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Title

Spatial assessment of environmental fate of Acid Mine Drainage (AMD) contaminants in engineered wetlands along the Varkenslaagte canal

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Declaration

Spatial assessment of environmental fate of acid mine drainage (AMD) contaminants in engineered wetlands along the Varkenslaagte canal has not been submitted for a degree or examination at any other university and that all the sources I have used and quoted have been acknowledged by complete references.

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Abstract

A major cause of environmental problems, in the vicinity of mine tailings in and around Johannesburg, is Acid Mine Drainage (AMD). In most research, engineered wetlands are used to ameliorate AMD with the use of vegetation to remove or extract heavy metals from the soil (i.e. phytoremediation). Phytoremediation has been defined as a technology that uses plants to extract or immobilize contaminants in soils and waters (Torresdey, 2007). The aim of this study was to assess and quantify the mass pool size of contaminants (macronutrients, micronutrients, non-essential trace elements) within and between a subset of paddocks from various compartments including sediments, aboveground biomass (shoots –stems and leaves), and belowground biomass (roots and rhizomes) of the two wetland plant species present (*P. australis* and *S. corymbosus*).

Analyses were done on the wetland paddocks *in situ* and *ex situ* applying different methods, water sample metal cations were analysed by ICP-MS and the major anion analysis by chromatography and Ion Chromatography (IC). The sediment and plant samples were subject to X-Ray fluorescence (XRF) analyses of major elements and trace elements. Although analysis was undertaken for numerous trace and metal elements, only a few macronutrients, micronutrients, and non-essential elements with significant importance to the West Wits Mining Operation were selected for this study. The stream water test strips yielded poor results for this extremely contaminated plume receiving environment this suggests that in this system they are not a useful substitute for conventional laboratory analyses. Of the elements tested, only S showed significant differences in concentrations in plants between paddocks, with the highest concentrations and mass in the downstream paddocks ww6 and ww7. These paddocks also had the greatest masses of S in sediments, and water concentrations were also highest in paddocks ww4, ww6 and ww7.

P. australis accumulated highest elemental mass than *S. corymbosus,* with the highest Zn mass of 93%. *P. australis* accumulated double the mass of U, Cu, Cl, Ca. In both plants, the roots consistently had highest elemental concentration with sequence often as follows roots> shoots> rhizomes.

Sediment element mass accumulation of most tested elements significantly increased with depth, except for Zn and U, which decreased with depth. There are few significant differences in the mass distribution of the elements analysed between paddocks, which is assumed to reflect either the heterogeneity in the underlying sediments following construction of the wetlands, or lateral inputs into the system as seepage from other TSFs.

Key words*:* AMD, Wetland, Varkenslaagte Canal, West Wits Mining Operation, metals, sediment, *S. corymbosus*, *P. australis*, ICP-MS, XRF.

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Acronyms

AGA	AngloGold Ashanti Limited
Al	Aluminium
As	Arsenic
Au	Gold
AMD	Acid Mine Drainage
C	Carbon
Cd	Cadmium
Cr	Chromium
Cl	Calcium
DWS	Department of Water and Sanitation
EC	Electrical Conductivity
Eh	Redox Potential
EMPr	Environmental Management Programme Report
Fe	Iron
GIS	Geographic Information System
GPS	Global Positioning System
Hg	Mercury
IC	Ion Chromatography
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
Km	Kilometres
K	Potassium
L	Litre
m	Metre
Mg	Magnesium
mm	Millimetre
MRD	Mine Residue Deposit

N	Nitrogen
Na	Sodium
NEMA	National Environmental Management Act
Ni	Nickel
P	Phosphorus
Pb	Lead
RS	Remote Sensing
V	Vanadium
S	Sulphur
SA	South Africa
USEPA	United States Environmental Protection Agency
T	Temperature
TSF	Tailings Storage Facility
U	Uranium
WHO	Word Healthcare Organization
WRG	West Rand Group
WW	West Wits
XRF	X-ray Fluorescence
Zn	Zinc

1 CHAPTER ONE

1.1 Introduction

Johannesburg is the largest city in Gauteng, the smallest province and the financial center of South Africa's economy. It owes its origin to the gold bearing rocks of the Witwatersrand Basin (Tutu *et al.,* 2008). The basin is situated in a northeast to south west direction and hosts seven major goldfields. The basin is home to one of the deepest mining operations in the world, known as TauTona Mine at approximately 3.9km deep (Coetzee *et al.,* 2006; AGA, 2009). Gold mining started in the basin in 1886 with Au being extracted from coarsely crushed ore using Hg amalgam and tailing were deposited in large dams (Tutu *et al.,* 2008). Since 1886 the extraction of gold resulted in significant mining operations in the Witwatersrand producing vast amounts of waste which were deposited in tailing storage facilities.

As proclaimed and in terms of section 21 of the Constitution of the Republic of South Africa everyone has the right to an environment that is not harmful to their health or well-being, and have their environment protected, for the benefit of present and future generations. This can be achieved by enforcing compliance with reasonable legislative and other measures that prevent pollution and ecological degradation, promote conservation and secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development.

Residual sulphide minerals, especially pyrite, in the tailings dumps are unstable when exposed to atmospheric oxygen and undergo oxidation resulting in the generation of acid mine drainage (AMD) and the subsequent release of heavy metals and metalloids (Jambor and Blowes, 1998). AMD is not a new phenomenon in South Africa. It has been considered one of the industries toughest problems to solve (Sracek *et al.,* 2010, Smith, 1997). AMD is a major cause of environmental problems (Akcil and Koldas, 2006; Sheoran and Sheoran, 2006).

Water characterized by AMD contamination may have an extremely low pH and elevated concentration of iron, sulfide and toxic trace metals (Sheoran and Sheoran, 2006). AMD is also characterised by high salinity (or high electrical conductivity), toxic heavy metals and in some cases radionuclides (Navarro *et al.,* 2008; Galvan *et al.,* 2012). AMD consequently has adverse negative impacts on groundwater and surface water, affects the

geochemistry of wetland sediments and is very harmful to aquatic species (Sheoran and Sheoran, 2006).

Water quality is affected mainly by mine tailings and spillages, especially from active slimes dams, reprocessed tailings, as well as from footprints left behind after reprocessing (Tutu, 2012). AMD does not only affect water, it may also degrade soil quality and allows heavy metal seepage into the environment thus leading to the complete degradation of fluvial ecosystems and their surrounding environments (Galvan *et al*., 2012).

The Central Rand goldfield of the Witwatersrand Basin, South Africa has been one of the most important gold mining areas in the world. It is, however, replete with gold mine tailings, which have contributed significantly to water pollution as a result of AMD (Tutu, 2012). AngloGold Ashanti Ltd Pty in partnership with the University of the Witwatersrand (Johannesburg, South Africa), School of Animal, Plant and Environmental Sciences, Ecological Engineering and Phytotechnology Programme, like many other South African mining companies, is in the progress of rehabilitating the historically impacted land (Weiersbye *et al.*, 2009).

The study area is situated about 75 km West of Johannesburg and about 8 km south of Carletonville, Johannesburg, South Africa at the West Wits AngloGold Ashanti Gold Mining property (Figure 1), refer to section 1.2 for site description.

During the 1980s tailing spills occurred in one of the slimes dams of the West Wits operation Varkenslaagte drainage area, resulting in vast amounts of waste pollution (I. Weiersbye, personal communication, 17 July 2013). Mining effluents (i.e. AMD) are one of the major causes of environmental problems globally which consequently has adverse effects on groundwater and surface water. The water from North Savuka complex tailing storage facilities (TSF) drains into the west boundary dam which flows into the Varkenslaagte Stream, which historical drained into the Wonderfontein Spruit while some of the water infiltrates into Dewatered Turffontein Dolomite outside the mines (Weiersbye *et al*., 2009). The Varkenslaagte drainage area is suspected to be extremely polluted, since the historically removed tailing spills or tailing footprints caused major pollution in the Varkenslaagte Wetland. In terms of Section 28 of the National Environmental Management Act (NEMA) (“duty of care”), it was decided that further post-clean-up (i.e. rehabilitated condition) soil surveys/sampling (fieldwork) would be conducted in the same area of the Varkenslaagte Wetland in October 2014.

Generally, in South Africa, the right to an environment not harmful to a person's health and wellbeing, and the right to the protection of the environment are explicitly documented in the Bill of rights (s24 of the Constitution of the Republic of South Africa 108 of 1996). Thus there is a need to understand the extent and types of contamination, and the potential risks associated with different land use activities not only those associated with mining activities but also industrial and municipal.

AngloGold in partnership with the Ecological Engineering and Phytoremediation Programme at University of the Witwatersrand has embarked on a journey to rehabilitate the historically impacted land in the Varkenslaagte Wetland associated with tailing spills. Today approximately 76,000m^3 of tailing spills has been removed from the Varkenslaagte Wetland as part of the rehabilitation of the Wetland (I. Weiersbye, personal communication, 17 July 2013) and over 40ha of indigenous trees have been planted as part of the rehabilitation programme (Dye and Weiersbye, 2010).

The deteriorating quality of water in rivers presents a major challenge to South Africa. Mining activities generate acid wastewater or AMD waste that is delivered into wetlands and rivers, transported by groundwater and surface water i.e. tributaries of catchments. Environmental problems such as water quality deterioration including acidification, eutrophication, increased salinity, increased turbidity and toxicity, present a serious threat to aquatic ecosystems (Sracek *et al*., 2004).

Slimes dams that are used to confine waste from gold mines produce AMD. AMD is produced when sulfide-bearing materials are exposed to air or water (Sracek *et al.,* 2004; Akcil and Koldas, 2006; Nsimba, 2013).

Mining effluents (AMD) are a major causes of environmental problems (Akcil and Koldas, 2006; Sheoran and Sheoran, 2006). AMD consequently has adverse negative impacts on groundwater and surface water, affects the geochemistry of wetland sediments and is very harmful to aquatic species. Water characterized by AMD contamination may have an extremely low pH (Sheoran and Sheoran, 2006).

1.2 Description of the study area

The Varkenslaagte study area is located at about 75 km west of Johannesburg within Gauteng Province, South Africa and about 7 km south of Carletonville, at the West Wits AngloGold Ashanti Gold Mining Operation (Figure 1). Towns close to the area are

Fochille and Potchefstroom, which are situated 12 km and 50 km respectively to the south and west of the study site situated between Gauteng and North West Province.

The West Wits operation receives most of the heavy rainfall during the wet season, usually between November and February, which decreases gradually towards winter seasons. Maximum average heavy rainfall peaks are mostly in summer between December and January. The rainfall is almost exclusively due to showers and thunderstorms, (refer to site description in chapter two for more detailed study area description or description of baseline environment). Wetlands are common along the streams and are vegetated mainly by the sedge species *Schoenoplectus corymbosus, Typha capensis* and by the reeds *Phragmites australis*.

Figure 1 A map showing the study area indicated by a red star on AngloGold Ashanti West Wits gold mine by Paul da Cruz, 2016

1.3 Concise description of the environment on site relative to the environment in the surrounding area

1.3.1 Vegetation

The Varkenslaagte Spruit flows from Gauteng Shale Mucina Mountain Bushveld through Carletonville Dolomite Grassland, North-West of the West Wits sub catchment (Mucina and Rutherford, 2006). The Varkensglaate study area (Figure 1) is situated within the savanna biome and the grassland biome vegetation unit (Mucina and Rutherford, 2006).

The majority of the grasslands surrounding the site (wetland) are transformed or ploughed. The dominant wetland vegetation species includes *S. corymbosus, P. australis and T. capensis.*

1.3.2 Geology and Soil

The area is characterised by Witwatersrand basin and accumulated sediments within the basin are collectively known as the Witwatersrand Supergroup and are made up of the West Rand Group (WRG) and the Central Rand Group (CRG) (McCarthy and Rubidge, 2005).

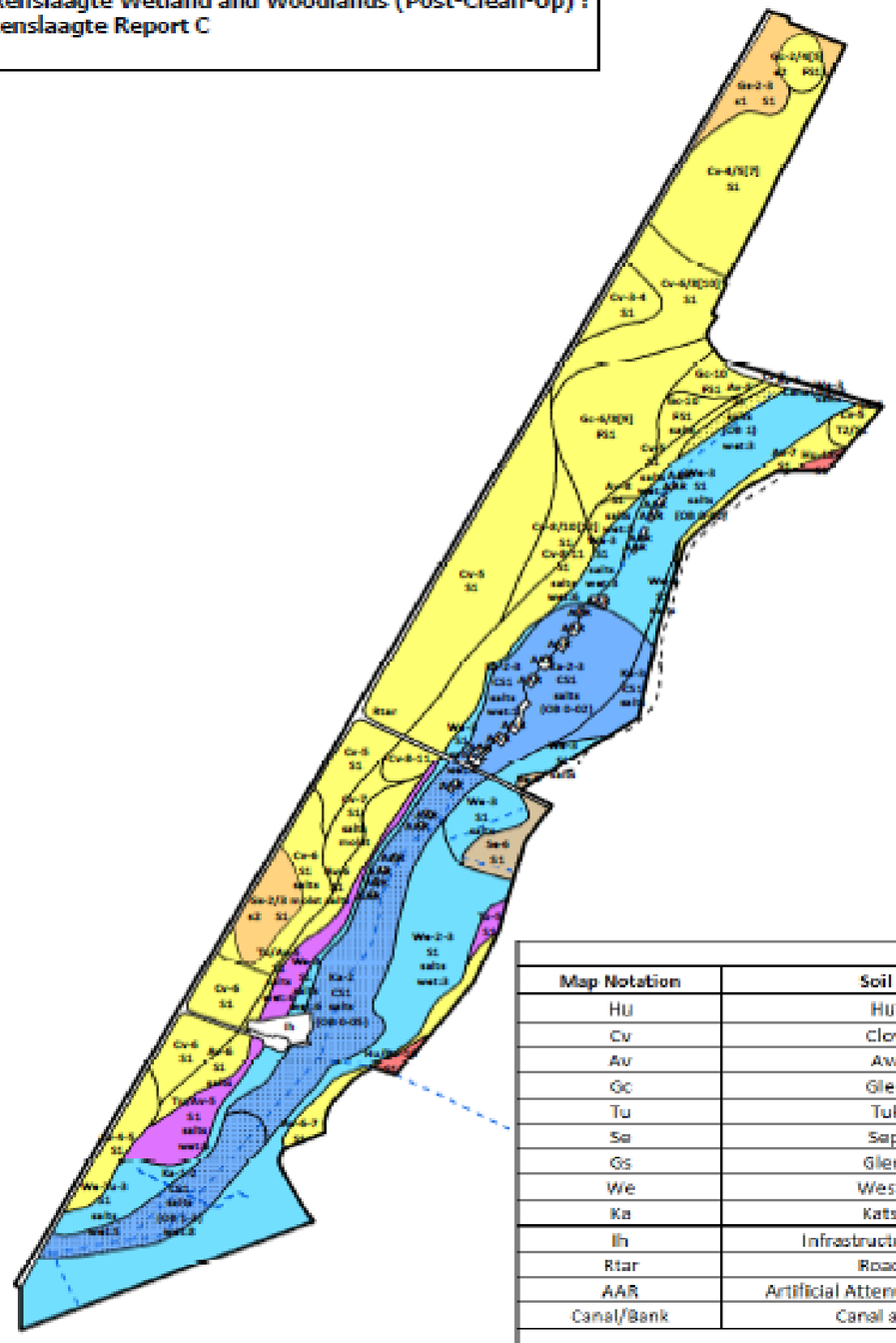
The Varkensglaate upper most wetland paddocks structure is characterised by apedal (Westleigh form); or weak to strong-blocky (Katspruit form) soil forms with very little clay material covering an area of 24 hectares detailed in Figure 2.

1.3.3 Topography

The general topography in the study area is very gently undulating and gentle concave. A localised high point lies to the northern boundary of the Varkenslaagte wetland site, and as such all topography surrounding this point slopes down away from the point.

Reed beds have invaded the wetland / drainage line to the north of the Varkenslaagte wetland. The Varkenslaagte study site is thus situated on very gently sloping ground with a south-facing aspect. As such all natural drainage is southwards, and this is expressed in the topography as a shallow valley develops just south to the Mangaan Drive access road south of the site. The wetland becomes more pronounced as it runs to the east of the Main Road Drive.

WEST WITS OPERATIONS : Varkenslaagte Wetland and Woodlands (Post-Clean-Up) :
Map Reference: REMS54-2 Varkenslaagte Report C
Map 2. Soil Mapping Units



Legend

- Boundaries
- Tailings
- Soils Outside Boundary
- Canal and Drains

Soils

- Hu
- Cv
- Av
- Gc
- Tu
- Se
- Gs
- We
- Ka
- AAR
- Canal/Bank
- Ih
- Rtar

0 50 100 200 300 400
Meters

red earth cc
soil and land use planning consultants

Varkenslaagte Wetland and Woodlands (Soils Legend)

Map Notation	Soil Form	Soil Horizons	Count	Area (ha)	Area (%)
Hu	Hutton	orthic A/red apedal B	2	0.44	0.43
Cv	Clovelly	orthic A/yellow-brown apedal B	17	33.80	32.67
Av	Avalon	orthic A/yellow-brown apedal B/soft plinthic B	7	5.79	5.60
Gc	Glencoe	orthic A/yellow-brown apedal B/hard plinthic B	4	7.77	7.51
Tu	Tukulu	orthic A/neocutanic B/unspecified wet material	3	3.55	3.43
Se	Sepane	orthic A/pedocutanic B/unspecified wet material	2	1.19	1.15
Gs	Glenrosa	orthic A/lithocutanic B	2	3.68	3.56
We	Westleigh	orthic A/soft plinthic B	10	24.94	24.11
Ka	Katspruit	orthic A/G-horizon	5	17.65	17.06
Ih	Infrastructure (human)		1	0.47	0.45
Rtar	Road (tar)		1	3.16	3.05
AAR	Artificial Attenuation Reed Bed		22	0.67	0.65
Canal/Bank	Canal and Bank		1	0.35	0.34
TOTALS			**77**	**103.46**	**100.00**

Figure 2: Shows the Varkenslaagte Wetland and Woodland soil mapping Unit by B.B. McLeroth (2014)

1.3.4 Climate

Climate for the wider Carletonville area is a strongly seasonal summer-rainfall region, with warm temperatures and very dry winters. The range in annual precipitation is given as 425.16 - 737.3 mm/year between 2005 -2013. Temperatures vary between 0°C and 30°C, with an average of 15.8 °C. The climate of the study area is discussed to detail in chapter 3.

1.3.5 AMD and Metal Precipitation

The wetland paddocks are situated on the foot of the mining dump. It is evident that the volume of seepage entering the system is as result of AMD leachate from the mine dump to the paddocks and subsequent canal, and thus forms surface precipitation and salt crusting (Figure 3).

Figure 3 : Metal precipitates at the edge of the engineered wetland southbound of the Varkenslaagte canal.

1.4 Wetlands as a solution for AMD treatment

South Africa is a water stressed country, and AMD continues to contribute to water pollution problems. The treatment of AMD has become a global challenge. It has been considered one of the industries toughest problems to solve (Smith, 1997). The rate of contaminant generation, along with the rate of contaminant transport will determine the concentration and volume of seepage and thus the extent of environmental impact (Coetzee *et al.*, 2006). Conventional clean up techniques are considered to be extremely expensive and have led to a search for more creative, cost effective and environmentally sound ways in which to treat the problem of AMD (Sheoran and Sheoran, 2006).

In the past decades, research efforts have been directed towards wetlands as an alternative low cost means of removing heavy metals from AMD (Sheoran and Sheoran, 2006). Constructed wetlands to treat AMD may provide a continuous, low-cost and effective solution to a growing problem for mining industries (Smith, 1997).

Studies have revealed positive results regarding the use of engineered wetlands to remediate human induced pollution (Fennessy and Mitsch, 1989; Faulwetter *et al.*, 2009). Use of these systems has been developed rapidly and treatment wetlands are now successfully employed to remove a diverse array of pollutants originating from almost every conceivable contamination source e.g. nitrate, S, or a consortium of pollutants typical to a specific source, e.g. primary treated domestic wastewater or AMD (Faulwetter *et al.*, 2009).

1.5 Varkenslaagte wetland for AMD treatment

The Varkenslaagte drainage area is known to be extremely contaminated (AGA, 2009; Lusilao, 2012), and the historical tailing spills, containing sulphate salts and metal pollution were removed from the study location during the 2011 and 2012 period. AngloGold Ashanti Ltd subsequently reshaped the Varkenslaagte canal, and established 20 shallow reed beds as an AMD attenuation and remediation system along it. This was established to reduce the strength effect of AMD as part of AngloGold Ashanti rehabilitation. The above-mentioned reed beds were constructed in different phases.

The 7 uppermost paddocks were engineered within the same period between 1st and 6th of December 2011 and the planting of reeds commenced on the 11th and was completed on the 19th of January 2012 (B.L. Dawson, personal communication, 14th of October 2013).

The planting and excavation started from the uppermost paddock (north) to the bottom (south) along the canal (B.L. Dawson, personal communication, 14th of October 2013).

Figure 4 : Example of the engineered remediation paddock along the Varkenslaagte canal

Spatial assessment and periodic monitoring of the engineered reed beds is essential to understand the fate of AMD contaminants in the Varkenslaagte, and test the efficacy of the engineered reed beds as a pollution control, AMD attenuation and remediation measure. After a year following construction of the attenuation reed beds, this study involved the evaluation of the elemental mass of macronutrients, micronutrients and trace metals associated with the major identified environmental compartments (water, sediment, and higher plant biomass), and determine whether the reed beds are contributing to the sequestration of mass from acid mine drainage, and improvement of surface water quality. The biomass component was further divided into above and below ground. The vegetation communities in the Varkenslaagte catchment primarily consist of planted terrestrial trees with relatively degraded areas (Figure 5).

