the slimes dam and the flowing direction of the water from the slimes dam into the canal.

West Wits receives an average rainfall of over 725 millimetres (mm) per annum. The rainfall is in the form of showers and thunderstorms, falling during the summer months between October and April (World Weather Online, 2004). Below is a graph showing the average rainfall in mm of West Wits.

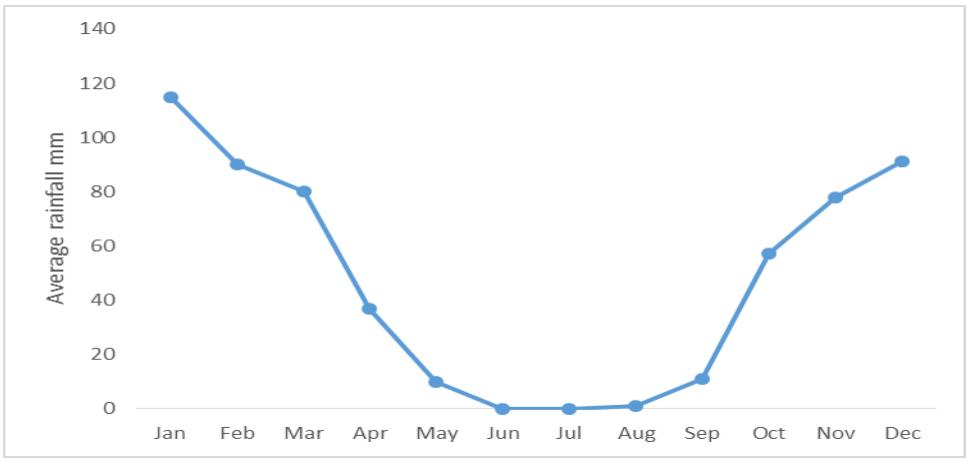


Figure 5: Average rainfall mm of West Wits

Figure 5 above shows the monthly minimum temperatures for the three coldest months of June, July and August were 1.1, -1.4 and 1.0˚C and the maximum temperatures for the three hottest month of December, January and

February were 26.4, 26.9 and 26.6°C respectively (Schulze et, al., 2008). Figure 6 below illustrates the annual high and low temperatures of Carletonville adopted from World Weather Online

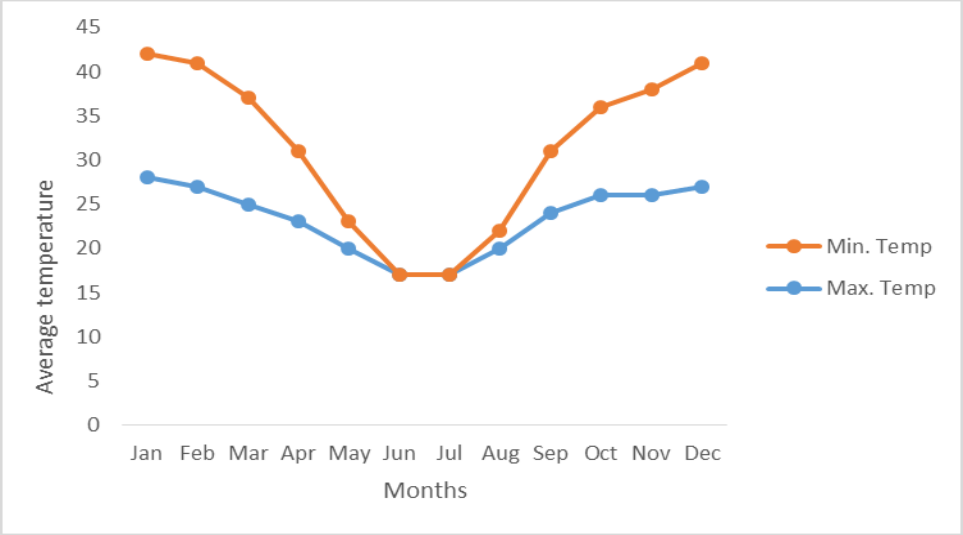


Figure 6: Average temperature 0°C for West Wits.

West Wits is comprised of a range of soil types with their associated soil properties and soil formation. According to Mucina and Rutherford (2006), the area consists of different types such as Hutton which is characterised of deep red soils without the surface rock. The Clovelly is another soil type is another type found in and around West Wits, and it consist of the yellow brown soils with gentle slopes mainly the shales. West Wits is also known to have Mispah soil type, which is characterised by shallow red soils with the surface rock. The natural vegetation present in the area consists of the savanna biome and the grassland Biome (Mucina and Rutherford, 2006).

The drainage line has approximately 17 adjacent constructed wetlands and for the basis of this study only 5 wetlands were selected. The wetlands receive most of the water from the base of the mine dump which is situated on the Western side of the Varkenslaagte drainage line. The sites are constructed adjacent to the canal and at the bottom of each wetland the water is channelled into another wetland. Some of the water enters the wetland through the seepage especially during the periods of heavy rainfall where the flood plain becomes saturated. The constructed wetlands are dry during seasons where there is no rain but sites towards the bridge are deeper than 50 cm and have water. The wetland plant species to be investigated in this study are *S. corymbosus, P. communis* and *T. capensis*.

The plants species have been grown inside and on the land surface on the eastern side of the mine dump where the water from the mine dump drains into the canal. Through the use of plants that have the ability to uptake some of the metals that seep directly from the mine dump the two processes called phytoremediation and bioaccumulation purposes. During the rainy seasons, more heavy metals and runoff water become channeled down the mine dump into the canal and into the constructed wetlands where the wetlands plant species have been grown. No water

cleaning process is present in the area except the bioaccumulation and phytoremediation by the plants that have grown within the sites.

3.2.1 Description of the sampling sites

Five sampling sites were selected along the Varkenslaagte canal and these were site1, 2, 4, 6 and 7, which in this study will be regarded as Sites 1, 2, 3, 4 and 5. The sites were dominated by the macrophyte species *T. capensis, S. corymbosus and P. communis* as shown in Figure 7a & 7b. Below is a table showing the grid references of the five sites sampled.

Table 2: Summary of descriptive characteristics and Grid References of the sample sites

Site 1: This is the first site along the canal, and it receives AMD water from the tailings dam. The pH of the water is much lower with a lot of salts crusting on the soil surface and on the base of the macrophytes as shown in fig. 7 a & b. the soil type dominating the site is mostly gravel with red oxides visible on the soil surface.	S: 26° 25' 49.44" E: 27° 22' 16.68"
Site 2: This is the second site along the canal and the characteristics of the site are similar to that of the first site. In the 1st and 2ndsites, the plants were much shorter with lot of element precipitation on the surface.	S: 26° 25' 50.16" E:27°.22' 16.12"
Site 3: This is site 4 along the canal, and on this site where there was rising temperature, pH and EC was observed. Crusting of salts on the surface was not much in this site and the plants were quite taller than they were in site1, 2 and 3 respectively.	S: 26° 25' 51.98" E: 27° 22' 14.58"
Site 4: This was the site 6 along the canal. It consists of many macrophytes species with lot of water in it. The pH in the canal is also close to 7.	S: 26° 25' 54.24" E: 27° 22' 13.45"
Site 5: Along the canal, there was site 7. The site consists of lot of rainwater which seeps directly from the sideways of the site after the rainy seasons with the almost neutral pH levels. This site also has tall macrophyte species dominated by the phragmites. The soil type dominating the site was mostly clay.	S: 26° 25' 55.44" E: 27° 22' 12.74"

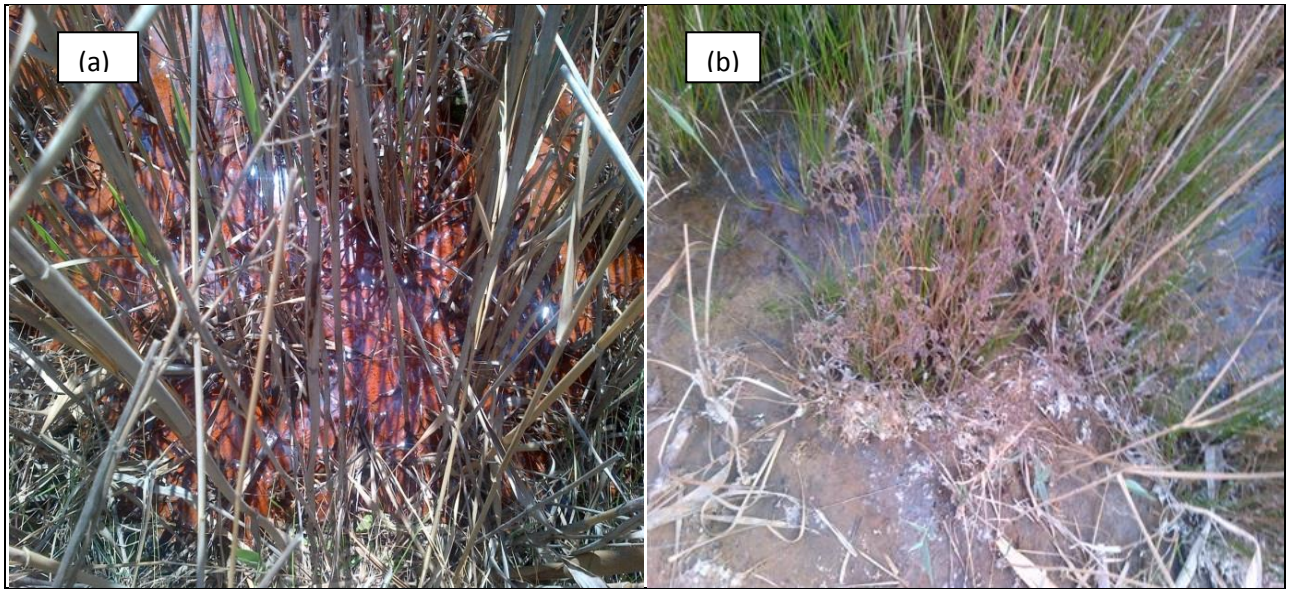


Figure 7: (a) Some *P. communis* species with AMD water flowing between the plants & (b) Metal precipitation on the soil crusts with some metals sticking to the basal part of the *S. corymbosus* and *P. communis species.*

As the sites and canal are situated on the foot of the mining dump, It is evident that AMD leaches from the mine dump to the canals and sites, and form lot of surface precipitation and salts crusting as shown in Figure 8 (a & b) below.

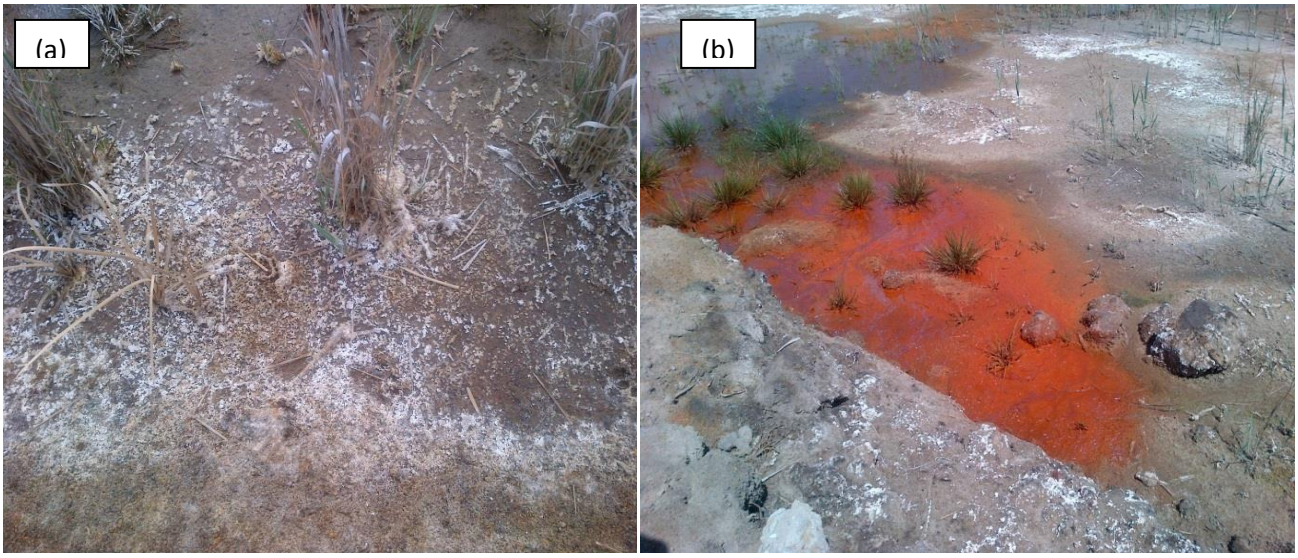


Figure 8: (a): some metal precipitates at the edge of the artificial wetland with some precipitates attached to the lower parts and leaves of the *P. communis spp* that fall off often become covered by the salts. (b) Some AMD and metal precipitation of the floodplain of the right side adjacent the canal.

3.2.2 Soil core sampling and sediments sample preparation

Soil cores were extracted from the selected five sites mentioned above. The sediment samples were only collected during the summer period of 2013 because the study was mainly focusing on the plant study but not on the sediments. A diameter of approximately 15 cm was measured and PVC (Polyvinylchloride) pipe was inserted into the soil using a hammer and a plank. The cores were pulled out using a long steel bar inserted on two holes made on the top sides on the pipe. The pipes were pulled out and wrapped in refuse bags to prevent the sediments from falling out of the pipes. The pipes with the contents were then taken to the laboratory and stored in the cold storage room at a temperature of 5°C before preparation and analysis. The cores were cut vertically into two halves. Sediments inside the pipe were separated into three distinctive parts of 0 - 2, 2-10 and 10 - 30 cm respectively using a plastic ruler.

A subsample of 20 g was extracted from the pipe and homogenised and mixed with distilled water and pH and electrical conductivity measured. Another sediment subsample of +/-20 g was extracted and preserved inside a plastic bag and freeze dried for 3 days. The freeze dried samples were milled using a mortar and pestle. The samples were further prepared and put inside the Spectroscout Geo+ XRF Fluorescence small containers with a thin film inserted in the middle of the containers. The thin film served as the base of the container and it was also polished before it was put into the Spectroscout Geo+ XRF Analyzer Pro for analysis. The sediments were analysed for presence and concentration of Fe, S, Mg, Cr, Mn, Co, Ni, Cu, Zn, Mo and Pb using the X-Ray Fluorescence.

3.2.3 Plants sampling and sample preparation

Samples of *Typha, Schoenoplectus* and *Phragmites spp* were collected from the five sampling sites. The two plants species of *Schoenoplectus and Phragmites spp* were sampled in all the five sites in summer 2013 and during the autumn season in 2014, three plant species of *Schoenoplectus, Phragmites* and *Typha spp* were sampled. In each site sampled, the plants species were sampled on the inflow of the site, on the midflow and also on the outflow of the site. Three plant samples were collected at each sampling point in the site. A total of nine samples were collected in each site and at the end of the sampling period, a total of 45 samples were collected in all the five sites in one sampling period. Three samples of each of the plants were collected per site, and a total number of 45 samples were collected from all the five sites in one season. The macrophyte species were uprooted using a spade, sampling plant material within a 20 cm^2. The plants were taken out of the sites and washed with local water followed by distilled water, for quantitative removal of soil and other foreign particles.

The plants were taken to the laboratory where they were thoroughly washed and rinsed with tap and distilled water. All the three plant species were measured for height using a plastic ruler. They were then separated into leaves, stems, rhizomes and roots. The fresh mass of the leaves, stems, rhizomes and roots were measured and recorded and placed in clean paper bags. The plants' leaves, roots, rhizomes and stems were also chopped into pieces of +/-

two cm and homogenised and a subsample of +/- 20 g was extracted from all the samples and freeze dried for a period of two-three days to ensure that the plants are much dry for easy grinding. The homogenised plants samples were milled in the using a Fritsch Pulverisette 6 Mill into pulverized powder before metals analysis.

3.2.4 Water sampling and water analysis

Three water samples in each site were collected. Water collection was only done once for this study and it was in summer period of 2013. A total number of 15 samples are collected from all five sites. The water samples were collected from five sites, and measurements were recorded at inflow, midflow and outflow of the sites. The parameters measured on-site include pH, Conductivity, Dissolved Oxygen and Redox Potential. Water measurements were done using Thermo Scientific Orion Star A329 portable pH/ISE/Conductivity/RDO/DO Meter. At each site of each study site, three water samples were collected in a sequence of inflow, mid-flow and outflow. 500 ml bottles were used to collect water and the bottles were kept in a fridge on-site at a temperature of 5°C. Later in the lab the water inside the bottles was filtered using 0.45 µm Millipore. The filtered water was divided into 250 ml water bottles and one 250 ml water bottle was acidified with 3 ml of nitric acid for metal analysis and the other was used for determining the total Nitrogen.

3.2.5 Total nitrogen analysis

Water was analysed for Total Nitrogen using a Merck Spectroquant Pharo 300. 10 ml of the water samples was taken from the water bottles using the graduated cylinder and transferred into 15 ml test tubes or test cells. One scoop of 0.5 mg of Potassium-persulfate (N-1K) was added into the cells. Six drops of sodium hydroxide (N-2K) were added into the sealable cells, and the contents of the cells were agitated. The cells were preheated in a Merck Spectroquant TR320 (thermoreactor) for one hour at a temperature of 120 °C. The test cells were then taken out of the thermoreactor to cool to room temperature. The cells were swirled for ten minutes, adding one scoop of 0.5 mg of N-3K into the reaction cell, and the cells were again shaken vigorously for one minute. 1.5 ml of the preheated samples were transferred into the cell and mixed. The cells were allowed to react for a time of ten minutes until taken to the Spectroquant Pharo 300 for absorbance and total nitrogen concentration analysis.

3.2.6 Data presentation

The raw water *in-situ* data will be presented in the form of table and line graphs indicating the average mean values of the physicochemical measures that have been measured in the field. The data that is presented include water, sediments and three plant organs namely roots, rhizomes and the leaves as well as metal ones. The graphs will be drawn by using the average mean concentrations of the selected elements of Mn, P, S, Cr, Mn, Fe, Co, Ni, Cu, Zn, Mo as well as Pb. Brief discussions of the selected elements accumulated in the water, sediments and plant parts is

presented in the form of minimum values, maximum and the mean average concentrations. The significant variations of all the selected elements are presented by means of the p = 0.05. Correlation matrixes portraying the relationship that exist between the elements and plants organs have been put in Appendix 1. Statistical analyses to determine the p-value as well as the correlations was done using the Statistical Analysis Package Version 10 and later converted into excel for formatting purposes. Further correlation details are shown in Appendix 1.

Chapter 4

4.1 Results and discussion

The following sub-section discusses the results obtained from different data collection tasks. The first section looks at *in situ* water measurements taken during the investigation periods of 2013. Section 4.1.1 will briefly discuss the trends of physico-chemical parameters measured in water. Section 4.1.2 will briefly discuss the Total Nitrogen (Nitrate, Nitrite and ammonium concentrations in water) results analysed from water samples collected from the sites. Section sediments whilst section three shows the results from the plants samples. Each of the elements and parameters is discussed separately.

4.1.1 Physico-chemical parameters

The summary of the physical chemical parameters for all the sampled sites are given in Table 3. It is only the Redox and Dissolved Oxygen (DO) that showed large variances between the sites.

Table 3: Summary of descriptive statistics of summer *in situ* water measurements in summer

Variable	Descriptive Statistics of Raw Water Data (n=15)							
	Mean	p-value	Median	Min.	Max.	Range	Variance	Std.Dev
Temperature (°C)	23.63	0.999	23.40	20.20	27.40	7.20	5.01	2.2429
pH	6.15	1.000	6.06	5.69	6.70	1.01	0.0934	0.3056
EC (mS/cm)	6.925	1.045	4.54	1.49	41.40	39.91	99.163	9.958
Redox (mV)	99.91	2.1725	94.40	54.40	194.30	139.9	1517.20	38.95
DO (%)	14.63	1.5675	6.11	0.0235	0.1436	0.7234	570.62	23.88

Water temperature plays a significant role in the physical, chemical and biological processes in the aquatic ecosystems. Processes such as chemical reactions, toxicity, microbial activities and solubility of oxygen and other natural gases are well known to be influenced by water temperature (United States Environmental Protection Agency, undated). According to Duffus (1980), water temperature in the aquatic ecosystems also influences the solubility of dissolved oxygen as well as its concentrations.

The mean average for water temperature was 23.63 ˚C. The minimum temperature of 20.2 °C was recorded on the outflow of Site 2 whilst the maximum temperature of 27.4 °C (Table 3, Figure 9) was recorded at the inflow of Site 4. In Site 1, temperature was increasing and dropped towards Site 2, and started to rise again in Sites 3 and 4 but in Site 5 temperature remained somewhat constant. The temperature values were slightly higher because sampling was done during the summer season and during this period metal precipitation on the soil surface was relatively high because of high evaporation rates accompanied by high temperatures. The summary of the water temperature in the sites indicate that there were significant variations. This is shown by the p value, the p value at $p \leq 0.05$ and calculated p value = 0.999.

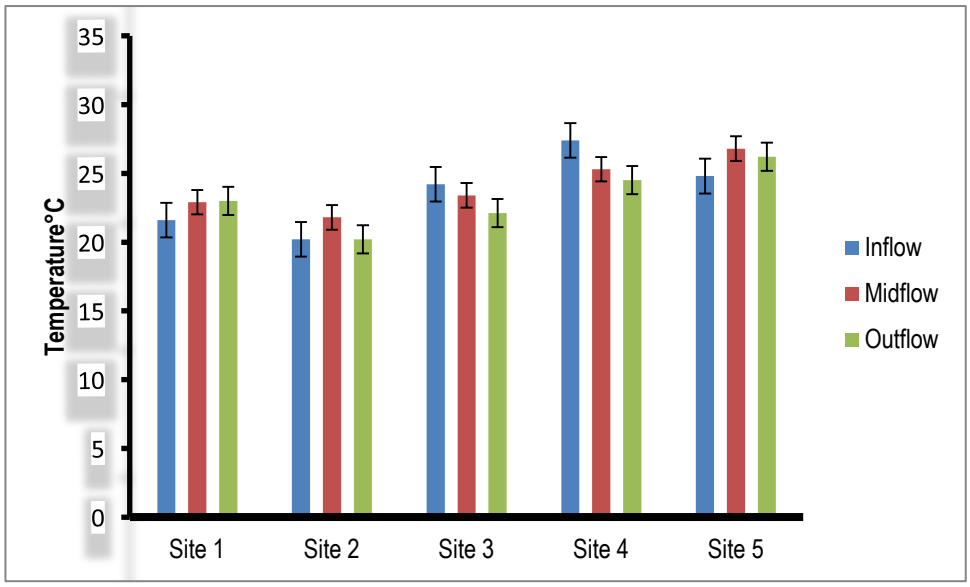


Figure 9: Differences in temperature °C measured in water at inflow, midflow and outflow for the five sites.

The average value for the pH measurements for the water was 6.15. The minimum pH measued in all the sites was 5.69 and the maximum pH measured was 6.70 (Table 3, Figure 10). The results indicate that the pH was closer to the neutral level at the top of the canal i.e. at Site 1, water pH began to drop towards the midflow of Site 2, where the water pH was found to be decreasing (Figure 10) and this could be the result of high seepage that takes place on the sideways of the sites.

In Site 1, the pH dropped in the middle of the site (Figure 10). In Site 2, pH dropped and started to rise again towards the bottom of the site as shown by the outflow line on the graph. In Site 3, the pH was observed to have increased but continued to drop towards the midflow and outflow of the site. In Site 4, the pH was observed to be low at the inflow of the site and rose towards the midflow and dropped again at the outflow of the site. In Site 5, the pH was observed to be neutral and suddenly dropped towards the midflow and outflow of the site. This was observed

simultaneously with massive orange precipitation which could mainly be sulphur and iron as accumulation of salts on the sides of the sites that gave colourless to white crust on the surface of the site. The prevalence of the anaerobic conditions on a shallow surface on the constructed wetlands is encouraged by the fine rock properties that are carried by water and wind down the stream which can also have impact on the pH of the water. As a result, they end up in the site and acting as a blanket that sits underneath the water. This prevents the exchange of gases between the surface of the water and the sediments. In the sites, there were significant variations of pH in water. This is shown by the p value, the p value at $p \leq 0.05$ and calculated p value = 1.000.

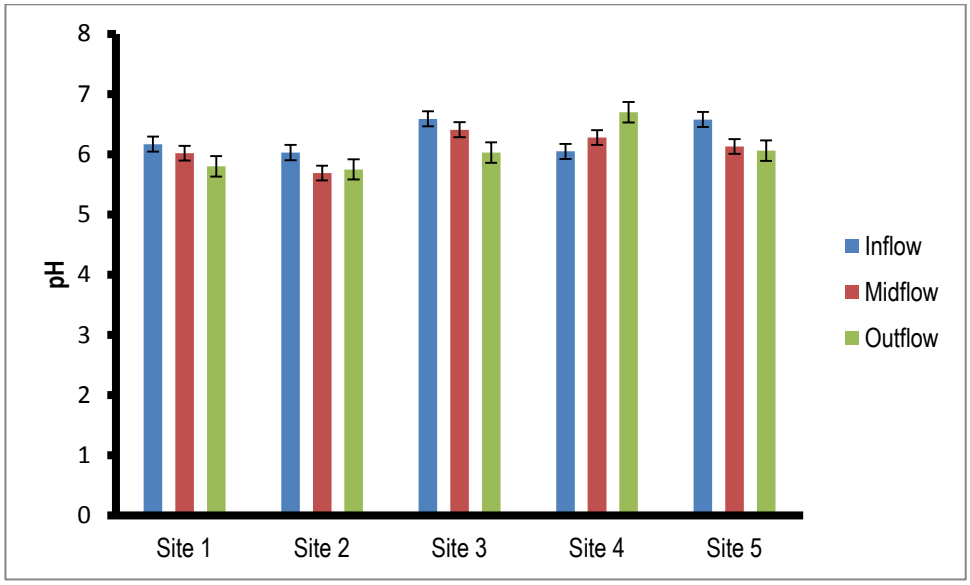


Figure 10: Differences in pH measured in water at inflow, midflow and outflow for the five sites.

Electrical Conductivity is defined as a measure of the electric current conveyed by a solution (Hershey and Sand, 1993). It is widely known as an estimate of total ion concentration. The average mean value for the EC was 6.925 µS/cm respectively. The minimum value for EC was 1.49 µS/cm and it was observed at the bottom of Site 1 as shown by the outflow (Table 3, Figure 11). The maximum value for EC was 41.40 µS/cm was observed at the middle of Site 3 (Figure 11). In the water samples, there were significant variations in the concentrations of EC accumulated in the water. This is shown by the p value, the p value at $p \leq 0.05$ and calculated p value =1.045.