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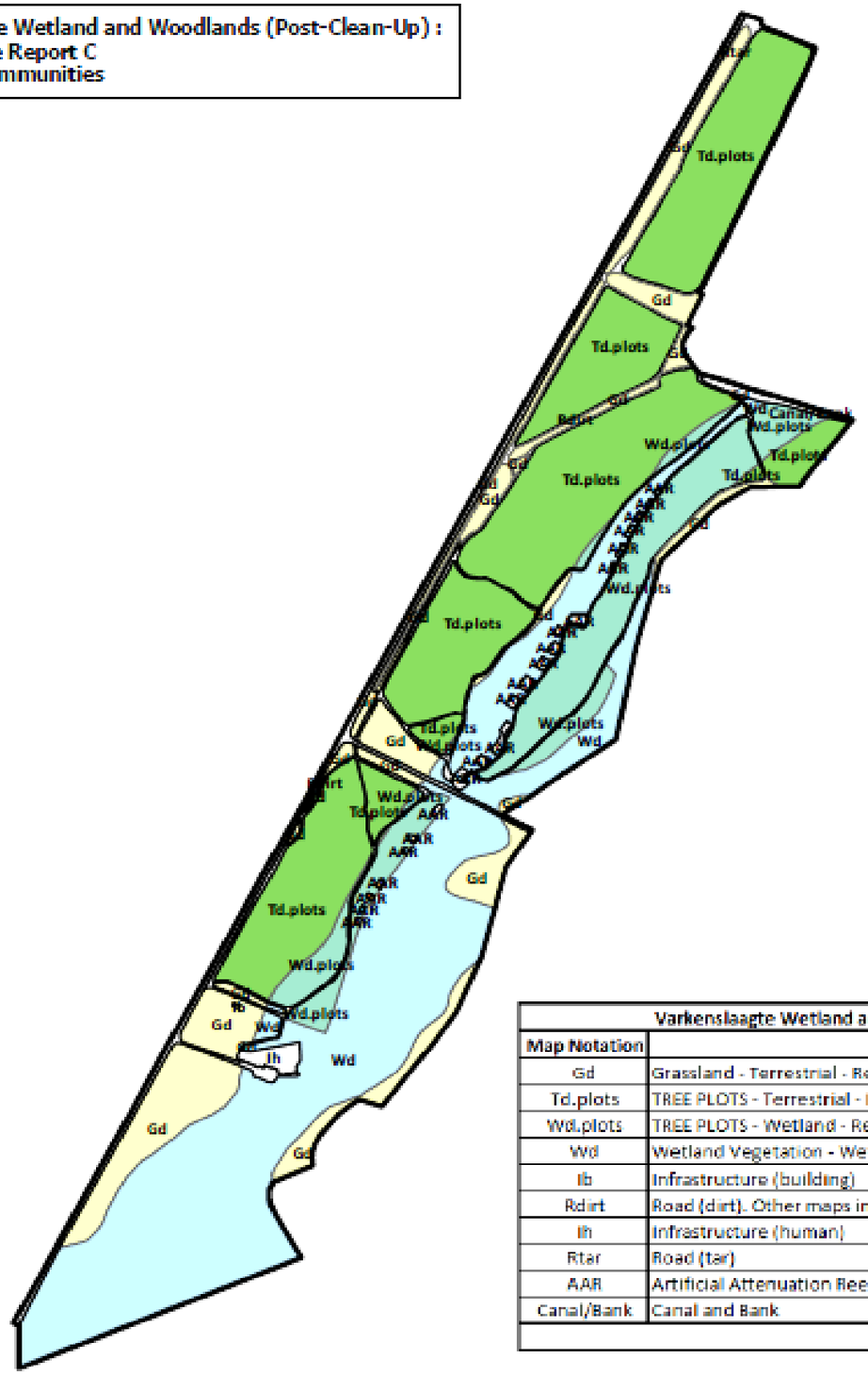
WEST WITS OPERATIONS : Varkenslaagte Wetland and Woodlands (Post-Clean-Up) :
Map Reference: REMS54-4 Varkenslaagte Report C
Map 4. Present Land Use / Vegetation Communities



Legend

- Boundaries
- Landuse
  - Gd
  - Td. plots
  - Wd.plots
  - Wd
  - AAR
  - Canal/Bank
  - Ib
  - Ih
  - Rdirt
  - Rtar

Varkenslaagte Wetland and Woodlands (Present Land Use / Vegetation Communities Legend)				
Map Notation	Explanation	Count	Area (ha)	Area (%)
Gd	Grassland - Terrestrial - Relatively Degraded	24	14.41	13.93
Td.plots	TREE PLOTS - Terrestrial - Relatively Degraded Areas	10	34.44	33.29
Wd.plots	TREE PLOTS - Wetland - Relatively Degraded Areas	9	11.47	11.09
Wd	Wetland Vegetation - Wetland - Relatively Degraded Areas	5	34.04	32.90
Ib	Infrastructure (building)	1	0.01	0.01
Rdirt	Road (dirt). Other maps in the set ignored minor dirt roads / tracks	2	4.44	4.29
Ih	Infrastructure (human)	1	0.47	0.45
Rtar	Road (tar)	1	3.16	3.05
AAR	Artificial Attenuation Reed Bed	22	0.67	0.65
Canal/Bank	Canal and Bank	1	0.35	0.34
TOTALS		76	103.46	100.00

Figure 5: Present land use, Vegetation communities and Wetland Paddocks (AAR) of the Varkenslaagte drainage line by B.B. McLeroth (2014)

1.6 Problem statement and rationale for the study

The historical tailing spills in the West Wits mining operation Varkenslaagte wetland have generated vast volumes of waste (AMD) since they occurred. The Varkenslaagte drainage is well known to be highly contaminated (AGA, 2009; Lusilao, 2012), which may constitute a threat to the environment.

The study seeks to address the efficiency of constructed engineered wetlands on mine tailings in the remediation of AMD and control of metals emanating from AMD. The use of plant species in constructed wetlands that accumulate significant amounts of metals is one of the significant methods in the reduction of AMD, since plants may reduce the production of AMD by element uptake and prevention of pH reduction (Stoltz and Greger, 2002).

Constraints to the use of engineered wetlands could include, amongst others, the use of plants taking up high levels of metals and concentrating them in their tissues which could pose a risk; if they are not harvested or burned, they could disperse metals to the environment which may be consumed by grazing animals or by metal leaching via litter degradation (Nyquist and Greger, 2009).

The rationale is therefore, an evaluation of the ability and the use of engineered wetlands to remediate AMD (AMD Attenuation, reducing down the volume) and to treat the already formed AMD, with special emphasis on the role of the wetland vegetation communities in Varkenslaagte Wetland (i.e. *P. australis* and *S. corymbosus*) in the remediation process.

The AMD attenuation reed beds implemented by AngloGold will to furthermore seek to prove that the use of this system (i.e. constructed treatment wetland) efficiently reduces a diverse array of pollutants originating from AMD in the Varkenslaagte Wetland. Consistent monitoring of this system is certainly essential in the assessment of the spatial distribution of metal pollutants in sediment, and vegetation.

1.7 Research aims, key question and objective

1.7.1 Aim

The aim of this study was to assess and quantify the mass pool size of contaminants (macronutrients, micronutrients, non-essential trace elements) within and between a subset of paddocks from various compartments including sediments, aboveground biomass (shoots –stems and leaves), and belowground biomass (roots and rhizomes).

1.7.2 Key questions

1.7.2.1 Is there any progressive capture of selected contaminants that are important in West Wits AMD *inter alia* Uranium (U), Copper (Cu) , Calcium (Ca), Sodium (Na), Sulphur (S), Chloride (Cl), Iron (Fe), Chromium (Cr), by biomass and substrata from paddock to paddock?

1.7.2.2 What is the pollutant pool sizes within and between the compartments?

1.7.2.3 Is there a seasonal change in water quality parameters across the paddocks?

1.7.3 Objectives

In order to achieve the above-mentioned aims and answer the key questions the following objectives were stated for the study:

1.7.3.1 Firstly to calculate concentrations and pool sizes of macronutrients, micronutrients and non-essential elements within the key environmental compartments of the selected five uppermost engineered wetland paddocks i.e. sediments and biomass (live dominant vegetation – shoots (leaves and stems), fine roots and rhizomes).

1.7.3.2 Secondly, compare water quality parameters across the wetland in different seasons.

1.8 Study approach

The approach to the spatial assessment of environmental baseline of AMD contaminants in engineered wetlands along the Varkenslaagte canal was based on summer sampling of sediments and vegetation, and monitoring of water quality parameters in winter and summer.

Furthermore, the study approach was to analyse the concentration of macronutrients, micronutrients, and non-essential trace elements in the water, sediment and vegetation samples to evaluate baseline contaminants allocation between the wetland compartments.

2 CHAPTER TWO

2.1 Literature review

2.1.1 Heavy Metals

Heavy metals are natural component of the earth's crust. The term "**heavy metal**" mainly includes transition elements and some metalloids, which have a relatively high density, which are toxic or poisonous at low concentration. Heavy metals may occur naturally in soil and rocks of an area or could be introduced to the environment (Meerkotter, 2011). They belong to a group whose hydro-geochemistry is influenced by human activities mostly in developing countries.

Numerous studies nationally and internationally reported that mining activities are the most obvious and major sources of metal pollutants in the environment and often release metals in soluble form. On a local scale in South Africa recent studies that have been reported include amongst others Durand (2012); Winde (2010); Tutu (2012); Lusilao (2013); Dye and Weiersbye (2010). Effluents from other human activities such as industrial waste from ports and refineries, agricultural activities and over usage of chemicals are also potential sources of heavy metal pollutants in the aquatic environment (Dallas and Day, 2004).

Heavy metals may exist in soil as ionic species that can be taken up by vegetation and consequently be passed to various consumers in the food chain. Heavy metals may have a negative impact on the surrounding environment such as biological processes and contamination of water.

Studies done in the Witwatersrand area, South Africa have reported that metal effluents released as AMD enter surface water bodies and the groundwater and pose a threat to humans and domesticated animals (Durand, 2012),while Tutu (2012) states that metals associated with mine effluents can travel hundreds of kilometers and also impact ecosystems.

According to Davies and Day (1998) pollutants may enter water bodies via non-point sources such as seepage, and surface runoff from the agricultural activities. Non-point source pollution can be contrasted with point source pollution, whereby a waste plume discharges into a water body at single location. Metal pollution has been intensively studied worldwide and tailings have significantly contributed to heavy metal pollution in the environment as result of AMD from gold mines e.g. Witwatersrand Basin, South

Africa. Active slimes dams and mining tailings are major sources of both point source pollution (i.e. spillages) and non-point source pollution (i.e. seepage). Tailing footprints left behind after reprocessing or remediation of tailing spills are a typical example of non-point source pollution and can adversely affect water quality.

Rivers serve as a transport medium and soils act as a reserve, while plants uptake heavy metals as micronutrients such as Cu and Zn (Davies and Day, 1998; Marschner, 1995). In most cases, the concentration of heavy metals in a soil is often reflected by a higher concentration of metals in plants. In most studies there is a correlation of metal levels in soil and plant samples.

There are certain methods used for remediation of metal pollution in the environment, some of which are more feasible and practical than others. A typical example of a method, which is often not practical, is simply covering the polluted soil material layer with a layer of unpolluted soil material or evacuating the polluted topsoil layer and replacing it with an unpolluted soil layer.

2.1.2 AMD

AMD is a phenomenon that is of concern worldwide. It is the tailings and waste rock deposits in the mining industry which are the sources of the metals which contribute intensively to AMD when these deposits contain sulphides (pyrite) (Nyquist *et al.,* 2009; Tutu, 2012; Lusilao, 2012; Sracek *et al.,* 2004; Akcil and Koldas, 2006). AMD is produced when sulphide bearing materials or sulphide minerals present in mine tailing are oxidized as they are exposed to water and atmospheric oxygen (Sracek *et al.,* 2004).The production of AMD usually- but not exclusively -occurs in iron sulfide-aggregate rocks (Akcil and Koldas, 2006).

Although this is a naturally occurring process mining can simply promote AMD by significantly increasing the quantity of sulfide exposed (Akcil and Koldas, 2006; Nsimba, 2013). The oxidation of pyrites releases dissolved iron and acidity into the water, which in turn releases other metal ions (Nyquist *et al.,* 2009). AMD is characterized by low pH and numerous metals may be present in vast amounts emanating from AMD production, such as Fe, Mn, and Zn, while other metals such as Cu, Cd, Hg, and other toxic elements are usually present in smaller amounts (Nyquist *et al.,* 2009; Akcil and Koldas, 2006).

AMD for many decades has been studied extensively worldwide as it represents a significant environmental and financial liability to mining industries. Authors amongst others who investigated the phenomena thoroughly include Akcil and Koldas (2006),

Sracek *et al.* (2004), Tutu *et al.* (2008), Coetzee *et al.* (2006), Ochieng *et al*. (2010), Nsimba (2013), Faulwetter *et al.* (2009), Sheoran and Sheoran (2006), Fennessy and Mitsch (1989) and Lusilao (2012).

AMD has an adverse negative impact on the surrounding environment, which varies in severity and magnitude, severely contaminating surface and groundwater, as well as soils and sediments. As mentioned previously in the introduction AMD has been recognized and categorized as a global issue, but in South Africa it is the specific mineralogy of the West Wits deposits and magnitude of the basin in Gauteng province, that make this a geographically interesting study area for heavy metal contamination. For instance, gold mining wastes in South Africa usually contain elevated concentrations of Pb, Cu, Ni, Zn, Cr, U, Hg, As, Fe, Co, and rare earth metals among others (Ochieng *et al.*, 2010).

AMD generation varies between mining operations; every mine has a unique AMD potential. Metal concentrations in AMD are variable and highly dependent on the mineral composition of the ore being mined (Akcil and Koldas, 2006), although the negative consequences are generally the same having impacts on surface water, groundwater, soils and ecosystems.

2.1.3 Metal transportation

AMD is a critical source of metal contamination. An adequate understanding of the fate and transport processes occurring (in this case, over the wetland) may assist in remediation efforts. Once the AMD mixes with surface water, a variety of chemical processes may occur, including neutralization of acidic inputs, oxidation, and precipitation of metal oxyhydroxides (Butler *et al.,* 2008). Transport of metals can be very complex. Precipitated metals can be transported downstream and settle to the streambed and wetland sediments (Butler *et al.,* 2008). Metals can also be taken up by plants from the surrounding contaminated media i.e. wetlands. Plants uptake metals as micronutrients (Marschner, 1995).

Butler *et al.* (2008) conducted a study to track the fate and transport of suspended solids and heavy metals in a tidal wetland using samples of vegetation (*Phragmites communis* and *Kandelia candel),* sediments and water. From the study it was noticed that these plants can tolerate high salinity. The results revealed that high levels of total Zn and Cu in the sediments were in the range of 4.6 $x10^4$–8.5 $x10^6$ mg/ kg and 5.1 $x10^3$–1.7 $x10^6$ mg/kg respectively, and water samples accumulated Zn of 0.5 mg/l and Cu of 0.03 mg/l.

Total Zn and Cu in the water, sediment, and aquatic plants (in this case *Phragmites communis*) showed significant variation in total metal concentration, whereby Butler *et al.* (2008) concluded that metals were significantly concentrated in the sediment substrate.

2.1.4 Effects of plant species in AMD wetland treatment

*P.s australis (*common reed) is a fast growing plant species (Goldblatt and Manning, 2000). It is a seasonally dormant species which normally grows in wetlands, standing shallow water or streams with high concentrations of nutrients. *P. australis* been used for many years in constructed wetlands for treatment of industrial wastewater containing metals (Ye *et al.,* 1998). The underwater stem has plenty of adventitious roots to supply the plant with water (Goldblatt and Manning, 2000). According to Torresdey (2007), living plants can accumulate large amounts of metals in the aboveground plant compartments.

Some plant species can tolerate environmental conditions with salinity e.g. *Phragmites communis, Kandelia candel* and *T. capensis* (Butler *et al.,* 2008). These plant species have the ability to tolerate some degree of salinity, accumulate large amounts of major elements, and trace metals *(*Butler *et al.,* 2008). Goldblatt and Manning **(**2000) have reviewed that plant species with plenty of adventitious roots, have the ability to extract water, reducing flow rates and contamination of shallow aquifers (Dye and Weiersbye, 2010).

Figure 6 : Photograph shows *P. australis* (common reeds) and *S. corymbosus* in a Varkenslaagte wetland paddock.

2.1.5 Engineered wetlands or wetland systems

Engineered Wetlands are also known as constructed wetlands or treatment wetlands (TWs). They are called treatment wetlands simply because of the capability to remove or reduce effluent pollutants from the environment (Figure 6).

The engineered wetlands system has been utilized for many decades; this system is now successfully employed to remove many effluent pollutants originating from almost every conceivable contamination source (Smith, 1997; Faulwetter *et al.*, 2009). Better understanding of this method has led to a great variety of designs and configurations in an effort to optimize the removal of a specific pollutant, e.g. nitrate and sulfate or pollutants emanating from specific sources e.g. primary treated domestic wastewater or AMD (Faulwetter *et al.*, 2009).

The removal mechanisms in natural or engineered wetlands involve physical removal processes (settling and sedimentation), chemical removal (adsorption and sorption),

filtration, volatilization, and biological removal processes (vegetation uptake) which are well documented in various studies e.g. Sheoran and Sheoran (2006) and Faulwetter *et al.* (2009). Wetland waters contaminated by AMD have extremely low pH and elevated concentration of iron, sulfide and other trace elements (Sheoran and Sheoran, 2006).

Figure 7: Example of Varkenslaagte engineered wetland treatment system and physical removal of the sediment core.

2.1.6 Usage of wetlands to remediate AMD

Wetlands constructed for the treatment of AMD vary based on the site characteristics. According to Smith (1997), constructed wetlands for effective AMD treatment are engineered in such a way that characteristics such as biochemical processing, loading rate, slope, substrate, vegetation, sediment control, neutralization and flow rate are controlled. Vegetation up take is perhaps the most essential path for heavy metals removal in wetlands. Perennial vegetation is an important characteristic in a wetland treatment. Wetland vegetation plays an important role in AMD remediation, and has an influence on other wetland characteristics such as stabilizing slopes and sediments (Fennessy and Mitsch, 1989). The *Typha* species are the most commonly used with the ability to tolerant acidic environments, and this plant has been used for many years in constructed wetlands for treatment of industrial wastewater containing metals (Ye *et al.,* 1998).

As mentioned in section 2.1.1, the most commonly used method for wetland treatment for AMD is phytoremediation, but a constraint with this method is that it takes

many years for trees to regenerate and grow, and thus to reduce heavy metal contamination (Meerkotter, 2011). In contrast, an advantage of this phytoremediation method is that it reduces the potential surface flow rate and contaminants through shallow aquifers and within the soil horizons into neighbouring lands and surface channels (Dye and Weiersbye, 2010). Substrate and vegetation are closely linked in constructed wetlands for AMD treatment, in that the substrate used is dependent upon the desired vegetation (Fennessy and Mitsch, 1989).

The constructed wetland for AMD treatment normally has ponds or engineered paddocks, which accumulate sediment loads and enhance growth for tolerant vegetation. The Varkenslaagte wetland paddocks in the study area are designed in a way in which they could accumulate surface water and thus reduce flow rate and provide a growth medium for *P. australis* (Figure 6 and Figure 7).

The other method with fewer detailed research studies for wetland treatments is the use of microbiology. Faulwetter et *al.* (2009) emphasized that microbial functional groups are the most important factor influencing wetlands treatment systems e.g. nitrifiers, denitrifiers, and sulfate reducing bacteria (SRB) responsible for removal of specific pollutants in treatment wetlands.

2.1.7 Biogeochemistry of engineered wetlands

Pollution removal in a wetland environment depends on the oxidation-reduction (redox) conditions as this affects the environmental chemistry of trace elements (Ritter *et al.*, 2002). Anaerobic conditions in a wetland occur when oxygen consumption in the biota exceeds diffusion of oxygen into the soil profile. Anaerobic environments such as wetland sediments are usually limited by electron acceptors and have a supply of electron donors. Redox potential measurement allows the characterization of the degree of reduction and the prediction of stability of various compounds that regulate the nutrients and metals availability in the sediments (DeLaune and Reddy, 2004).

Reductants (electron acceptors) in wetland sediments are organic matter and inorganic ions such as NH_4^+, Fe^{2+}, and S^{2-} whereas oxidants are inorganic compounds such as O_2, NO_3^-, MnO_2, and FeOOH (DeLaune and Reddy, 2004). High redox potential is associated with an oxidized environment and promotes aerobic processes such as nitrification.

In contrast, lower redox potentials are linked to reduced conditions and promote anaerobic processes such as sulfate reduction (Faulwetter *et al.*, 2009). When pyritic

minerals are exposed to the atmosphere in the presence of water, oxidation of sulphide produces sulphuric acid and releases heavy metals and other AMD pollutants (Sheoran and Sheoran, 2006; Tutu, 2012). When pyritic minerals are reduced under anaerobic conditions the release of heavy metal and other pollutants will be minimized.

Element mobility in sediments and water is affected by changes in pH and redox potential (Eh) (Morgan and Stumm, 1996). Various factors and processes such as temperature, runoff and decay processes influence the pH of natural water. pH is the controlling factor in the reduction of sulphate. Sulfate reducing bacteria are naturally occurring in wetlands (Faulwetter et *al.,* 2009). Sulfate reduction (SRB and SRP) require pH ranges near neutral for optimal sulphate reduction (Sheoran *et al.,* 2010).

Oxygenation reactions often lead to a decrease in the pH while processes such as denitrification and sulfate reduction increase pH (Morgan and Stumm, 1996), which may efficiently minimize acidity and metal toxicity (Sheoran *et al.,* 2010).

2.1.8 Engineered wetland constraints

The constraint that affects AMD wetland treatment is insufficient wetland management and monitoring. It is not conclusive that wetland treatment for AMD is effective and has no effects on the biodiversity which is highly dependent on the wetland for survival. A long-term concern of AMD wetland treatment is the ability of the wetland to sustain the continuous AMD and wastewater emitted into it, without discharging toxic substance back in the environment. A possible solution will be a continuous monitoring of AMD wetland treatment and applying specific regulation that enforces the treatment of AMD.

2.1.9 Major and trace elements that are significant to West Wits Mining Operations

As explained previously AMD is the most negative impact from mining activities which affects quality aspects of the receiving environment such air, water, soil, animals etc. AMD formation occurs over the medium to long term but its effects if not properly managed persist even after mining closure, i.e. the West Wits Mining operation is a typical example. AMD has been reported as a major persistent challenge in this operation and elements explained below in the literature are reported to be highly elevated, while other selected elements not explained in detail will also be investigated in this study.

2.1.9.1 U

Uranium is a naturally occurring radioactive mineral present in certain types of rocks and soil throughout the world (Anke *et al.,* 2009; Fayek, 2011). It can be used as an

abundant source of concentrated energy (Anke *et al.,* 2009). Uranium occurs in most rocks in concentrations of 2 to 4 parts per million and is as common in the earth's crust as tin, tungsten and molybdenum (Fayek, 2011). Uranium is commonly found in very small amounts in rocks, soil, water and plants (Anke *et al.,* 2009). Uranium is weakly radioactive and contributes to low levels of natural background radiation in the environment (U.S. Environmental Protection Agency, 2006).

Uranium is one of the radioelements whose mobility in soils may vary strongly depending on soil type and physico-chemical properties (Echevarria *et al* 2001). Human induced activities such as mining, refining processes, coal combustion and other fuels, usage of phosphate fertilizers and nuclear power production are the major sources of U in the environment (Davies and Day, 1998).

Human activities enhance U contamination. Uranium pollutants lead to contamination of groundwater, surface water and thus in turn accumulate in plants and animals (Anke *et al.,* 2009; USEPA, 2006). Living organisms are highly exposed to U if they live in close proximity to industrial areas, which process U or mining activities. Uranium can be taken up by animals in different ways, e.g. it could be inhaled or swallowed, and when it enters the body it may cause development of cancer and kidney failure (Leggett, 1989; USEPA, 2006).

The transfer of U to the food chain of humans is significantly affected by the geological origin of the soil and the groundwater basin as well the drinking water reservoir (Anke *et al.,* 2009). The South African water quality guidelines set by then the Department of Water Affairs and Forestry (DWAF, 1996a) do not provide a water quality guideline for U, but a concentration of 70 µg/l is deemed acceptable for the drinking water limit (USEPA, 2006).

Uranium can be accumulated by plants and soil, and uptake by plants is a process which could lead to adverse effects in ecosystems. U in plants is accumulated in the root system which in most cases is found to be higher than in the shoot system (Roivainen, 2011). Uranium concentration in plants may vary between different plant species. According to Shahanden and Hossner (2002) dicotyledonous plant species (coniferous trees) tend to accumulate more U than monocotyledonous plants species (herbs and ferns). Soil pH is one of the most important factors affecting the behavior of U in soil, however according to Echevarria *et al.,* (2001) and Anke *et al.,* (2009) clay content and organic material have no significant effect on sorption.

2.1.10 Uranium in the study area, West Wits, Gauteng

Mining activities are the major source of U in the study area. The exploration of U in South Africa started in 1944 through the Manhattan Project from the United States of America (USA) in the Witwatersrand area and was supported by the South African Chamber of Mines and Great Britain. In 1959 South Africa produced 4 950 t U with more than 150 000 t U between 1945 and 1990, which made South Africa the fourth largest global provider of uranium (Coetzee., *et al* 2006). Mining activities of U may affect the availability of water (quantity aspect) or the quality of water in the area (pollution aspect). This may result in the deterioration of water quality.

The quality aspect of water affected by mining activities is caused by seepage from slime dams, uncontrolled leakage from pipelines, canals and tailing storage facilities. The major sources of U in the area include amongst others, tailings facilities used for sinkholes in the dewatered compartments, windblown tailing dust deposition and seepage from storage dams (Sutton and Weiersbye, 2008).

According to Coetzee *et al*. (2006), mining operation fluvial sediments found in dams, wetlands and the streambed itself frequently contain significant elevated U concentrations, sometimes even exceeding those in tailings deposits. Such sediment may, under certain environmental conditions, release U back into the water. Uranium in gold reefs of SA occurs mainly in brown-blackish tetravalent minerals such as uraninite (Coetzee *et al*., 2006). Uranium in the area has an extreme impact on stream water.

2.1.10.1 Cu

Copper is a naturally occurring common metallic element in the rocks and minerals of the earth's crust. Sources of this heavy metal in the aquatic environment are due to weathering processes or from the dissolution of Cu minerals and native Cu (DWAF, 1996 d). Copper occurs in four oxidation states, namely 0, I, II and III. The two most common forms are cuprous copper (I) and cupric copper (II); cuprous copper is unstable in aerated aqueous solutions and will normally be oxidized to cupric copper (USEPA, 2006; DWAF, 1996 d).

Effluent discharge and wastewaters contain heavy metals such as Cu (Barakat, 2010; Davies and Day 1998). Copper enrichment like that of all other heavy metals may occur as a result of anthropogenic activities such as liquid effluent from mining, sewage treatment plant effluents, agricultural runoff and groundwater contamination from the use of Cu as fungicides and pesticides in the treatment of soils (DWAF, 1996a).

Aquatic plants take up mineral nutrients over their entire submerged surface whereas terrestrial plants acquire their minerals via a root system from a soil (Larcher, 2001). Copper is an important micronutrient required by plants for growth in specific tolerable amounts, when it is taken up as Cu^{2+} , but extreme uptake of Cu minerals by plants may cause plant deficiency in tissues (Marschner, 1995). Plants differ in their susceptibility to Cu deficiency, however Cu deficiencies are rare in plants because they require very little thereof (Meerkotter, 2003).

Copper deficiency may occur in animals including humans; many of the symptoms seen in Cu deficient animals also occur in humans (IPCS, 1996). Like all elements, too much Cu can also lead to undesirable effects. The review by World HealthCare Organization on International Programme on Chemical Safety 1996 showed that, for decades Cu has been recognized as an essential trace metal. However, concern has been over possible Cu toxicity in particularly liver cirrhosis in infants and children resulting from consumption of high levels of Cu in milk stored in brass vessels.

According to DWAF, (1996) South African water quality guidelines for aquatic ecosystems, the target water quality range for Cu in aquatic ecosystems is less than 0.3 µg/l in soft water and below 0.8 µg/l in medium soft water.

2.1.10.2 Mercury (Hg)

Mercury is a heavy metal that is of quite rare geological occurrence and its concentration in the environment is normally very low. There are few studies of its concentration in the atmosphere or its emission in the developing countries, especially in Africa (Gichuki and Mason, 2013; Masekoameng *et al.,* 2010). South Africa is among the largest producers of this metal in the world ranking, responsible for 12% of global production (Masekoameng *et al.,* 2010). Most developed countries have established monitoring programs, but little work has yet been done in developing countries especially in Africa (Gichuki and Mason, 2013).

Mercury occurs in three oxidation states in the natural aquatic environment, namely as the metal, as mercury (I) and as mercury (II) (DWAF, 1996 b; USEPA, 1986). It is nonflammable; slightly volatile at room temperature but it releases toxic vapor or is significantly more volatile especially when heated (WHO, 2000).

This element is also found as organo-mercurial salts, the most important of which is methyl mercury (WHO, 2000; USEPA, 1986). Sources of Hg deposits are supplied by secondary sources, and additional quantities of Hg are obtained as a byproduct of gold

mining (where Hg is used to form an amalgam before being burnt off) and other metal ores (e.g. Cu, zinc). Mercury present in gold ore may be released to the environment (e.g., in disposed air pollution control wastes and spent ore tailings) (WHO, 1996).

There are many ways in which Hg may occur at high concentrations which include, in water bodies from industrial pollution or in industrial activities discharging Hg or compounds thereof (USEPA; 1986; WHO, 2000) The major sources of Hg are industrials that use it in their processes (Dabrowski *et al.,* 2008) or in their products such as painting industrials, pharmaceuticals, medical, and dental industries and the electrical equipment industry (DWAF, 1996 b).

Like other metals it may be bio-accumulated by plants and intake of Hg by animals may occur via food, air and water (DWAF, 1996 b). Coal combustion may significantly contribute to substantial Hg emission (Dabrowski *et al.,* 2008). The most common form of Hg found in aquatic organisms is lipid soluble (readily passes through plant and animal membranes) and is stored within the bodies of organisms. In aquatic animals, bio-accumulated Hg is stored in fatty tissues (DWAF, 1996 b).

The most common and primary manner in which animals including humans may be exposed to Hg is through inhalation of the released vapor (WHO, 1996). According to the World Health Organization (2007) virtually no elemental Hg is absorbed from the gastrointestinal route or through skin. The other ways in which humans might be exposed to methyl mercury is through the consumption of contaminated water or eating contaminated fish and shellfish more especially in a population that relies heavily on the consumption of predatory fish. The chronic exposure might be as a result of continuous exposure to elemental Hg which accumulates in the body or damages the nervous system or kidney and children may also extract it from breast milk and are more likely to develop respiratory failure (WHO, 2007).

2.2 Soil and water characterization

2.2.1 pH

According to Golterman *et al,* (1997), water quality parameters such as pH and electrical conductivity are essential indicators of water quality. The pH is defined as -log (H^+) which is a measure of hydrogen ion activity and an indicator of hydrogen ion concentration present in a solution (DeLaune and Reddy, 2004; DWAF, 1996 d; WHO,

1996). The pH is a good indicator of acidification in a sample of water (Ngwenya, 2006). Most raw water or mineral water pH lies generally within narrow range of 6.5 - 9.5 (Morgan and Stumm, 1996; DWAF, 1996 a).

The pH of natural water is influenced by various factors and processes including temperature, acid mine drainage, acidic precipitation, runoff, and decay processes (WHO, 1996). Oxygenation reactions often lead to a decrease in pH and processes such as denitrification and sulfate reduction tend to increase pH (Morgan and Stumm, 1996).

The pH of pure water (that is, water containing no solutes) is neutral 7.0, the number of H^+ and OH^- ions are equal (DWAF, 1996 d). The average pH of water in South Africa is between 6 and 8.5. Metals may become highly toxic in lower pH, whereas a change in pH value below seven (i.e. decrease to below 7) changes water chemistry and may convert non-metallic ions to toxic (Ngwenya, 2006; DWAF, 1996 d). For example the ammonium ion can be converted to highly toxic ammonia (Dallas and Day, 2004). Consequently pH affects the fitness of water for ecological, industrial and domestic supply. In low pH conditions some toxic metals are soluble and therefore mobile (Dallas and Day, 2004; DWAF, 1996), whereas at high pH condition this mobility is decreased, but some metals are normally soluble throughout the entire pH range such as U (Tutu, 2005).

2.2.2 Redox potential

Oxidation and reduction are processes also called redox reactions. It is also known as oxidation- reduction potential, reduction potential and is abbreviated as pE or E_h. Redox (pE) is defined as –log (e^-), and is a measure of the tendency of a chemical species (compounds) to acquire electrons and thereby be reduced (Morgan and Stumm, 1996). Reduction potential is measured in volts (V), or millivolts (mV) and E_h is the more common expression of soil redox potential. Redox potential measurement allows the characterization of the degree of reduction and the prediction of stability of various compounds that regulate the nutrient and metal availability in the soils and sediments (DeLaune and Reddy, 2004).

Anaerobic environments such as wetland soils are usually limited by electron acceptors and have a supply of electron donors. Electron acceptors in wetland soils are organic matter and various compounds including inorganic ions such as NH_4^+, Fe^{2+}, and S^{2-} whereas oxidants are inorganic compounds such as O_2, NO_3^-, MnO_2, and FeOOH (DeLaune and Reddy, 2004).

2.2.3 Redox potential and pH Relationship

Element mobility in soil and water is affected by changes in pH and redox potential (Eh) (Morgan and Stumm, 1996). According to Morgan and Stumm, (1996) and DeLaune and Reddy, (2004), redox potential increases with increased activity of the oxidized component, decreases with the increasing activities of the reduced component and increases with an increase in hydrogen ion H^+ activities (or decrease in pH). An alkaline acidic solution is a result of higher concentration of hydrogen ions [H^+] indicated by lesser pH values therefore it decreases the redox potential. The pH is inversely related to the EC, the proton (H^+) or hydroxyl ion (OH^-) contributes extensively to the EC concentration if all other constraints are constant; solutions of high pH therefore often have lower EC concentrations (Morgan and Stumm, 1996).

2.2.4 Electrical conductivity (EC)

Electrical conductivity is a measure of the total amount of dissolved material in water (Dallas and Day, 2004; WHO 1996). EC (also known as specific conductance or conductivity), is simply the measure of the ability of water to conduct an electric current. Conductivity is often expressed as microsiemens per centimeter ($\mu S\ cm^{-1}$) or $mS\ m^{-1}$. According to a classification system of DWAF, (1996a) pH and electrical conductivity are classified as Group A indicators.

According DWAF (1996a) and Golterman *et al* (1997) group A indicators are those that are considered extremely important and should always be monitored for water quality studies and domestic water supplies. Total dissolved solids concentration is a measure of the quantity of various inorganic salts dissolved in water, which is proportional to EC in the water. EC is much easier to measure and it is used to estimate the TDS concentration of water (DWAF, 1996a; WHO, 1996).

The amounts of electric charges on ions have an influence on conductivity due to presence of ions in water. According to DWAF (1996a) and WHO (1996) EC in water is due to the major cations (Ca, Mg, Na, and K) and anions (carbonate, nitrate, bicarbonate, Cl and sulphate).

Most organic compounds dissolved in water do not dissociate into ions, and as a result do not affect EC. The EC depends on the geological formation which water was or is in contact with, that is the dissolution of minerals rocks, soils and from plant decomposing materials. The conductivity of most freshwaters ranges from 10 to 1,000 $\mu S\ cm^{-1}$ but may exceed 1,000 $\mu S\ cm^{-1}$, especially in polluted waters (WHO, 1996). This occurs normally as

a result of large quantities of surface runoff, discharging effluent into the streams or rivers. Electrical conductivity can be measured *in situ* or in the laboratory.

2.3 Environmental compartments

The vegetation, water and sediments according to Ross (1994) and Gandois *et al.* (2010) are the major environmental compartments which are relatively important for analysis of element mass pool size in wetlands, floodplains or ecosystems. Trace elements and major elements can cycle through the water body, soil and the biosphere like the phosphorus cycle through an ecosystem, since most of these elements do not exist in a gaseous state (Meerkotter, 2011). Pollutants enter the environmental body either through intentional application, through use in the chemical industry or degradation processes of other products (Semple *et al.,* 2003).

Pollutants enter water bodies via various pathways such as seepage, spills and surface runoff (Davies and Day, 1998). Rivers, as one of the major environmental compartments, act as a transport medium for organic matter, major ions, trace metals and major elements.

The load transport of contaminants depends on the flow of water, which is linked to rainfall patterns and intensity. During the dry season the transport of trace elements and major elements are in most studies shown to be much lower than in the wet season. Soil and sediments on the earth's surface can act as reservoirs for accumulation of heavy metal pollutants (Ross, 1994). Heavy metal may exist in soil as ionic species that can be taken up by plants. Vegetation uptakes and accumulates major ions, trace metals and heavy metals as micronutrients such as Cu and Zn (Marschner, 1995; Gandois *et al.*, 2010).

In some studies, microorganisms have been included as a key environmental compartment for assessment e.g. (sulphate-reducing prokaryotes and sulphate-reducing bacteria) which reduce sulphate to sulphide (Sheoran *et al.,* 2010; Sheoran and Sheoran *et al.,* 2006) and generate alkalinity in polluted media, playing a key role in controlling the flow of contaminants in wetlands used for water treatment (Sheoran *et al.,* 2010). These microorganisms also provide a measurable amount of heavy metal uptake and storage (Sheoran and Sheoran *et al.,* 2006).

2.4 Phytoremediation technology

Phytoremediation is a remediation process that entails the use of plants to partially or substantially remediate selected substances in contaminated soil, sludge, sediment,

groundwater, surface water and wastewater (USEPA, 1999). Torresdey (2007) defined phytoremediation as a technology that uses plants to remove, extract, transform, or immobilize metal contaminants in soils and waters. It is also referred to as green remediation or vegetative remediation. UNEPA, (2010) reported that there are a number of different ways in which the removal of metal contaminants from water and soil can be remediated such as phytoextraction, and phytodegradation. Phytoextraction, also known as phytoaccumulation, is the process by which metal elements in soil are taken up by plant roots and translocated to the aboveground biomass of the plant (USEPA, 1999).

2.4.1 Phytoremediation constraints

As it is with any technology, phytoremediation has several advantages and disadvantages when compared to conventional remediation processes. A constraint with regard to this method is that the removal of the contaminated vegetation is likely to cause pollution to site(s) where it is dumped (Meerkotter, 2011). The use of plant species to extract heavy metals from the soil, i.e. phytoremediation, is very practical. In this process, living plants can accumulate high amounts of metals in the aboveground plant parts.

The constraint with the use of the phytoremediation method is that it takes many years for trees to develop and thus to reduce AMD (Dye and Weiersbye, 2010) and again it takes years to remove metals sufficiently from soil and thus might make this method less plausible to commercial farmers (Meerkotter, 2011). Phytoremediation may however, be feasible or applied to highly contaminated sites where human contact is less frequent i.e. in mining wetlands, tailing footprints, and slimes dam spillages. Phytoremediation techniques may include the introduction of trees that are extremely vigorous and capable of high rates of transpiration (Dye and Weiersbye, 2010). The study seeks to address the use of specific plant species for AMD attenuation i.e. the use of reed beds to reduce the strength effect of AMD. In the Witwatersrand Basin, some phytoremediation programmes have been undertaken by AngloGold Ashanti through the Ecological Engineering and Phytoremediation Programme with the University of the Witwatersrand. Some successes have been reported (Dye *et al*, 2008; Dye and Weiersbye, 2010; Weiersbye *et al*, 2009).

In general, phytoremediation has been used around the world as a cost effective method of accumulating the heavy metals from various heavy metals contaminated sites. Several vegetation species are well known for their potential cleanup of metal contaminants. Some macrophyte vegetation species such as *S. corymbosus,* and *P. australis* are also known for their role of metal uptake, and translocate them in various components such as shoots (leaves and stems) and below ground biomass (rhizomes and

roots). These plants absorb metal contaminants from the sediments and water column and either trap them in their rhizosphere or translocate a smaller portion into the above ground biomass.

The advantages of phytoremediation include economical and low cost technology, with minimal disruption to the environment. This method does not involve waiting for new plant communities to colonize the site. This process does not require a need for disposal sites as plants take up contaminants, thus reducing risk of continuous spread of contaminants.

Notwithstanding the mentioned advantages, the disadvantages amongst others include:

- Dependent on the environmental growth conditions (climate, geology, altitude, temperature) required by the plant,
- Success depends on tolerance of the plant to the pollutant,
- Risk of contaminants collected in senescing tissues being released back into the receiving environment.
- Remediation time is longer since it is dependent on plant development and growth, and
- Possibility for environmental damage due to leaching of soluble contaminants.

2.5 Other phytoremediation technologies

Conventional techniques commonly applied for the remediation of heavy metals in wastewater treatment systems include physicochemical remediation, phytoremediation and microbial remediation (Matheickal et al., 1987).

2.5.1 Microbial remediation process

Microbial bioremediation is defined as the process by which micro-organisms are stimulated to rapidly degrade hazardous organic contaminants to environmentally safe levels in soils, subsurface materials, water, sludge, and residues. Microbes deal with poisonous chemicals by applying enzymes to convert one chemical into another form and taking energy or utilizable matter from this process. The chemical transformations generally involve breaking of large molecules into several small molecules in simpler form. Bioremediation may all be referred to as rhizodegradation which is the process

where compounds are broken down in the rhizosphere through microbial activity (USEPA, 1999). In this process, organic substances such as fuels or solvents are broken down (consumed and digested) or biodegraded by microorganisms such as bacteria or Eukaryotes (fungi, protists) which are more sensitive to metal toxicity than bacteria (USEPA, 1999).

2.5.2 Fungi in bioremediation

Bioremediation involves the use of plants or micro-organisms, viable or not, natural or genetically engineered to treat environments contaminated with organic molecules that are difficult to break down (xenobiotics) and to mitigate toxic heavy metals, by transforming them into substances with little or no toxicity, hence forming innocuous products (Dobson and Burgess, 2007). As in phytoremediation, *in-situ* bioremediation technologies are more economical and release fewer pollutants into the environment. This method can be can be applied *in-situ,* at the contaminated site, or *ex-situ*, involving removal of contaminated material to be treated elsewhere.

2.5.3 Algae in bioremediation

The term alga refers to a large and diverse assemblage of organisms that contain chlorophyll and carry out oxygenic photosynthesis. Algal biomass in most instances is insignificant in comparison with vegetation biomass. Surface algal mats are a key environmental compartment for assessment of metal remediation as it is known that some algae are cable of removing heavy metals, since the presence of functional groups such as carboxyl, sulfate, amino and hydroxyl groups in the algal cell wall makes it an ideal biosorbent for heavy metal removal (Nsimba, 2013).