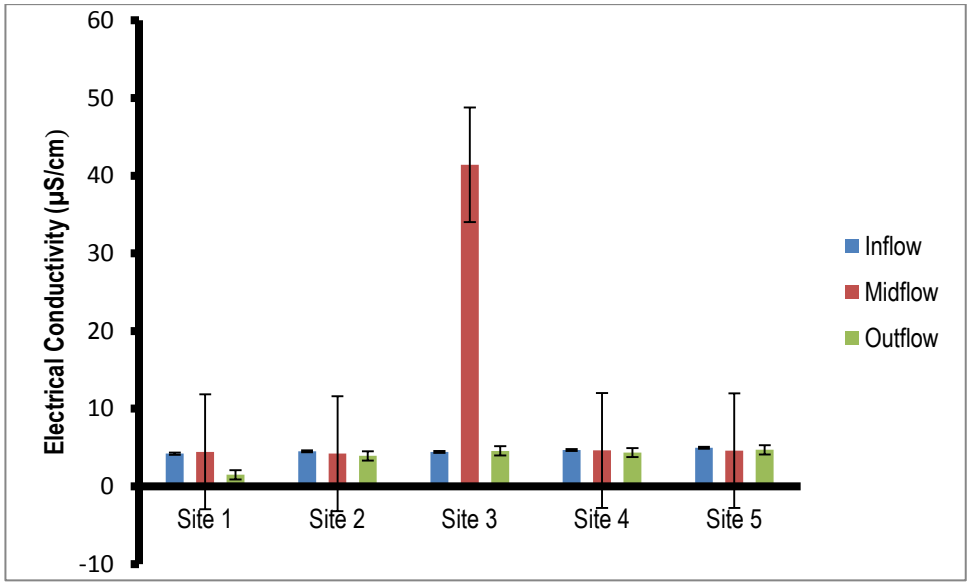


Figure 11: The differences in electrical conductivity measured in water at inflow, midflow and outflow for the five sites

The mean average value for Eh measured in all the sites was 99.91 mV. The minimum and maximum values for Eh were 54.4 and 194.30 mV respectively (Table 3, Figure 12). There were significant variations in redox measured in water. This is indicated by the p value, the p value at $p \leq 0.05$ and calculated p value = 2.1725. In all the sites, the highest Eh values were measured in Site 3, followed by Site 4 and Site 5. Site 2 was observed to have the lowest Eh values followed by Site 1.

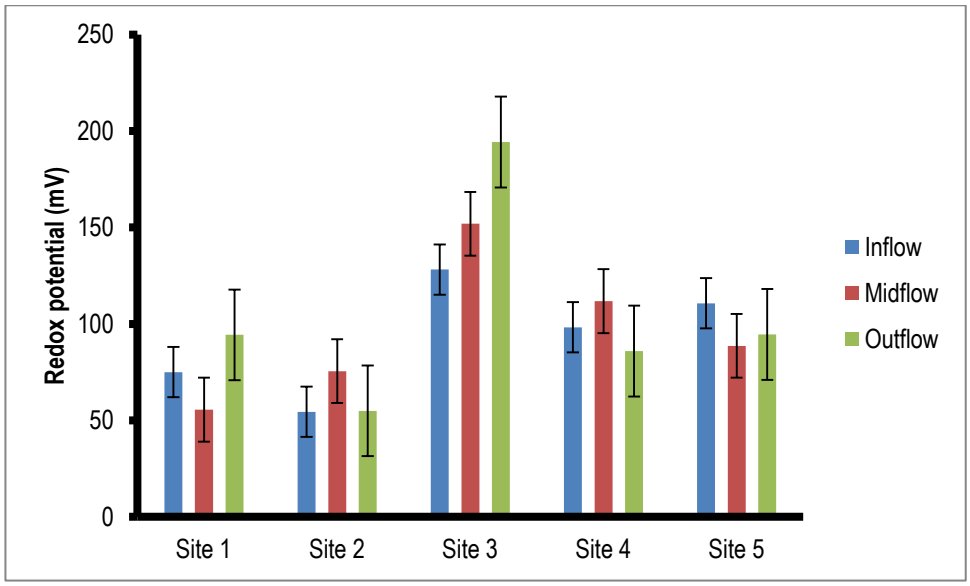


Figure 12: Differences on redox potential measured in the water samples at inflow, midflow and outflow for the five sites

In some of the sites, higher dissolved oxygen measurements were measured and such sites were mostly comprised of tall and more vigorous *P. communis.* On the site, site 6 and 7 were found to be having tall *P. communis spp*, while the other constructed sites with short *P. communis* and *S. corymbosus spp* it was found that such sites have massive growth and development of algae that are believed to contribute significantly in the consumption of oxygen (Wetzel, 1983). Figure 12 above describes the measured variations between DO. The major variations in DO concentrations were due to massive algal growth within the sites where the water measurements were taken.

When measurements were taken, inflow of Site 3 and midflow of site four, peak DO values of 0.7234 and 0.1436 mg/l respectively were observed and the peak values were shown by the inflow and midflow (Table 3, Figure 13). The DO mean average value obtained was 0.1463 mg/l respectively. The minimum DO value was 0.0235 mg/land it was observed on the top of Site 2 as shown by the inflow line on the graph. The maximum DO value was 0.7436 mg/land it was observed in the middle of Site 4 as shown by a midflow line in graph. In the sites, there were significant variations in the concentrations of DO measured in the water. Higher DO concentrations were obtained when the pH was at a depleting rate. This is shown by the p value, the p value at $p \leq 0.05$ and calculated p value =1.5675.

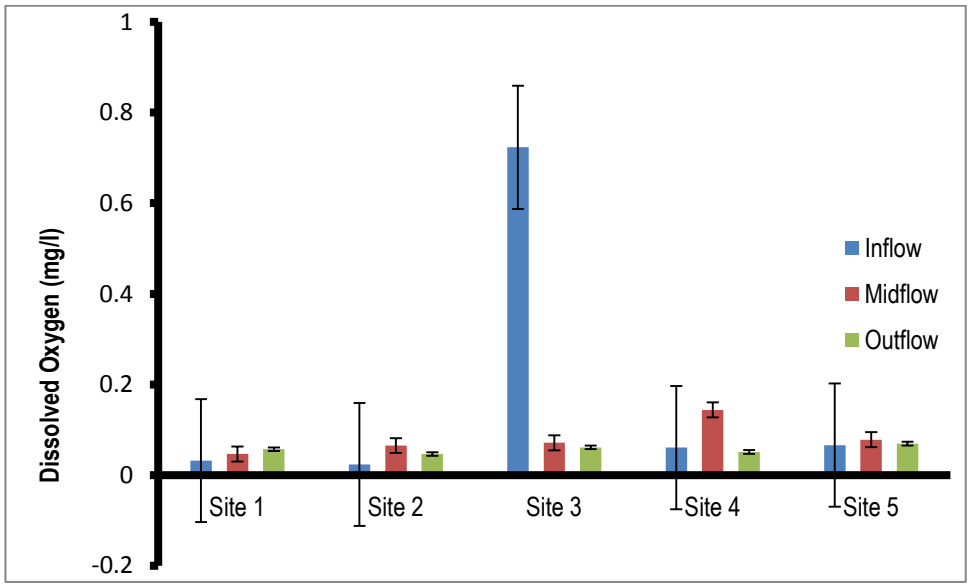


Figure 13: Differences in dissolved oxygen mg/l measured in water at inflow, midflow and outflow for the five sites

4.1.2 Nitrite, Nitrate and Ammonium concentrations in water

This sub-section briefly discusses the concentrations of nitrate, nitrite and ammonium measured in water. The samples were collected from five sites, and in each site a sub-sample of water was collected from the inflow, midflow and outflow of the constructed wetlands. The data is presented by means of inflow, midflow and outflow lines in the

graphs. A descriptive summary of the statistics of nitrite, nitrate and ammonium are shown in the table below, with a sample size of 15 (n=15).

Table 4: Summary of descriptive statistic of nitrite, nitrate and ammonium in water for the five sites

Variable	Descriptive Statistics of Nitrite, Nitrate and Ammonium in water (n=15)							
	Mean	p-value	Median	Min.	Max.	Range	Variance	Std.Dev
Nitrite (mg/l)	0.57	0.0003	0.00	0.00	5.00	5.00	1.80	1.34
Nitrate (mg/l)	0.06	1.000	0.06	0.04	0.08	0.04	0.00	0.01
Ammonium (mg/l)	0.01	1.000	0.01	0.00	0.03	0.03	6.82	0.01

During the investigation period in summer 2013, nitrite concentration was below the detection point in site two, three and four. The average mean concentration value of nitrite was 0.57 mg/l only in Site 1 and 5 were the only sites where nitrite was detected. The maximum nitrite concentration detected was 5 mg/l (Table 4, Figure 14). Nitrite is generally unstable in water bodies and this explains why it was not detected in a number of samples. There was no significant variation in the concentration of nitrite measured in water samples. This is shown by the p-value, the p value at $p \leq 0.05$ and calculated p-value=0.0003.

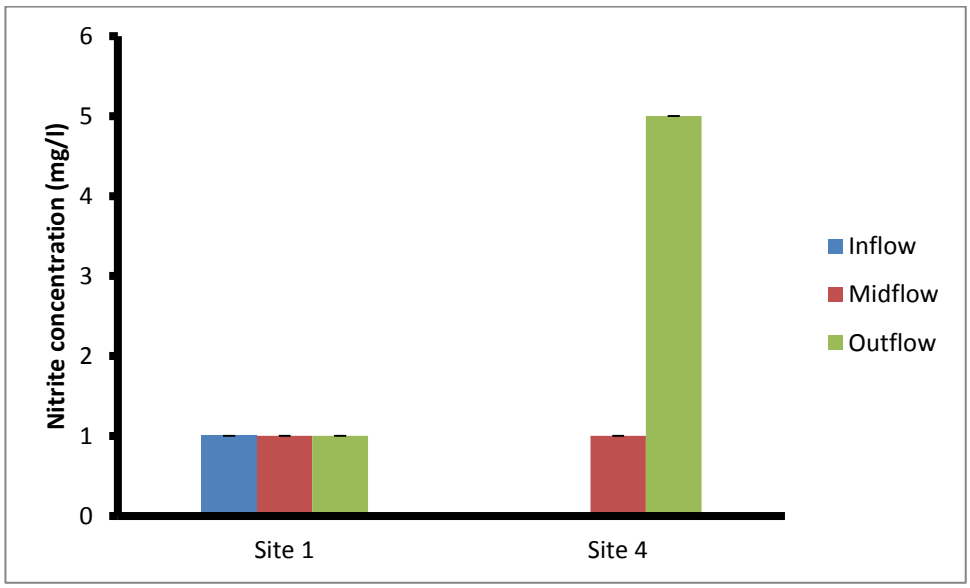


Figure 14: Differences in nitrite concentration measured in water at inflow, midflow and outflow for the five sites

The minimum and maximum concentration values for nitrate in the water were 0.04 and 0.08 mg/l respectively (Table 4, Figure 15). There were significant variations in the concentration of nitrate measured in water. This is shown by the p value, at $p \leq 0.05$ and calculated p value = 1.000. Nitrate measured in the water of the sampled site.

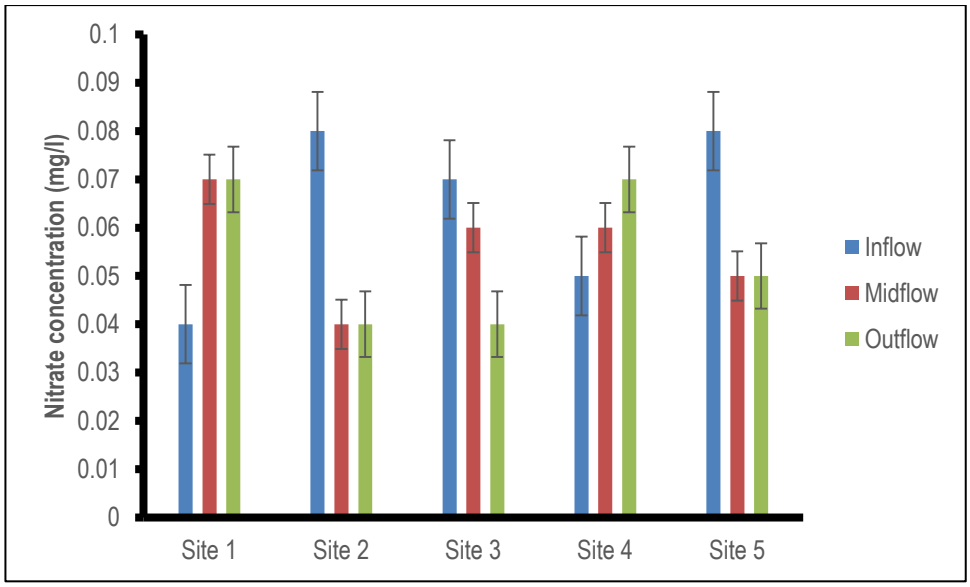


Figure 15: Differences in nitrate concentration measured in water at inflow, midflow and outflow for the five sites

The minimum values of ammonium were not detected. The maximum concentration was 0.03 mg/l (Table 4, Figure 16). There were significant variations in the concentrations of Ammonium measured in the water. This is shown by the p value, the p value at $p \leq 0.05$ and calculated p value = 1.000.

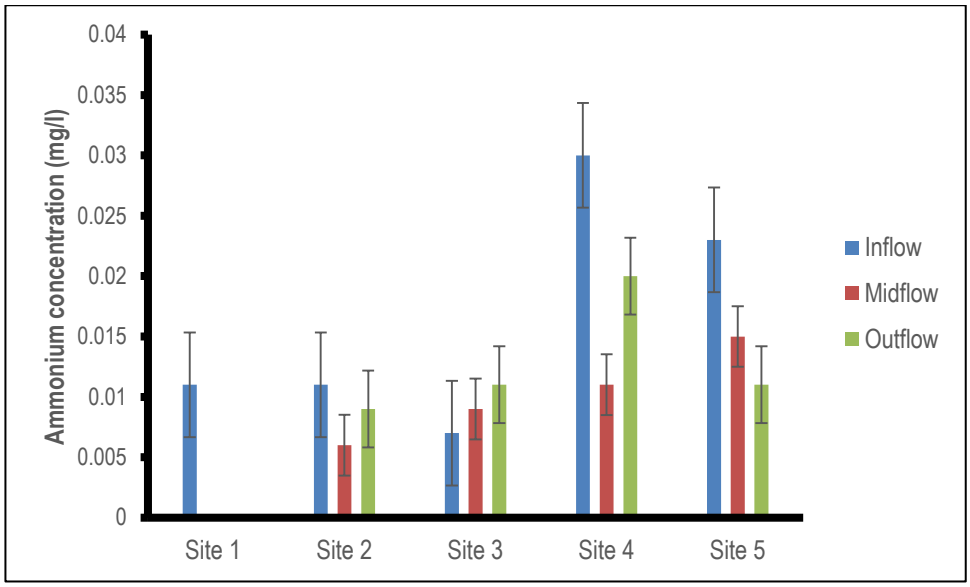


Figure 16: Differences in ammonium concentration measured in water at inflow, midflow and outflow for the five sites

When comparing the measured pH with the Target Water Quality Range, the pH was found to be within the acceptable range but only in those sites where the pH was below 6 as the toxic effects associated with dissolved metals including lead, were likely to occur at a pH of less than 6. When comparing the values of EC in the water it was found that, EC was within the acceptable range and no health effects would be caused by EC and EC was < 45 mS/cm. When comparing the concentrations of ammonia measured for the basis of this study with the South African guidelines on fresh water, the Target Water Quality Range of ammonia measured in all the sampled sites was within the acceptable range where there will be no health or aesthetic effects caused by ammonia. Nitrite and nitrate in water were within the acceptable range.

4.2 Concentrations of elements measured in Water

The water was analysed for the presence of selected elements for this study and below is the brief discussion of the analysed elements in the water from the sampled sites.

4.2.1 Comparison between elements

Table 5: Descriptive statistics of various element concentrations in water

Element	Descriptive statistics of various element concentrations in water (n=15)							
	Mean	p-value	Median	Min.	Max.	Range	Variance	Std.Dev
Zn (mg/l)	0.039	0.999	0.016	0.009	0.267	0.258	0.0042	0.065
Ni (mg/l)	0.032	0.999	0.004	0.000	0.282	0.282	0.0052	0.072
Fe (mg/l)	0.1967	0.042	0.0365	0.000	2.23	2.23	0.3434	0.330
Mn (mg/l)	1.399	1.597	0.799	0.268	5.90	5.632	2.043	1.429
Cu (mg/l)	2.46	9.3542	1.5478	0.881	14.08	13.196	10.630	3.260
Mg (mg/l)	219.433	1.000	194.1	132.4	369.60	237.20	5468.234	73.948
Co (mg/l)	232.55	0.999	243.251	112.11	335.33	223.22	7064.27	84.049
S (mg/l)	1209.35	0.000	783.60	584.40	5839	5254.60	1710541	1307.88

4.2.2 Individual trends of the elements

This section highlights the individual trends of all the elements analysed in water samples. The elements measured in water consisted of metals and non-metals. Firstly, table 5 above shows the descriptive summary of the parameters of the elements analysed and each of the elements are discussed individually by means of a graph showing the mean, minimum and maximum concentration values of the elements.

Total value of Zinc (Zn) in soil depends on the chemical composition of the soil. Zinc consists of approximately 80mg/kg on the earth's crust. Zn plays significant roles in plants including the protein synthesis, activation of enzymes, oxidation and stimulation of reactions and carbohydrates metabolism. The movement of Zn in the plants and soil depend on a number of factors including the acidity of the soils, the organic matter content of the soil, types of soil and the total concentration of Zn in the soil (Mousavi, 2011).

Zinc deficiency is commonly experienced in eroded, calcareous and weathering acidic soils. Calcareous soils serve a good example where Zn and Fe deficiency occurs. The deficiency of Zn in sandy soils results from the lack of organic matter due to limited root growth. In cold weather conditions, Zn availability becomes limited because there is general lack of microbial activity and limited release of Zn by microorganisms in the soils (Abdou *et al*., 2011; Alam *et al*., 2010; Terhan and Sekhon, 1977) cited by Mousavi, 2011. Zn is widely to be deficient in the younger leaves since it is an immobile element (Mousavi, 2011).

Excessive amounts of Zn results in plants toxicity. Excess amounts of Zn in plants reduce the growth and development of leaf and root. The reduction in the leaf growth and development result in the reduced growth and development of chloroplasts which, reduces the production of NADPH in the plants. Another impact of Zn toxicity in the chloroplasts cells include the reduction in the activity of RUBP carboxylase enzyme and reduced photosystem II. Higher amounts of toxicity further reduce the production of ATP which gives rise to the declining photosynthetic activity. Because Zn interferes with P and Fe uptake, it availability in abundant amounts reduce the uptake of this elements (Mousavi, 2011).

The mean concentration value for Zn in the water was 0.0389 mg/L. The minimum and maximum concentration values for Zn in water were 0.009 and 0.267 mg/L (Table 5, Figure 18). There were slight significant variations in the concentrations of Zn in water. This is shown by the p value and, the p value at p value ≤ 0.05 is $p \geq 0.05$ and calculated p value = 0.999.

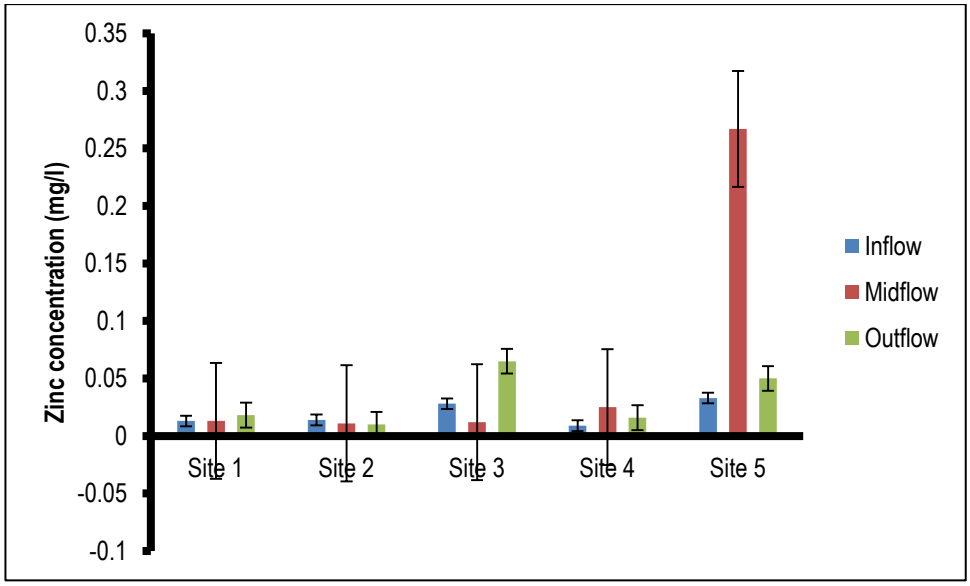


Figure 17: variations in zinc concentration in water at inflow, midflow and outflow for the five sites

Nickel (Ni) is an element required by plants and is widely used in many anthropogenic activities. Ni occurs in minute concentrations in the soil and surface water. It is released into the environment through various anthropogenic activities such as mining, burning of fossil fuels. In addition, Ni can also be emitted from vehicles and through municipal as well as industrial wastes (Chen *et al*., 2009). The uses of Ni varies from being used as a catalyst in the chemical and food industry, being a component of electrical batteries to being used as a raw material in the metallurgical and electroplating industries (Chen *et al.,* 2009).

Ni is required by plants but excess amount of Ni can have varying negative impacts (Chen *et al.,* 2009). Reduction in yield of many field crops and inhibited growth and development are the two common negative impacts of Ni on the plants. Other negative effects of Ni on plants include necrosis, chlorosis and wilting (Seregin & Kozhevnikova, 2006). These effects can be observed on young leaves of plants that have been treated by excess amounts of Ni. When Ni is applied in higher amounts than required by plants, it tends to produce the dark spots on the leaf margin of the plants which alter rates of transpiration and the water potential of the leaves become decreased. According to Bethkey *et al.,* (1992) photosynthesis is another process in plants that is disrupted by Ni element. In the chloroplast cells, excess Ni can disorganise the photosynthetic apparatus, destroy the mesophyll cells and the epidermis tissue as well as decreasing the chlorophyll content of the leaves. Further, it also interferes with antioxidant enzymes and cause oxidative stress in plants. Furthermore, Ni is also known to perform better than other essential elements in the soils such as K, Na, Ca, Mg, Fe, Cu, and Zn as well as Mn. Consequently, Ni inhibits their intake and result in their deficiency (Taiz *et al.,* 2002).

Ni concentrations in the water were found to have an average mean concentration value of 0.032 mg/L. The minimum concentration value for Ni in water was not detected and the maximum concentration value was 0.282 mg/L (Table 5, Figure 19). There were no significant variations in the concentrations of Ni accumulated in the water. This is shown by the p value and for Ni the p value, the p value at $p \leq 0.05$ and calculated the p value = 0.032.

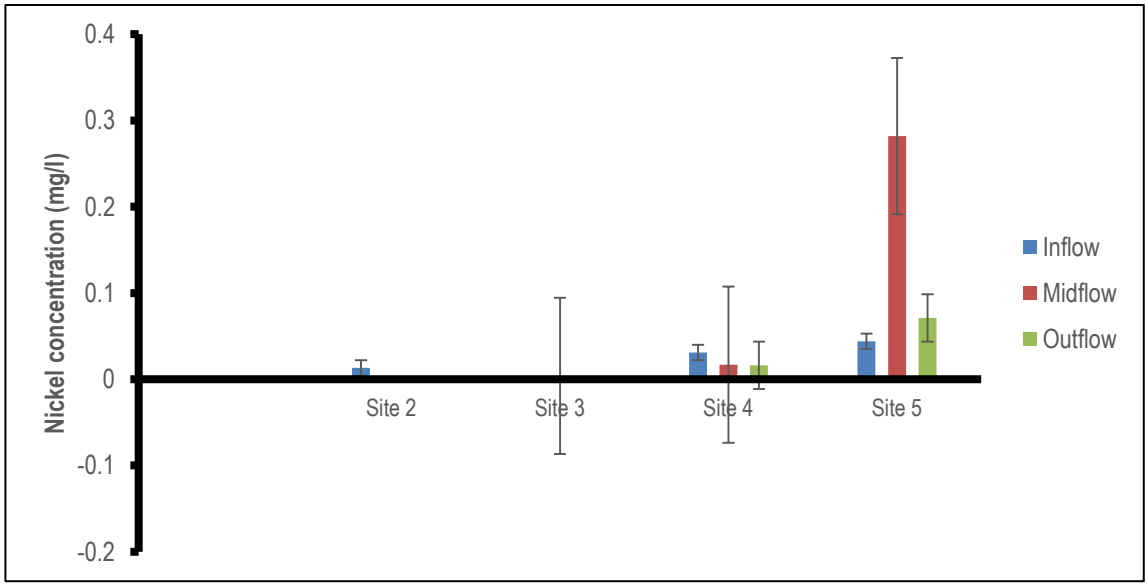


Figure 18: Differences in nickel concentrations measured in water at inflow, midflow and outflow for the five sites

Fe is one of the micronutrients that play an enormous role in the body of humans and in plants. Primarily, it serves as a transporting media of proteins by distributing the haemoglobin and myoglobin and secondarily it is required for the normal functioning of enzymes (Vuori, 1995). It is therefore, the 4th abundant micronutrient because it can be sourced from various activities of mining, peat production, agriculture. Fe is needed by both the aquatic and terrestrial ecosystems to sustain the food resources of the organisms. The average mean concentration value for Fe was 0.1967 mg/l. The minimum concentration value for Fe in water was not detected and maximum concentration value for Fe was 2.23 mg/l (Table 5 Figure 20). There were significant variations in the concentration of Fe accumulated in the water. This is shown by the p value, the p value at $p \leq 0.05$ and calculated p value = 0.1967.

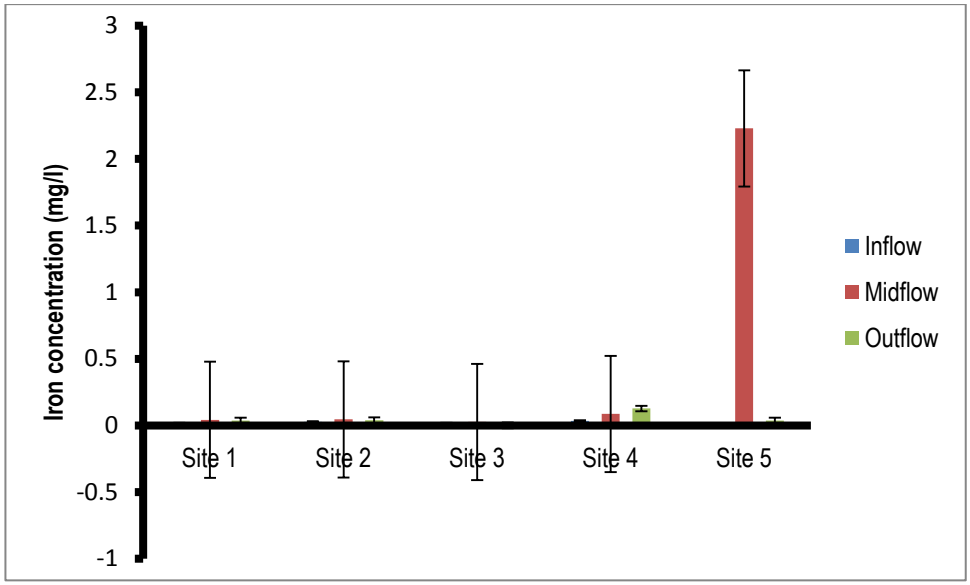


Figure 19: Variations in iron concentration in the water at inflow, midflow and outflow for the five sites

Manganese (Mn) is also an essential trace element required by both the living plants and animals for the physiological functioning and performance. Its abundance in food, water soils and rocks makes it to be the 12th most abundant element on the earth's crust. Various sources of Mn include food stuffs such as cereals, nuts, fruits and vegetables and grains. In addition, Mn is widely known for its use in various activities such as iron and steel production, matches and fireworks production and is it used as an oxidant when producing hydroquinone. In the agricultural sector, Mn is widely used as a fertilizer and as a fungicide. Further, the most common Mn fungicides are Mancozeb and Maneb (Santamaria, 2008).

Furthermore, Mn plays a crucial role in the metabolic functioning of plants and animals. In animals, Mn contributes towards the development of skeletal system, activation of certain enzymes and acts as an antioxidant and protects the cell from being damaged. Consequently, in plants, it activates gluconeogenic pyruvate carboxylase and isocitate dehydrogenase enzymes and it also protects the mitochondrial membrane through superoxide dismutase (Santamaria, 2008).