3 CHAPTER THREE

3.1 Methods and Materials

3.1.1 Study approach

The study approach involved an *in situ* and *ex situ* assessment of water quality within the entire 7 uppermost engineered wetland paddocks which were established simultaneously within the same few weeks. The study further involve sampling of sediments and biomass (dominant plants – leaves, biomass, and rhizomes) from a subset of 5 selected paddocks within the canal that is ww1,ww2,ww4,ww6 and ww7, from the uppermost 7 paddocks (Figure 8). The dominant vegetation biomass at the site has been identified as the reed species *P. australis* and the sedge species *S. corymbosus*.

3.1.2 Sampling technique

The field sampling was based on an initial site inspection meeting and the guidance of my supervisor and co-advisor from the University of the Witwatersrand and as well as input from Anglo Gold Ashanti employees. The site inception meeting was done on the 14th of October 2013.

The two uppermost paddocks (1 and 2) were extremely wet and consist of two plant species of the same density (*P. australis and* the sedge species *S. corymbosus*). The fourth paddock was selected as per the geographical location it is centered within the uppermost seven (7) paddocks, was partially wet towards the edge of the inflow and partially dry towards the edge of the outflow. The two bottom paddocks (6 and 7) were completely dry and consisted of *P. australis* species only. The selection showed contrast in terms of water availability and species type within the paddocks (Figure. 8).

Figure 8 : Site locality map of the uppermost wetland paddocks

3.1.3 Outline of research methodology

The research methodology involved *in situ* field measurements, collection of field samples and laboratory analyses to determine the elements present in water, sediments and biomass (dominant plants – leaves, roots, and rhizomes) and determine the mass pool of contaminants in the upper drainage system as displayed in Figure 9. For the purpose of the study historical aerial photographs and soil mapping (Bruce McLeroth, 2011) were used to simulate the spatial extent of tailings distribution along the Varkenslaagte drainage line (Figure 10).

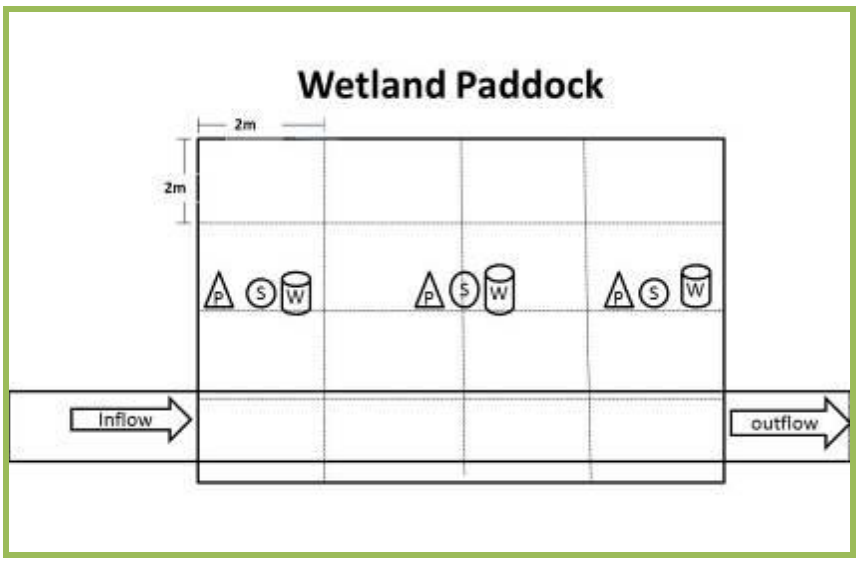


Figure 9: Schematic diagram representing spatial research design of engineered wetland paddocks with horizontal surface inflow and outflow structure including water sampling zone (W), pattern of vegetation sampling (P), sediment sampling zone (S)

The total mass allocation / pool size of macronutrients, micronutrients and non-essential trace elements in the paddocks were estimated, focussing on those that were released as a result of historical tailing spills. The Varkenslaagte research site diagram (Figure 10) was designed with reference from West Wits Operation-Varkenslaagte Wetland Soil Maps.

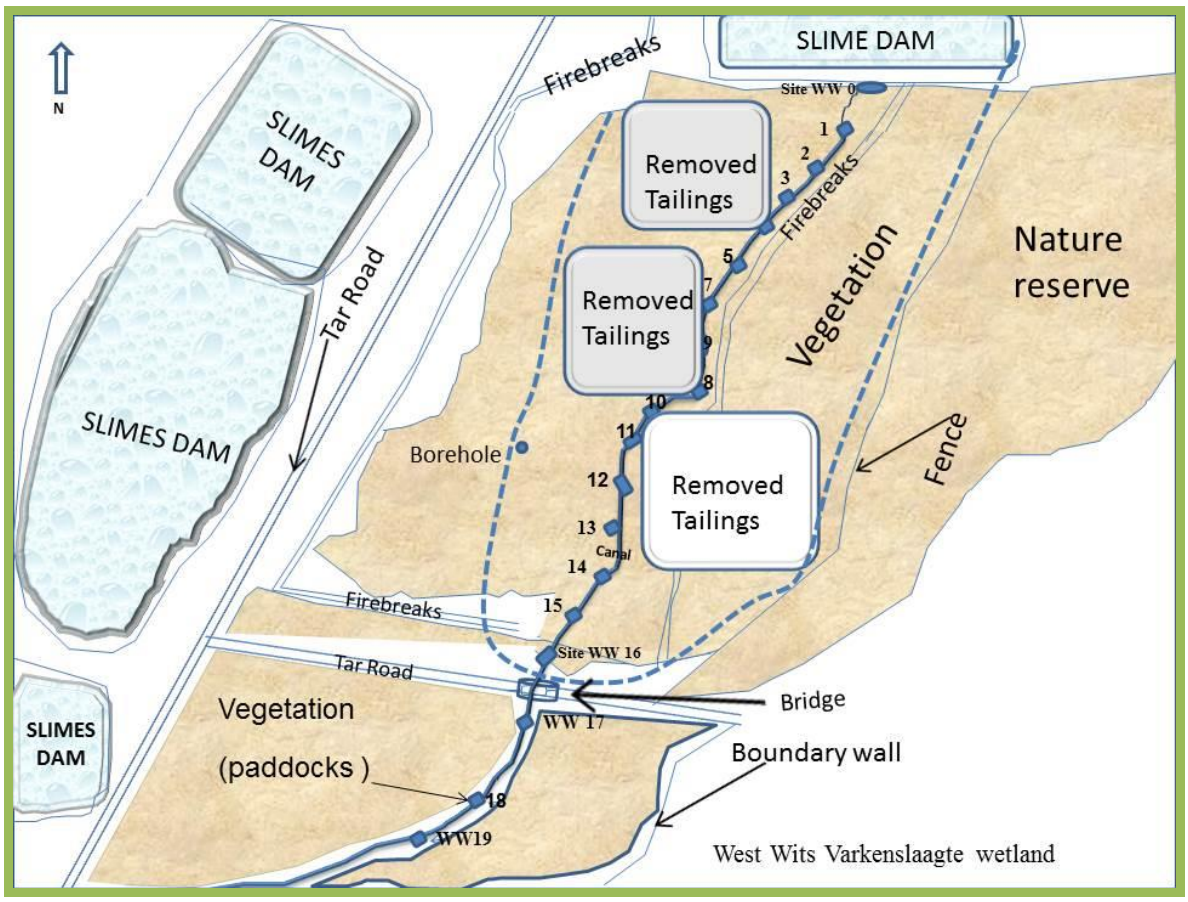


Figure 10 : Illustrates the entire engineered wetland paddocks (sites) (WW1-WW19) along the canal at the Varkenslaagte wetland research site

Table 1 : Shows the detailed compartments and analyses done on the samples on site and in the laboratory

	Analyses						
Compartments	In s*itu* YSI	Redox Meter Thermo Scientific	*In situ* Text Strips	XRF	ICP-OES	IC	ICPMS
Sediments		X		X			
Shoots (leaves and Stems), roots and rhizomes				X			
Water	X	X	X		X	X	X

Figure 11 : Photos respectively show the canal and an engineered wetland paddock at the Varkenslaagte Wetland West Wits research site

3.1.4 Site description

The Varkenslaagte Wetland (26°25’53.44” S, 27° 22’14.24” E) is one of the three surface water sub catchments of the West Wits Operations (Mponeng, Savuka and TauTona) (Figure 12). The area is located at approximately 75km west of Johannesburg within Gauteng province in South Africa. All the samples used in this study were collected from the Varkenslaagte canal (refer to Table 5 for sampling coordinates).

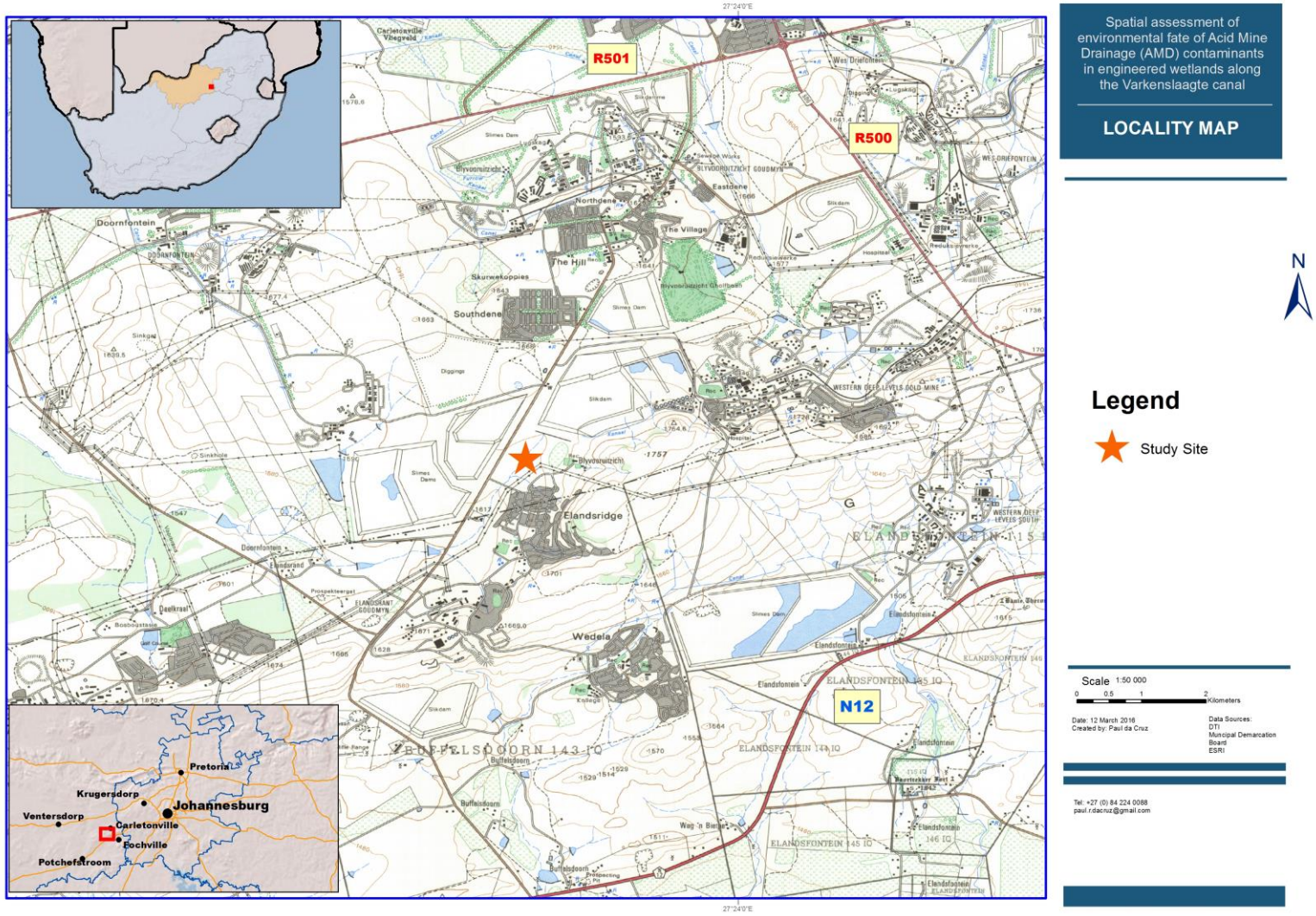


Figure 12 : Locality map of the study area (indicated by a red star) on the AngloGold Ashanti West Wits gold mine by Paul da Cruz, 2016

The West Wits operation during the wet season receives most of the heavy rainfall during a four-month period between November and February, which decreases gradually towards the winter season. Maximum average heavy rainfall peaks are mostly in summer between December and January (Figure 13). Average monthly rainfall ranges between 0 - 300 mm/month from 2005-2012 (Figure 13). Annual rainfall ranges from 500-800 mm per year (Figure 15). Maximum temperature peaks in the study side are between November and January and minimum temperatures occur in winter between June and July (Figure 14). Data used to represent the graphs were requested and obtained from the South African weather service, which is gratefully acknowledged.

The Varkenslaagte Spruit flows from Gauteng Shale Mucina Mountain Bushveld through Carletonville Dolomite Grassland, North-West of the West Wits sub catchment (Mucina and Rutherford, 2006). The stream drains into the Wonderfontein Spruit, a tributary of the Mooi River which finally discharges into the Vaal River. Water from North Savuka complex tailing storage facilities (TSF) drains into the west boundary dam and then into the Varkenslaagte stream, which historically drained into the Wonderfontein Spruit while some of the water infiltrates into dewatered Turffontein Dolomite outside the mine (AGA, 2009; Weiersbye *et al.*, 2009).

The 7 uppermost paddocks were established within a one week period between 1st and 6th December 2011 and the planting of reeds commenced on the 11th and was completed on the 19th January 2012 (B.L. Dawson, personal communication, 14 October 2013). The planting and excavation started from the uppermost end (north) to the bottom (south) along the canal (B.L. Dawson, personal communication, 14 October 2013).

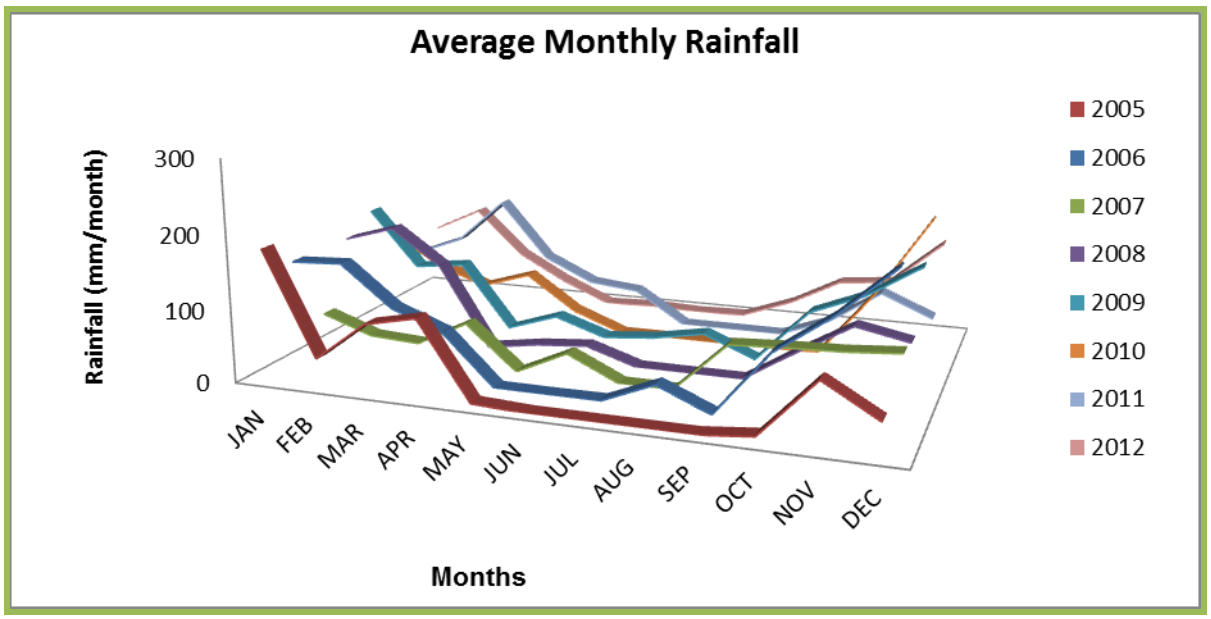


Figure 13: Average monthly rainfall (mm/month) between 2005 to 2012, data for station [0474680 9] Carletonville, West Wits Operation, measured at 08:00 (Data provided by SAWS)

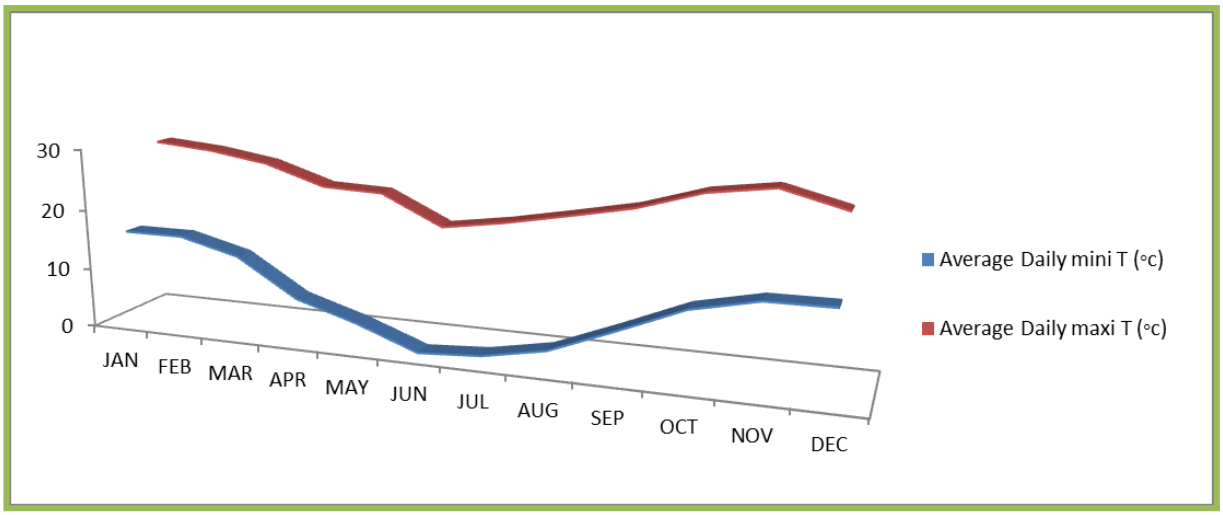


Figure 14: Average daily temperature (◦C) in 2013; data for station [0474680 9] Carletonville, West Wits Operation, measured at 08:00 (Data provided by SAWS)

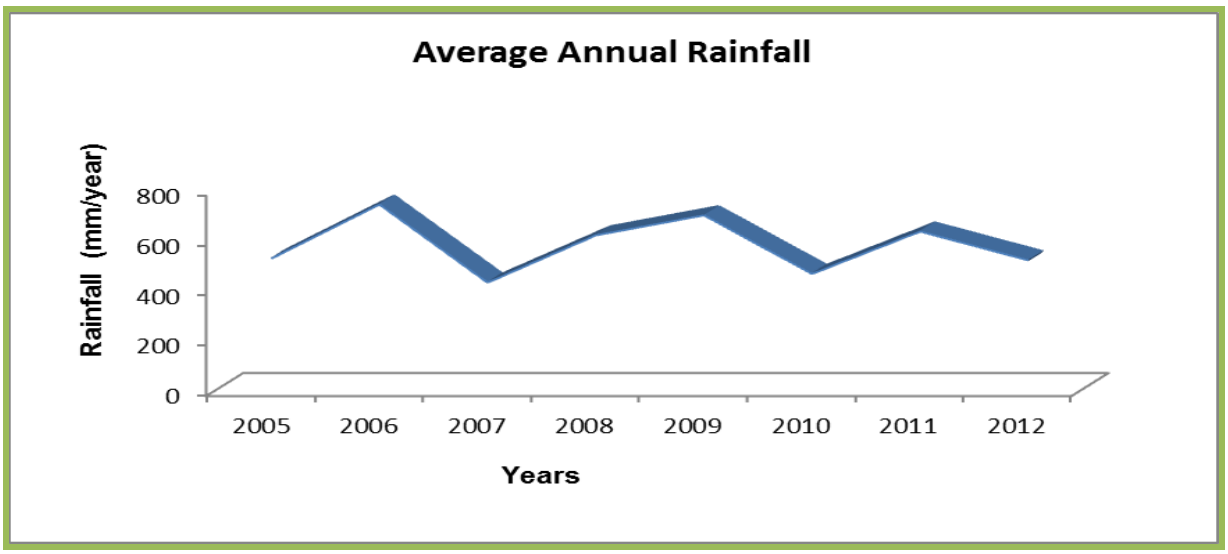


Figure 15: Average annual rainfall mm/year from 2005 to 2012 data for station [0474680 9] Carletonville, West Wits Operation, measured at 08:00 (Data provided by SAWS).

3.2 Field procedure and laboratory preparation

3.2.1 Field procedure

Technical sampling of sediments, plants and surface water samples was carried out at the Anglo Gold Ashanti West Wits Operations Varkenslaagte engineered wetland on the 27th to 28th of November 2013 and 4th of December 2014 during the rainfall season. Samples were consequently labeled as follows: date, sample number, wetland name, wetland paddock and coordinates (GPS). Anglo Ashanti general laborers were employed to assist in technical sampling to fast track the process in order to minimize variation in field sampling dates.

3.2.2 Water Sampling Procedure

The water levels in the reed beds were moderately high with a lot of lateral seepage on the reed beds banks (Figure 16). The *in situ* water quality parameter i.e. pH, dissolved oxygen and electrical conductivity, water hardness, nitrite and nitrate and redox potential measurements and water sampling were undertaken on the 4th of December 2014.

Figure 16 : Photograph shows moderately high water level and AMD lateral seepage

3.2.2.1 Vegetation sampling

Plants compartments of two species (leaves, roots and rhizomes) were randomly sampled within each of the 5 selected paddocks (WW1, WW2, WW4, WW6 and WW7), with 5 replicates for dry standing biomass, on the 27th of November 2013. The harvested *S. corymbosus and P. australis* plant species were separated into aboveground (leaves) and belowground (roots and rhizomes).

The reed beds were dug out with a garden spade up to a minimum depth of 10cm to a maximum of 30cm in order to harvest the belowground biomass rhizomes, roots and leaves. The reeds samples were washed with water from the paddocks and rinsed with deionized water following the same sampling protocol applied by Karunaratne *et al.* (2004).

The plants samples were rinsed on site in a big jar and bucket in order to minimize loss of essential hairy and fine roots (Figure 17) and the harvested samples were placed in packaging paper bags, which were accurately labeled according to the sites harvested (paddocks). The samples were stored in a refrigerator below 5^{o} C immediately after sampling.

Figure 17: *In situ* sampling and rinsing of plant species

3.2.2.2 *In situ* YSI Professional plus Calibration procedure

A two-point calibration of pH 4 and 7 were used. A 5-milliliter beaker was filled with the buffers and the sensors were rinsed with distilled water and calibrated with these pH buffers. The calibration pH values of the buffer solution were displayed and outputs for each buffer shown were accepted.

Nitrate was calibrated and the NO_3 millivolt calibration point was displayed. The mV span between 1 mg/l and 100 mg/l values was 90 to 130 mV. For electrical conductivity about 30 ml of 0.01M potassium chloride (KCI) solution was added to a glass beaker. The sensor was placed in the solution, gently moving the sensor up and down to remove any air bubbles that may have been trapped in the conductivity sensor. The temperature was recorded while the EC electrode was placed into the solution and electrical conductivity readings displayed were accepted.

3.2.2.3 *In situ* water quality measurements and sampling

To determine the total major elements and trace element concentrations, water samples were collected spatially at the selected uppermost 7 paddocks. Three water

samples were collected from each of the selected uppermost paddocks (i.e.at the inflow, middle and outflow at no particular depth, but roughly in close proximity to the sediment core-sampling locality; Table 5). Additional water samples were collect at Mangaan Drive and Main Road Drive. A total of 17 water samples were collected.

Water samples were taken using 500ml acid washed plastic bottles. All the bottles were washed in a solution of 2 liters of distilled water containing 5 drops of nitric acid measured using a pipette and then vigorously rinsed with distilled water only. The sample bottles were allowed to dry and then sealed with their lids. When taking a water sample the lids were removed just before the bottles were vigorously rinsed with the water from the paddocks, at least five times and then completely submerged. The bottles were sealed while submerged, taking care to leave little to no air bubbles. Immediately on site the samples were placed in a mobile refrigerator.

The protocol was followed in order to minimize the volatilization and loss of Hg, known to be present in the reed beds (Lusiloa, 2012). Samples for total major elements and trace element concentration analyses were stored at 5°C during transportation and thereafter following laboratory procedure.

3.2.2.4 *In situ* stream water indicator paper / test strips analysis

The *in situ* water samples were measured across the entire wetland canal at the inflow, middle and outlet of the engineered paddocks for analyses of nitrate (NO_3^-), nitrite (NO_2^-), sulphate (SO_4^{2-}), sulfite (SO_3^{2-}), H_20 hardness and pH-Fix using indicator papers (Table 3) to identify which anions are most available in the samples and thus selecting those which will be subject to laboratory analysis.

Other water quality parameters measured *in situ* include Electrical Conductivity (EC), Dissolved Oxygen (DO), pH and Nitrate (NO_3-), using a portable YSI Professional Plus multiparameter water quality meter model 12C102662A. The YSI (logging) data sondes were dipped in the surface water for 60 seconds, the sondes measured multiple parameters i.e. EC, DO, pH. The handheld logger/display of YSI displayed the data and it was recorded.

MACHEREY-NAGEL GmbH & Co. in Germany is the international manufacturer and distributor of the MACHEREY-NAGEL test strips brand. Indicator papers with varying ranges (Table 3) were used to measure ions in the wetland via the colour change range. The indictor papers were dipped in the surface water of the wetland and then

removed for comparison against a colour key code (Figure 18). This attempt for using the test strips was to assess their utility based on analytical cost savings.

Table 2 : The pH colour change range of the pH indicator paper

pH	<3	4	5	6	7	8	9	>10
Colour								
	Very Acidic	Acidic		Neutral		Basic	Very Basic	

Table 3 : The test strips used

Test strip	Range
Nitrate	10 – 500 mg/l NO_3^-
Nitrite	1 – 80 mg/l NO_2^-
Sulfate	200 – 1600 mg/l SO_4^{2-}
Sulfite	10 – 100 mg/l SO_3^{2-}
Water Hardness	0^od – 25^od H_2O
pH-Fix	0 – 14 PT

Figure 18: Shows the colour change range of the indicator paper

Table 4: *In situ* measurements of water quality parameters of the entire wetland paddocks

***(In situ)* measurements of water quality parameters (YSI)**					
pH	EC (µS.cm^{-1})	DO (mg.l^{-1})	NO_3^- (mg.l^{-1})	E*h* (mV)	
***(In situ)* measurements of water quality parameters (Indicators paper)**					
SO_4^{2-} (mg.l^{1})	NO_2^-(mg.l^{-1})	NO_3^-(mg.l^{-1})	NO_2^-(mg.l^{-1})	(SO_3^{2-})	pH-Fix

3.2.2.5 Sediment samples

Sediment samples were obtained in the five selected paddocks and were collected using core tubes, installed as deep as possible to a maximum of 30 cm, depending on the depth reached by each of the cores (Figure 19). The comparison of sediment depth profile data will give information about enrichment or depletion processes between sediments layers. In each of the five uppermost paddocks, three replicated core sediment samples were collected from the inflow, middle and outlet areas (Figure 19). A diameter of 15 cm was measured and PVC (Polyvinyl chloride) pipe was inserted into the wetland sediment

using a hammer and a plank. The cores were pulled out using a long steel bar inserted through two holes on the top of the core tubes and wrapped with plastic bags to prevent the sediments from falling out of the cores and to prevent oxidation reactions. The sediment samples were stored in the refrigerator (5°C) immediately after field sampling before preparation and analysis.

Table 5: The sediments sample ID Description and GPS

Paddock ID	Co-Ordinates	Distance between paddocks (m)	Accuracy (m)
WW1.1	S 26°26'49.0''	3.8 (Inflow to middle)	3
	E 27°22'16.6''		
WW1.2	S 26°22'49.0''	(Middle)	3
	E 27°22'16.2''		
WW1.3	S 26°25'49.1''	3.82 (Middle to Outflow)	3
	E 27°22'16.2''		
WW2.1	S 26°22'48.9''	5.8 (Inflow to middle)	3
	E 27°22'15.9''		
WW2.2	S 26°25'49.7''	(Middle)	3
	E 27°22'15.7''		
WW2.3	S 26°25'49.9''	5.25 (Middle to Outflow)	3
	E27°22'15.6''		
WW4.1	S 26°25' 51.4''	11.60 (Inflow to middle)	3
	E 27°22' 14.4''		
WW4.2	S 26°25' 51.7''	(Middle)	3
	E 27°22' 14.1''		
WW4.3	S 26°25' 52.2''	11.40 (Middle to	3

	E 27°22’ 14.0’’	Outflow)	
WW6.1	S 26°25’53.8’’ E 27°22’ 13.2’’	4.7 (Inflow to middle)	4
WW6.2	S 26°25’54.0’’ E 27°22’13.3’’	(Middle)	3
WW6.3	S 26°25’ 54.1’’ E 27°22’ 13.1’’	6 (Middle to Outflow)	4
WW7.1	S 26°25’ 54.7’’ E 27°22’ 12.7’’	5.7 (Inflow to middle)	3
WW7.2	S 26°25’ 54.8’’ E 27°22’12.7’’	(Middle)	3
WW7.3	S 26°25’ 55.0’’ E 27°22’ 12.6’’	4.5 (Middle to Outflow)	3

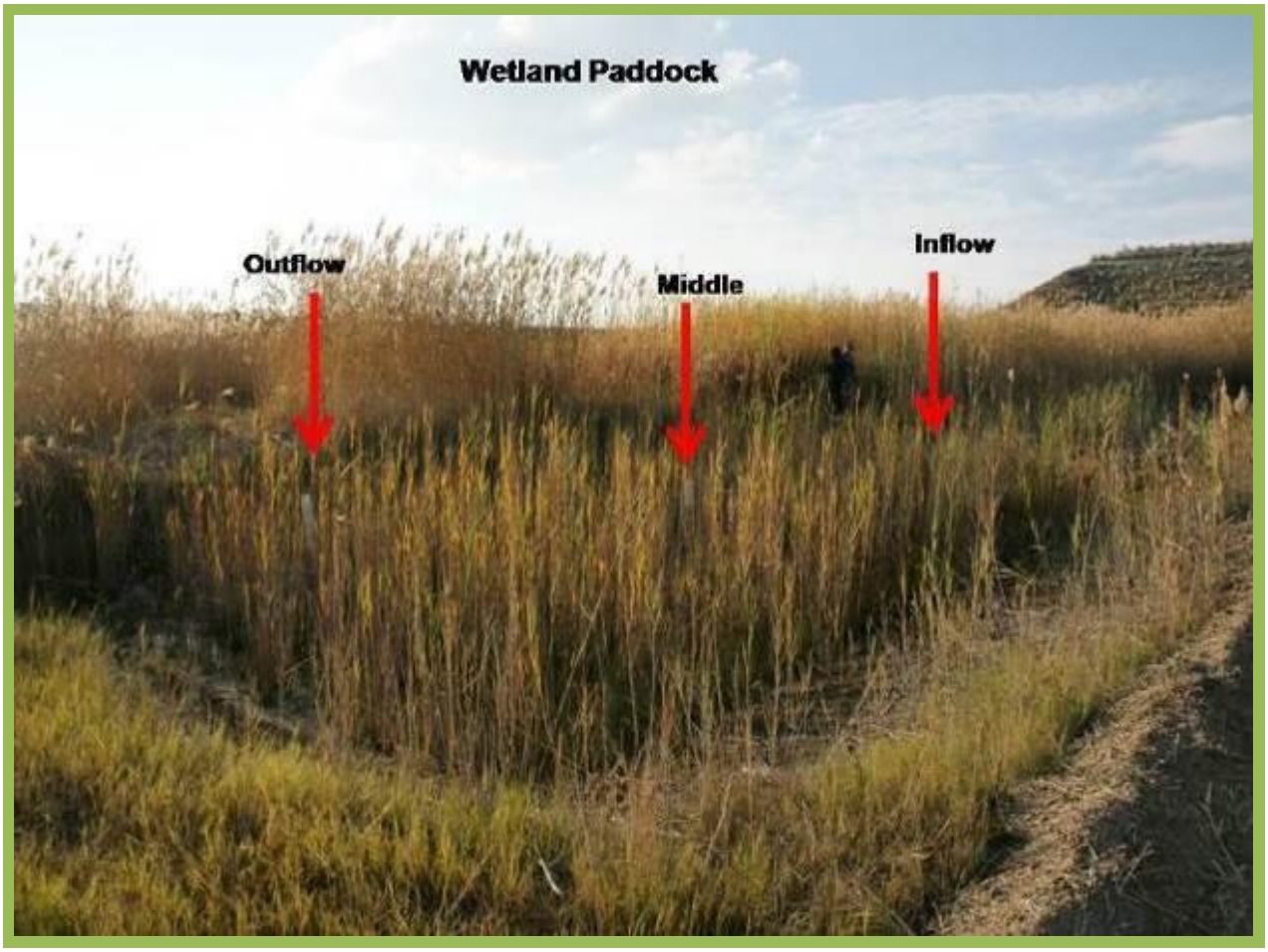


Figure 19: Shows representation of spatial sampling of sediments using core tube installation method

3.2.3 Laboratory procedure (Analysis)

Sampling preparation was carried out at the University of the Witwatersrand EEPP laboratory, Johannesburg. After preparation the samples were then sent to the Department of Chemistry Laboratory for trace element and major element analyses.

3.2.3.1 Vegetation sample preparation and laboratory analysis

The samples were vigorously washed in the laboratory with deionized water and stored in a refrigerator below 5^{o} C following a method applied by Hoeing (2001). The samples were separated into above ground shoots (dead + live) and below ground biomass. The roots were separated from the rhizome and also large roots were peeled. The samples were rinsed ten times in distilled water vigorously with rubbing of leaves and scrubbing of roots to remove microscopic soil or tailings dust particles in plant samples (Figure 20). The samples were shaken and patted dry on a paper towel, and the fresh mass was weighed.

Figure 20 : Example of rinsed plant roots

The shoot and finer roots thinner than 5mm were chopped into small (1 cm) pieces using ceramic knifes, these pieces were well mixed into bags (Figure 21). The chopping method using a ceramic knife was used in preference to the Wiley mill grinding technique to minimized the tendency of substances to vaporize, i.e. volatile substances such as NH_3, CO_2, CO, CH_4, K, Na, Pb and Hg which could be lost using the higher temperatures of the Wiley mill.

Figure 21 : A chopped representative sample of *S. corymbosus* rhizomes

The *S. corymbosus* and *P. australis* belowground and aboveground biomass samples were separated. The whole plant belowground (rhizomes and roots) and aboveground (shoots-leaves and stems) fresh mass was weighed using a scale or balance. After chopping a representative sample of 20g of fresh mass was sub-sampled and placed in the paper bags (Figure 21) and complete samples with a mass of less than 20 g were weighed for fresh mass as well and placed in the paper bags.

These sub-samples were weighed for fresh mass and subject to freeze-drying for two to three days. The freeze-dried sub-samples were weighed again for dry mass. The homogenized plant samples were milled using the Fritsch Pulverisette 6 mill into pulverized powder.The freeze-dried fine power samples were subject to elemental analysis using X-Ray Fluorescence at the University of the Witwatersrand Chemistry Department.

All the remaining plant samples from the wetland paddocks were placed in paper bags and freeze-dried. These freeze-dried samples were weighed and stored in a cold room for any further analysis of elements or studies.

3.2.3.2 Sediment samples

The cores were cut into two equal halves and the sediment was subsampled at different depths from the topsoil to the lower soil (subsoil) i.e. from intervals 0-2 cm, 2-10cm, and 10-30cm depending on the depth of sediments harvested in each core using a plastic ruler (Figure 22). Due to financial constraints only 3 replicates were considered for analyses from each interval core depth. The sediment sub-samples were placed in plastic bottles and sealed in plastics bags, which were labeled according to depth intervals and sampling sites.

Figure 22: Example of core sediment intervals profile

The acidity, redox potential and conductivity determinations were measured in each depth interval of the sediment cores. All measurements were carried out in 2:1 sample suspensions (100 mL liquid/50 g sediments) after vigorously mixing sediment samples with deionized water. The two pairs of pH electrodes (i.e. glass and calomel electrodes) were calibrated against two buffer solutions pH 4.0 and pH 7.0. Sediment suspensions were analysed for pH, EC and redox potential with a Thermo Orin Scientific multiparameter Sn GO1658 instrument.

The subsamples were measured for redox, pH and electrical conductivity. Another sediment subsample of +/-30g was extracted and preserved inside a plastic bag and freeze-dried for 2 days. The freeze dried samples were milled using a mortar and pestle.

After 48 hours of freeze drying, samples were then ground using an agate mill into a homogenous fine powder. The samples were subject to X-Ray fluorescence (XRF) analyses for major elements, trace elements and nitrogen and S in the Chemistry Department, University of the Witwatersrand.

3.2.3.3 Core sediment bulk density

Seven core sediment samples were collected from seven uppermost wetland paddocks to estimate the bulk density. The measurements undertaken in the laboratory to calculate the bulk density of the sediment are as follows:

1. Inner diameter of the sediment core;
2. Height of the sediment core;
3. Total mass of sediment core (dry);
4. The sediment core volume

The volume of the soil was calculated by measuring the height of the core plastic tube with a tape measure to the nearest mm. The diameter of the core was measured with a ruler and halved to get the radius. The core volume was calculated using the equation below:

$$\text{Height core sediment (cm)} * \pi * \text{r(cm)}^2 = \text{cm}^3$$

The formula used to calculate the bulk density is given by:

$$\frac{Core\ Sediment\ Dry\ Weight\ (g)}{Core\ Sediment\ Volume\ (cm^3)} = g/cm^3$$

The masses of PVC pipe and plastic bags were also subtracted to obtain the actual dry mass value. The inner diameter of the plastic pipe used for coring was 15cm; hence the radius of the sediment cores was 7.5cm.

To assess the mean concentration of the whole paddocks, a depth weighting function was applied. To know the actual number sediment cores to the whole paddock, the area of paddock was divided by sediment core area as guided by the equations below.

$$\text{Area of the paddock } (m^2) = \text{Length} * \text{Width}$$

$$\text{Sediment cores area } (m^2) = \frac{\pi 7.5^2}{10000}$$

$$\text{Total number of sediment cores per paddock} = \frac{\text{Area of the paddock } (m^2)}{\text{Sediment cores area } (m^2)}$$

The total mass pool accumulation was estimated by multiplying the measured mass pool per core by the equivalent number of sediment cores per paddock. The results of these calculations are presented in section 4.4.

3.2.3.4 *Ex situ* water samples

Water samples were immediately filtered with a 0.45µm membrane filter using a Sigma-Aldrich vacuum filtration system with a Nalgene hand pump. Prior to the filtering process of each sample the filter rig was rinsed in solution of 2 liters of distilled water containing 5 drops of nitric acid measured using a pipette and then vigorously rinsed with distilled water only. The filtered samples were divided in half and decanted into plastic bottles washed as above. For sample preservation and metal cation analyses to avoid adsorption losses (Hoenig, 2001), one half was acidified with a drop of nitric acid, measured using a pipette (a single drop per sample bottle) for the metal cation analysis by ICP-MS.

The other half was not acidified for the anion analysis by spectrophotometry and chromatography. The halves were placed in the deep freezer, the lids were left very loose and when frozen the lids were tightened up. The filter papers used were stored together with the water samples in the deep freezer.

These samples were sent to the Chemistry Department of the University of the Witwatersrand for analysis. Major anion analysis, Cl, nitrate, nitrite, ammonium phosphate and sulphate ions were measured using Ion Chromatography (IC). Total concentration of all major elements was determined using ICP-OES and trace elements were determined using ICP-MS.

3.2.4 Mass allocation of wetland compartments

3.2.4.1 Sediment samples

The overall mass allocation for each depth interval was normalized to the wetland paddock surface area and volume. The total mass of the interval layer for all the wetland paddocks combined was determined along with the average for each wetland paddock. The average was determined in kg and g, and then the total load of mass (elements) in the interval layer of sediments for each paddock was also determined.

3.2.4.2 Plant samples

The overall mass allocation of each plant was normalized to the wetland paddock biomass which was estimated by calculating the number of individual plants per species within a paddock using photographs taking during field sampling (Figure 7). The total numbers of individual plants were also verified using the Google earth image. The total load in dry mass of element in both plants species was estimated and total load in dry mass of the plant species compartment was calculated (i.e. leaves, roots and rhizomes). To estimate the total element mass pool in the plant species, the dry mass per plant analysed was multiplied by the total number of plants of that species per paddock, and then multiplied by the elemental mass concentration. The results of elemental mass pool analysis in the two plant species are presented in section 4.2.9.

3.2.5 Statistical analysis

The statistical analysis was based on the secondary and primary raw data. The mean, median, mode, standard deviation, and standard error were calculated with Formula Function in Excel for all water samples.

Firstly, plant elemental data were pooled based on the different sites (e.g. paddock ww1 to paddock ww7) i.e. to observe trends and patterns. Secondly comparisons were based on plant compartments (i.e. shoots, roots and rhizomes) to get an indication of the elemental storage in the plant compartments in both vegetation, and compared using single factor ANOVA and followed by a t-Test: Paired Sample for Means. Furthermore, the plants *S. corymbosus and P. australis* were compared to each other using One Way ANOVA.

Secondly pooled sediment core data from the paddock sites were also used to compare elemental concentrations of sediments against depth and by paddocks using single factor ANOVA and followed by a t-Test: Paired Sample for Means. All the tests were done using excel data analysis tool for scientific data analysis.

3.2.5.1 Summary of descriptive statistics for core sediments

The descriptive statistics for sediments in the paddocks were analyzed according to the following levels

- First level, the overall wetland paddocks: 3 replicate concentrations per paddock were aggregated to sediment core masses using bulk density to obtain one value per core. This provides the mass and weighted concentration for upscaling to each individual paddock.

- Secondly, masses were calculated for the sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. This provides the mean for the study site against depth of the uppermost Varkenslaagte Wetland paddocks with all measurements combined.

3.2.5.2 Summary of descriptive statistics for vegetation species

The descriptive statistics for *P. australis* were analysed as follows:

- The overall mass per paddock i.e. using mean of 4 replicate plants per paddock, upscaled to all plants of this species in the paddock. This provides the overall mass and weighted concentration for *P. australis* in each individual paddock for the whole plants.

- The overall for the plant compartments (mean of N=20 individual plants) across all wetland paddocks. This provides the mean concentration across the study site in plant compartments including paddock ww7.