The involvement of Mn in the process of photosynthesis makes it to act as an antioxidant-cofactor. Although Mn plays an important role for plants, its availability in higher amounts in plants can result in the overall change in the performance and functioning. In plants, excess Mn results in the reduction of biomass and also reduces rates of photosynthesis in the plants. It is also known for the inhibition of enzymic activity and restricts the uptake, transport, and utilisation of other essential elements such as Ca, Mg, Fe and P (Millaleo *et al.,* 2010).

The average mean concentration of Mn in water was 1.399 mg/l, whilst the minimum and maximum concentration for Mn in water was 0.268 and 5.90 mg/l respectively. There were significant variations in the concentration of Mn

measured in the water (Table 5, Figure 21). The significant variations are illustrated by means of the p value at $p \leq 0.05$ and calculated p value = 1.597.

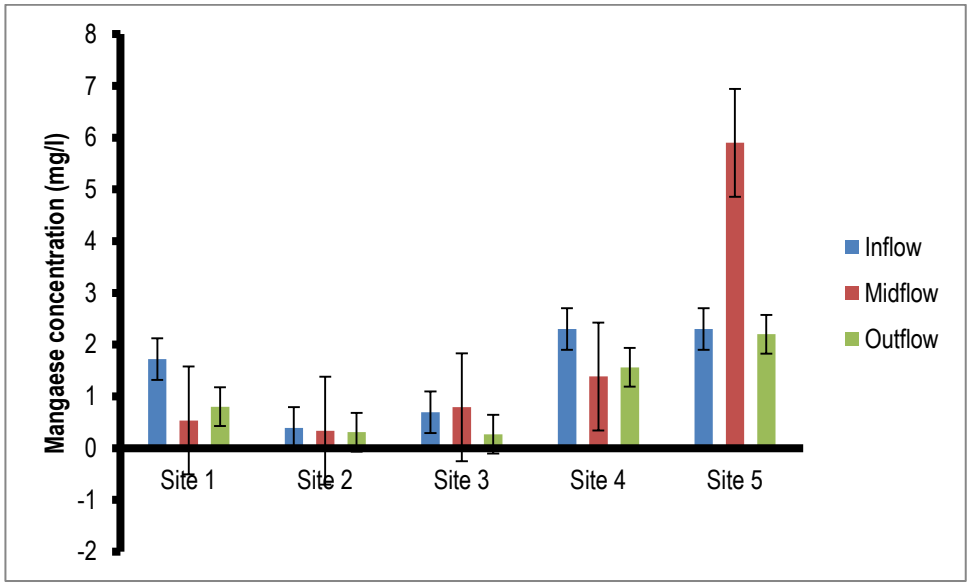


Figure 20: Variations in manganese concentration in water at inflow, midflow and outflow for the five sites

Copper (Cu) is another element required by plants in minute amounts. Excess amount of Cu in the plants reduce various processes of the functioning and performance of the plants. Cu is well-known for its various uses (Küpper & Kroneck, 2005). For instance, it can be used as pesticide. In addition, Cu can also inhibit both photochemical and biochemical reactions of the most important process such as photosynthesis. Although Cu as a known heavy metal required by plants, there are some plant species that have over time developed the strategies to deal with the toxic levels of Cu. Such strategies have been reviewed by Goldsbrough (2002) and cited by (Küpper & Kroneck, 2005).

Furthermore, Cu tolerance mechanisms in plants include efflux pumps, sequestration in cells and binding of elements inside the cells by strong ligands like amino acids. Another mechanisms developed by plants include the translocation of metals from the shoots and store them in certain parts of the plants. Furthermore, Baker (1981) highlighted these plant types as excluders (Küpper *et al*., 2009).

The average mean concentration value for Cu in the water was 2.46 mg/l. The minimum and maximum concentration for copper in water was 0.881 and 14.08 mg/l respectively (Table 5, Figure 22). There were no significant variations

in the concentration of Cu accumulated in the water. This is shown by the p value, the p value at p≤ 0.05 and calculated p value = 9.3542.

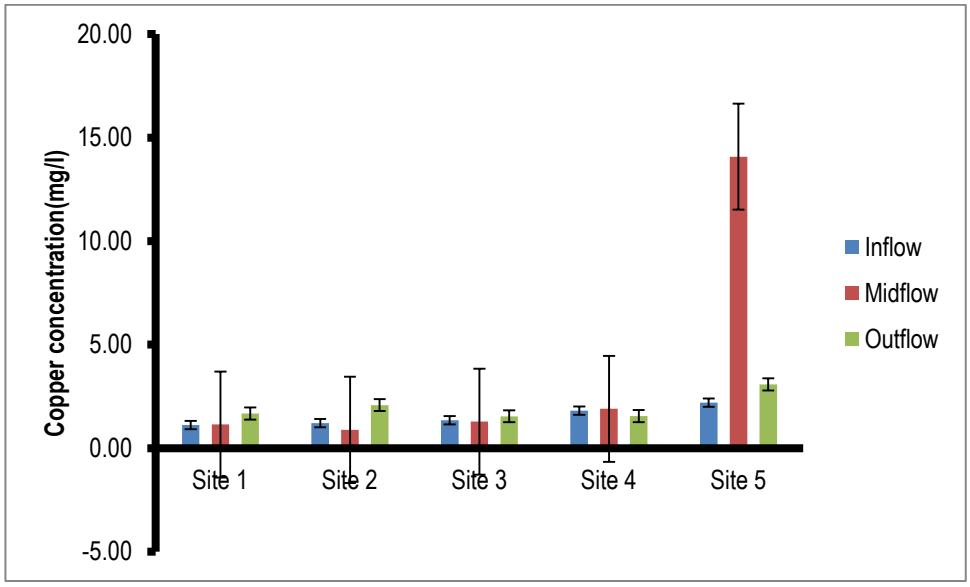


Figure 21: Variations in copper concentrations measured in water at inflow, midflow and outflow for the five sites

Magnesium (Mg) is another major nutrient required in the life cycle of plants and also in animals. In plants, magnesium is added in the form of fertilizers and it helps in the increase of yields. Plants take up magnesium from the soil in the form of Mg^{2+}. Magnesium takes three forms of occurrence in the soil of exchangeable ions, non-exchangeable ions and water soluble forms (Wilkinson, 1990). When Mg is in excess supply in the environment, it can have a negative impact on a variety of physiological functioning of many processes in the ecosystem that benefit from each other mutually. In addition, Mg in the environment affected by a number of factors including pH, microbial activities, and proportions of other micro nutrients present in the rhizosphere (Huber & Jones, 2013).

According to Huner & Hopkins (2009), Mg can become unavailable in soils that are sandy, light and strongly acidic due to leaching. It is therefore, required by the plants in large amounts. Mg has been well documented for its crucial role in photosynthesis and its involvement in the formation of chlorophyll pigment. The common Mg deficiencies include necrosis and chlorosis which involves the release of Mg from the older leaves into the younger leaves that are active for the role of photosynthesis. Its role in the plants also assist in the enzyme activity where it is regarded as the activator of ribulo-biphosphate carboxylase (RuBisCO) and phosphoenolpyruvate carboxylase (PEPC), the most important enzymes required for the process of carbon fixation (Huner & Hopkins, 2009).

During the study period, the mean average concentration for Mg was 219.433 mg/l in water. The minimum concentration for Mg in water was 132.4 mg/l and the maximum concentration for Mg in water was 369.60 mg/l (Table 5, Figure 23). In the water, there were significant variations in the concentrations of Mg accumulated in water. This is shown by the p value, at $p \leq 0.05$, and for Mg the calculated p value = 1.000.

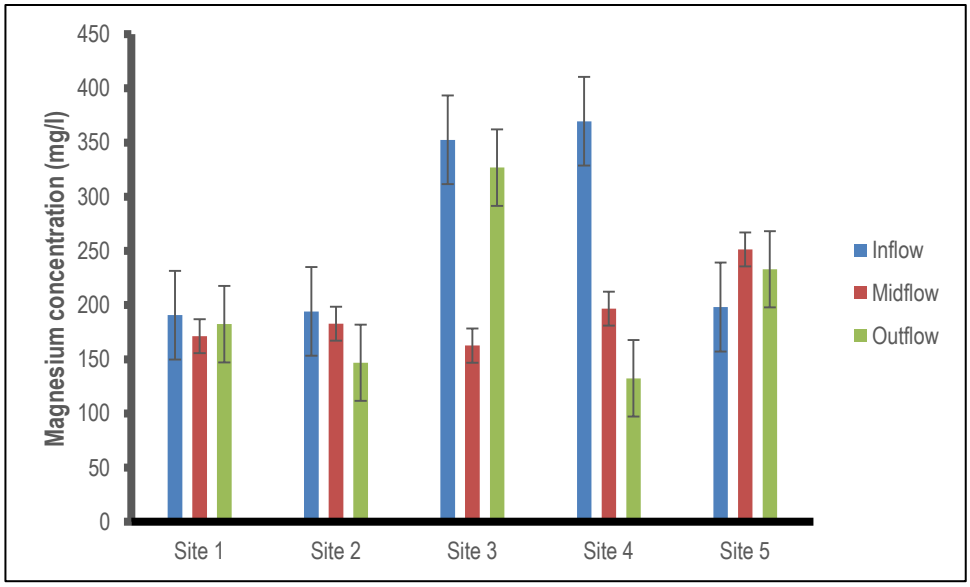


Figure 22: Variations in magnesium concentration measured in water at inflow, midflow and outflow for the five sites.

Cobalt (Co) is a naturally occurring element, and is found in air, surface water, toxic waste leachates, soil and sediment as well as in groundwater. Sources of cobalt include natural and anthropogenic. Among other anthropogenic sources of Co, is phosphate fertilization and mining (WHO, 2006). Co is also an element required by the plants for the processes of nitrogen fixation by the blue-green algae and it also contributes towards the biological processes of the rhizobium and roots such as symbiosis. Co is a constituent of nitrogen-fixing bacteria in higher plants especially in the legumes. However, the essentiality of Co does not make it to be required by animals in the ionic forms for nutrition supplementation, it forms part of the microbes inside the stomach of most ruminants for example, horses and cows (Smith, 1987). In aquatic environments, Co concentrations in a range of 5-20 mg/L have detrimental impacts on the cyanobacterial performance (WHO, 2006).

The mean average concentration value of Co in water was 232.55 mg/l. The minimum and maximum concentration was 112.11 and 335.33 mg/l respectively (Table 5, Figure 24). During the investigation period, there were significant variations in the concentration of Co accumulated in the water samples. This is shown by the p value at the p value is $p \leq 0.05$ and calculated p-value = 0.999.

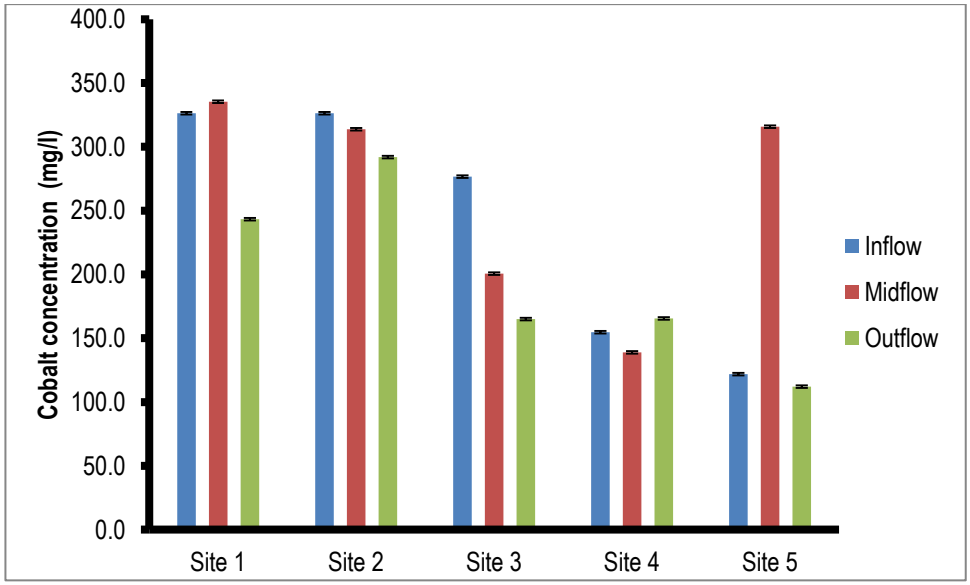


Figure 23: Variations in cobalt concentration in water at inflow, midflow and outflow for the five sites

Sulphur is an essential, non-metal, micro element required for the growth and development of the plants. It is present in many proteins, chloroplast membrane lipids, co-enzymes and vitamins. It plays a crucial role of defence mechanism against herbivory and various forms of pathogens in the plants. It can also be used as an anti-microbe (Falk, 2007). The average mean concentration value for S accumulated in the water was 1209.35 mg/l. The minimum and maximum concentration values for S analysed on water were 584.4 and 5839 mg/l respectively (Table 5, Figure 25). There were significant variations in the concentration of S accumulated in the water of the constructed wetlands. This is shown by the p value = 0.05 at the $p \leq 0.05$ and the calculated p = 0.000.

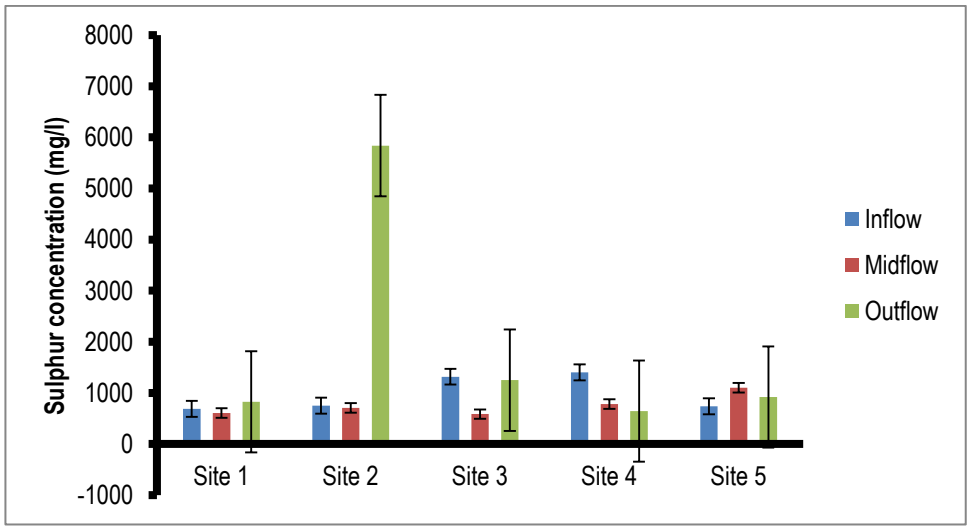


Figure 24: Variations in sulphur concentration in water at inflow, midflow and outflow for the five sites

4.3 Concentrations of selected elements in sediments

Table 6 below illustrates the summary of the descriptive statistics of the sediment samples from the selected five sites. The mean concentration values of the elements are also shown in a graph below to compare the levels of element concentration in the sediments of the sampled site.

Table 6: Summary of descriptive statistics of elements accumulated by the sediments

Variable	Descriptive statistics of elements accumulated by sediments n=15							
	Mean	p-value	Median	Min.	Max.	Range	Variance	Std.Dev
Mg (µg/g)	0.1887	1.000	0.2027	0.039	0.321	0.282	0.0072	0.09
P (µg/g)	487	2.679	526.33	145.37	649	503.63	17973.47	147.39
S (µg/g)	1891.46	0.000	1813.67	519.67	3927	3407.33	629351.4	793.32
Cr (µg/g)	192.03	1.371	167.25	97.13	282.67	185.53	3450.42	58.74
Mn (µg/g)	1081.76	0.000	873.33	222.17	2807	2584.83	628712.2	792.91
Fe (µg/g)	7.23	0.9455	7.01	3.16	11.59	8.43	3.48	1.93
Co (µg/g)	29.51	1.6718	27.47	9.53	51.83	42.3	112.41	10.60
Ni (µg/g)	59.23	0.0001	60.33	20.67	75.67	55	175.61	13.25
Cu (µg/g)	38.37	0.1889	39.43	16.00	41.60	30.1	50.44	7.10
Zn (µg/g)	39.11	0.0022	40.23	10.5	50.27	39.77	94.29	9.71
Mo (µg/g)	1.73	0.6704	1.6	0.000	3.5	3.5	1.380	1.175
Pb (µg/g)	29.20	0.0210	29.67	8.53	41.3	32.77	55.71	7.464

4.3.1 Individual trends of elements in the sediments of the sampled sites

Mg was measured in all the sampled sites in the form of magnesium oxide (MgO). The average mean concentration for Mg accumulated in the sediments was 0.1887 µg/g, as shown in (Table 6). The minimum and maximum concentration for Mg analysed on the sediments was 0.039 and 0.321 µg/g respectively (Table 6, Figure 26). There were significant variations in the concentration of Mg accumulated on the sediments of the sampled sites, and the significant difference was shown by means of p value, at $p \leq 0.05$, and the calculated p value is $p = 1.000$.

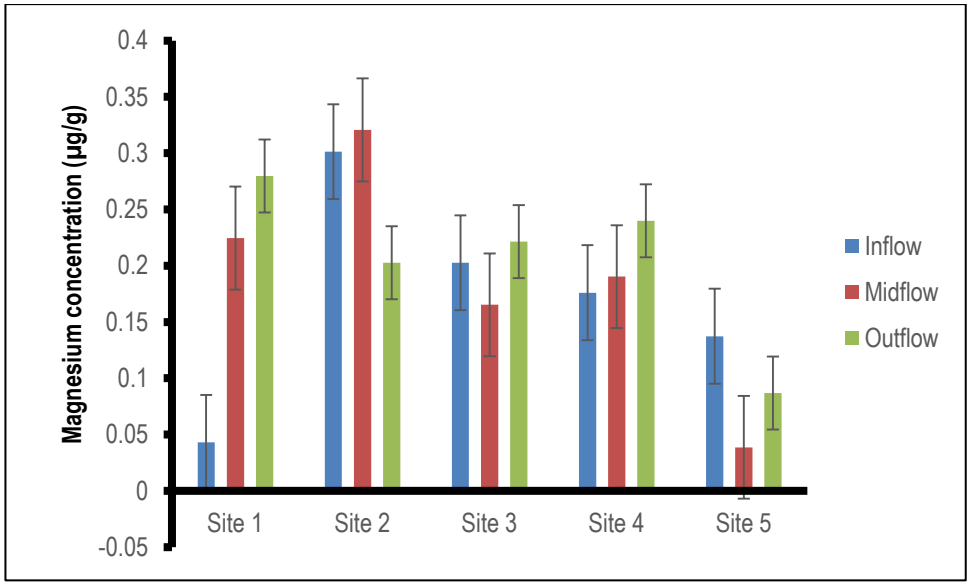


Figure 25: Variations in magnesium concentrations measured in sediments at inflow, midflow and outflow for the five sites

Phosphorus (P) is a macro element that contributes about 0.2% of the dry biomass. P is the main component of phospholipids, nucleic acids and Adenosine Triphosphate (ATP), which plays a significant role in the process of photosynthesis. According to Schachtman *et al.,* (1998), plants cannot be grown if P is not present in the soil to support growth and development of the plants. In addition, it is also regarded as another essential macro element that can also limit growth of the plants. P is present in the soil in the rhizosphere. In many agricultural practices, P is applied in the form of a fertilizer and is released much faster and it supports the growth of higher plants. In the soil, it has been observed that more that 80% of the P in the soil become unavailable for the plant uptake, because of the processes of adsorption, P being converted in an organic form and due to precipitation of P (Schachtman *et al*., 1998). P in the soil can be found in the form of organic P and mineral P.

The average mean concentration value for P accumulated in the sediments was 487 µg/g (Table 6). The minimum and maximum concentration values for P were 145.37 and 649 µg/g respectively (Table 6, Figure 27). There were significant variations in the concentrations of P accumulated in the sediments. This is shown by the p value at $p \leq 0.05$ and the calculated $p = 2.679$.

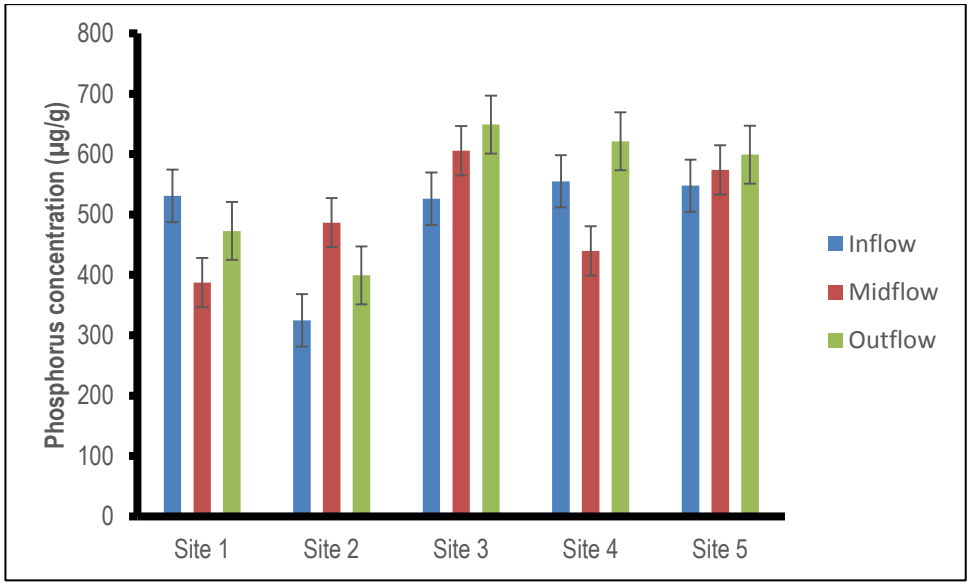


Figure 26: Variations in phosphorus concentration measured in sediments at inflow, midflow and outflow for the five sites

The average mean concentration value of Sulphur on sediments was 1891.46 µg/g (Table 6). The minimum and maximum concentration values of S were 519.67 and 3927 µg/g respectively (Table 6, Figure 28). In the sediments of the sampled sites, there were no significant variations in the concentrations of S accumulated in the sediments. This is shown by the p value at $p \leq 0.05$ and the calculated p value = 0.000.

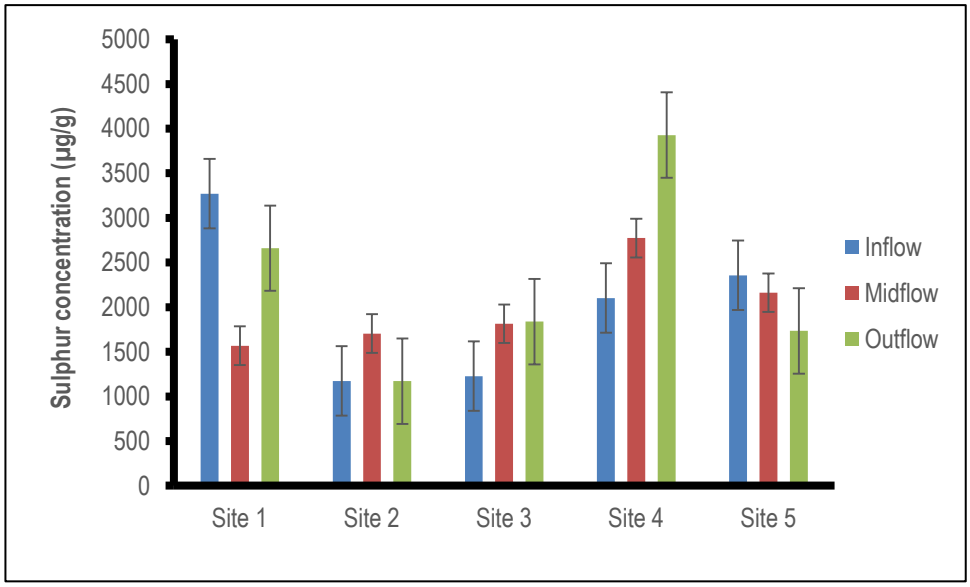


Figure 27: Variations in sulphur concentrations measured in sediments at inflow, midflow and outflow for the five sites

In the environment Cr takes two forms of Cr (III) as a trace nutrient and Cr (VI) as non-essential and toxic. The two forms of Cr in the environment have different impacts on the plants as well as in the contaminated lands. Cr (III) is regarded as trace nutrient and less mobile and it can be transported by soil organic matter whilst Cr (VI) is toxic to plants and other living organisms because of its ability to associate with oxygen in the environment. Cr has various negative impacts on plants since it significantly contributes towards reductions in the yield, mutagenesis, inhibit the activities of enzymes and retarded plant growth and development of the leaves (Shanker *et al.,* 2005). Cr negatively impacts the photosynthetic ability of the plants and inhibits growth and development, drastically reducing yields on many crops (Shanker *et al*., 2005).

The average mean concentration value for Cr was 192.03 µg/g (Table 6). The minimum and maximum values for Cr concentration obtained from the sediments of the sampled sites were 97.13 and 282.67 µg/g respectively (Table 6, Figure 29). There were significant variations in the concentrations of Cr in the sediments and this is shown by the p value at $p \leq 0.05$, and the calculated p value = 1.3714.

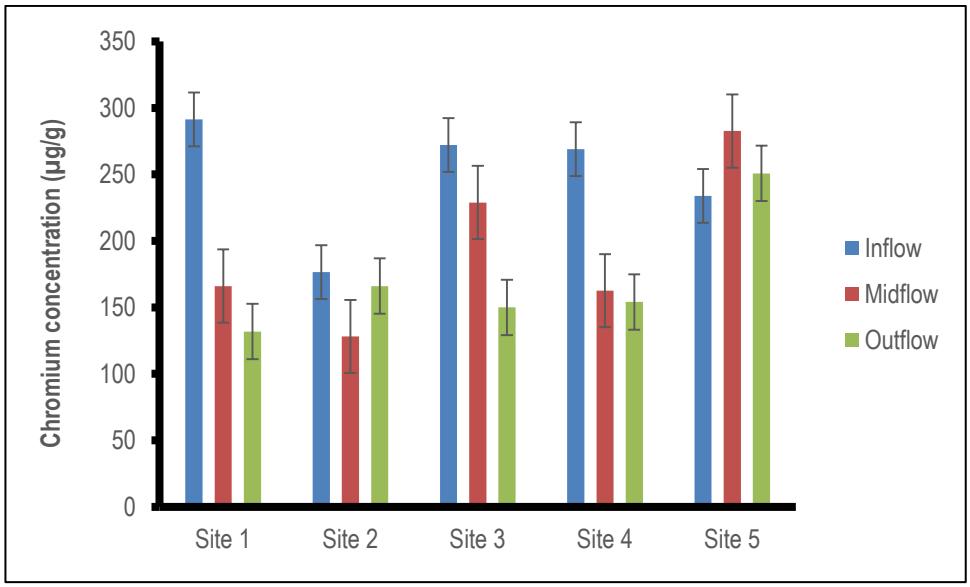


Figure 28: Variations in chromium concentration measured in sediments at inflow, midflow and outflow for the five sites.

Mn was measured in the sampled sediments of all five sites as manganese oxide (MnO). The average mean concentration value for Mn was 1081.76 µg/g (Table 6). The Minimum and maximum concentration values for Mn measured in all the sites were 222.17 and 2807 µg/g respectively (Table 6, Figure 30). There were no significant

variations in the concentration of Mn accumulated in the sediments of the sampled sites. This is shown by the p value at $p \leq 0.05$ and the calculated $p = 0.000$.