The descriptive statistics data for *S. corymbosus* were analysed as follows:

- The overall mass per paddock i.e. using mean of 4 replicate plants per paddock, upscaled to all plants of this species in the paddock. This provides the overall mass and weighted concentration for *S. corymbosus* in each individual paddock for the whole plants.

- The overall for the plant compartments (mean of N=16 individual plants) across all wetland paddocks. This provides the mean concentration across the study site in plant compartments, excluding paddock ww7 this paddock did not have species *S. corymbosus*.

3.2.6 Data presentation

Numerous data were produced during this study, more predominantly from X-Ray fluorescence (XRF), ICP-MS scans, as well as *in situ* test strips and YSI data. The raw data were processed and presented in the form of figures and tables. The data presented in this chapter focus on the elements selected as having the greatest importance to West Wits AMD; these elements are Zn, U, S, Fe, Cu, Cr, Cl and Ca. This does however not suggest that these elements are of greater concern or that the other elements are less important. The actual data tables and graphs for all elements of interest can be found in the appendixes.

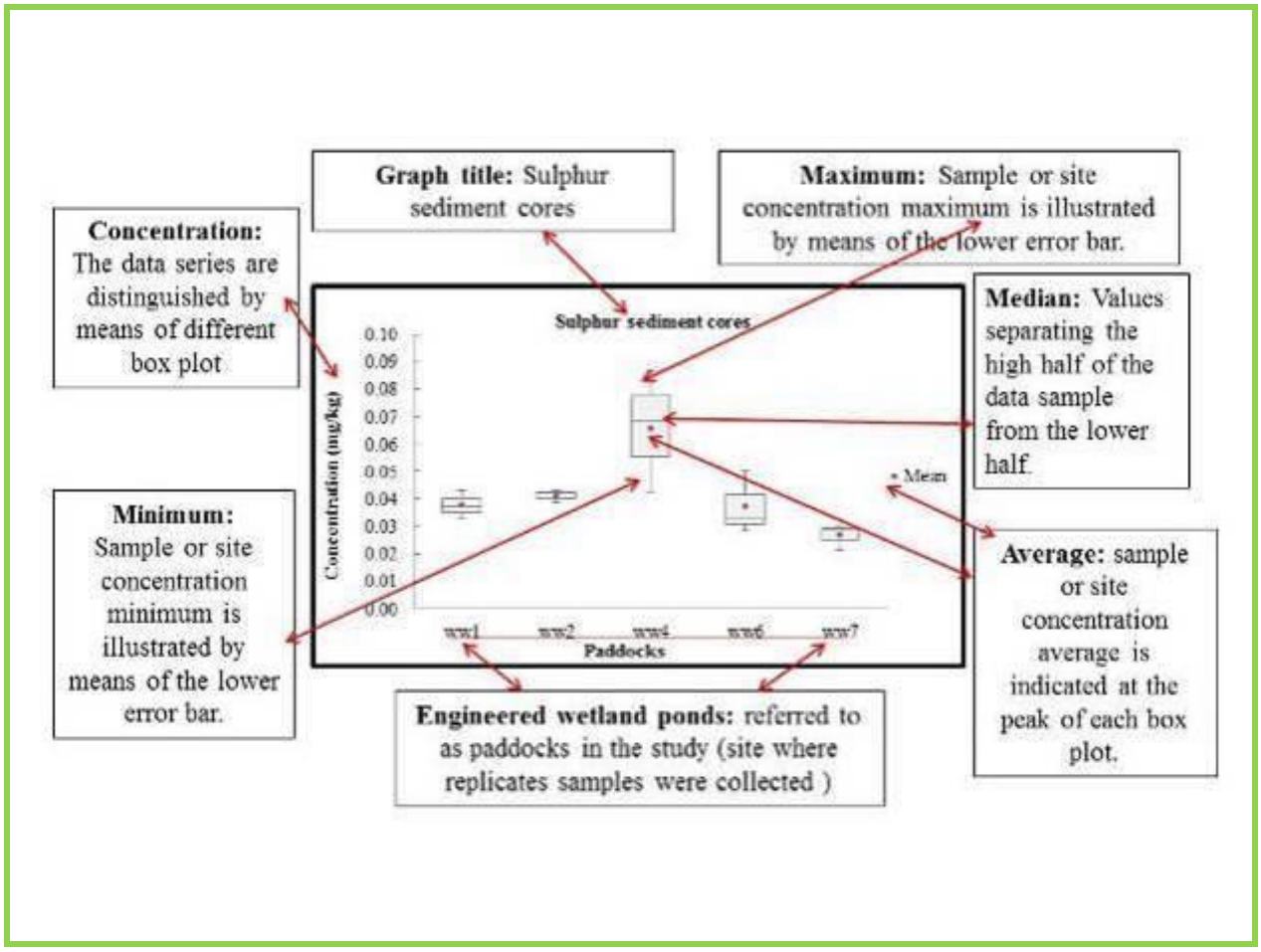


Figure 23: An example of the results that are illustrated by means of box plots

Box and whisker charts (box plots) are commonly used in the display of statistical analysis. In this study the customised box plot charts were created using the stacked bar or column charts and error bars (Figure 23). The customary error bars were calculated as the mean plus/minus one standard deviation. In less complex form, the box plot diagram shows the range from the first to the third quartile, and the median divides this large box, the interquartile range, into two boxes, for the second and third quartile. The whisker spans

the first quartile, from the second quartile box down to the minimum, and the fourth quartile, from the third quartile box up to the maximum.

The paddock site codes used were ww1, ww2, ww4, ww6 and ww7 which represent the sites where samples were collected. In some instances shoots, roots, and rhizomes were used representing plant compartments and sediment intervals were used to represent sediment substrata. The statistical description of the test strip and YSI data were presented only in the form of linear graphs and tables in appendix E as the results showed the chemical parameters tested were below detection limits.

The *in-situ* data are presented as linear plots. In some instances, the mean is plotted on the line graph showing the trends along the paddocks. Box plots are used to display the element mean concentrations and ranges. The raw data from all water, sediments and plants can be found in appendixes A to D. The total calculated load of metal pollutant is illustrated in the form of tables and charts. The aforementioned are briefly interpreted in the results section.

3.2.7 Calculation of the mass pool in a set of paddock compartments

The major part of the study was to calculate the element mass concentration and mass in sets of wetland paddock compartments in both vegetation species and sediments cores. The calculation of elemental mass in vegetation species at the lowest level involved the estimate of the sample concentration and mass in leaves, rhizomes and fine roots per vegetation species of the uppermost wetland paddocks. To convert the vegetation concentrations of an element to grams of dry mass, the element concentration values were multiplied by the dry mass of each vegetation compartment to obtain the actual element mass. The calculation of this was performed using Microsoft excel 2010 to determine the actual gram (g) dry mass allocation for vegetation compartments.

The estimation of mass pool at the highest levels in both vegetation species is the overall value for the uppermost paddocks combined above and belowground biomass. The element concentration was multiplied by the dry mass of each wetland vegetation compartment and summed to estimate the actual element mass pool size in both vegetation species per paddock.

Sediment dry weight and estimated volume were used to determine approximate bulk density of the sediments.

The bulk density calculated was used to express the total mass pool per paddock. Core sediment concentration of each element expressed in milligrams per gram dry mass can easily be converted into the total mass of each compartment when using the dry mass of cores of known surface area and volume. Brady (1990) has applied this method in soil studies.

3.2.8 Secondary data

Secondary data were used to compare water quality values in different seasons; the data were obtained from previous studies done by Joubert (2013) and Omo-Okoro (2015).

4 CHAPTER FOUR

4.1 Results and interpretation of *in situ* data analysis

The following section presents the results of in *situ* data analysis of this study compared to previous studies of water samples along the Varkenslaagte canal. Water samples for this study were collected on the 20th December 2013 while other studies by Joubert were collected on 31st May and 26th July 2013.

4.1.1 Dissolved Oxygen (DO)

This study showed lower DO values than the study by Joubert (2013). There was no trend or pattern observed (Figure 24).

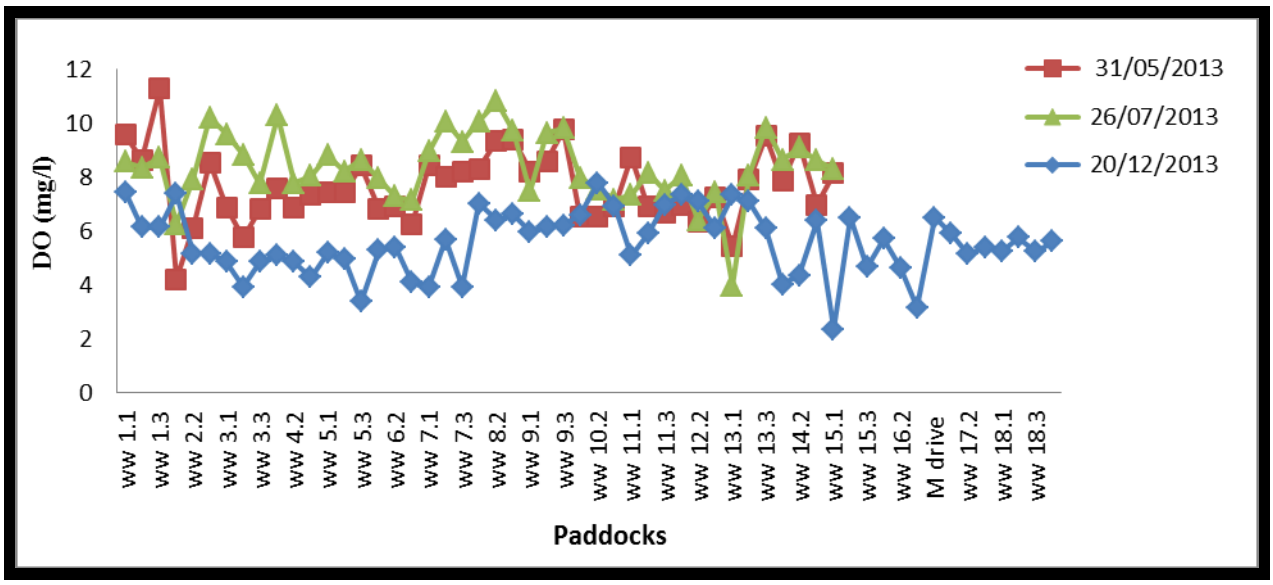


Figure 24 : *In situ* stream water Dissolved Oxygen (DO) values along the engineered wetland in different seasons

4.1.2 pH

This study found higher pH values from paddock ww1 to paddock ww10 than the study by Joubert (2013). These pH values ranged from 5.82 to 7.48 and low values occur from paddocks ww10 to ww12. Joubert (2013) pH data showed a minimum value of 3.65 and a maximum of 7.62. The pH values from the current study showed a slight decreasing trend down the stream to paddock ww15 while Joubert (2013) found lower pH values in paddocks ww2 to ww9, which are not apparent to this study (Figure 25).

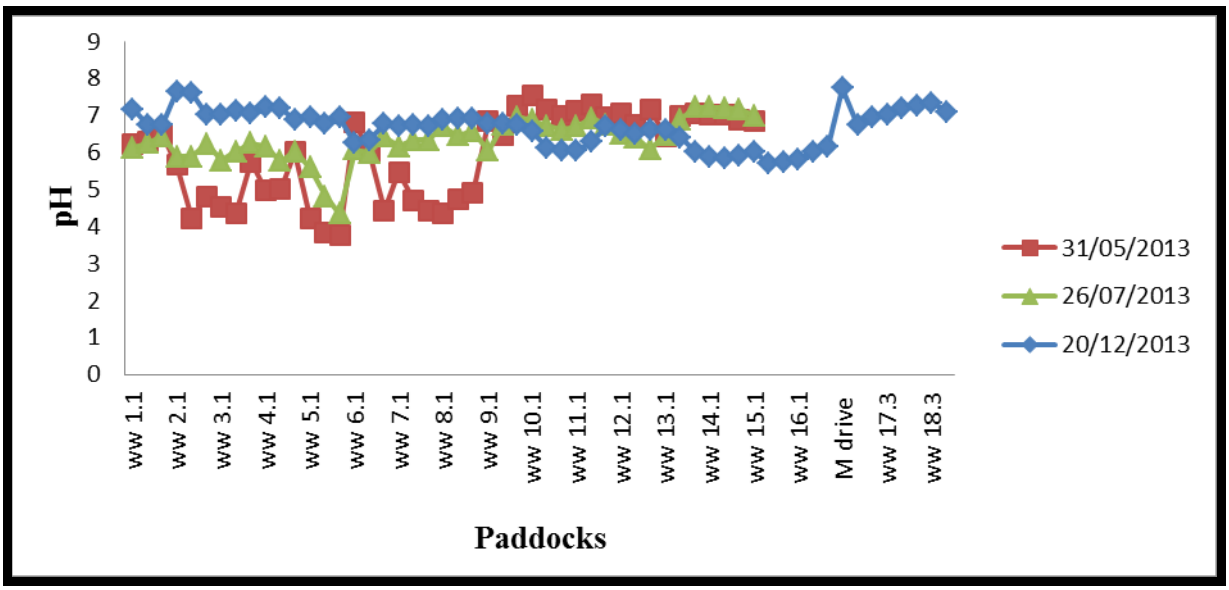


Figure 25: *In situ* stream water pH values along the engineered wetland in different seasons.

4.1.3 pH probe and pH Fix *(0-14PT)*

The test strips showed a drop in pH-Fix values in paddocks ww8, ww12, Mangaan Drive and paddock ww16 after the bridge. The pH probe and pH-Fix values obtained from the paddocks were found to have a range of 4 to 8 (Figure 26). The pH-Fix values had no trend in the lower values observed. The pH probe values showed a slight decreasing trend down the stream with lowest values observed in paddock ww15 but an increase thereafter.

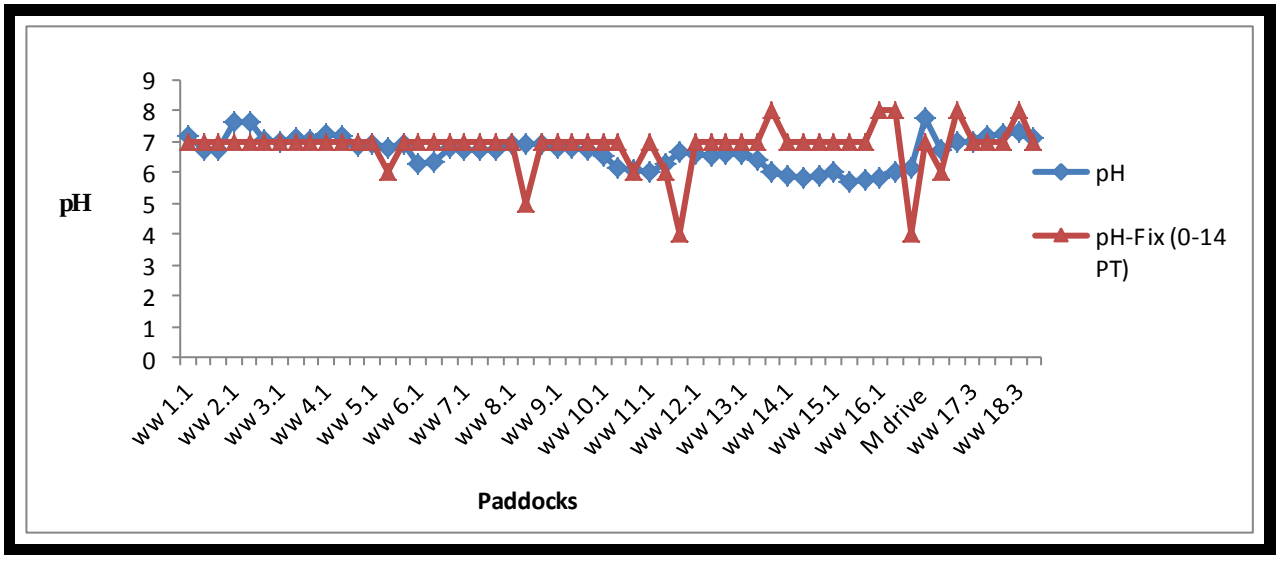


Figure 26: In situ stream water pH probe data and pH-Fix test strip data pattern along the engineered wetland paddocks.

4.1.4 Electrical Conductivity (EC)

This study found higher EC values than the study by Joubert (2013). In this study, EC values dropped below 1000 µs/cm at Mangaan Drive and towards the main road (Figure 27). This study showed decreasing EC values from paddock ww8 to paddock ww15 , which was also apparent to a lesser degree in the July data from Joubert (2013). Joubert (2013) July data show a similar pattern although the May data do not.

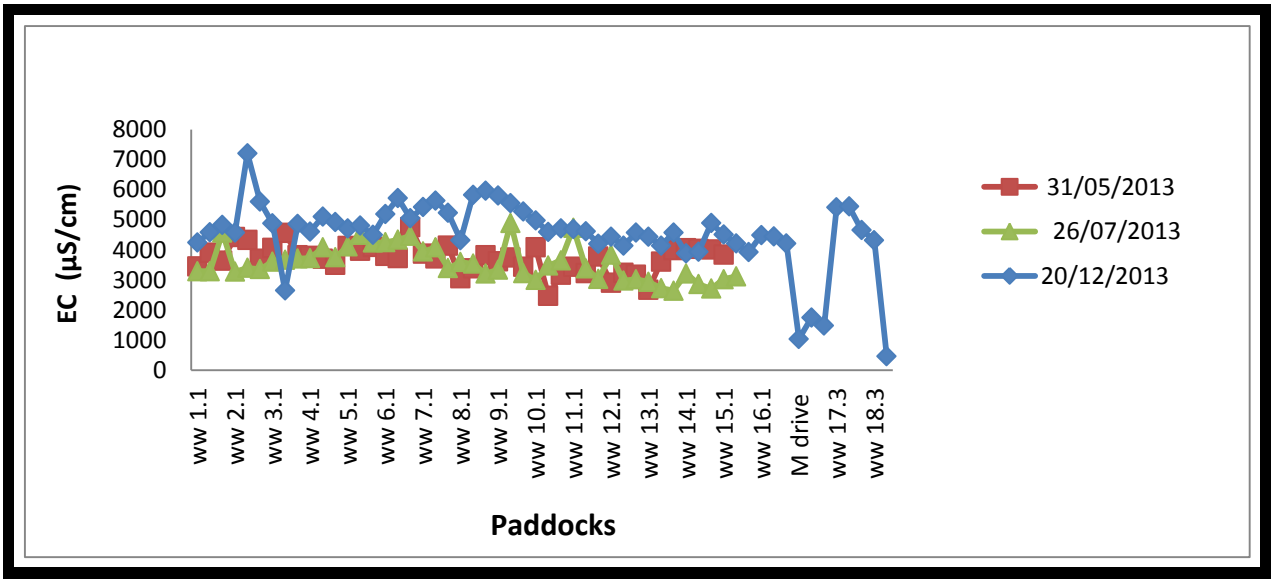


Figure 27: In situ stream water EC values along the engineered wetland paddocks in different seasons.

4.1.5 Redox Potential (Eh)

Eh values are lower then the previous studies by Joubert (2013). The values showed a decreasing trend along the stream in winter while there is a slight decrease in Eh in summer which peaks again further downstream after the Mangaan Drive (Figure 28).

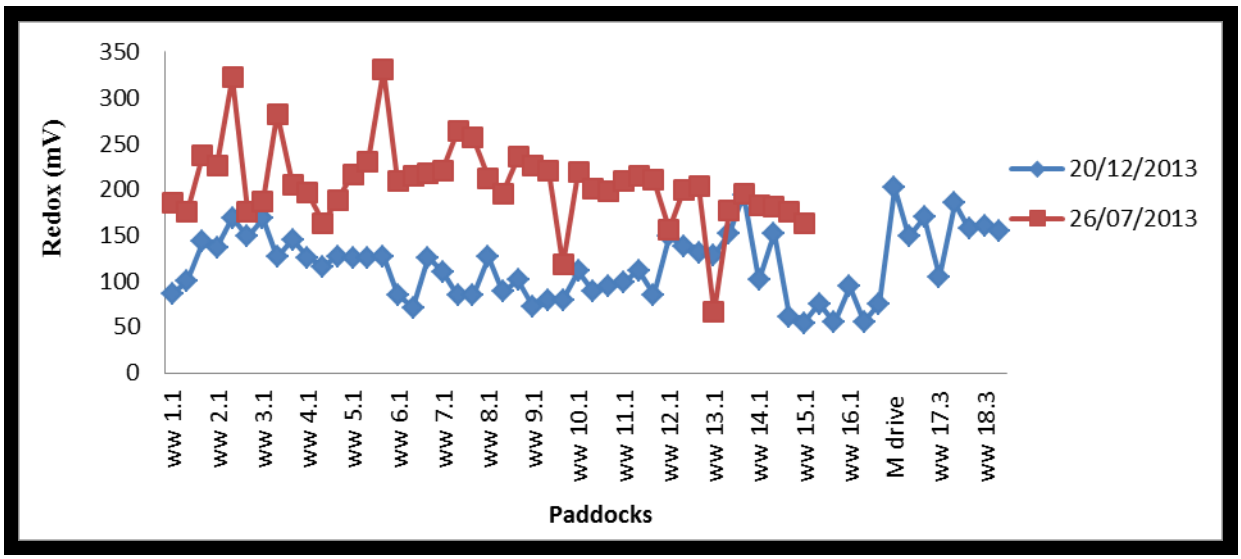


Figure 28: *In situ* stream water Redox potential values along the engineered wetland paddocks in different seasons.