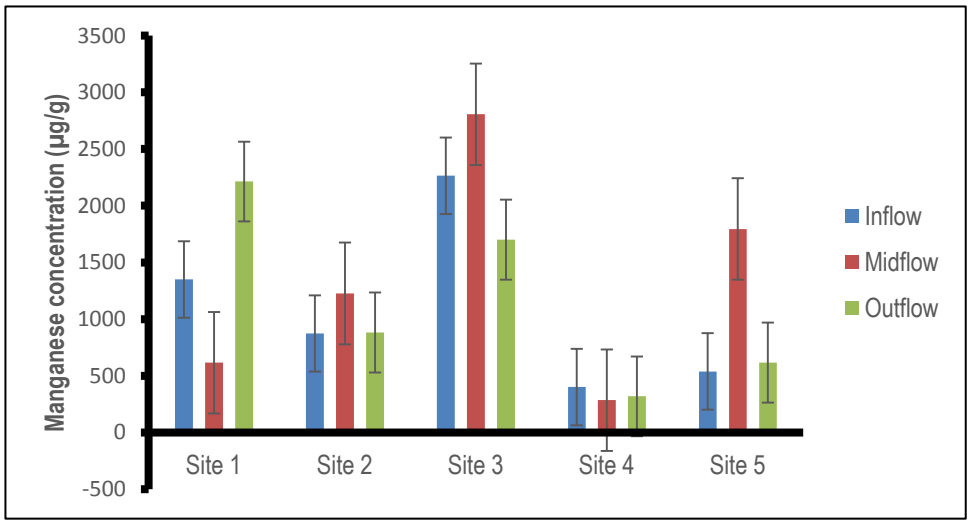


Figure 29: Variations in manganese concentrations measured in sediments at inflow, midflow and outflow for the five sites

The average mean concentration value for Fe measured in the sediments of all five sites was 7.23 µg/g. Fe was accumulated in the sediments as iron oxide (FeO). The minimum and maximum concentration values for Fe were 3.16 and 11.59 µg/g respectively. There were significant variations in the concentration of Fe accumulated in the sediments of the constructed wetlands. This is shown by the p value at $p \leq 0.05$ and the calculated $p = 0.9455$.

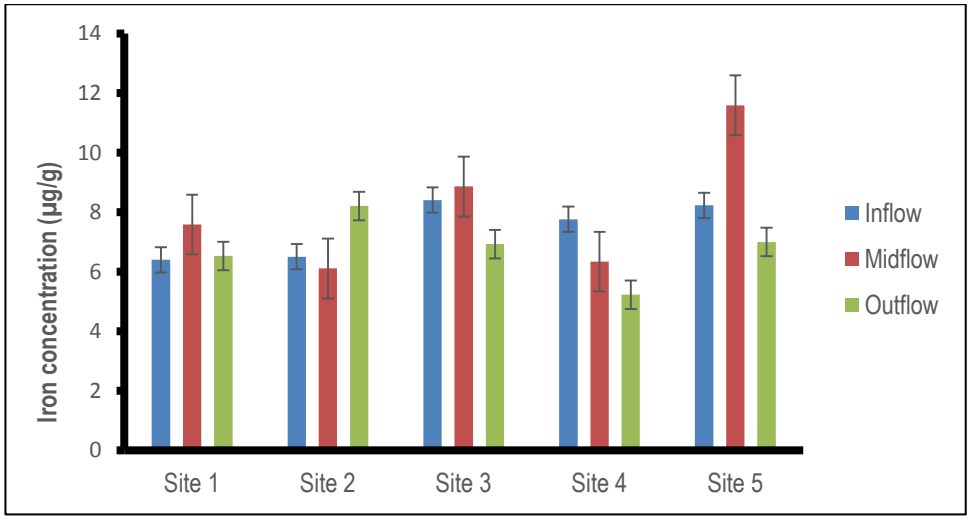


Figure 30: Variations in iron concentration measured in sediments at inflow, midflow and outflow for the five sites

The average mean concentration value of Co measured on the site was 29.51 µg/g (Table 6). The minimum and maximum concentration values for Co obtained in the sediments of the sampled sites were 9.53 and 51.83 µg/g respectively (Table 6, Figure 32). There was no significant variations in the in the concentrations of Co accumulated in the sediments of the sampled sites. This is shown by the p value at $p \leq 0.05$ and the calculated $p = 1.6718$.

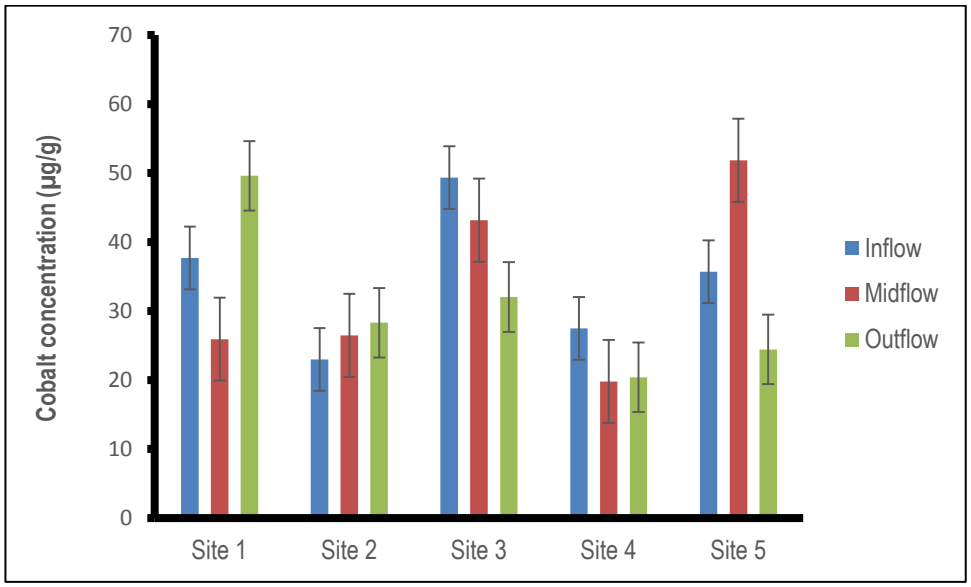


Figure 31: Variations in cobalt concentration measured in sediments at inflow, midflow and outflow for the five sites

The concentrations of Nickel (Ni) measured in the sediment samples of all the five sites were varying slightly. The average mean concentration value for Ni accumulated in the sediments of the sites was 59.23 µg/g. The minimum and maximum concentration of Ni measured from the sediments was 20.67 and 75.67 µg/g (Table 6, Figure 33). There were no significant variations in the concentration of Ni in the sediments of the sites, and this is indicated by the p value at $p \leq 0.05$, and the calculated $p = 0.0001$.

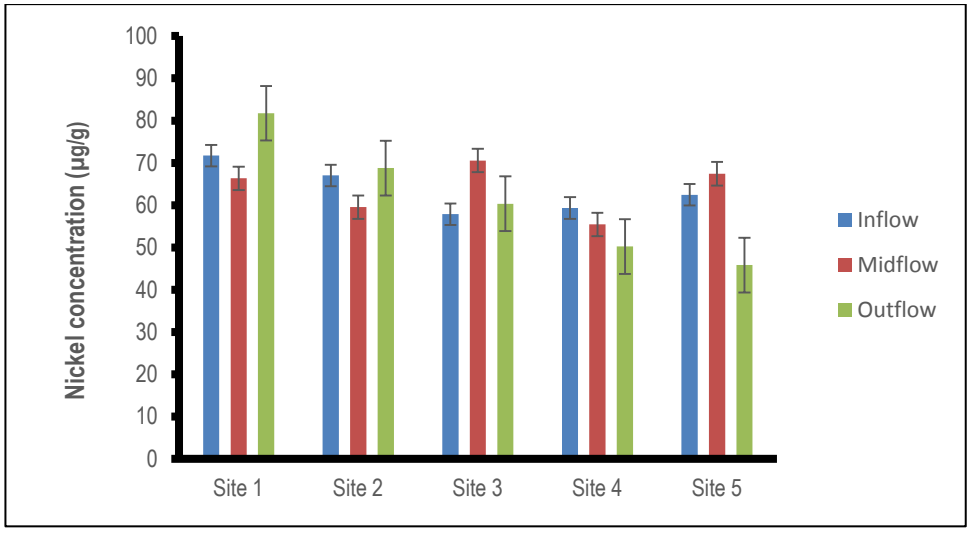


Figure 32: Variations in nickel concentration measured in sediments at inflow, midflow and outflow for the five sites

The average mean concentration value for Cu accumulated on the sampled sediments of all five sites was 38.37 µg/g. The minimum and maximum concentration for Cu was 16 and 41.60 µg/g (Table 6, Figure 34). There were significant variations in the concentration of Cu accumulated in the sediments of the sampled sites. This is shown by the p value at $p \leq 0.05$ and the calculated $p = 0.1889$.

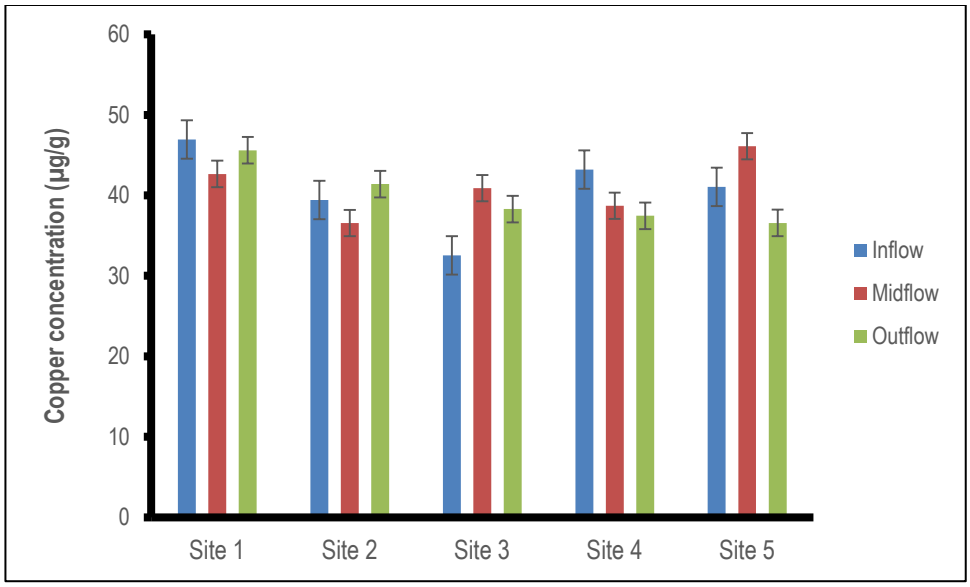


Figure 33: Variations in copper concentrations measured in sediments at inflow, midflow and outflow for the five sites

On the sampled sediments, the average mean concentration value for Zn was 39.11 µg/g. The minimum concentration of Zn was 10.5 µg/g (Table 6). The maximum concentration of Zn measured in the sampled sediments of all five sites was 50.27 µg/g (Table 6, Figure 35). The significant variations in the concentrations of the sediments

were indicated by means of the p-value. There were no significant variations in the concentrations of Zn accumulated in the sediments. This is shown by the p value at $p \leq 0.05$ and the calculated $p = 0.0022$.

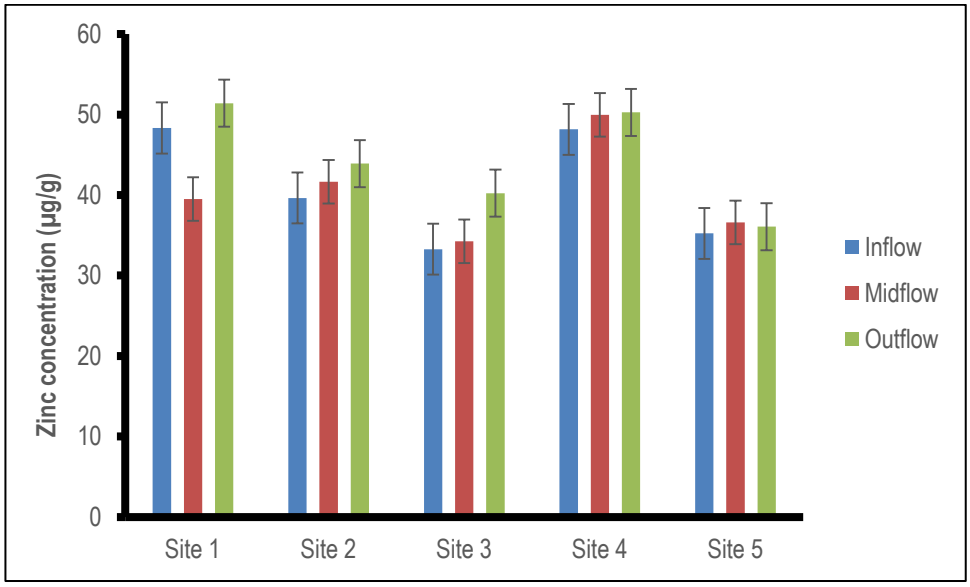


Figure 34: Variations in zinc concentration measured in sediments at inflow, midflow and outflow for the five sites

Molybdenum (Mo) is a rare microelement and according to (Fortescue, 1992) only 1.2 mg/kg of the soil contains molybdenum. In soils the oxidation states ranges between II to VI. According to Brookings, (1987), Mo in the oxidation state IV occurs in the anoxic conditions. In anoxic soils with a pH of 4 - 8, Mo occurs in the form of Mo (VI) anions and salts. Molybdenum toxicity is often seldom in field conditions. Molybdenum deficiency occurs in many plant species of herbs, crops and trees and the lack of nitrate reductase catalyses the first stages of nitrate assimilation (Zimmer & Mendel, 1999). Molybdenum deficiency in crops result in the delayed flowering stage, inhibit tasselling, anthesis and the development of sporogenous tissues which gives rise to poor yield (Agarwala *et al.,* 1978).

In this study, Molybdenum was found to have an average mean concentration value of Mo of sediments accumulated in the sampled sites was 1.73 µg/g (Table 6). The minimum concentration value of Mo in the sediments was not detected and the maximum concentration value for Mo was 3.5 µg/g respectively (Table 6, Figure 36). This indicates that there are significant variations in the concentrations of Mo accumulated in the sediments of the sampled sites. This is shown by the p value at $p \leq 0.05$ and the calculated $p = 0.6704$.

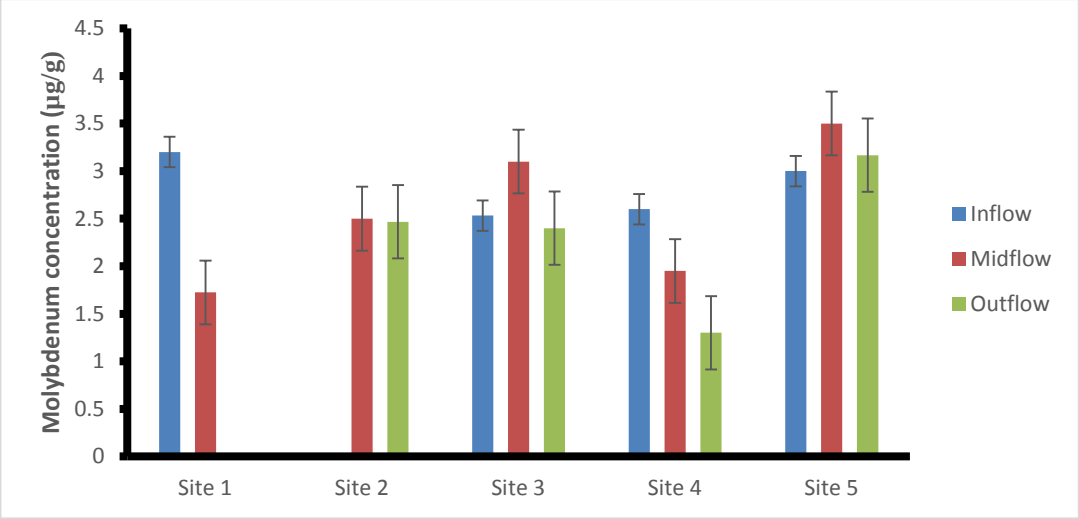


Figure 35: Variations in molybdenum concentrations measured in sediments at inflow, midflow and outflow for the five sites.

The wide spreading use and demand of Lead (Pb) by anthropogenic activities give rise to the alarming rates of contaminated environment by Pb. Various sources of Pb have been documented, and they include metals smelting, paints, pesticides and phosphate fertilizers. Automobile exhausts released from crashed alkyl derivatives in gasoline are the most common source of Pb (Singh *et al.,* 1997). The soil is another source of Pb. In the soil, Pb is found in the form of soluble and insoluble salts and is strongly bound by the colloidal organic molecules.

According to Zimdahl and Koeppe, (1977) as cited by Singh *et al.,* (1997), Pb can be absorbed by the plants in two major forms; it can be taken up through the soil or by the air. Dust fumes that contain the aerosol Pb can be deposited on the leaves of the plants in the form of vapour and become absorbed by the plants. Pb is another toxic element when absorbed by the plants in higher quantities, although there are some plants that can able to withstand the Pb contaminated sites. Its accumulation in the plants is influenced by factors of species, plant cultivar, plant organ, the presence of other metal ions in the environment. Higher concentrations of Pb in plants have some negative impacts on the physiological processes such as photosynthesis. Pb excess in the soil reduces nitrates in the roots of most plants. It also has impacts nodulation, inhibits N-fixation as well as the assimilation of ammonium in the nodules of the roots (Singh *et al.,* 1997).

The average mean concentration for Pb measured in the sampled sediments was 29.20 µg/g (Table 6). The minimum and maximum concentration of Pb measured from the sediments was 8.53 and 41.3 µg/g respectively (Table 6, Figure 37). The results of the concentration values of Pb accumulated in the sediments of the sampled sites

show that there is no significant difference in the concentrations of Pb. This is shown by the p value at $p \leq 0.05$ and the calculated p value= 0.0210.

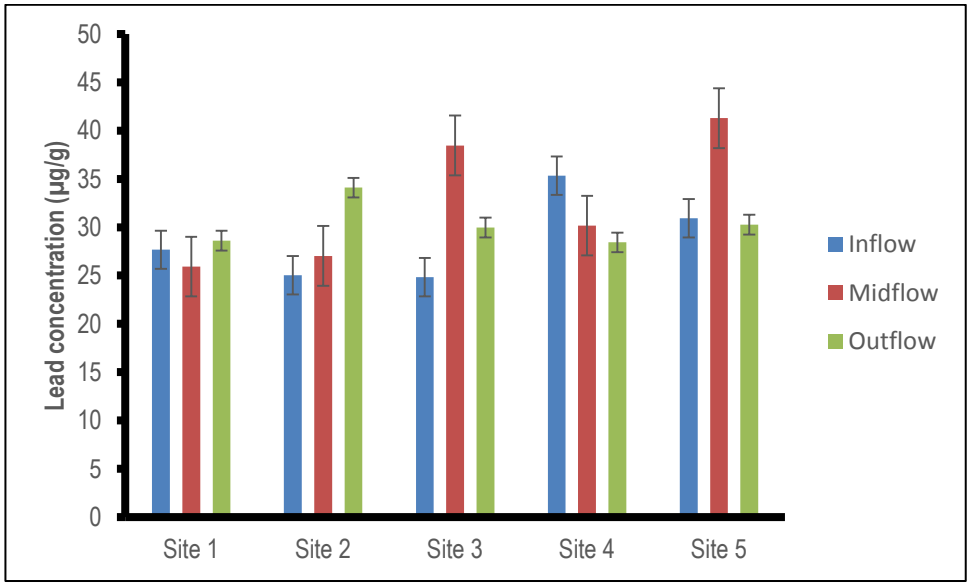


Figure 36: Variations in lead concentrations measured in sediments at inflow, midflow and outflow for the five sites

4.4. General comparison of elements accumulated by plants between seasons

The following section compares the elements accumulated by the plants between the two seasons of summer and autumn. The discussion will be supported by means of the graphs indicating the average levels of elements concentration in the plants between the two seasons. The plants species that will be used for comparison are the *P. communis*, *S. corymbosus* and the *T. capensis*.

The average concentration for Zn accumulated by the plants in autumn and summer seasons were in a range of 124.83 and 230.47 µg/g respectively. The result for higher accumulation of Zn in summer could be that the new shoots are sprouting and as a result more Zn is taken up by the plants as compared to autumn when the plants shed their leaves.

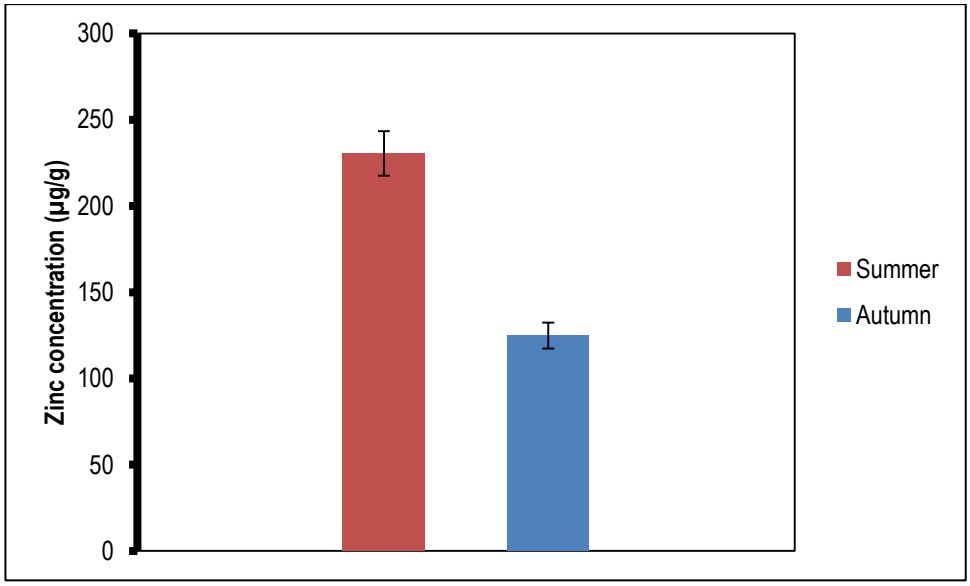


Figure 37: Variation in average Zn concentrations accumulated by plants in autumn and summer seasons

When plant samples were analysed for Ni content, the higher average conentration for Ni was measured in summer. The average concentration for Ni accumulated by the plants during the autumn and summer seasons were 36.36 and 49.55 µg/g respectively. Ni accumulation in the plants in the two seasons follow that of Zn.

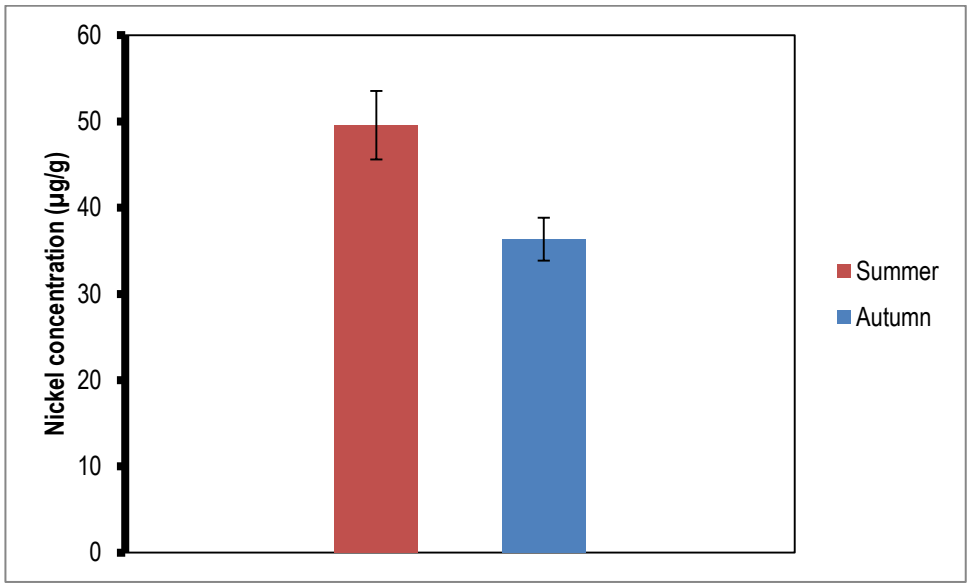


Figure 38: Variations in mean Ni concentration accumulated by the plants in summer and autumn seasons

During the investigation period, it was observed that Fe was accumulated by the plants as FeO. Higher amount of Fe in the plants was accumulated in summer season. The average concentration for Fe accumulated by the plants

during summer and autumn seasons was 25657.92 and 24807.34 µg/g respectively. Unlike for Zn, Fe accumulation in the two seasons did not differ much.

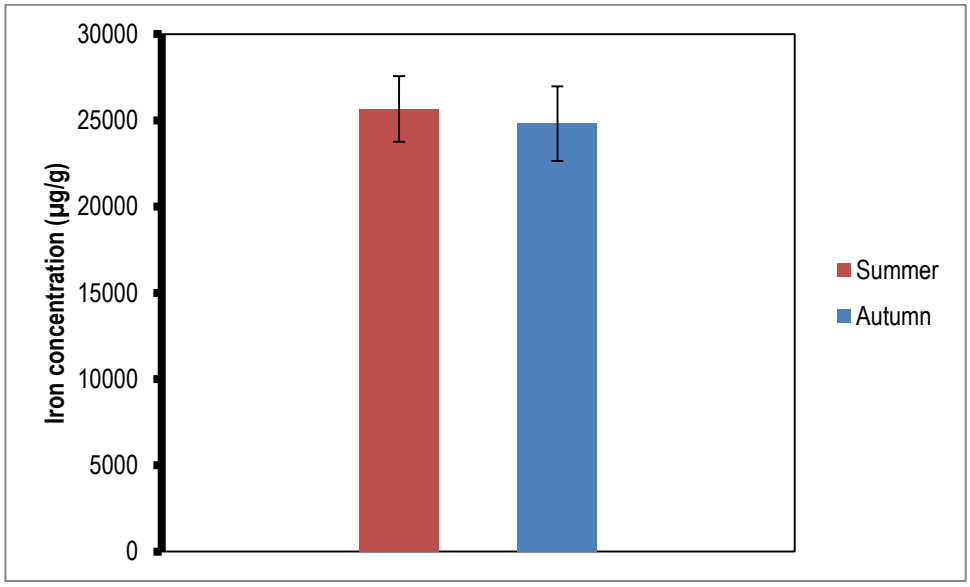


Figure 39: Variations in mean Fe concentration accumulated by the plants in summer and autumn seasons

Plants accumulated Mn in the form of MnO. Mn was highly accumulated by the plants during summer season and this also follows the trends of Zn and Ni. The average concentration for Mn accumulated by the plants were 5984.96 and 3950.78 µg/g in summer and autumn seasons respectively.

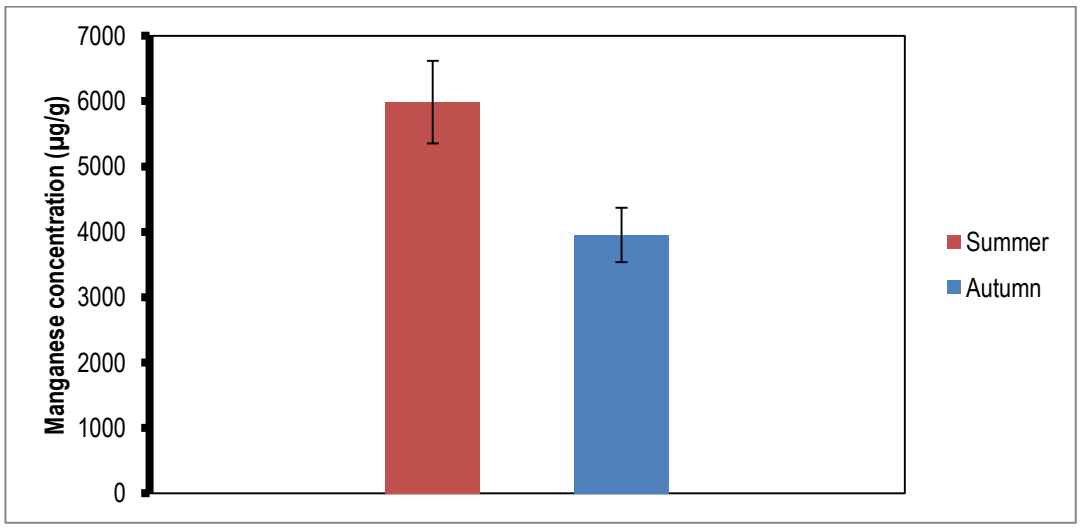


Figure 40: Variations in mean Mn concentration accumulated by the plants in summer and autumn seasons

In all the sampled plants, Cu was highly accumulated by the plants in summer than in autumn in the same way as Zn, Ni and Mn. The average concentration for Cu accumulated in the plants sampled during the summer and autumn months was 78.30 and 45.63 µg/g.

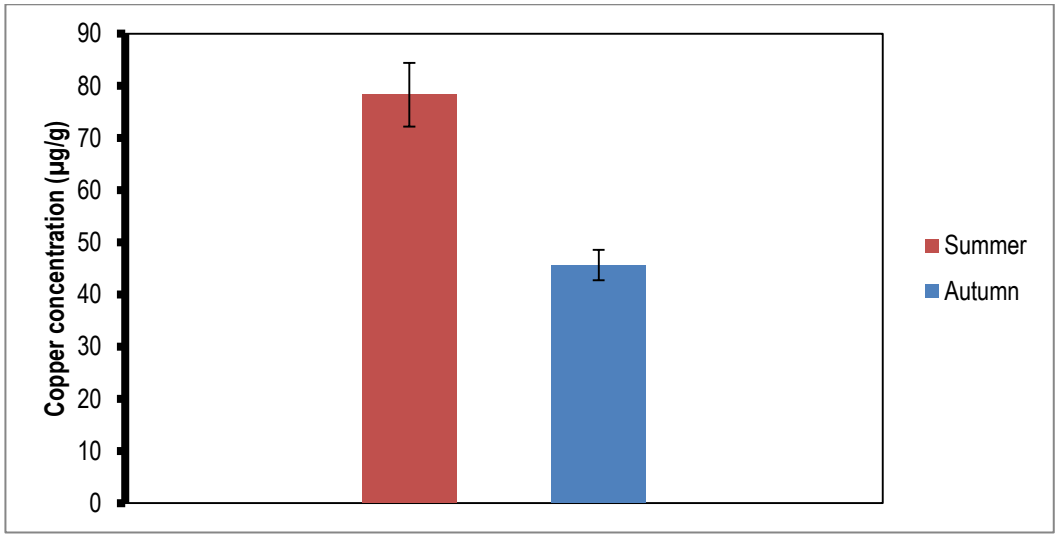


Figure 41: Variations in mean Cu concentration accumulated by plants in summer and autumn seasons

During autumn and summer seasons, the average concentration for Mg accumulated in the plants was 18561.31 and 14976.21 µg/g respectively. Higher concentration of magnesium was accumulated by the plants during autumn. When the plants were measured for Mg, seasonal differences in the accumulation of Mg were not very pronounced.

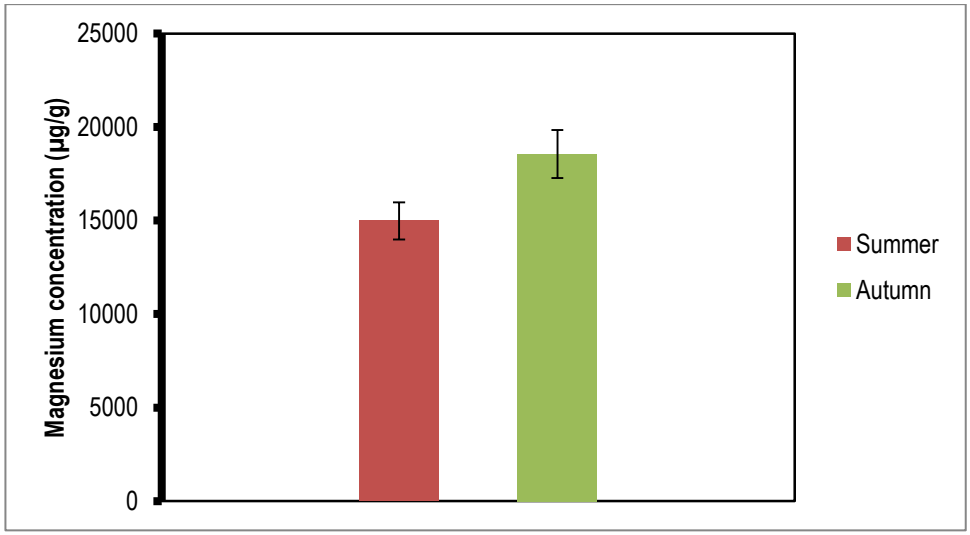


Figure 42: Variations in mean Mg concentration accumulated by plants in summer and autumn seasons

During the sampling periods, harvested plants were measured for the presence of Co. After the plants have been measured for the metals accumulated by the plants, it was observed that the plants harvested in summer have accumulated and stored in them higher amounts of Co than in autumn, and this also follows the trends like that of Zn, Ni, Cu and Mg. The average concentration for Co measured in summer was 58.13 µg/g, whilst the average concentration of Co in autumn was 43.24 µg/g.

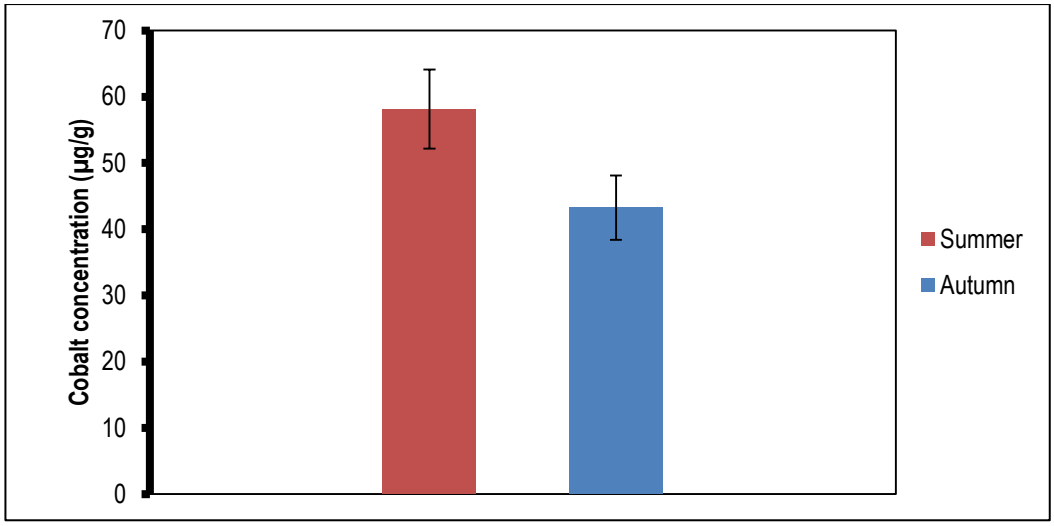


Figure 43: Variation in mean Co concentration accumulated by plants in summer and autumn seasons

In summer, the plants have accumulated higher concentrations of S. The average concentration of S in the plants was 22826.03 µg/g in summer whilst in autumn it was 22749.46 µg/g. The uptake of S is also not very pronounced and for the mean values, they do not differ much between the plants measured from the sites.

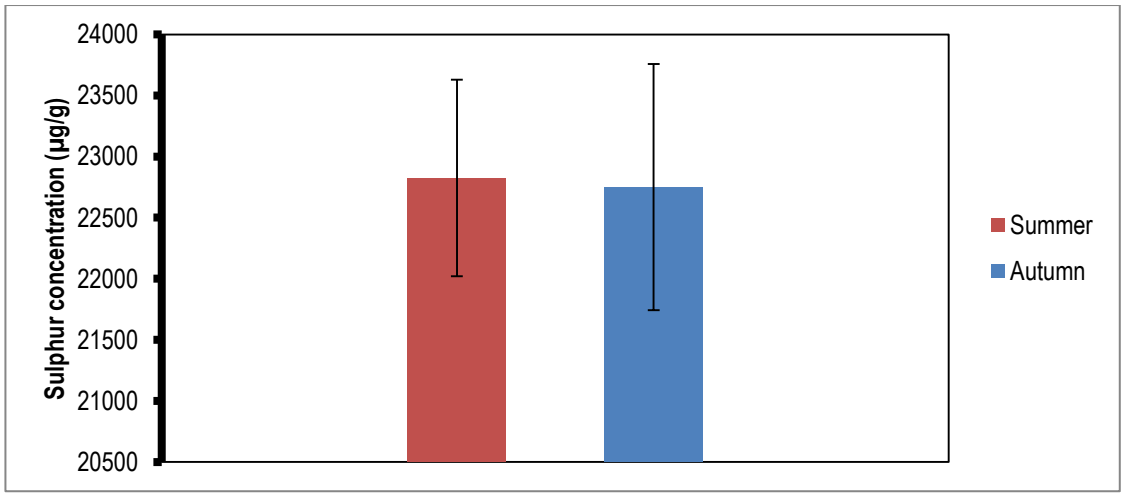


Figure 44: Variations in mean S concentration accumulated by plants in summer and autumn seasons

P was highly accumulated by the plants in autumn. The average concentration for P accumulated by the plants in autumn was 3759.61 µg/g whilst in summer it was 3487.44 µg/g.

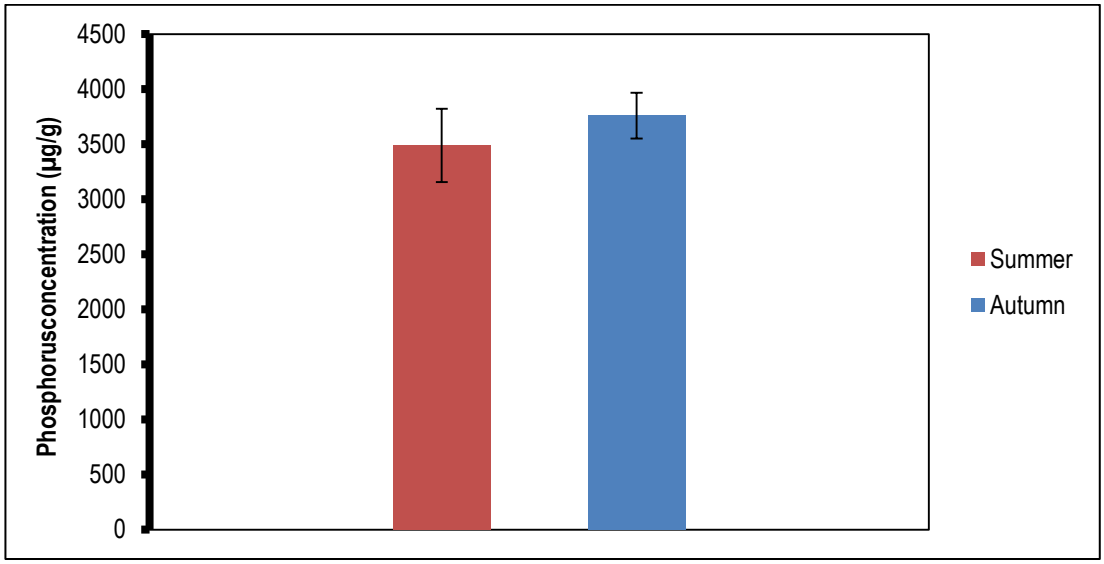


Figure 45: Variations in mean P concentration accumulated by plants in summer and autumn seasons

Higher amounts of Cr were measured in the plants in autumn. The average concentration for Cr in autumn was 68.76 µg/g and in summer it was 64.82 µg/g.

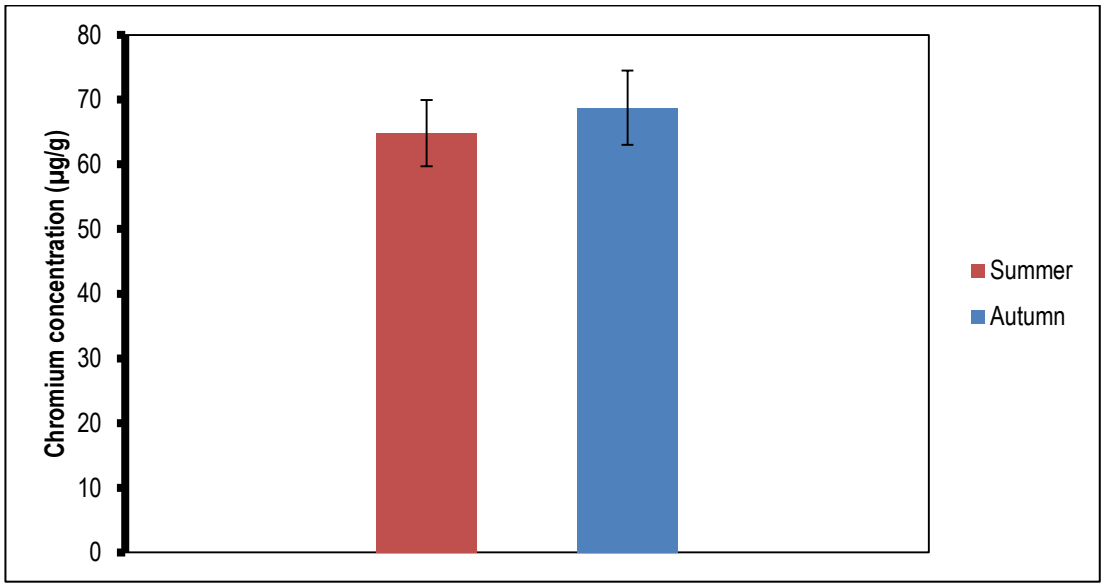


Figure 46: Variations in mean Cr concentration accumulated by plants in summer and autumn seasons

In the plant sampled measured for Mo in autumn and summer, it was found that Mo was highly accumulated by the plants in autumn. The average concentration for Mo measured in autumn was 0.09 µg/g and in summer was 0.07 µg/g.

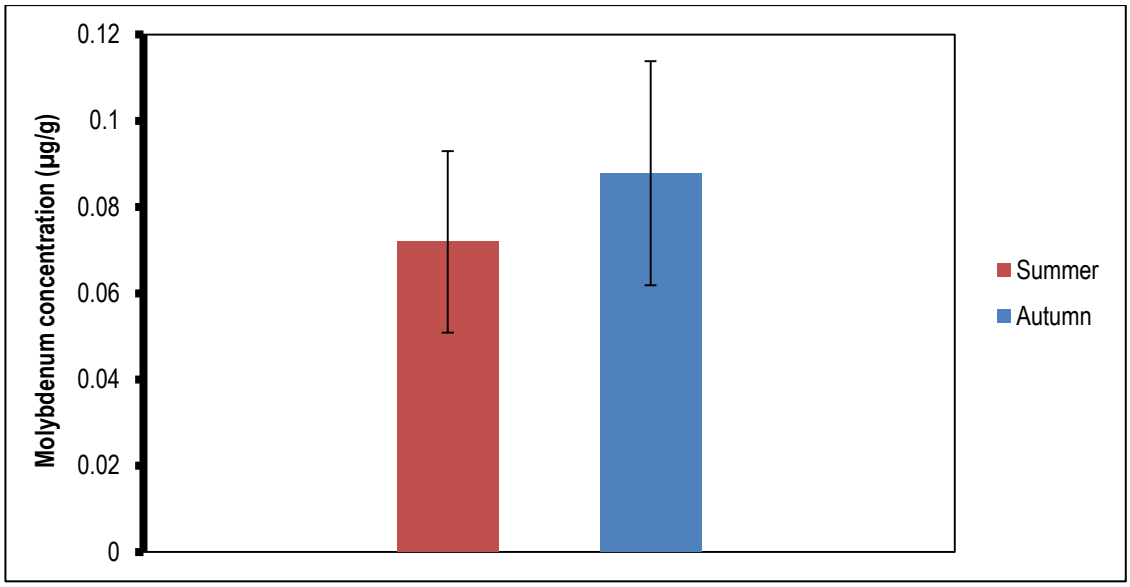


Figure 47: Variations in mean Mo concentration accumulated by plants during summer and autumn seasons

The average concentration for Pb in summer and autumn months was 12.082 and 12.084 µg/g respectively. Higher concentrations for Pb were measured in the plants in summer.

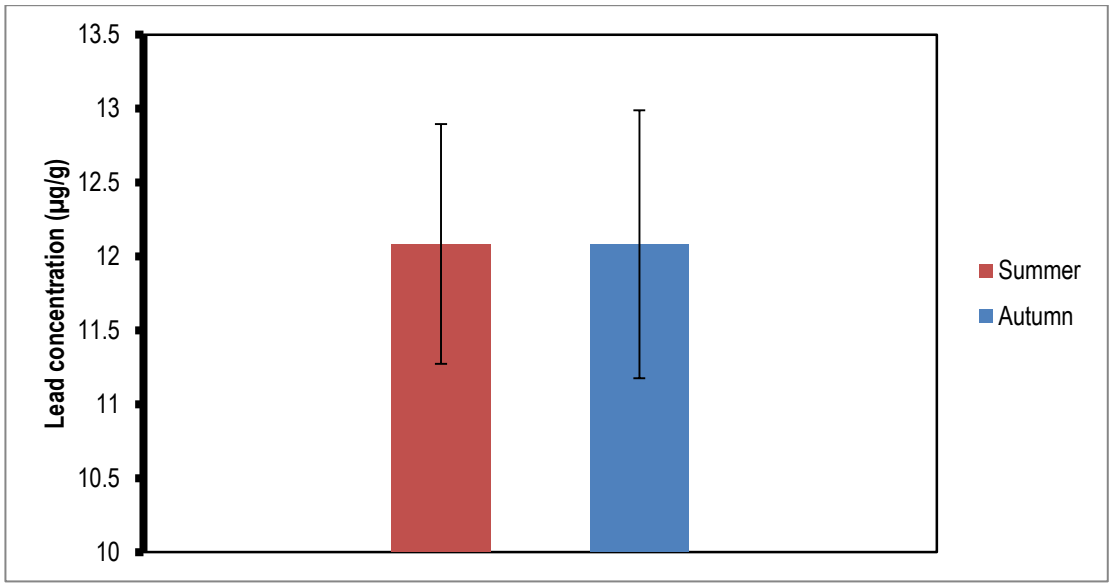


Figure 48: Variations in mean Pb concentration between autumn and summer seasons

4.5 Correlation of elements

The correlation coefficients was performed in order to determine the relationship between the levels at which the elements were absorbed by the three different organs of the three species of *Schoenoplectus, Phragmites* and the *Typha spp*. The concentrations of elements in the water, sediments and plants was compared by the use of the significant variance at p = 0.05. When the significant correlations co-efficient was performed, there was a number of significant correlations that were found to take place between the rhizomes, roots, leaves, water and sediment. A correlation was found between sediments and plants, where Magnesium was negatively correlating with Cr and Fe and positively correlating with P, S, Mn, Co and Ni. Their associated r values were (Cr) r = -0.06143 and (P) r = -0.6232. The r values for the positive correlations were (P) r = 0.7598, (S) r = 0.6406, (Mn) r = 0.8714, (Co) r = 0.8021 and (Ni) r = 0.6704.The p values were significant at p = 0.0500 and all the p values were in arrange of p = 0.000 and 0.0015 (Refer to appendix 1 attached).

P was found to be having both the negative and positive correlations with the elements in water, sediments and plants organs. The negative correlations were found between the concentrations of Cr, Fe and Pb while the positive correlations were obtained between the concentrations of Mn and Co. The r values of the elements with negative concentration correlations were (Cr) r = -0.6488, (Fe) r = -0.7973 and (Pb) r = -6581, while the correlation for the elements with positive concentrations of elements were (Mn) r = 0.07636 and (Co) r = 0.7841. Their p values were significant at p = 0.0500 and their p values were in arrange of p = 0.000 and p = 0.009 in water, sediments and also in plants. Cr was also found to have a negative correlation with Mn and positive correlations with Fe and Pb.

The correlations were significant at p = 0.0500 and the p values were in a range of p=0.000 and p=0.038. The correlations were (Mn) r = -0.5389, (Fe) r = 0.8005 and (Pb) r = 0.8584 respectively in water, sediments and plants. In sediments, Mo was found to have a positive correlation with total N in water, and the correlation was (Mo) r = 0.6109 and the (N) p = 0.016. In water, it was found that there is a positive correlation between DO and NH_{4+} and the r value were (Do) 0.6431 while the p value = 0.010. pH was found to have positive correlations with Eh, NO_3^+ and NH_4^+ in water and the r values were (Eh) r = 0.5353, (No_3^+) r = 0.5469 and (NH_4^+) r = 0.5994 consecutively while their respective p values were p = 0.040, p = 0.035 and p = 0.018 respectively (Refer to appendix 1 attached).

4.6 Accumulation of elements by plant organs

The following section discusses the accumulation of elements by the plant organs. Elements accumulated by the plants organs of leaves, rhizomes and roots are shown in graphs with their levels of concentrations. The discussion will also highlight the specific plant organs which tend to accumulate certain metals than the other organs of the plants. All the elements analysed in the plants samples are discussed individually by means of graph showing the amount of elements accumulated by the leaves, rhizomes and roots of *P. communis* all the sampled site.

In all the sites, in the leaves, highest average concentration for Zn in autumn was 99.01 µg/g and the lowest average concentration in autumn in the leaves was in autumn was 66.21 µg/g (Figure 50). The highest and lowest average concentration of Zn in summer in the leaves was 145.81 and 123.75 µg/g respectively. In the rhizomes, the highest average concentration for Zn in autumn was 151.48 µg/g and the lowest average concentration for Zn was 77.19 µg/g. In summer, the highest average concentration for Zn was 213.2 µg/g and the lowest average concentration was 129.36 µg/g. In the roots, the highest average concentration for Zn was 425.77 µg/g and the lowest average concentration value was 261.29 µg/g in summer. In autumn, the highest and lowest average concentration for Zn in the roots was 228.36 µg/g and 107.12 µg/g respectively as illustrated in fig 49.

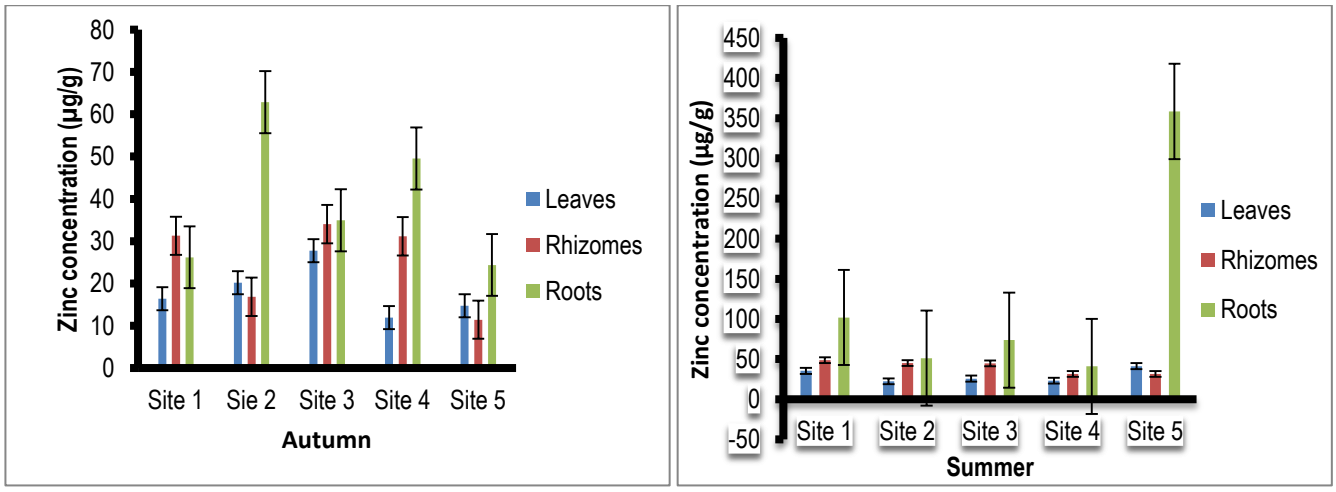


Figure 49: Variations in Zn accumulated in leaves, rhizomes and roots

The highest average concentration for Ni in autumn in the leaves of the plants was 21.37 µg/g whilst the lowest concentration for Ni was 13.04 µg/g. In summer, the highest average concentration for Ni was 14.92 µg/g and the lowest concentration for Ni was 13.37 µg/g in the leaves of the sampled plants. In the rhizomes of the plants samples in autumn, the highest average concentration for Ni was 27.79 µg/g and the lowest average concentration for Ni was 20.01 µg/g.

In summer on the other hand, the highest average concentration for Ni was 43.56 and the lowest average concentrtaion for Ni in the rhizomes was 29.92 µg/g. Ni was accumulated in high amount in the roots of the plants. In autumn, the highest average concentration of Ni in the roots was 72.67 µg/g and the lowest average concentration was 61.83 µg/g. in summer, the highest average concentration for Ni was 111.54 µg/g and the lowest average concentration was 64.94 µg/g.

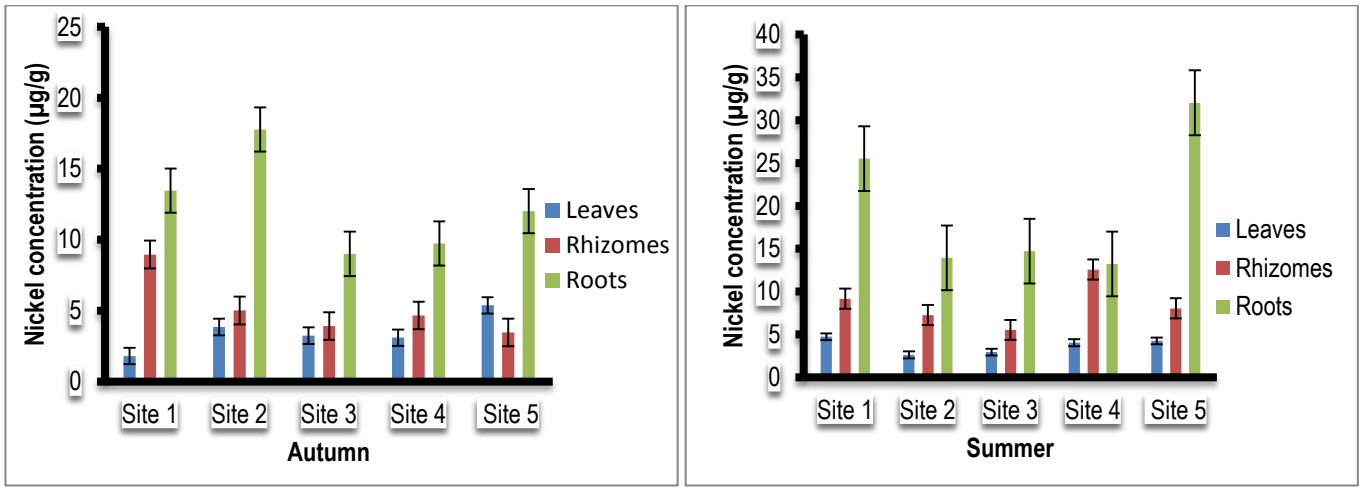


Figure 50: Differences in Ni concentration accumulated in the leaves, rhizomes and roots

Fe was highly accumulated by the roots in higher amounts than it was in the leaves in both autumn and summer seasons. In autumn, in the leaves of the plants, the highest average concentration for Fe was 7800 µg/g whilst the lowest average concentration for Fe was 5100 µg/g. In summer, the highest average concentration for Fe accumulated by the leaves of the plants was 5400 µg/g and the lowest average concentration for Fe was 4500 µg/g in the leaves. In autumn, the highest average concentration for Fe accumulated by the rhizomes of the plants was 19800 µg/g and the lowest average concentration for rhizomes was 15800 µg/g.

In summer, the average concentration for Fe accumulated in the rhizomes was 28500 µg/g whilst the average lowest concentration for Fe in the rhizomes was 14600 µg/g. In autumn, the highest average concentration for Fe was 58600 µg/g and the lowest average concentration for Fe in the roots of the plants was 42100 µg/g. The highest average concentration for Fe accumulated by the plants roots in summer was 57600 µg/g and the lowest average concentration for Fe accumulated by the plants roots in summer was 33500 µg/g respectively.