4.1.6 Nitrate (NO_3^-) mg/l probe data and Nitrate (NO_3^-) test strips 10-500(mg/l)

The nitrate probe concentrations ranged from 0 to 1.0 mg/l while the nitrate test strip values ranged from 0 to 25 mg/l (Figure 29).

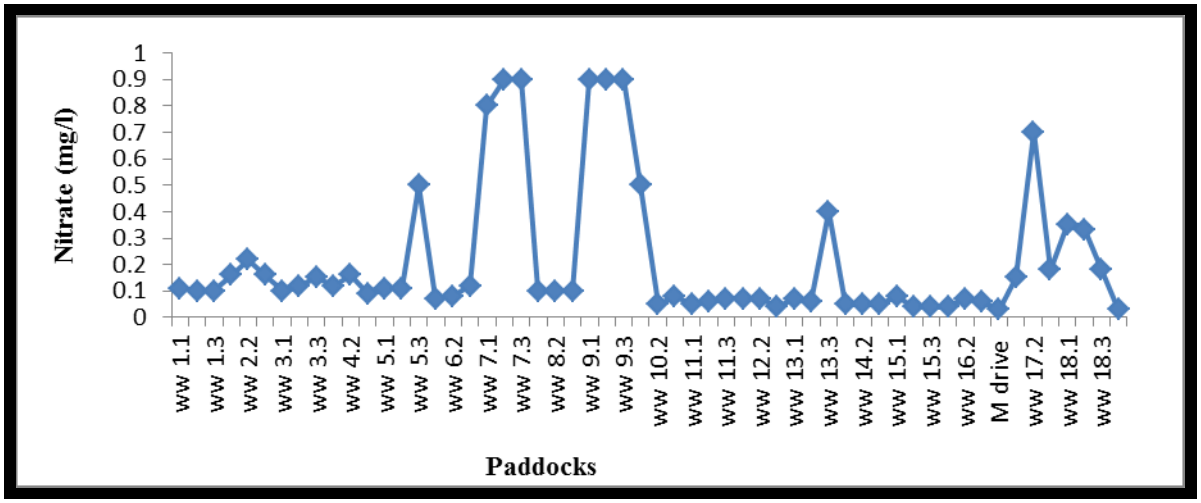


Figure 29 : *In situ* stream water nitrate probe values along the engineered wetland paddocks.

There is no clear trend or pattern observed in nitrate probe values with peaks in paddocks 7 and 9. The test strips however showed an apparent increase in the lower paddocks but much variability between adjacent paddocks (Figure 30).

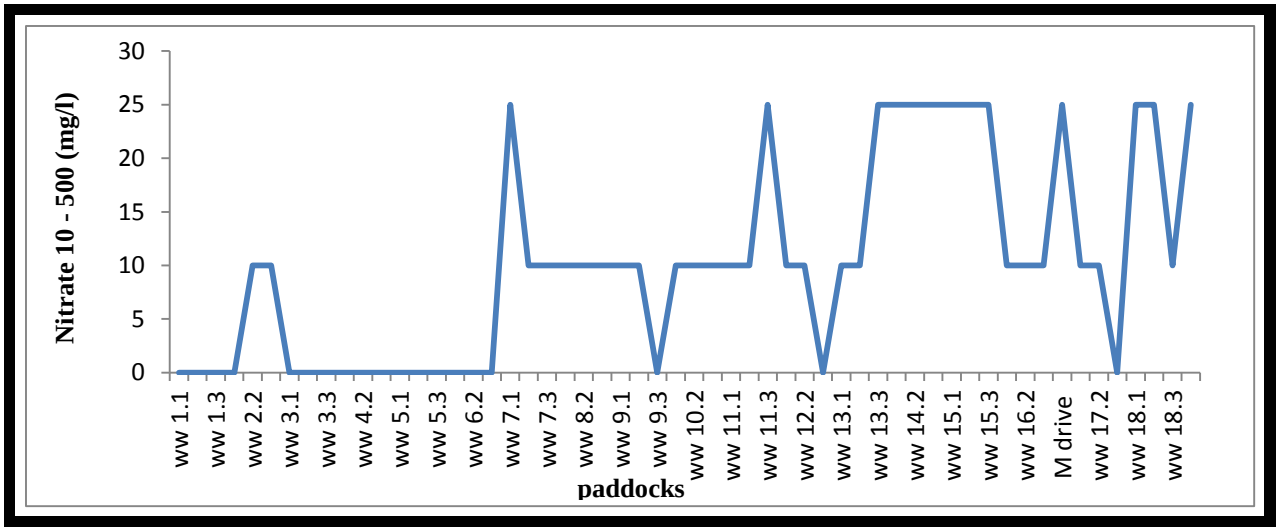


Figure 30: In situ stream water nitrate test strips values along the engineered wetland paddocks.

4.1.7 Nitrite (NO_2^-) Concentration

The test strips provide "categorical" data, in the range between 0 -5 mg/l. The concentration is zero at all paddocks down to ww13 except for peaks in paddocks ww7 and ww11 (Figure 31). Higher values were generally observed below paddock 13.

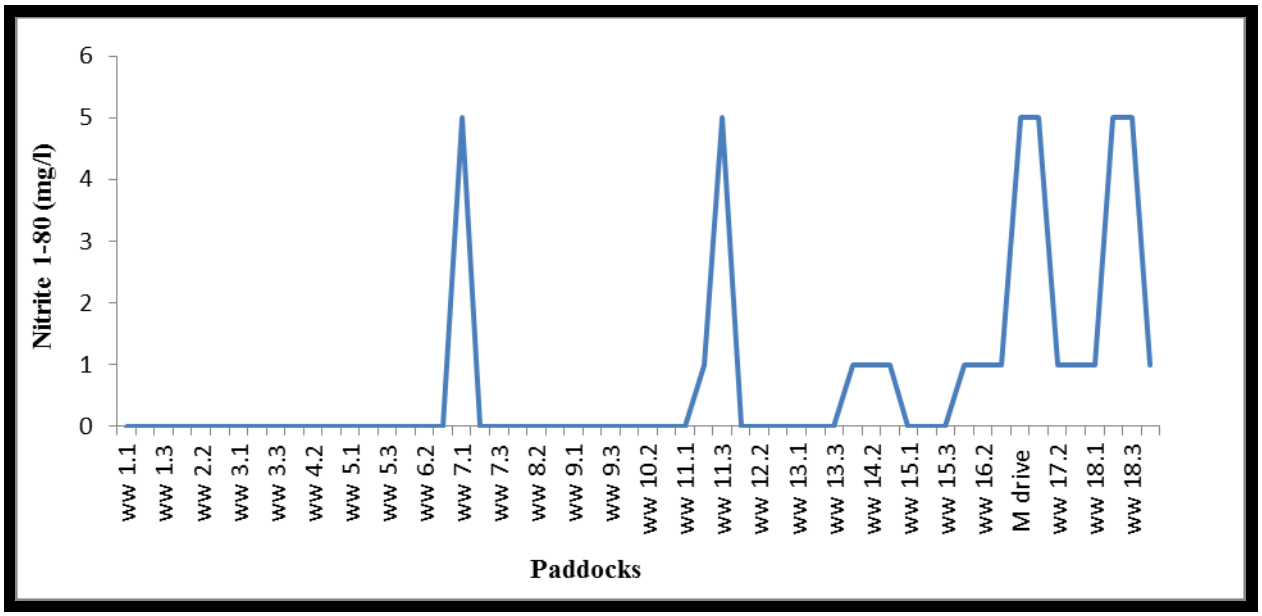


Figure 31: *In situ* stream water nitrite test strip data along the engineered wetland paddocks.

4.1.8 Sulphate (SO_4^{2-})

Sulphate values from paddock ww1.1 to ww7.3 of the uppermost wetland paddock had a consistent concentration at or above the maximum range of 1600 mg/l below

paddock ww8.1, the concentration fluctuated but showed a sharp decrease below Mangaan Drive. The highest values above the target water quality range were observed at the uppermost paddocks ww1 to ww7 and in paddocks ww9, ww12, ww14, and ww16 (Figure 32).

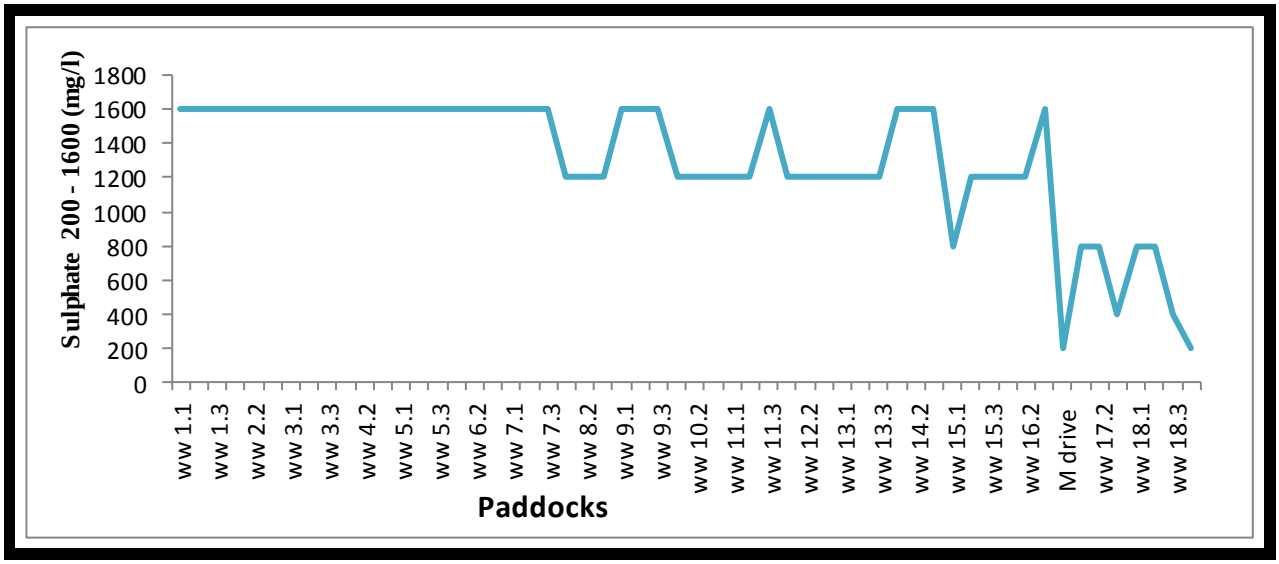


Figure 32: *In situ* stream water sulphate test strip data along the engineered wetland paddocks.

4.1.9 Sulfite (SO_3^{2-})

The stream water sulfite test strip concentration was below detection limits from paddocks ww1 to ww13. Detectable concentrations were found only in paddocks ww14, ww17 and ww18 after Mangaan Drive (Figure 33).

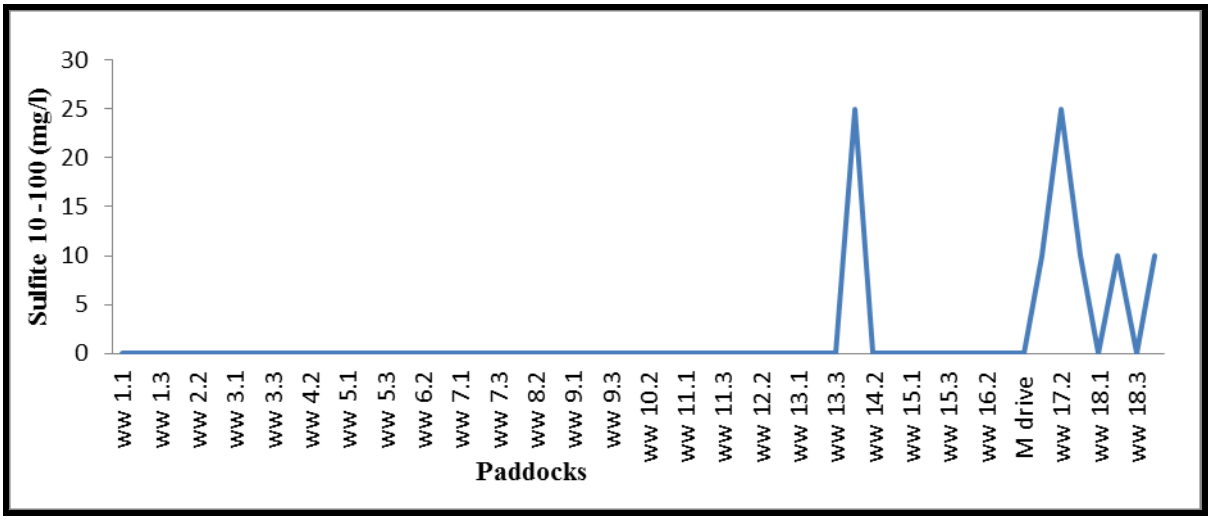


Figure 33: *In situ* stream water sulfite (SO_3^{2-}) test strip data along the engineered wetland paddocks.

4.1.10 H_20 Hardness

In situ stream water H_20 hardness test strips data along the engineered wetland paddocks provide "categorical" data. The H_20 hardness concentrations were outside the detection range of the test strips throughout from paddock ww1 to paddock ww18.

4.2 Sediments analysis of Eh, pH and EC by paddock

4.2.1 Redox Potential (Eh)

The levels of redox potential in sediment cores varied between 248 mV to 379 mV. There was no clear pattern observed, but generally the lower values were found in paddock ww1 and higher values in paddocks 6 and 7(Figure 34).

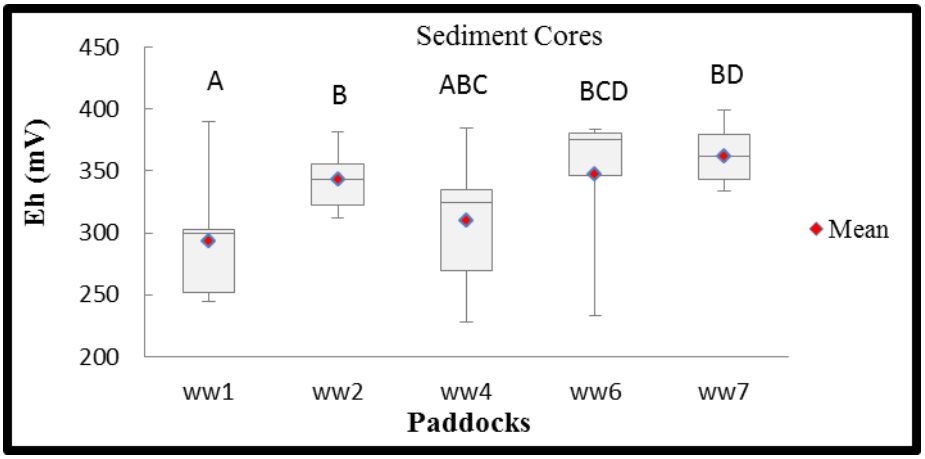


Figure 34: Mean Eh values in sediment cores across the uppermost paddocks (N = 3 sediment cores x N = 3 interval depths) in the Varkenslaagte canal. The paddocks marked with same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

4.2.2 pH

The range of pH in the sediment cores varied between 5.7 and 7.2. The mean values of pH by paddocks ranged from 6.2 to 7.0. There were no extreme pH values recorded (Figure 35). The pH showed a decreasing pattern down the stream with lower values observed in paddock ww6 and ww7. The pH of the 3 uppermost paddocks (ww1 and ww2) were significantly higher than the values at the 2 bottom paddocks (ww6 and ww7).

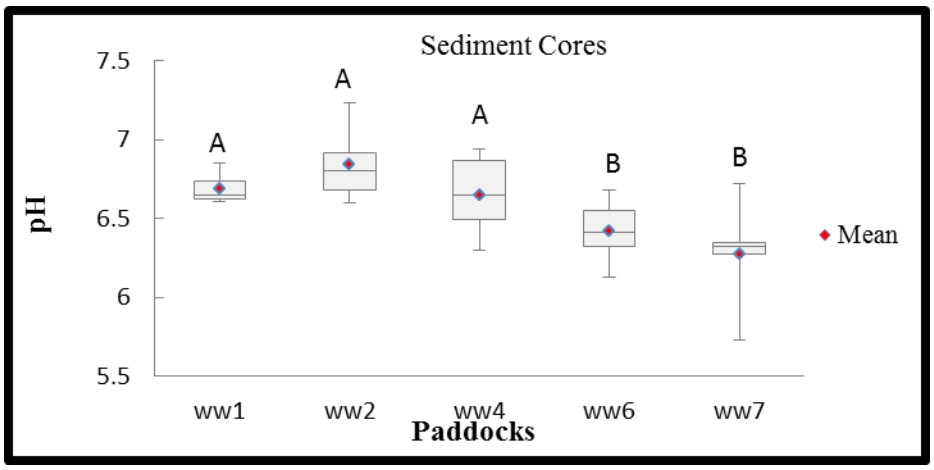


Figure 35: Mean pH values in sediment cores across the uppermost paddocks (N = 3 sediment cores x N = 3 interval depths) in Varkenslaagte canal. The paddocks marked with same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

4.2.3 Electrical Conductivity

The levels of electrical conductivity by paddocks varied between 317µs/cm and 1170 µs/cm in the sediment core samples (Figure 36). The mean values of EC by paddocks ranged from 432.3 µs/cm to and 637.0 µS/cm. There were no significant differences in EC (P value = 0.652, One Way ANOVA at $p \leq 0.05$).

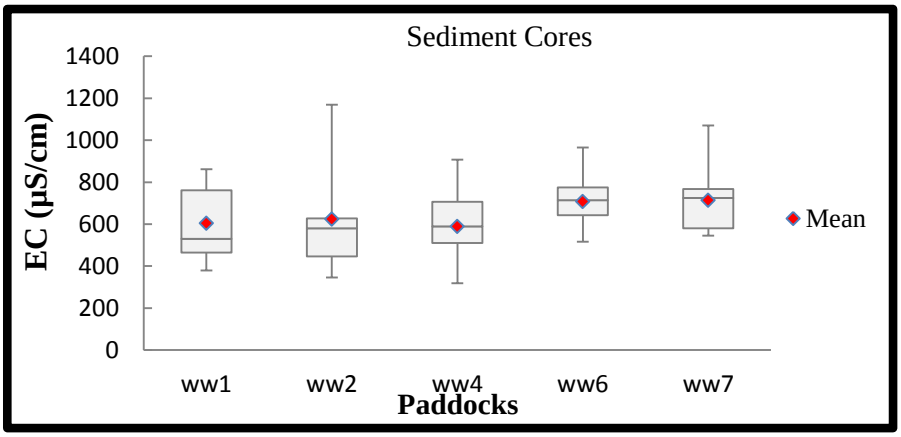


Figure 36: Mean values of EC in sediment cores across the uppermost paddocks (N = 3 sediment cores x N = 3 interval depths) in the Varkenslaagte canal. There was no significant difference in EC between paddocks (P value = 0.652), One Way ANOVA at $p \leq 0.05$.

4.3 Sediment analysis of Eh, pH and EC by interval depth

4.3.1 Redox potential

The redox potential showed no significant difference in concentration down the sediment profile across the uppermost wetland paddocks (Figure 37).

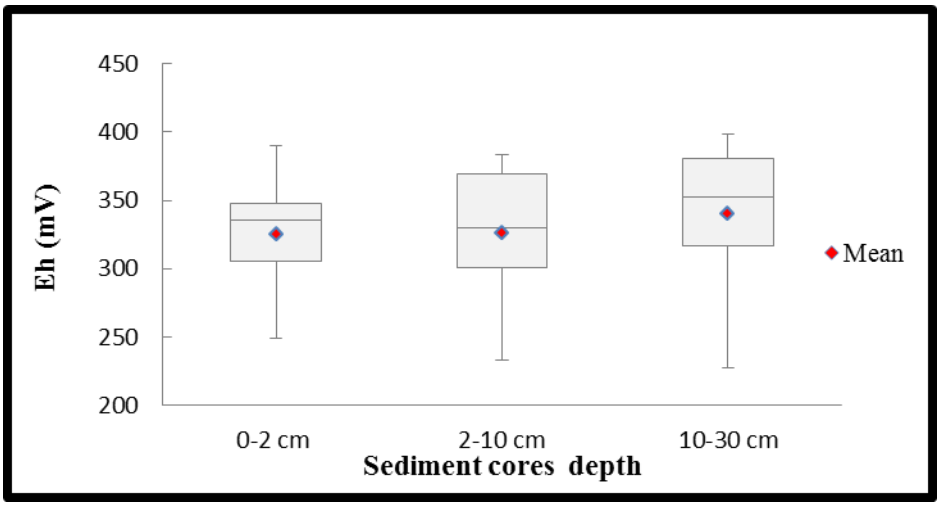


Figure 37: Mean values of Eh in sediment cores interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. There is no significant difference in Eh concentration between intervals (P value = 0.652), One Way ANOVA at $p \leq 0.05$.

4.3.2 pH

In general, there was no significant difference in the pH values with depth in all the sediment samples across the uppermost wetland paddocks (Figure 38). The pH values by interval depth ranged from 5.7 to 7.2.