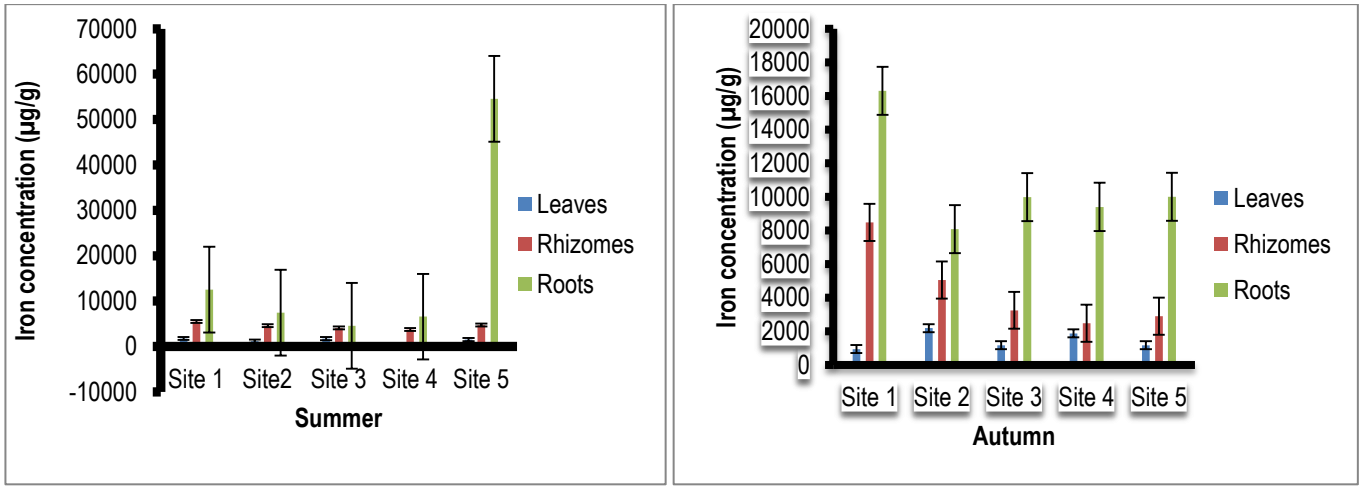


Figure 51: Differences in Fe concentration accumulated by the leaves, rhizomes and roots

In autumn, the highest average concentration for Mn was 7513.60 µg/g and the lowest average concentrationfor Mn was 3399.22 µg/g accumulated by the leaves of the sampled plants. In Summer the highest and lowest average concentration for Mn accumulated in the leaves of the plants were 3810.30 µg/g and 2457.63 µg/g. Both in autumn and summer seasons, rhizomes were the second highest accumulator of Mn. In autumn, the highest average concentration for Mn measured in the rhizomes was 3147.75 µg/g and the lowest average concentration for Mn was 1357.70 µg/g respectively.

In summer, the highest average concentration for Mn in the rhizomes was 5851.30 µg/g and the lowest average concentration for Mn was 1944.23 µg/g respectively. The roots of the sampled plants were the highest accumulator of Mn in both seasons. In autumn, the highest average concentration for Mn was 4795.22 µg/g whilst the lowest average concentration for Mn was 1652.96 µg/g. in summer, the highest average concentration for Mn was 24375.40 µg/g and the lowest average concentration for Mn in the roots of the plants was 6346.00 µg/g.

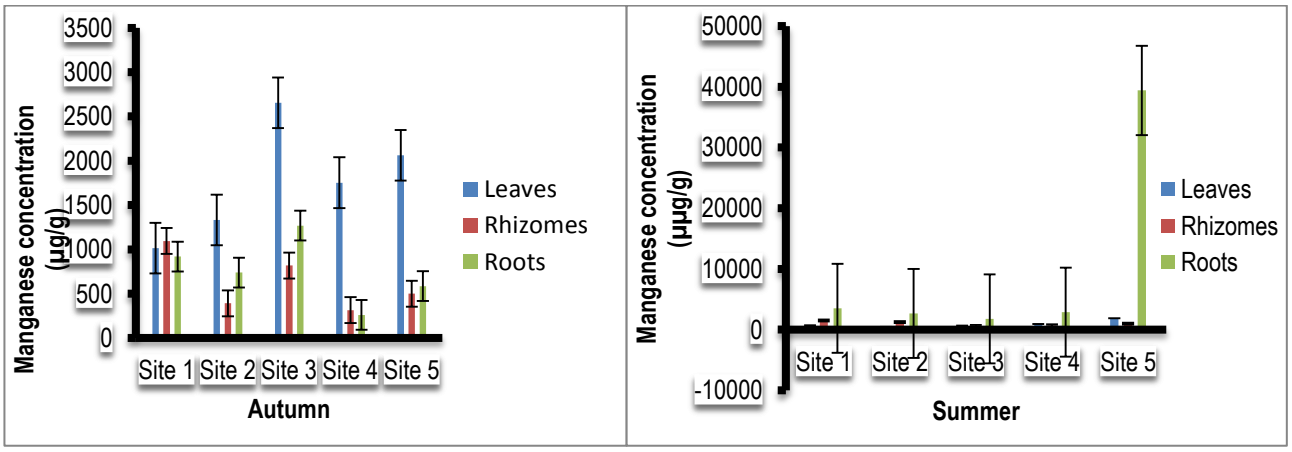


Figure 52: Differences in Mn concentration in the leaves, rhizomes and roots

Cu was another element that have been highly accumulated by the plant roots. The highest average concentration for Cu in autumn was 24.16 µg/g and the average lowest concentration for Cu measured in the leaves of was 16.71 µg/g. In summer the highest average concentration for Cu measured in the leaves of the sampled plants was 30.26 whilst the average lowest concentration for Cu in the leaves of the plants was 20.68 µg/g. The highest average concentration for Cu measured in the rhizomes of the plants in autumn was 51.02 µg/g and the average lowest concentration for Cu in the rhizomes was 36.96 µg/g.

In summer, the highest average concentration for Cu in the rhizomes of the sampled plants was 66.70 µg/g and the lowest average concentration for Cu was 44.66 µg/g. the rhizomes of the sampled plants in both autumn and summer seasons, were the highest accumulator of Cu. The highest average concentration of Cu was measured during summer and the highest average concentration for Cu in summer in the roots was 180.98 µg/g and the lowest average concentration for Cu measured in the roots of the plants was 99.62 µg/g. In autumn, the highest average concentration for Cu measured in the roots of the plants was 98.40 µg/g and the lowest average concentration for Cu on the other hand was 49.53 µg/g respectively.

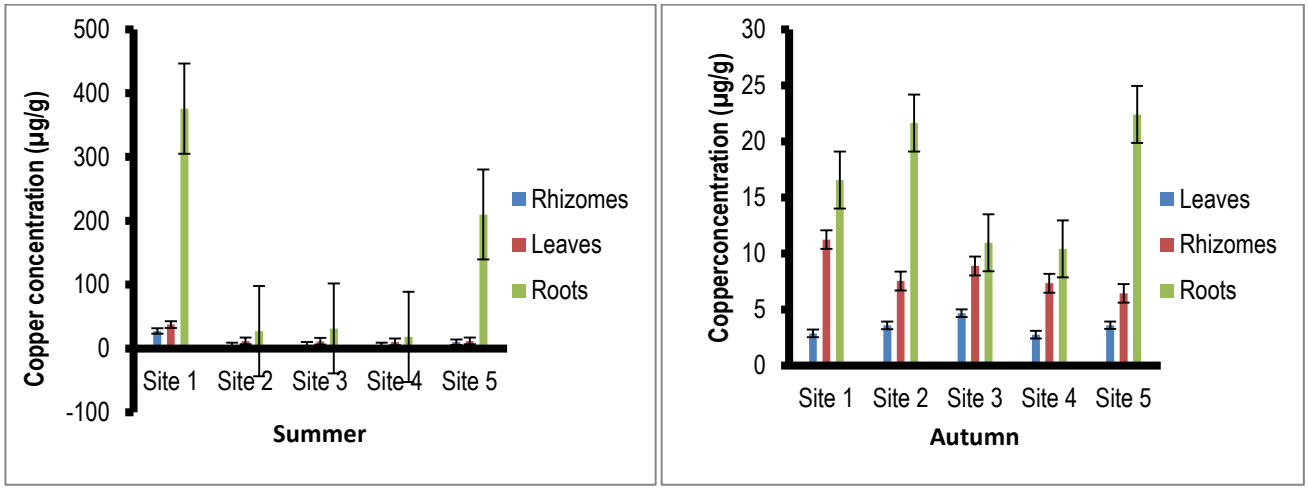


Figure 53: Differences in Cu concentrations measured in the leaves, rhizomes and roots

In the leaves Mg was accumulated in the form of MgO. The leaves in both the autumn and summer seasons were the highest accumulator of Mg.The highest average concentration for Mg in autumn was 29200 µg/g and the lowest average concentration for Mg was 18800 µg/g. In summer, the highest average concentration for Mg accumulated in the leaves of the plants was 25800 µg/g and on the other hand, the lowest average concentration for measured in the leaves was 20400 µg/g. The highest average concentration for Mg in the rhizomes of the plants in autumn was 17300 µg/g and the lowest average concentration for Mg was 10100 µg/g.

The highest average concentration for Mg measured in the rhizomes of the plants in summer was 11100 µg/g and the lowest average concentration for Mg in the rhizomes was 5900 µg/g. Autumn plant roots were analysed for the presence of Mg and the highest and lowest average concentration for Mg measured were 17800 and 13500 µg/g respectively, whilst in summer, the highest ang lowest average concentration for Mg measured in the roots of the plants were 17100 and 9500 µg/g.

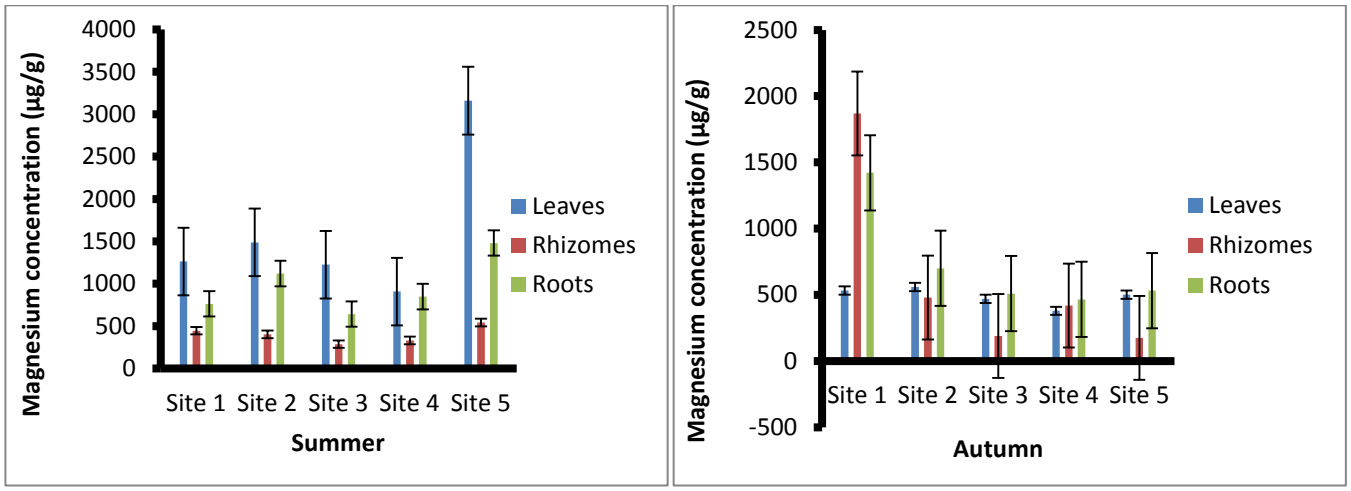


Figure 54: Variations in Mg concentrations measured in the leaves, rhizomes and roots

In the leaves, Co was measured in autumn and summer seasons. During the autumn season, the highest and lowest average concentration for Co were 23.48 and 10.53 µg/g respectively. On the other hand, summer sampled plants were measured for the presence of Co and the highest average and lowest concentration for Co were 13.36 and 9.41 µg/g. Rhizomes were the second highest accumulator of Co in both autumn and summer seasons.

The highest average concentration for Co in autumn measured in the rhizomes of the plants were 22.88 and 18.57 whilst the highest and lowest average concentration for Co were 56.91 and 28.6 µg/g respectively in summer. The roots of the selected plants for this study were able to accumulate high amounts of Pb much higher than the amount accumulated in the leaves and rhizomes. In autumn season, the highest and lowest average concentration for Pb measured in the roots were 131.65 and 68.35 µg/g respectively. In summer, the highest and lowest average concentration for Pb measured in the roots of the sampled plants were 145.00 and 77.59 µg/g respectively.

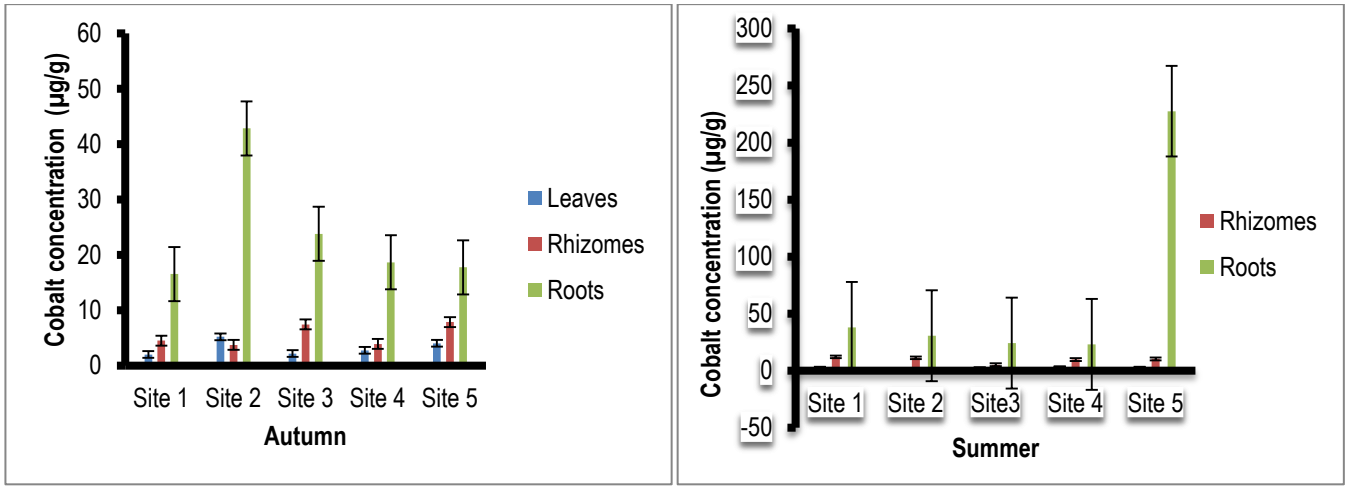


Figure 55: Variations in Co concentrations measured in leaves, rhizomes and roots

The highest and lowest average concentration of Cr in the leaves in autumn were 96.22 and 21.84 µg/g. In summer the highest and lowest concentration accumulated in the leaves of the plants was 42.10 and 29.30 µg/g respectively. The highest and lowest average concentration for Cr accumulated in the rhizomes of the plants in autumn was 96.4 and 41.78 and on the other hand, the highest and lowest average concentration for Cr in summer was 93.81 and 46.66 µg/g respectively. Cr was also another element that was highly accumulated by the plant roots. Plant samples collected in autumn were observed to have accumulated higher amounts of Cr when compared to the amounts of Cr acccumulated by the plants during summer season. The highest and lowest average concentration for Cr measured in the roots of the plants in autumn was 155.22 and 113.61 µg/g. The highest and lowest average concentration for Cr measured in the roots of the plants in summer was 114.01 and 79.6 µg/g respectively.

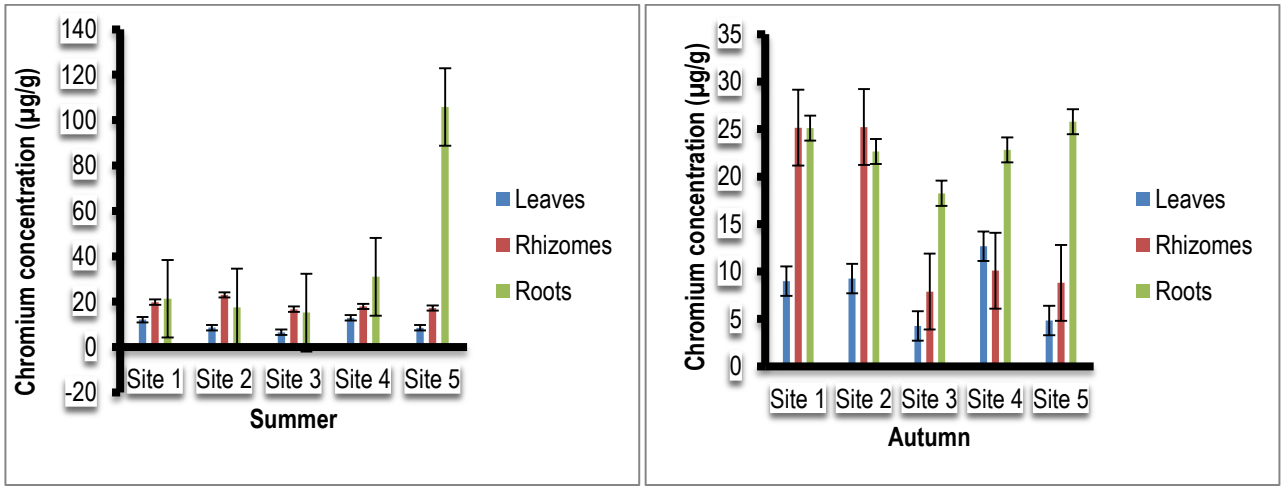


Figure 56: Variations in Cr concentrations measured in leaves, rhizomes and roots

Mo is an element that was accumulated by the plant leaves in relatively lower amounts and at some sites, the amount of Mo was not detected. In the leaves of the plants in autumn, the highest and lowest average concentration for Mo measured in the leaves of the plants was 0.71 and 0.2 µg/g.

In summer, the highest and lowest average concentration for Mo was 0.27 and 0.1 µg/g respectively. In the rhizomes of the plants, the highest and lowest average concentration for autumn and summer seasons was 0.3 and 0.1 µg/g as well as 0.85 and 0.1 µg/g respectively. In autumn, the concentration for Mo in the roots was not detected while in summer the highest average concentration for Mo was 0.2 µg/g and the lowest average concentration for Mo was below the detection limit.

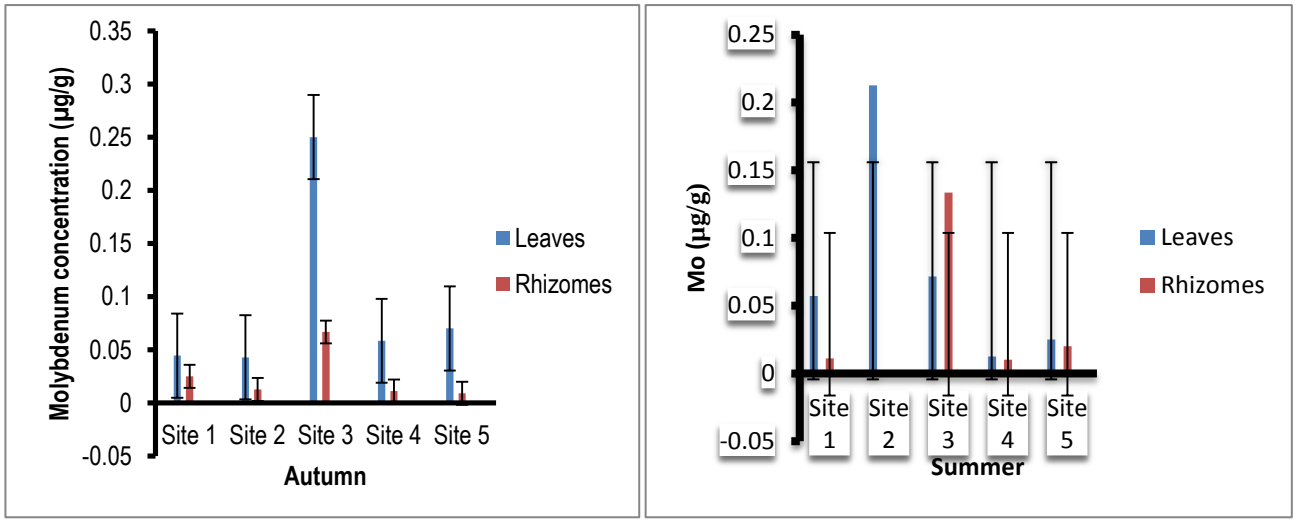


Figure 57: Differences in molybdenum accumulated in the leaves, rhizomes and roots

Pb is another metal that was accumulated by the plant roots in lower amounts. The highest and lowest average concentration for Pb in autumn in the leaves was 6.18 and 4.46 µg/g. In summer, the highest and lowest average concentration of Pb measured in the leaves was 4.77 and 4.50 µg/g. In the roots, the highest average concentration for Pb measured in autumn in the rhizomes of the sampled plants was 8.63 and 6.36 µg/g. In summer, the highest and lowest average concentration for Pb measured was 13.64 and 7.38 µg/g respectively. During the autumn and summer seasons, the average concentration of Pb measured in the roots was much higher than the amount of Pb measured in the leaves and rhizomes. In autumn, the highest and lowest concentration for Pb was 27.68 and 19.00 µg/g respectively.The highest and lowest average concentration for Pb measured in the roots of the plants in summer was 27.77 and 17.77 µg/g respectively.

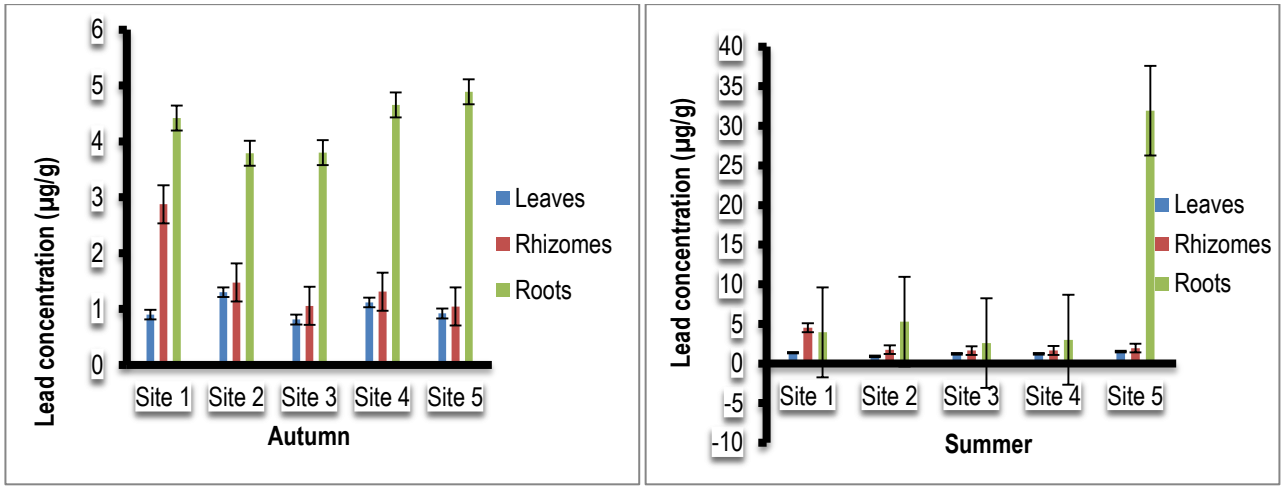


Figure 58: Differences in lead concentration accumulated in the leaves, rhizomes and roots

S is an element that was accumulated by the plant roots in higher amounts when compared to all the other elements analysed in the plants samples in both autumn and summer seasons. In autumn, the highest and lowest average concentration for S measured in the leaves of the plants was 34341.25 and 20128.14 µg/g. The highest and lowest average concentration for S measured in the leaves of the plants in summer was higher than the average concentration measured in autumn, and the highest and lowest average concentration measured in the leaves was 35772 and 24652.50 µg/g respectively.

The highest and lowest average concentration for rhizomes in autumn was 20697.67 and 10104.36 and the highest and lowest average concentration for S measured in the rhizomes of the plants in summer was 20233 and 16615.75 µg/g respectively. In the roots, the highest and lowest average concentration for S measured in autumn was 29246.67 and 20802.22 and in summer, the highest and lowest average concentration for S was 24464.44 and 19655.56 µg/g respectively (Figure 60).

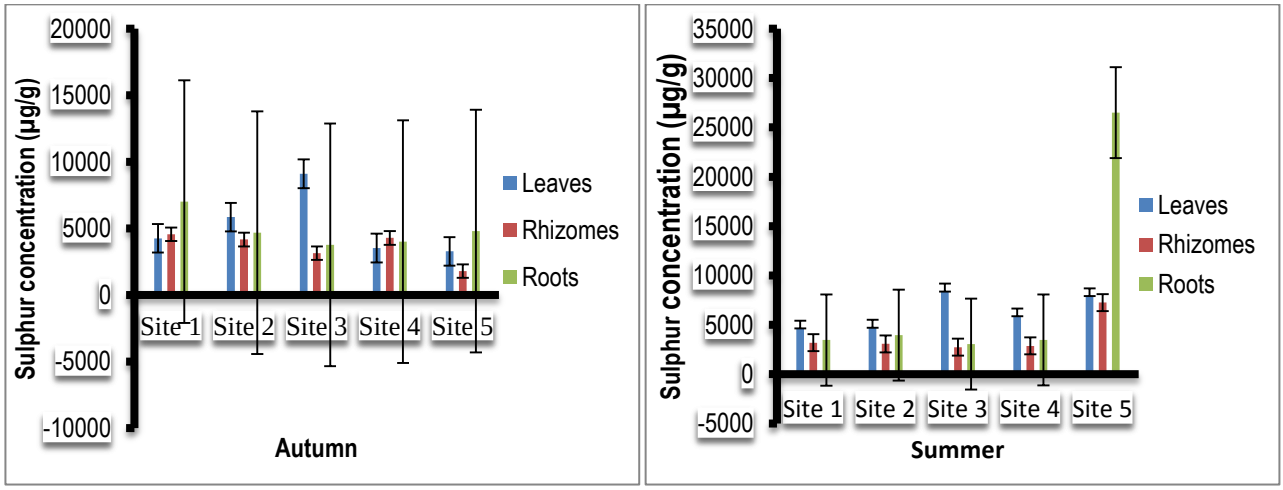


Figure 59: Differences in sulphur concentration accumulated in the leaves, rhizomes and roots

P is a non-metal accumulated by the plant leaves in higher amounts. The highest and lowest average concentration amounts of P in autumn was 3942.67 and 2650.14 µg/g respectively. In summer the highest and lowets average concentration for P measured in the leaves of the plants was 6223.70 and 3146.43 µg/g. In autumn, P was highly accumulated by the rhizomes of the plants. The average highest and lowest concentration for P was 9205.44 µg/g and 3070.09 µg/g respectively. In summer, the highest and lowest average concentration for P accumulated in the rhizomes of the plants was 5640.40 and 1554.80 µg/g.

P was found to have been accumulated by the plants roots in lower amounts in both autumn and summer seasons. In autumn, the highest and lowest average concentration for P measured in the roots of the plants was 2972.25 and 1749.43 µg/g respectively, whilst on the other hand, the highest and lowest concentration for P accumulated by the roots of the plants was 3537.56 and 2315 µg/g respectively.

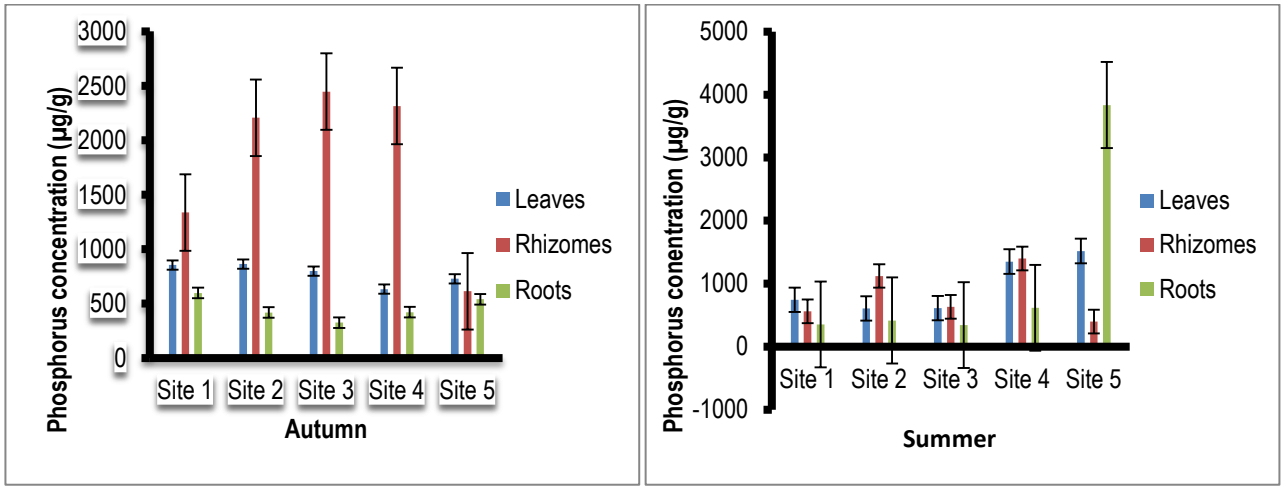


Figure 60: Differences in P concentration accumulated in the leaves, rhizomes and roots

4.7 Comparison of elements in water with the international organisations

Table 7 illustrate the current drinking water quality guidelines by international organisations, and for the basis of this study, the levels of elements in water were compared with the water quality guidelines to indicate whether the level of elements in water were either above or below the required or acceptable levels. The last column indicates the concentration levels of elements measured in water measured in the samples sites for this study.

Zinc concentration in water was within the acceptable range of 267 µg/l, and when compared with the international guidelines for water quality standards which were above 500 µg/l. Fe, Ni, Mn and Cu were found to have higher concentrations above the acceptable levels in water when compared to the international water quality guidelines. In the water samples, Fe, Ni, Mn and Cu were found to have been in higher amount than the acceptable levels of international water quality standards with the concentration of 2230, 282, 5900 and 14080 µg/l respectively. The units for the elements concentrated in water were illustrated in µg/l in this section to easily compare with international guidelines as the standards were expressed in µg/l rather than in mg/l.

Table 7: Current drinking water quality guidelines (µg/L-1) for elements in water in this study

Heavy metal	WHO[a]	USEPA[b]	ECE[c]	FTP-CDW[d]	PCRWR[e]	ADWG[f]	NOM-127[g]	This study
Zinc		500		5000	5000	3000	5000	267
Iron		300	200	300		300	300	2230
Nickel	70		20		20	20		282
Manganese	100	50	50	50	500	500	150	5900
Copper	2000	1300						14080

[a], World Health Organisation (WHO 2011); [b], United States Environmental Protection Agency (USEPA, 2011); [c], European Commission Environment (ECE, 1998); [d], Federal-Provincial-Territorial Committee on Drinking water (CDW), Health Canada (FTP-CDW, 2010); [e], Pakistan Council of Research in Water (PCRWR, 2008); [f], Australian Drinking Water Guidelines (DDWG, 2011); [g], Norma Official Mexicana NOM-127-SSA1-1994 (DOF, 1994).

4.8 Comparison of three macrophyte species in element accumulation

The three plants species *S. corymbosus, T. capensis and P. communis sampled in summer and autumn* were analysed in order to determine their levels of element accumulation. In all the three plant species, roots, rhizomes and leaves were analysed for the same number of metals and Table 8 illustrates the highest average concentration of all the elements accumulated by the plants.

Table 8: Summary plants organs with their elements concentrations accumulated by plant parts roots, rhizomes and leaves.

Element	Roots			Rhizomes			Leaves		
	S. corymbosus	*P. communis*	*T. capensis*	*S. corymbosus*	*P. communis*	*T. capensis*	*S. corymbosus*	*P. communis*	*T. capensis*
Mg (µg/g)	1.5745	0.9493	3.639	2.73	0.549	2.306	4.003	0.706	4.556
P (µg/g)	4292.5	1999	30.56	16720	2019.5	3583	7369.5	2240.5	6199.33
S (µg/g)	25436.67	27890	36720	28930	15170	13680.8	43563.33	31333.33	32692.5
Cr (µg/g)	181.267	164.15	131.867	86.867	140.55	64.6	31.8	108.67	36.4
Mn (µg/g)	4468.33	5412	4505.33	3362.5	3157	4005.5	4002.23	9437.1	17466.67
Fe (µg/g)	6.532	7.504	6.748	2.9195	3.3125	1.4659	0.69	1.74	0.67
Co (µg/g)	78.9	98.4	232.933	29.2	41.4	30.05	18.35	17.07	36.23
Ni (µg/g)	60.267	80.7	88.067	31.133	38	22.025	18.8	29.2	26.67
Cu (µg/g)	136.45	101.85	76.867	62.6	52.167	61.9	26	26.87	25
Zn (µg/g)	173.8	294.025	226.2	135	184.167	207.45	174	76.83	94.88
Mo (µg/g)	0	0	0	0	0	0.4	0.8	0.2	0.8
Pb (µg/g)	25.6	28.85	35	10.8	10.3	6.225	5.5	11.467	5.2

The highest mean average concentrations of the elements accumulated by the plants were compared among themselves to indicate the plants that accumulated most of the elements in their different parts. Out of the three macrophyte species selected for phytoremediation purposes, roots of *Typha species* were observed to have accumulated higher concentrations of elements. *Typha species* were also found to have accumulated most of the metals such as Mg, S, Co, Ni and Pb. The highest average mean concentrations of Mg, S, Co, Ni and Pb accumulated by the roots of *Typha species* was 3.64, 36720, 232.93, 88.06 and 35.00 µg/g.

The roots of the *Phragmites spp* were found to be the best accumulators of Mn, Fe and Zn. The highest average mean concentrations for Mn, Fe and Zn that were taken up by the *Phragmites spp* roots was 5412, 7.504 and 294.025 µg/g respectively (Table 8). *Schoenoplectus spp* was amongst the selected species for phytoremediation. The elements accumulated by the roots of *Schoenoplectus spp* include P, Cr, and Cu. The highest average mean concentrations for P, Cr and Cu accumulated by the *Schoenoplectus spp* was 4298.5, 181.267 and 136.45 µg/g respectively.

The rhizomes of the three macrophyte species were also analysed in order to quantify the concentration of elements accumulated. Amongst the three species studied, the *Schoenoplectus spp* was found to be the highest accumulator of most elements and translocate them in the rhizomes. The metals that were accumulated by *Schoenoplectus spp* in relatively higher concentrations than the other two species of *Phragmites* and *Typha* include Mg, P, S, Cu and Pb. The highest mean average concentration was of Mg, P, S, Cu and Pb accumulated by the rhizomes of *Schoenoplectus spp* was 12.73, 16720, 28930, 62.6 and 10.8µg/g respectively. The second macrophyte species that accumulated most of the elements into its rhizomes was the *Phragmites spp*. A total of four elements were highly accumulated and these include Cr, Fe, Co and Mo. The highest average mean concentration of 140.55 µg/g for Cr was accumulated by the rhizomes of the *Phragmites spp*. Fe was taken up in relatively higher concentration and approximately 3.3125 µg/g was accumulated. The highest average mean concentration for Co and Ni was 41.4 and 38.00 µg/g was also accumulated in the rhizomes of the *Phragmites spp*.

The rhizomes of *Typha spp* were found to have accumulated only three types of elements of Mn, Zn and Mo. The highest average mean concentration for Mn was 4005.5 µg/g. The highest average mean concentration of Zn was 207.45 µg/g, and in that sense, the rhizomes of *Typha spp* became the highest accumulator of Mo when compared to the other two plants species of *S. corymbosus* and *P. communis* which have accumulated lower amounts of Mo especially the *Schoenoplectus spp* which had no Mo accumulation and *Typha spp* on the other hand has accumulated only 0.4 µg/g of Mo into their rhizomes.

Based on the investigations and observations made during the study periods, it was found that the leaves of these macrophyte species did accumulate a reasonable amount of molybdenum when compared with the roots and rhizomes. The leaves of *Typha* and *Schoenoplectus spp* had accumulated the same amounts of Mo whilst the *Phragmites spp* had taken up Mo in lower amounts. In the leaves of *Typha spp,* four different types of elements namely Mg, Mn, Co and Mo have been accumulated and their average mean concentrations were found to be 4.556, 17466.67, 36.23 and 0.8 µg/g respectively (Table 8). *Phragmites spp* accumulated into their rhizomes, Cr, Fe, Ni, Cu

and Pb in various concentrations. The average mean concentration for Cr accumulated was 108.67 µg/g, Fe and Pb concentrations were the only two elements that were accumulated two times higher in the *Typha spp* than in the *Schoenoplectus* and *Phragmites spp*, and their average mean concentration was 1.74 and 11.467 µg/g respectively, when compared to 0.69 and 0.67 µg/g of Fe and Pb accumulated by the *Schoenoplectus* and *Phragmites spp* respectively.

Ni was found to be highly accumulated by the leaves of the *Phragmites spp* with an average mean concentration of approximately 29.2 µg/g as illustrated in Table 8. Amongst the selected elements accumulated by the three different plants species of *Schoenoplectus*, *Typha* and *Phragmites spp*, P, S and Zn were also selected to be investigated for their quantities that could be accumulated in different parts of the plants. The leaves of *Schoenoplectus spp* were found to have high elements content and their average mean concentration was approximately 7369.5, 43563.33 and 174 µg/g respectively, and in this case Zn concentrations were two times higher in *Schoenoplectus spp* than the other two macrophyte species of *T. capensis* and *P. communis*.

4.9. Bioconcentration of elements in the water, sediments and plant species

Since the amounts of elements enter the aquatic ecosystem after being washed from the mine dump, the elements (some of which are toxic) become accumulated in the water column, in the sediments and also uptaken by the plants which then pose some health threats when accumulated in higher amounts. According to Zayed (1998), Bioconcentration factor (BCF) is described as the measure of amount of the element accumulated in the plant from its surrounding environment that is in contact with. It can be obtained by dividing the trace element concentration in plant tissues harvested by the initial concentration of the element in the external nutrient solution. In this study, the amount of elements taken up by the plants leaves, rhizomes and roots was compared with the amount of elements in the sediments. This resulted into BCFs for the different types of plant organ (Table 9 and 10). The BCF for the shoots, rhizomes and leaves were calculated from the elements accumulated by the plants in both the summer and autumn seasons. Translocation factor (TF) on the other hand is defined as the ratio of element concentration in the root to shoot (Lorestani *et al*, 2011).

Table 9 and 10 below show the TF and BCF of quantified elements in the study. In both seasons, elements that were mostly taken with high BCF were S, Mg, Zn, Mn, P, Cu, Ni and Co and elements that were mostly taken with highest TF were P, Mg, S, Cr, Zn, Pb, Cu and Mn. In autumn, the plant organs that were found to have highest BCF were the leaves of *T. capensis* and *P. communis* as well as the roots of *S. corymbosus.* In summer, the highest BCF were obtained in the roots and rhizomes of *P. communis* and *S. corymbosus*. In summer, the plant species with the highest BCF and TF was *S. corymbosus,* and in autumn, *T. capensis* was the plant species with the highest BCF

while the *S. corymbosus* was the plant species with the highest TF. The TF is affected by season and the autumn season was found to have the highest TF than the summer season. In general, BCF is also affected by season as the highest BCF was found in summer and this is assumed to be influenced by the active growth of the plants in summer as most of the elements are used by the plants during processes such as photosynthesis which is active in green leaves compared to when the plant leaves start drying up and photosynthesis ceases and most element loss occurs, as the leaves become brittle.

The results of the TF and BCF indicate that the studied plants accumulate higher concentration of the selected elements in reasonable amounts except the amounts of Mo. The plants with high BCF are suitable for accumulating the levels of elements and can be used to decontaminate soils. Although the plants showed high BCF, they still do not meet the criteria of being the hyperaccumulators. The plants accumulated levels of elements such as Cu, Zn and Pb in reasonable amounts with higher BCF (92.40, 200.79 and 17.55 in summer and 30.65, 76.14 and 11.98 in autumn) but the concentration of these elements were not greater than 1000 mg/kg to be regarded as hyperaccumulators. They can be regarded as moderate accumulators (Sukumaran, 2013). These plants are suitable to be applied in contaminated soils for phytoremediation processes (Nazir *et al*., 2011).

Table 9: Summary of plants species of *P. communis* and *S. corymbosus* showing Translocation Factor (TF) and Bio concentration Factor (BCF) in summer.

Species name	Plant organ	Mg	P	S	Cr	Mn	Fe	Co	Ni	Cu	Zn	Mo	Pb
P. communis	Leaves (TF)	1.21	1.32	0.95	0.16	0.02	0.08	0.06	0.1	0.14	0.30	--	0.16
P. communis	Rhizomes (TF)	0.41	0.52	0.53	0.35	0.13	0.30	0.18	0.27	0.26	0.41	--	0.33
S. corymbosus	Leaves (TF)	2.16	1.67	1.65	0.15	0.42	0.12	0.13	0.22	0.24	0.48	--	0.32
S. corymbosus	Rhizomes (TF)	1.06	2.42	1.34	1.43	0.77	0.79	0.61	0.81	0.76	0.78	--	0.96
P. communis	Roots (BCF)	125.21	0.05	0.01	12.18	253.51	14.44	92.30	39.3	92.40	200.79	0	17.55
P. communis	Rhizomes (BCF)	51.25	59.93	128.08	4.23	32.98	4.35	16.63	10.5	24.28	82.80	0.24	5.77
P. communis	Leaves (BCF)	151.05	0.06	228.58	2.00	6.23	1.13	5.66	3.96	12.86	59.28	1.09	2.73
S. corymbosus	Roots (BCF)	129.18	95.55	166.59	12.44	108.98	10.79	55.14	19.6	48.14	135.55	0	9.23
S. corymbosus	Rhizomes (BCF)	0.05	231.14	223.10	12.44	84.45	8.55	33.40	15.9	36.34	106.27	1.03	8.84
S. corymbosus	Leaves (BCF)	278.52	0.11	274.72	1.29	45.33	1.29	7.05	4.27	11.716	65.58	2.67	2.92

Table 10: Summary of plant species of *P. communis, S. corymbosus* and *T. capensis* and sediments samples showing the translocation factor (TF) and bio-concentration Factor (BCF) in autumn

Autumn	Plant organ	Mg	P	S	Cr	Mn	Fe	Co	Ni	Cu	Zn	Mo	Pb
P. communis	Leaves (TF)	1.26	1.80	1.29	0.44	1.02	0.21	0.17	0.29	0.26	0.35	---	0.37
P. communis	Rhizomes (TF)	0.29	0.01	0.34	0.32	0.23	0.25	0.18	0.25	0.28	0.32	---	0.20
S. corymbosus	Leaves (TF)	2.26	1.37	1.68	0.09	2.13	0.13	0.28	0.35	0.35	0.97	---	0.30
S. corymbosus	Rhizomes (TF)	1.18	3.49	1.00	0.42	0.52	0.31	0.33	0.45	0.64	0.72	---	0.40
T. capensis	Leaves (TF)	1.55	1.93	0.78	0.07	4.16	0.08	0.15	0.24	0.36	0.49	----	0.16
T. capensis	Rhizomes (TF)	0.66	0.89	0.27	0.15	0.69	0.19	0.10	0.17	0.54	0.81	-----	0.16
P. communis	Roots (BCF)	58.93	45.53	135.54	10.09	32.57	9.88	32.51	15.76	30.65	76.14	0	10.64
P. communis	Rhizomes (BCF)	17.10	0.27	46.62	3.27	7.62	2.48	5.76	3.88	8.44	24.66	0.06	2.21
P. communis	Leaves (BCF)	74.53	81.89	175.34	4.42	33.29	2.09	5.67	287.8	8.10	26.64	1.94	3.98
S. corymbosus	Roots (BCF)	87.12	77.22	128.88	10.01	34.45	9.03	23.91	11.35	22.86	41.36	0	7.93
S. corymbosus	Rhizomes (BCF)	102.88	269.32	128.84	4.22	17.95	2.80	7.96	5.05	14.63	29.86	0	3.16

S. corymbosus	Leaves (BCF)	196.83	105.80	216.11	0.94	38.22	1.19	6.80	3.95	8.02	40.01	2.36	2.35
T. capensis	Roots (BCF)	161.51	105.79	185.30	7.90	36.30	9.18	57.18	16.21	19.94	46.75	0	11.98
T. capensis	Rhizomes (BCF)	106.05	60.95	50.71	1.21	25.19	1.75	5.88	2.79	10.68	38.02	0.73	1.96
T. capensis	Leaves (BCF)	250.78	131.58	145.35	0.53	151.10	0.71	8.35	3.86	7.10	22.91	1.03	1.78

Chapter 5. Conclusion and Recommendations

5.1 Conclusions

The results obtained in this study shows that selected macrophyte species of *S.corymbosus, T. capensis* and *P. communis* significantly contribute towards the accumulation of elements arising from acid mine drainage site. West Wits has extensive AMD sites, and constructed wetlands have been made with macrophyte species grown in them to uptake and translocate in them the available elements that are channelled into the constructed wetlands by means of seepage, percolation and runoff. pH results of the sediments varied from sample to sample.

The result indicated a decrease in the pH levels towards the bottom of the canal and it was observed that there was rainwater seeping sideways of the site which could be diluting the concentration of elements in the soil and lowering the pH levels of the water in the sites. The results of plants, sediments and water samples revealed varying concentration levels of the elements. The analysed plants have also shown that although there are many elements taken up by the plants, there is no single plants that uptake multiple elements and accumulate in the plant organs of leaves, rhizomes and roots.

The results also indicate that Mg, S, Fe and Co were higher in water that in sediments and plants. While Cr, Mo and Pb were found to have higher concentrations in sediments than there are in water and plant organs. On the other hand, P, Mn, Ni, Cu and Zn were in relatively higher concentrations in the plant organs that they are in the water and sediments.

When comparing the levels of element accumulated by the plant species, it was found that the levels accumulation varied and was influenced by the type of plant species and plant organ part, as well season. Some of the elements were highly accumulated by specific plant species in specific season, and others were accumulated in higher amounts in specific organs of the plants. The reason for this could be due to physiological functioning of the species.

In this study, the use of plants to decontaminate the polluted sites is the main concept and as a result, the plants used for this study were determined as whether they are the hyperaccumulators based on the levels of metals they have accumulated. According to Nazir *et al.,* (2011), the shoots of such plants should possess the hyperaccumulation level (Pb, Cu, Zn) > 1000 mg/kg. In another study conducted by Sukumaran (2013), a hyperaccumulator is described as a plant with a BCF > one. Based on the definition by Sukumaran (2013), all the

three macrophyte species assessed are hyperaccumulators of the selected elements investigated in the study although they have been accumulated at various levels. These macrophyte species can as well be used for decontaminating the polluted soils and they have the potential of being used for phytostabilisation and phytoextraction.

5.2 Recommendations

The results obtained from this investigation shows that the three macrophyte species of *Schoenoplectus, Phragmites* and *Typha spp* are effective in the phytoremediation process and can significantly contribute in the processes of phytoextraction and phytostabilisation. Their growth rates and fast spreading within the constructed wetlands make the uptake of metals to be increased and accumulate higher concentrations. In addition, species such as *Cyperus haspen* can also be grown in the constructed wetland to increase the metals uptake by the plants in decontaminating the AMD polluted soils. Aquatic and wetland plants that can withstand high metal toxicity, the pH of the water can be increased as more of the metals will be taken up by the plants instead of being released into the medium due to lower pH conditions.

Typha species should always be harvested before the winter season in order to avoid the release of metals back into soil and later the rhizomes. This is because the aboveground part of *Typha* dies off completely during the winter months when the temperature conditions drop. The shoots of *Phragmites spp* should also be harvested after the autumn months as the leaves will dry up during winter and release metals into the rhizomes and roots. Harvesting of the aboveground shoots of *Typha* and *Phragmites spp* will promote more plant canopy during the spring seasons when the new leaves come up and higher accumulation of elements. Harvesting of the shoots of these species can yield a considerable amount of metals which can later be extracted in the metallurgical plants, and this can be seen as a way to treat AMD through the use of macrophyte species and reduce the levels of elements in the soil as well as in the water.

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Appendix 1: Summary of correlation matrix of elements in water, sediments, leaves, rhizomes and rhizomes

CORRELATION MATRIX OF ELEMENTS IN WATER, SEDIMENTS, ROOTS, RHIZOMES AND LEAVES				
Variable	Correlating Variables	Valid N	Correlation Co-efficient	p value
Magnesium	Phosphorus	15	.7598	p=0.001
	Sulphur	15	.6406	p=0.010
	Chromium	15	-.6143	p=.015
	Manganese	15	.8714	p=.000
	Iron	15	-.6232	p=.013
	Cobalt	15	.8021	p=.000
	Nickel	15	.6704	p=.006
Phosphorus	Chromium	15	-.6488	p=.009
	Manganese	15	.7636	p=.001
	Iron	15	-.7973	p=.000
	Cobalt	15	.7841	p=.001
	Lead	15	-.6581	p=.008
Chromium	Manganese	15	-.5389	p=.038
	Iron	15	.8005	p=.000
	Lead	15	.8584	p=.000
Manganese	Iron	15	-.7044	p=.003
	Cobalt	15	.8173	p=.000
	Nickel	15	.6145	p=.015
	Lead	15	-.5644	p=.028
Iron	Cobalt	15	-.5775	p=.024
	Lead	15	.8350	p=.000
Cobalt	Nickel	15	.8303	p=.000
	Molybdenum	15	-.5262	p=.044
Nickel	Copper	15	.6279	p=.012
	EC	15	-.5563	p=.031
Sulphur	Copper	15	.6003	p=.018
	Zinc	15	.7279	p=.002
Zinc	Molybdenum	15	.5946	p=.019

	Total Nitrogen	15	.6285	p=.012
Molybdenum	Total Nitrogen	15	.6109	p=.016
pH	Redox	15	.5353	p=.040
	Nitrite	15	.5469	p=.035
	Ammonium	15	.5994	p=.018
DO	Ammonium	15	.6431	p=.010
Nitrite	Ammonium	15	-.5590	p=.030

Appendix 2: Summary of descriptive statistics of elements accumulated by the sediments

Position on site	Descriptive statistics of elements accumulated by the sediments								
	Fe (mg/l)	Mn (mg/l)	Cu (mg/l)	Zn (mg/l)	Ni (mg/l)	P (mg/l)	Mg (mg/l)	S (mg/l)	Co (mg/l)
P 1 WW1	0.015	1.719	1.12	0.013	0	0	190.7	686.9	326.2
P1 WW2	0.042	0.535	1.14	0.013	0	0	171.2	605.6	335.3
P1 WW3	0.036	0.799	1.67	0.018	0	0	182.4	823.8	243.3
P2 WW1	0.027	0.389	1.21	0.014	0.013	0	194.1	749.1	326.3
P2 WW2	0.045	0.336	0.88	0.011	0	0	182.9	703.6	313.8
P2 WW3	0.039	0.308	2.08	0.01	0	0	146.8	5839	292.0
P4 WW1	0.015	0.691	1.35	0.028	0	0	352.5	1316.4	276.7
P4 WW2	0.026	0.79	1.28	0.012	0.004	0	162.6	584.4	200.7
P4 WW3	0	0.268	1.54	0.065	0	0	326.9	1248.4	165.0
P6 WW1	0.035	1.8	1.80	0.009	0.031	0	369.6	1399.4	154.8
P6 WW2	0.086	1.383	1.89	0.025	0.017	0	196.8	783.6	138.8
P6 WW3	0.127	1.561	1.55	0.016	0.016	0	132.4	644.2	165.5
P7 WW1	0.01	2.3	2.19	0.033	0.044	0	198.2	736.4	121.9
P7 WW2	2.23	5.9	14.08	0.267	0.282	0	251.4	1100.4	315.9
P7 WW7	0.037	2.2	3.08	0.05	0.071	0	233	919	112.1

Appendix 3: Summary of descriptive statistics of elements accumulated in water

Variable	Descriptive statistics of elements in water n = 15							
	Mean	**Median**	**Min.**	**Max.**	**Range**	**Variance**	**Std. error**	**Std. dev**
Fe (mg/l)	0.185	0.036	0	2.23	2.23	0.321	0.146	0.567
Mn (mg/l)	1.399	0.799	0.268	5.90	5.632	2.043	0.369	1.429
Cu (mg/l)	2.457	1.548	0.881	14.076	13.196	10.629	0.842	3.260
Zn (mg/l)	0.039	0.016	0.009	0.267	0.258	0.004	0.017	0.065
Ni (mg/l)	0.032	0.004	0	0.282	0.282	0.005	0.019	0.072
P (mg/l)	0	0	0	0	0	0	0	0
Mg (mg/l)	219.433	194.10	132.40	369.60	237.20	5468.237	19.09	73.948
S (mg/l)	1209.347	783.60	584.40	5839	5254.60	1710541	337.69	1307.876
Co (mg/l)	232.553	243.251	112.11	335.327	223.217	7064.274	21.70	84.049

Appendix 4: Summary of descriptive statistics of elements accumulated by the leaves of *S. corymbosus*

Variable	Descriptive statistics of *S. corymbsus* leaves in summer

	Mean	median	Mode	Range	*Min.*	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	3.42	3.05	3.04	4.95	1.47	6.41	1.48	0.3	1.22
P (µg/g)	4953.31	4615.5	10800	8586	2214	10800	6214229	623.21	2492.84
S (µg/g)	35184.38	32710	45870	36250	22470	58720	1.05	2564.6	10258.38
Cr (µg/g)	13.65	7.5	0	46	0	46	238.59	3.86	15.45
Mn (µg/g)	3194.4375	3105	3212	3072	1882	4954	906061.6	237.97	951.87
Fe (µg/g)	0.54	0.47	0.26	1.02	0.2	1.21	0.09	0.07	0.3
Co (µg/g)	12.6625	12.25	12.6	16.1	6	22.1	15.85	0.995	3.98
Ni (µg/g)	14.78125	13.9	12.9	16.4	8.1	24.5	18.38	1.07	4.29
Cu (µg/g)	27.84375	26.3	21.4	20	21	41	37.66	1.53	6.14
Zn (µg/g)	158.5375	160.3	159.3	150.5	96.3	246.8	1253.16	8.84	35.4
Mo (µg/g)	0.275	0.15	0	1.7	0	1.7	0.2	0.11	0.45
Pb (µg/g)	5.075	4.8	4.5	6.1	2.3	8.4	3.47	0.47	1.86

Appendix 5: Summary of descriptive statistics of elements accumulated by the rhizomes of *S. corymbosus*

Variable	Descriptive statistics of *S. corymbosus* rhizomes in summer

	Mean	Median	Mode	Range	Min.	Max	Variance	Std.Error	Std.Dev
Mg (*µg/g)*	1.84	0.97	1.48	2.47	0.259	2.73	0.44	0.13	0.66
P (µg/g)	5033.5	4104	4363	12729	12729	4363	12729	711.01	5033.5
S (µg/g)	20432.29	20870	20870	20985	20985	20870	20985	1131.94	20432.29
Cr (µg/g)	97.33	98.7	178.5	170.6	170.6	178.5	170.6	11.14	97.33
Mn (µg/g)	4258.16	3249.5	13370	12576.2	12576.2	13370	12576.2	730.93	4258.16
Fe (µg/g)	2.62	2.5	4.66	3.58	3.58	4.66	3.58	0.21	2.62
Co (µg/g)	45.30	39.55	108.5	94.4	94.4	108.5	94.4	5.36	45.3
Ni (µg/g)	39.80	39.9	64.8	46.8	46.8	64.8	46.8	2.66	39.8
Cu (µg/g)	61.64	57.25	80.5	66.7	66.7	80.5	66.7	3.65	61.64
Zn (µg/g)	185.45	169.5	218.8	316.5	316.5	218.8	316.5	17.52	185.45
Mo (µg/g)	*0.071*	0	0	1.2	1.2	0	1.2	0.05	0.07
Pb (µg/g)	11.25	12.2	8.7	12.7	12.7	8.7	12.7	0.73	11.23

Appendix 6: Summary of descriptive statistics of elements accumulated by the roots of *S. corymbosus*

	Descriptive statistics of elements accumulated in the roots of *S. corymbosus* in summer								
Variable	Mean	median	Mode	Range	Min.	Max.	Variance	Std.error	Std.Dev
Mg (µg/g)	1.58	1.58	0	1.73	0.61	2.34	0.28	0.13	0.53

P (µg/g)	2937.53	2651	0	3912	1635	5547	1348130.51	281.61	1161.09
S (µg/g)	21452.35	20310	0	19910	15800	35710	25949581.6	1235.49	5094.07
Cr (µg/g)	95.83	86.6	0	235.5	43.7	279.2	2637.6	12.46	51.36
Mn (µg/g)	7757.88	6799	0	21882	1908	23790	36045216	1456.13	6003.77
Fe (µg/g)	4.67	4.83	0	7.72	2.31	10.03	3.77	0.47	1.94
Co (µg/g)	105.57	80	0	268.8	34.8	303.6	5924.9	18.67	76.97
Ni (µg/g)	72.24	57.9	0	92.9	27.1	120	1009.33	7.71	31.77
Cu (µg/g)	115.26	113.1	0	225.2	56.6	281.8	3248.3	13.82	56.99
Zn (µg/g)	333.98	319.4	0	517.4	144.4	661.8	15445.7	30.14	124.28
Mo (µg/g)	0	0	0	0	0	0	0	0	0
Pb (µg/g)	16.54	15.3	0	14.2	9.7	23.9	18.01	1.03	4.24

Appendix 7: Summary of descriptive statistics of elements accumulated by the leaves of *P. communis*

	Descriptive statistics of *P. communis* leaves in summer								
Variable	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.error	Std.Dev
Mg (µg/g)	1.5717	1.490	1.721	3.201	0.726	3.927	0.448	0.150	0.670
P (µg/g)	3988.2	3942	6199	4854	1687	6541	2288370	338.258	1512.736

S (µg/g)	25020.5	23965	16700	36740	10320	47060	68647573	1852.668	8285.383
Cr (µg/g)	18.795	0	0	103	0	103	1004.902	7.088	31.700
Mn (µg/g)	3767.6	3687	4077	6219	1124	7343	4153915	455.737	2038.116
Fe (µg/g)	0.418	0.252	0.252	1.427	0.103	1.53	0.163	0.090	0.403
Co (µg/g)	9.215	7.4	0	27.9	0	27.9	51.806	1.609	7.198
Ni (µg/g)	12.395	9.35	7.5	29	4.1	33.1	68.674	1.853	8.287
Cu (µg/g)	26.17	24.5	32.6	27.7	11.9	39.6	50.995	1.597	7.141
Zn (µg/g)	214.17	127.85	95.6	143.5	63.5	207	1398.684	8.357	37.372
Mo (µg/g)	0.09	0.1	0.1	0.4	0	0.4	0.010	0.023	0.102
Pb (µg/g)	4.165	3.1	3.1	8.4	1.4	9.8	6.046	0.550	2.459

Appendix 8: Descriptive statistics of elements accumulated by the rhizomes of *P. communis*

	Descriptive statistics of *P. communis* rhizomes in summer								
Variable	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.error	Std.Dev
Mg (µg/g)	1.5717	1.490	1.721	3.201	0.726	3.927	0.448	0.150	0.670
P (µg/g)	3988.2	3942	6199	4854	1687	6541	2288370	338.258	1512.736
S (µg/g)	25020.5	23965	16700	36740	10320	47060	68647573	1852.668	8285.383

Cr (µg/g)	18.795	0	0	103	0	103	1004.902	7.088	31.700
Mn (µg/g)	3767.6	3687	4077	6219	1124	7343	4153915	455.7365	2038.116
Fe (µg/g)	0.418	0.252	0.252	1.427	0.1026	1.53	0.163	0.090	0.403
Co (µg/g)	9.215	7.4	0	27.9	0	27.9	51.806	1.609	7.198
Ni (µg/g)	12.395	9.35	7.5	29	4.1	33.1	68.674	1.853	8.287
Cu (µg/g)	26.17	24.5	32.6	27.7	11.9	39.6	50.995	1.597	7.141
Zn (µg/g)	124.17	127.85	95.9	143.5	63.5	207	1396.684	8.357	37.372
Mo (µg/g)	0.09	0.1	0.1	0.4	0	0.4	0.014	0.023	0.102
Pb (µg/g)	4.165	3.1	3.1	8.4	1.4	9.8	6.046	0.550	2.459

Appendix 9: Summary of descriptive statistics of elements accumulated by the roots of *P. communis*

	Descriptive statistics of *P. communis* roots in summer								
Variable	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	1.133	1.168	1.224	1.361	0.578	1.939	0.093	0.064	0.305
P(µg/g)	2634.217	2709	2737	2262	1573	3835	261198.91	106.567	511.076
S (µg/g)	22843.91	23640	24500	15970	14500	30470	20127607	935.475	4486.38
Cr (µg/g)	99.457	88.10	73.70	132.20	44.30	176.50	1302.413	7.525	36.089

Mn (µg/g)	13338.30	8876	4285	36761	2629	39390	1.10	2152.03	10320.8
Fe (µg/g)	4.619	4.287	3.98	4.662	2.043	6.705	1.464	0.252	1.210
Co (µg/g)	130.630	121.10	62.70	248.70	48.70	297.40	4099.532	13.351	64.028
Ni (µg/g)	107.061	99.70	53.90	152.40	51.70	204.10	1592.699	8.322	39.909
Cu (µg/g)	163.552	161.80	101.10	220.50	79.00	299.50	3508.729	12.351	59.235
Zn (µg/g)	365.691	322.80	250.30	645.90	167.90	813.80	26437.27	33.903	162.595
Mo (µg/g)	0	0	0	0	0	0	0	0	0
Pb (µg/g)	23.248	1.759	24.10	33.10	14.40	47.50	71.125	1.759	8.434

Appendix 10: Summary of descriptive statistics of elements accumulated by the leaves of *S.corymbosus*

	Descriptive statistics of *S. corymbosus* leaves in autumn								
Variable	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	2.926	2.806	0.00	2.833	1.775	4.608	0.903	0.254	0.950
P (µg/g)	3949.571	3725	0.00	6271	1343	7614	3663002.30	511.511	1913.897
S (µg/g)	33792.86	31155	0.00	54740	18160	72900	1.63	3411.408	12764.32
Cr (µg/g)	12.60	9.55	0.00	48.3	0.00	48.30	234.335	4.091	15.308
Mn (µg/g)	3303.50	3115.50	0.00	3723	1129	4852	1401135.50	316.356	1183.696

Fe (µg/g)	0.627	0.573	0.00	0.8961	0.2749	1.171	0.082	0.076	0.285
Co (µg/g)	15.821	11.05	11.10	47.10	5.20	52.30	164.313	3.426	12.818
Ni (µg/g)	17.65	13.25	9.90	46.70	7.00	53.70	143.178	3.198	11.966
Cu (µg/g)	23.314	24.60	25.80	25.00	10.90	35.90	38.441	1.657	6.200
Zn (µg/g)	119.721	131.25	0.00	175.70	46.00	221.70	2169.703	12.449	46.580
Mo (µg/g)	0.279	0.10	0.00	1.50	0.00	1.50	0.194	0.118	0.441
Pb (µg/g)	5.114	4.80	5.20	6.00	3.20	9.20	2.327	0.408	1.526

Appendix 11: Summary of descriptive statistics of elements accumulated by the rhizomes of *S. corymbosus*

Variable	Descriptive statistics of elements accumulated by *S. corymbosus* rhizomes in autumn								
	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	1.946	1.884	2.311	3.092	0.748	3.84	0.664	0.246	0.815
P (µg/g)	12796.091	14010	20830	19532	2488	22020	51466644	2163.050	7174.026
S (µg/g)	25640.91	23740	38620	23970	14650	38620	66173049	2452.699	8134.682
Cr (µg/g)	72.009	68.90	90.90	133.00	20.80	153.8	1323.801	10.970	36.384
Mn (µg/g)	1975.20	1810	1510	2685.90	703.10	3389	908835.88	287.439	953.329
Fe (µg/g)	1.873	1.931	1.931	2.757	0.636	3.393	0.738	0.259	0.859

Co (µg/g)	23.545	21.40	9.80	31.00	9.80	40.80	114.953	3.233	10.722
Ni (µg/g)	28.745	26.20	41.90	25.80	16.10	41.90	88.119	2.830	9.387
Cu (µg/g)	54.127	58.70	60.60	25.60	40.40	66.00	77.244	2.650	8.789
Zn (µg/g)	113.709	126.4	154	99.6	54.40	154	1258.903	10.698	35.481
Mo (µg/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pb (µg/g)	8.764	8.40	11.80	7.70	4.10	4.10	6.593	0.774	2.568

Appendix 12: Summary of descriptive statistics of elements accumulated by *S. corymbosus* in roots

	Descriptive statistics of elements accumulated by *S. corymbosus* roots in autumn								
Variable	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	1.394	1.32	0	1.57	0.79	2.36	0.19	0.12	0.44
P (µg/g)	3104.46	3054	0	2821	1507	4328	858681.44	257.01	929.65
S (µg/g)	21703.08	20720	0	22450	12030	34480	48753106	1936.55	6982.34
Cr (µg/g)	144.6	150.6	0	158.8	69.3	228.1	2185.03	12.96	46.74
Mn (µg/g)	3207.154	3021	0	4336	1175	5511	2646454.5	451.19	1626.79
Fe (µg/g)	5.11	4.539	0	6.887	2.9	9.786	4.25	0.57	2.06

Co (µg/g)	59.88	64.2	0	81.1	27.1	108.2	624.72	6.93	24.99
Ni (µg/g)	54.65	52.2	0	41.2	39	80.2	168.39	3.6	12.98
Cu (µg/g)	71.59	60.6	0	143.3	36	179.3	1520.5	10.81	38.99
Zn (µg/g)	133.28	149	0	125.5	69.4	194.9	1302.43	10.01	36.09
Mo (µg/g)	0	0	0	0	0	0	0	0	0
Pb (µg/g)	18.59	15.8	0	18.4	12.8	31.2	18.4	1.7	6.12

Appendix 13: Summary of descriptive statistics of elements accumulated by the leaves of *P. communis*

Variable	Descriptive statistics of elements accumulated by *P. communis* leaves in autumn								
	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	0.816	0.609	0.00	4.02	0.375	4.395	0.781	0.20	0.88
P (µg/g)	2252.474	2183.00	0.00	5147.00	1247.00	6394.00	1322693.90	263.85	1150.08
S (µg/g)	20203.11	15340.00	0.00	47671.00	9499.00	57170.00	1.28	2593.57	11305.10
Cr (µg/g)	43.65	26.10	0.00	151.90	0.00	151.90	1801.89	9.74	42.45
Mn (µg/g)	2120.53	1272.00	0.00	12251.10	578.90	12830.00	7692653.70	636.30	2773.56
Fe (µg/g)	0.809	0.77	0.00	1.98	0.2662	2.245	0.25	0.12	0.50
Co (µg/g)	9.711	8.10	3.40	26.90	0.00	26.90	57.60	1.74	7.59
Ni (µg/g)	15.15	12.30	9.80	32.20	4.90	37.10	69.06	1.91	8.31

Cu (µg/g)	17.35	15.50	23.10	20.90	8.50	29.40	42.86	1.50	6.55
Zn (µg/g)	58.74	62.30	26.50	68.00	26.50	94.50	416.69	4.68	20.41
Mo (µg/g)	0.17	0.10	0.10	2.00	0.00	2.00	0.20	0.10	0.45
Pb (µg/g)	6.38	5.50	3.50	10.80	2.60	13.40	9.13	0.69	3.02

Appendix 14: Summary of descriptive statistics of elements accumulated by the rhizomes of *P. communis*

Variable	Descriptive statistics of elements accumulated by *P. communis* rhizomes in autumn								
	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	0.44	0.044	0.00	0.55	0.17	0.725	0.05	0.08	0.21
P (µg/g)	1738.38	1680.50	0.00	1378.00	974.00	2352.00	180036.84	150.02	424.31
S (µg/g)	127580.00	13290.00	0.00	13060.00	5270.00	18330.00	17875478	1494.8	4227.94
Cr (µg/g)	76.76	68.65	0.00	195.30	6.50	201.8.	3067.56	19.58	55.39
Mn (µg/g)	1152.41	1007.40	0.00	1685.60	441.4	2127.00	354661.62	210.55	595.53
Fe (µg/g)	2.82	2.21	0.00	3.03	10.20	4.05	0.94	0.34	0.97
Co (µg/g)	23.43	24.65	0.00	29.00	7.00	36.00	92.06	3.39	9.59
Ni (µg/g)	30.38	33.45	0.00	27.90	12.20	40.1	77.60	3.11	8.81
Cu (µg/g)	42.94	46.55	0.00	42.30	18.4	60.7	180.24	4.75	13.43

Zn (µg/g)	129.15	116.35	0.00	232.8	47.7	280.50	5395.06	25.97	73.45
Mo (µg/g)	0.01	0.00	0.00	0.10	0.00	0.10	0.00	0.01	0.04
Pb (µg/g)	8.40	9.00	0.00	6.70	5.10	11.80	4.45	0.75	2.11

Appendix 15: Summary of descriptive statistics of elements accumulated by the roots of *P. communis*

	Descriptive statistics of elements accumulated by *P. communis* roots								
Variable	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.Error	Std.Dev.
Mg (µg/g)	0.88	0.87	0	0.96	0.43	1.39	0.11	0.08	0.33
P (µg/g)	1699.86	1672.5	0	1268	1048	2346	156920.90	105.87	396.13
S (µg/g)	21195	21810	0	22080	11810	33890	34556796	1571.10	5878.50
Cr (µg/g)	135.36	134.15	0	166.40	59.20	225.60	1507.88	10.38	38.83
Mn (µg/g)	2815.47	2257	0	10406.40	993.60	11400	6789485.90	696.39	2605.66
Fe (µg/g)	5.19	5.15	0	3.56	3.94	7.50	0.73	0.23	0.85
Co (µg/g)	75.59	70.75	0	117.50	27.10	144.6	1288.76	9.59	35.90
Ni (µg/g)	70.5	68.5	0	80.6	43.7	124.3	378.2	5.2	19.45
Cu (µg/g)	89.11	94.05	0	111.4	40	151.4	872.53	7.89	29.54
Zn (µg/g)	227.83	177.1	0	413.4	81.9	495.4	19077.91	36.91	138.12
Mo (µg/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Pb (µg/g)	23.16	22.85	21.10	15.70	14.30	30.00	32.70	1.53	5.72

Appendix 16: Summary of descriptive statistics of elements accumulated by *T. capensis* leaves in autumn

Variable	Descriptive statistics of elements accumulated by *T. capensis* leaves in autumn								
	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	4.01	3.74	0.00	4.33	2.28	6.61	2.10	0.40	1.45
P (µg/g)	5290.08	5505	0.00	5490	2206	7696	2436189.20	432.90	1560.83
S (µg/g)	24476.69	24950	0.00	23753	96.70	42360	84266657	2545.99	9179.69
Cr (µg/g)	7.72	0.00	0.00	64.80	0.00	64.80	336.18	5.09	18.34
Mn (µg/g)	14065.54	11850	0.00	13570	7660	21230	31373123	1553.49	5601.17
Fe (µg/g)	0.400	0.26	0.00	1.40	0.13	1.53	0.14	0.10	0.37
Co (µg/g)	20.92	19.3	0.00	41.30	5.20	46.50	115.44	2.98	10.74
Ni (µg/g)	18.6	16.4	13.60	21.70	7.50	29.20	40.20	1.76	6.34
Cu (µg/g)	22.23	23.2	23.80	24.9	12.40	37.30	53.83	2.03	7.34
Zn (µg/g)	73.81	66.70	0.00	83.60	46.20	129.80	525.31	6.36	22.92

Mo (µg/g)	0.13	0.00	0.00	1.00	0.00	1.00	0.11	0.09	0.33
Pb (µg/g)	4.17	3.80	3.30	6.90	2.20	9.10	3.40	0.51	1.84

Appendix 17: Summary of descriptive statistics of elements accumulated by the rhizomes of *T.capensis*

Variable	Descriptive statistics of elements accumulated by *T. capensis* rhizomes in autumn							
	Mean	Median	Range	Min.	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	2.00	1.87	2.53	0.96	3.49	0.71	0.25	0.84
P (µg/g)	289.09	2522	4024	1197	5221	2049596	431.66	1431.64
S (µg/g)	10092.55	8751	14537	5173	19710	24616527	1495.95	4961.5
Cr (µg/g)	20.66	16.50	64.60	0.00	64.60	605.41	7.42	24.61
Mn (µg/g)	2770.73	2318	6065	1282	7347	3019121	523.89	1737.56
Fe (µg/g)	1.17	1.07	2.18	0.33	2.51	0.56	0.23	0.75
Co (µg/g)	17.40	9.40	67.5	3.40	70.90	370.97	5.81	19.26
Ni (µg/g)	15.89	15.7	29.2	6.00	35.20	89.38	2.85	9.45
Cu (µg/g)	39.51	30.5	59.5	20.4	79.90	416.41	6.15	20.40
Zn (µg/g)	144.79	122	240.5	65.7	306.20	6045.75	23.44	77.75

Mo (µg/g)	0.11	0.10	0.60	0.00	0.60	0.03	0.05	0.18
Pb (µg/g)	5.44	5.50	7.50	1.80	9.30	6.20	0.75	2.49

Appendix 18: Summary of descriptive statistics of elements accumulated by *T.capensis* roots in autumn

Variable	Descriptive statistics of elements accumulated by *T.capensis* in autumn								
	Mean	Median	Mode	Range	Min.	Max.	Variance	Std.Error	Std.Dev
Mg (µg/g)	2.59	2.62	2.78	3.11	1.14	4.25	0.69	0.23	0.83
P (µg/g)	2742	2700	0	2149	1520	3669	296859	151.00	544.85
S (µg/g)	31203.85	29120	0	22630	19530	42160	47552276	1912.56	6895.82
Cr (µg/g)	114.17	108.10	0	92.90	67.70	160.60	919.99	8.41	30.33
Mn (µg/g)	3378.85	2906	0	3519	1951	5470	1584464.10	349.12	1258.76
Fe (µg/g)	5.20	4.83	0	6.89	2.51	9.40	4.17	0.57	2.04
Co (µg/g)	143.17	130.6	0.00	288.30	54.50	342.80	6081.94	21.63	77.99
Ni (µg/g)	78.05	73.50	0.00	38.00	59.40	97.40	134.09	3.21	11.58
Cu (µg/g)	62.45	61.1	0.00	60.30	31.00	91.30	331.56	5.05	18.21
Zn (µg/g)	150.65	152.4	0.00	231.40	77.00	308.40	3451.31	16.29	58.75
Mo (µg/g)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pb (µg/g)	28.08	26.50	0.00	34.10	12.40	46.50	87.91	2.60	9.38

Appendix 19: Summary of descriptive statistics of elements accumulated by sediments

Variable	Descriptive statistics of elements accumulated by the sediments n = 15							
	Mean	**Median**	**Min.**	**Max.**	**Range**	**Variance**	**Std. Error**	**Std.Dev**
Mg (µg/g)	0.19	0.20	0.04	0.32	0.28	0.01	0.22	0.09
P (µg/g)	487.00	526.33	145.37	649.00	503.63	17973.47	34.62	134.07
S (µg/g)	1891.46	1813.67	519.67	3927	3407.33	629351.40	204.83	793.32
Cr (µg/g)	192.03	167.25	97.13	282.67	185.53	3450.42	15.17	58.74
Mn (µg/g)	1081.76	873.23	222.17	2807	2584.83	628712.20	204.73	792.91
Fe (µg/g)	7.26	7.01	3.16	11.59	8.43	3.48	0.48	7.86
Co (µg/g)	29.51	27.47	9.53	51.83	42.30	112.41	2.74	10.60
Ni (µg/g)	59.23	60.33	20.67	75.67	55.00	175.61	3.42	13.25
Cu (µg/g)	38.37	39.43	16	46.1	30.10	50.44	1.83	7.10
Zn (µg/g)	39.11	40.23	10.5	50.27	39.77	94.29	2.51	9.71
Mo (µg/g)	1.73	1.60	0.00	3.50	3.50	1.38	0.30	1.17
Pb (µg/g)	29.20	29.97	8.53	41.30	32.77	55.71	1.93	7.46

Appendix 20: Summary of descriptive statistics of raw data for all elements in all substrate samples

Position on site	Mg (µg/g)	P (µg/g)	S (µg/g)	Cr (µg/g)	Mn (µg/g)	Fe (µg/g)	Co (µg/g)	Ni (µg/g)	Cu (µg/g)	Zn (µg/g)	Mo (µg/g)	Pb (µg/g)
P 1 WW1 0-2 cm	0.445	642.00	5030.00	<5.1	2541	4.677	58.7	80.8	46.4	61.1	<0.3	29.80
P 1WW1 2-10 cm	0.38	514.5	3227	<5.1	841.5	5.049	25.8	72.4	46.4	52.4	<0.3	27.60
P 1 WW1 10-30 cm	0.129	436.1	1559	291.4	666.5	9.491	28.6	62	48	31.5	3.2	25.60
P 1 WW2 0-2 cm	0.296	434.1	1963	143.5	565.8	6.546	21.9	67.6	37.2	42	<0.4	25.40
P 1 WW2 2-10 cm	0.153	386.1	1402	191	742.3	8.388	29.2	67	48.3	38.6	2.3	27.60
P 1 WW2 10-30 cm	0.22	318.4	1225	162.3	505.6	7.968	27	63.5	42.4	37.1	<0.4	24.20
P 1 WW3 0-2 cm	0.378	617	4282	119.5	4168	6.058	80.8	89.4	48.3	55.5	<0.3	31.40
P 1 WW3 2-10 cm	0.241	328.5	1039	144.1	258.5	7.008	18.4	74.1	42.9	47.3	<0.4	25.80
P 2 WW1 0-2 cm	0.371	390.9	1838	110.6	1743	6.074	31.7	65.3	35.5	44.2	<0.4	26.60
P 2 WW1 2-10 cm	0.213	291.4	445.7	269.2	319.3	7.429	18.2	70.1	43.5	34.9	<0.4	24.10
P 2 WW1 10-30 cm	0.32	291.5	1236	149.7	557.4	6.02	19.1	65.7	39.3	39.8	<0.4	24.40
P 2 WW2 0-2 cm	0.444	950	3044	109.8	2493	5.629	40.7	68.3	40.9	49.9	<0.3	28.50
P 2 WW2 2-10 cm	0.265	300.1	1050	135.5	1093	6.716	24.9	56.5	34.8	38.5	2.5	28.00
P 2 WW2 10-30 cm	0.253	209.7	1018	139	92.7	5.995	13.8	53.8	34	36.6	<0.4	24.60
P 2 WW3 0-2 cm	0.202	423.1	1226	130	1292	6.882	30.8	62.3	39	43.7	2.2	32.10
P 2 WW3 2-10 cm	0.21	357.5	1222	163.1	832.7	6.854	25.5	65.5	40.5	42.9	2.2	39.00
P 2 WW3 10-30 cm	0.196	417.2	1067	204.8	519.4	10.89	28.6	78.5	44.7	45.1	3	31.20
P 4 WW1 0-2 cm	0.288	498	1968	133.1	4097	7.321	56.4	68.7	38.4	44.1	2.1	26.50
P 4 WW1 2-10cm	0.159	523	1108	220.3	2337	8.286	42.3	64.2	35	29.5	2.3	23.90
P 4 WW1 10-30 cm	0.161	558	607.7	463	359.1	9.62	<7.6	40.7	24.2	26.2	3.2	24.10

P 4 WW2 0-2cm	0.221	777	2775	265.4	4011	9.95	50.2	72.1	42.3	36.1	3.3	40.10
P 4 WW2 2-10cm	0.153	537	1516	205.7	1543	7.847	33.5	62.6	38.6	32.8	2.8	33.80
P 4 WW2 10-30 cm	0.122	503	1150	215.3	2867	8.793	45.8	77	41.8	33.9	3.2	41.50
P 4 WW3 0-2cm	0.308	708	2069	122.7	3279	5.747	41.2	60.5	35.4	44.8	2.3	26.10
P 4 WW3 2-10 cm	0.203	638	1793	151	1069	7.06	26.7	57.4	40.5	38	<0.4	29.70
P 4 WW3 10-30cm	0.153	601	1654	176.2	754.4	7.978	28.2	63.1	39	37.9	2.5	34.10
P 6 WW1 0-2cm	0.19	531	2323	230.9	302.3	6.279	31.7	57.4	45.8	62.7	2.4	30.10
P 6 WW1 2-10cm	0.18	532	2085	189.9	371	7.23	22.8	54.2	39.4	44.8	<0.4	32.80
P 6 WW1 10-30cm	0.158	602	1901	386	530	9.779	27.9	66.4	44.4	37	2.8	43.10
P 6 WW2 0-2cm	0.194	534.1	3483	149.1	375.3	6.417	25.1	55.2	39.9	58.5	1.5	29.70
P 6 WW2 2-10 cm	0.193	343.7	2985	135.7	185.7	5.486	14.7	56.1	36.1	53.1	<0.3	27.20
P 6 WW2 10-30cm	0.184	441	1856	202.8	295.2	7.124	19.6	55	40.1	38.3	2.4	33.60
P 6 WW3 0-2cm	0.264	734	3821	145.4	421.7	4.824	32.2	58.3	41.2	68.9	1.3	29.20
P 6 WW3 2-10cm	0.264	601	5208	132.7	290.4	4.996	15.3	43.5	32.2	40.8	<0.3	27.40
P 6 WW3 10-30 cm	0.192	528.6	2752	183.9	243.9	5.878	13.7	48.9	39	41.1	<0.4	28.70
P 7 WW1 0-2cm	0.199	677	3411	214	1042	8.327	47.4	66.6	51.9	47.4	3.1	35.60
P 7 WW1 2-10cm	0.114	481	1760	232.7	280.4	8.298	30	62	35	28.6	3	27.70
P 7 WW1 10-30 cm	0.099	485	1899	254.5	290.4	8.067	29.7	58.8	36.3	29.7	2.9	29.50
P 7 WW2 0-2 cm	0.116	545	2870	258.1	849	9.208	38.5	54.4	45.3	39.9	2.9	34.20
P 7 WW2 2-10cm	<0.054	663	1809	336.3	3545	13.86	75.4	76.9	49.4	38.3	5.1	50.60
P 7 WW2 10-30 cm	<0.052	514	1809	253.6	989.9	11.71	41.6	71	43.6	31.6	2.5	39.10
P 7 WW3 0-2 cm	0.13	700	2272	277	828.2	6.82	25.3	43.8	36.3	43	2.7	28.50
P 7 WW3 2-10 cm	0.131	560.9	1785	198.1	247.2	5.213	19.1	39	34	38.9	3.6	28.50
P 7 WW3 10-30 cm	<0.04	537	1147	277.2	771.9	8.967	28.9	54.7	39.4	26.3	3.2	33.80