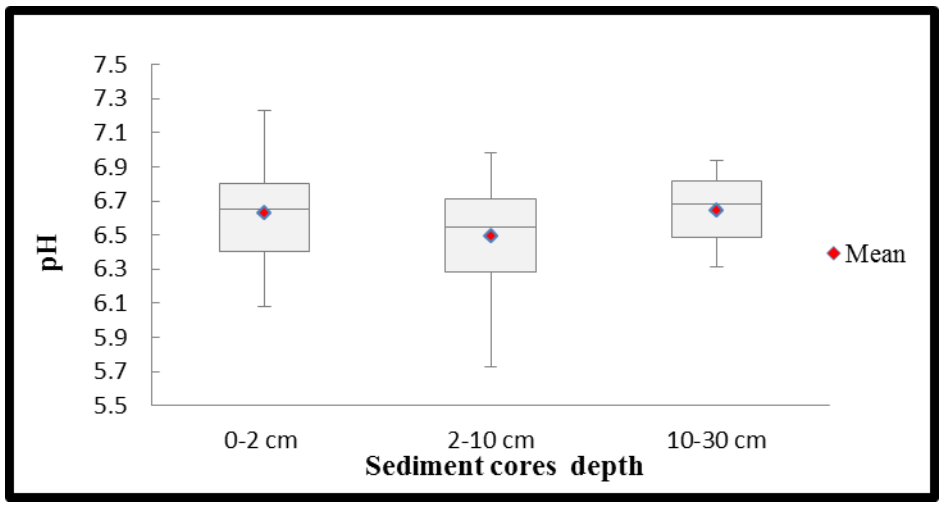


Figure 38: Mean values pH in sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. There is no significant difference in pH between intervals (P value = 0.277), One Way ANOVA at $p \leq 0.05$.

4.3.3 Electrical conductivity

EC showed an apparent decrease with depth in sediment cores but differences were only significant between the uppermost (0-2cm) and lower (10-30cm) intervals (Figure 39).

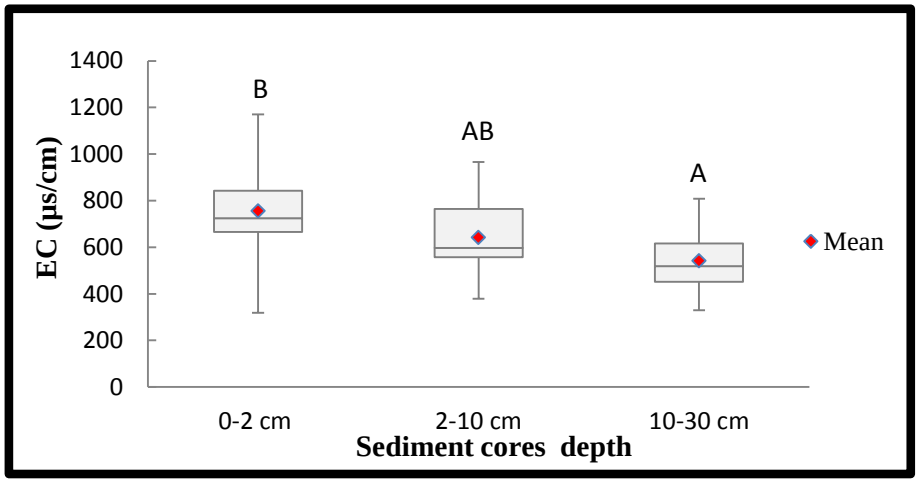


Figure 39: Mean EC in sediment cores depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. Those marked with the same letter do not differ significantly from each other after pairwise comparison One Way ANOVA, at $p \leq 0.05$.

4.4 Plants and sediments weighting function

This section of results presents the calculated mass pool of macronutrients, micronutrients, and non-essential elements and quantifies their distribution in the wetland compartments. As described in chapter 3 this section provides element mass allocation in a set of paddock compartments. The calculation was performed using Microsoft Office Excel 2010 to calculate the total sum of element mass pools in a set of wetland paddocks i.e. sediment core intervals and vegetation species.

The total number of plants per paddock was calculated using the area photographs and dated Google earth images to estimate the number of individual plants per paddock and to present mean concentrations per plant, a weighting function was applied using the mass of each compartment. For sediment cores, mean concentrations for the whole sediment core were weighted by depth interval (Table 6), assuming a constant bulk density down the core in the whole paddock to 30cm. The table below presents the measurements undertaken in the laboratory to calculate the bulk density of the sediment (Table 7). The bulk density was given in g/cm^3. However, the mass pool was converted so that the data will be presented in mg in section 4.8.

Table 6: Interval depth weighting function

Sediments core depth interval	Depth weighing function
0-2 (cm)	2cm
2 -10 (cm)	8 cm
10 -30 (cm)	20 cm

Table 7: Sediment core bulk density

Paddock	Total Dry Mass (kg)	Diameter (cm)	Radius	Height core sediments (cm)	Cores volume (cm3)	Bulk Density (g/cm^3)
			R	H	$h * \pi * r^2$	Dry mass (g)/ Core volume (cm^3)
WW 1	1.76	15	7.5	15	2650.72	0.66
WW 2	2.57	15	7.5	20	3534.29	0.73
WW 4	1.09	15	7.5	10	1767.15	0.62
WW 6	1.60	15	7.5	20	3534.29	0.45
WW 7	1.67	15	7.5	20	3534.29	0.47

As mentioned previously the analysis was undertaken for numerous trace and metal elements, but only a few were selected with significant importance to the West Wits Mining Operation, namely Fe, Ca, S, Cl, Cr, Cu, Zn , and U. Sodium and Hg are not reported further as these elements were below detection limits.

4.5 Plant element concentrations and mass

The followings sections present box plots of elemental concentrations for plant compartments, mass accumulation and weighted elemental concentration in both plant species per paddocks. Average elemental mass per plant in a paddock in both plant species is also presented. The raw data and calculations can be found in appendix B of the elements tested in both plant. It should be noted that Na and Hg were below detection limits in both plant species.

4.5.1 Elemental mean concentration in both plant species by plant compartments

This section presents the elemental concentrations in plant compartments. Zinc was the only element that showed significantly higher concentrations in the rhizomes of *S. corymbosus* than in the roots and shoots (Figure 40). There was no significant difference in Zn between shoots and roots.

Figures 41 and 43-44 present the mean elemental concentrations of U, Fe, and Cu respectively. These elements were the only elements that showed significantly higher concentrations in the roots of *S. corymbosus* than in the shoots. Uranium showed no significant difference between shoots and rhizomes while Fe and Cu showed significant differences between the shoots and rhizomes. Chromium showed no significant difference in the mean concentration between rhizomes and roots, but both were significantly higher than the shoots (Figure 45).

Concentrations of S, Cl, and Ca are presented in Figures 42, 46 and 47 respectively. The mean concentrations of these elements were significantly higher in the shoots than in the roots of *S. corymbosus.* Calcium and Cl concentrations were significantly higher in the roots than the rhizomes.

The mean concentrations of S showed no significant variation in the plant compartments of *P. australis* (Figure 50). Figures 48, 49 and 51-53 present the concentrations of Zn, U, Fe, Cu and Cr respectively, these elements were found to have significantly higher concentrations in the roots of *P. australis* than in the shoots and for Zn, U and Cu, higher also than the rhizomes. Uranium and Cu showed no significant difference between rhizomes and shoots, while Zn and Fe were significantly higher in the rhizomes than shoots. Chloride was the only element that did not show significant differences between roots and shoots of *P. australis* (Figure 54), but shoots were significantly higher than rhizomes. Calcium showed significant higher concentrations in the shoots compared to roots and rhizomes of *P. australis* (Figure 55). In general, the key findings are that *P. australis* roots had significantly higher mean elemental concentrations than the shoots for trace metals but not for S, Cl or Ca, while roots generally also had higher concentrations than rhizomes but fewer significant differences.

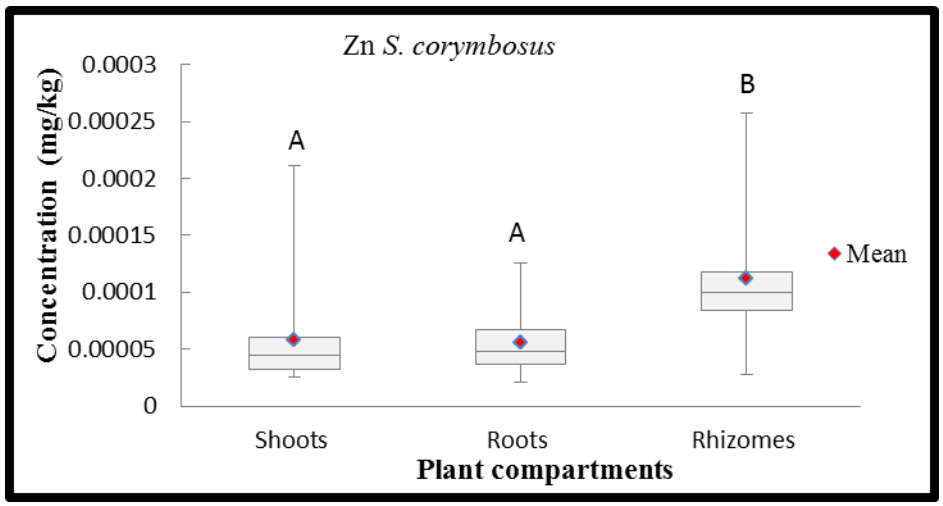


Figure 40: Concentration of Zn in *S. corymbosus* compartments (N=16); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

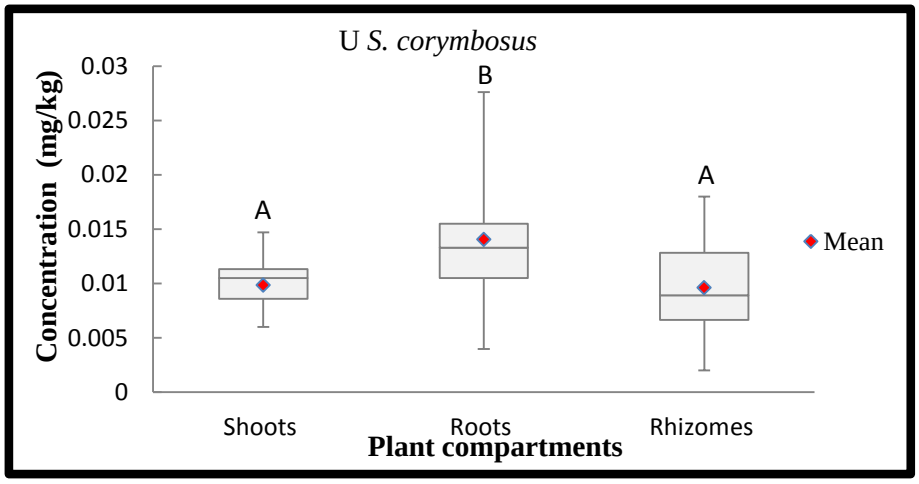


Figure 41: Concentration of U in *S. corymbosus* compartments (N=16); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

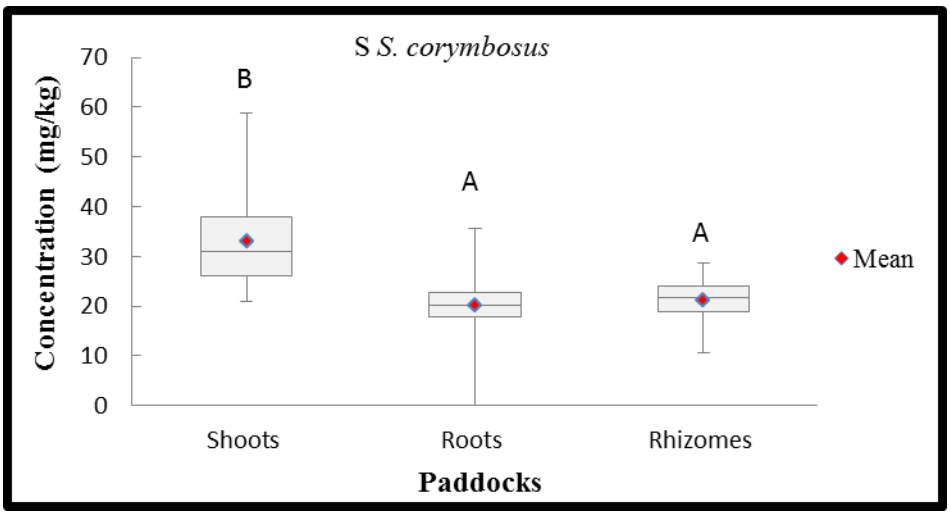


Figure 42: Concentration of S in *S. corymbosus* compartments (N=16); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

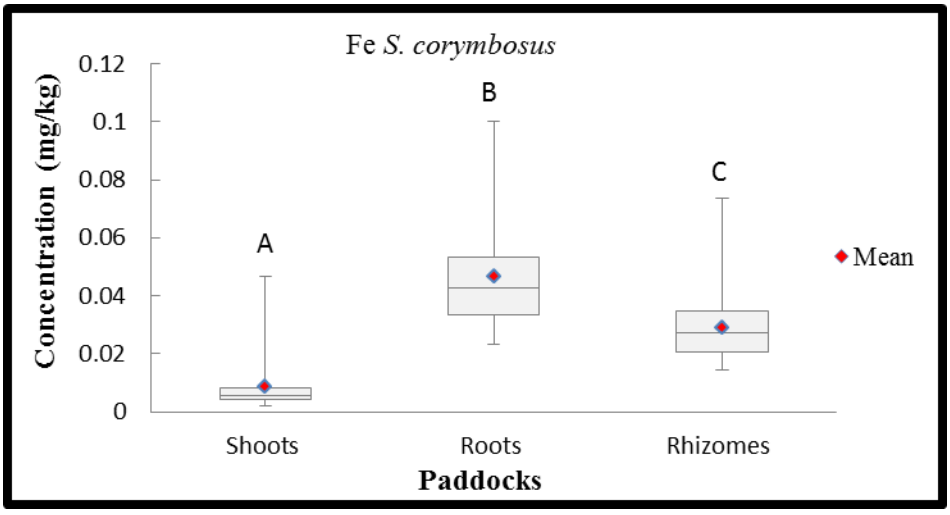


Figure 43: Concentration of Fe in *S. corymbosus* compartments (N=16); all differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

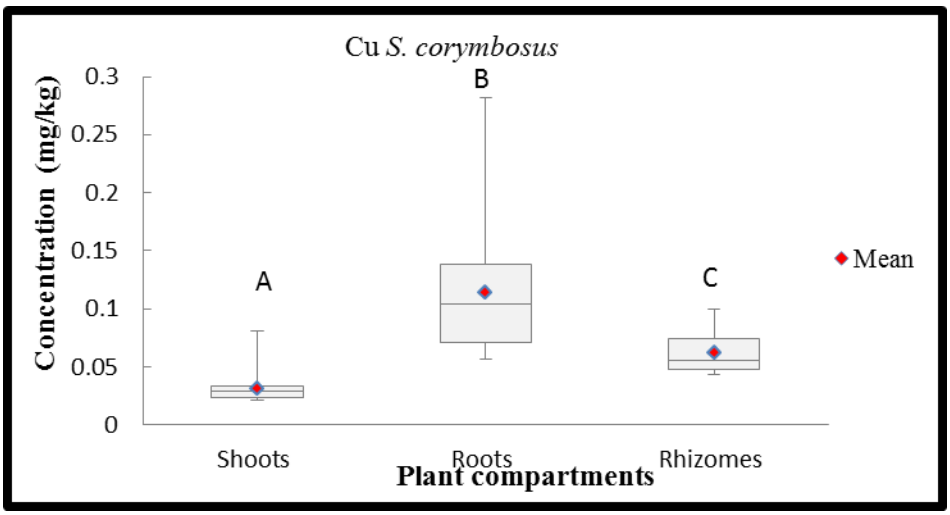


Figure 44: Concentration of Cu in *S. corymbosus* compartments (N=16); all differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

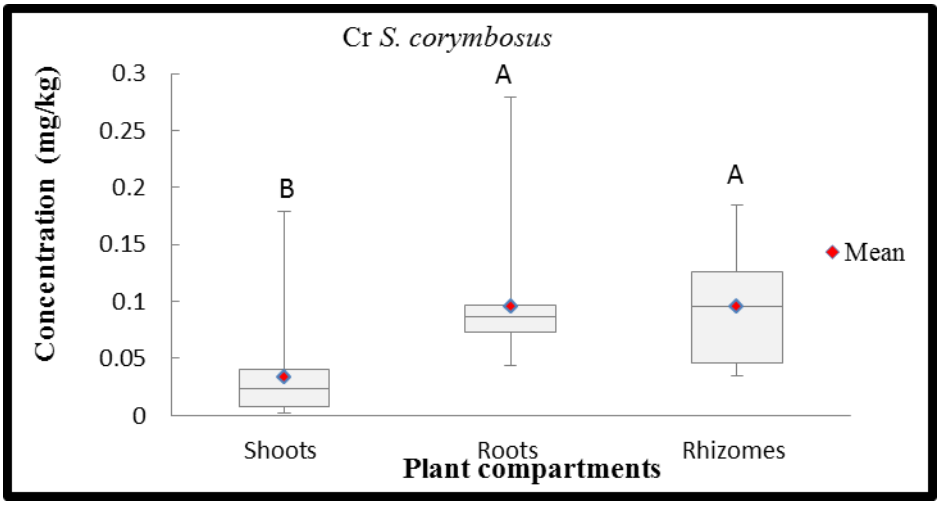


Figure 45: Concentration of Cr in *S. corymbosus* compartments (N=16); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

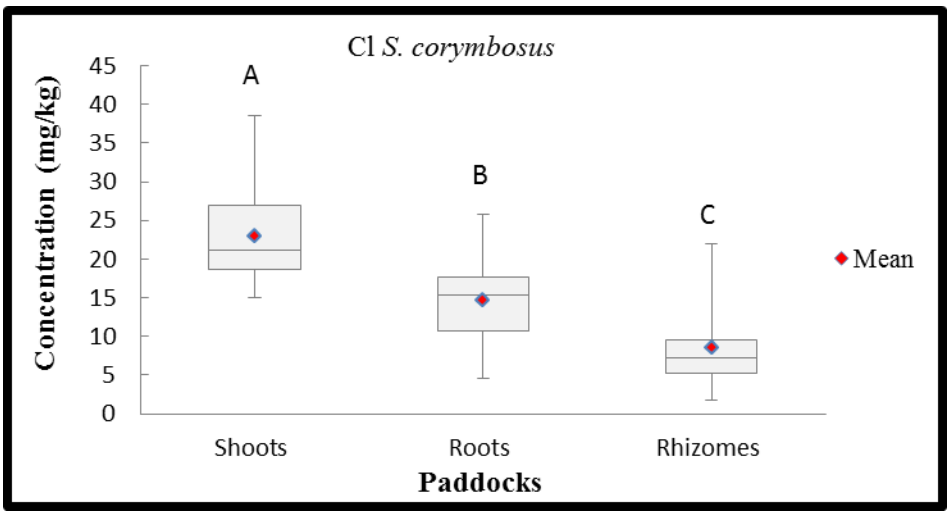


Figure 46 : Concentration of Cl in *S. corymbosus* compartments (N=16); all differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

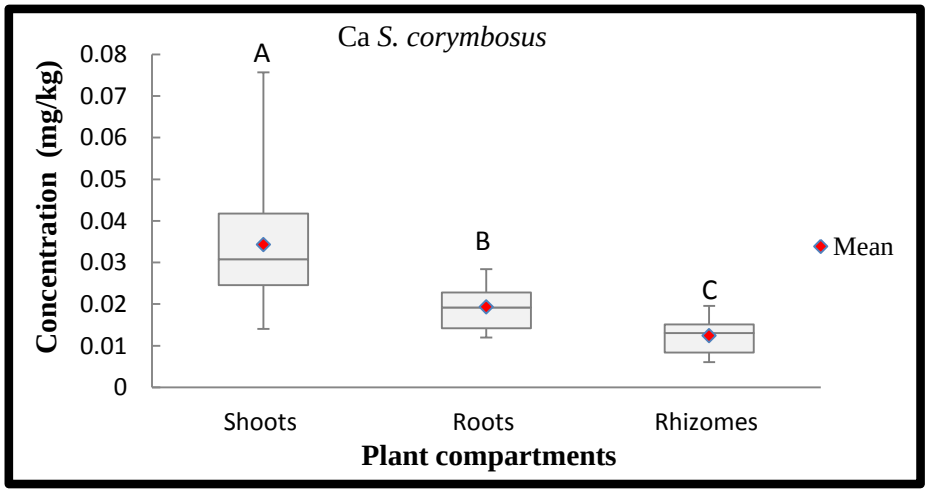


Figure 47 : Concentration of Ca in *S. corymbosus* compartments (N=16); all differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

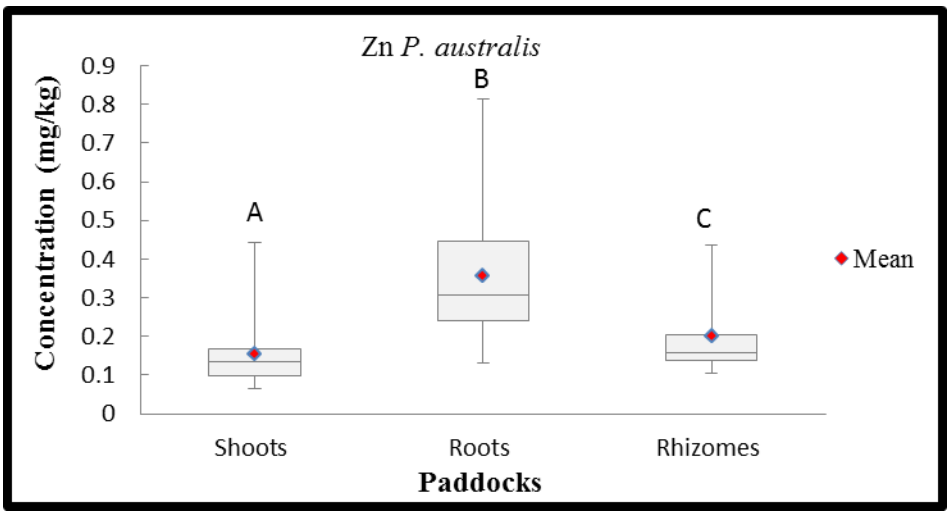


Figure 48: Concentration of Zn in *P. australis* compartments (N=20); all differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

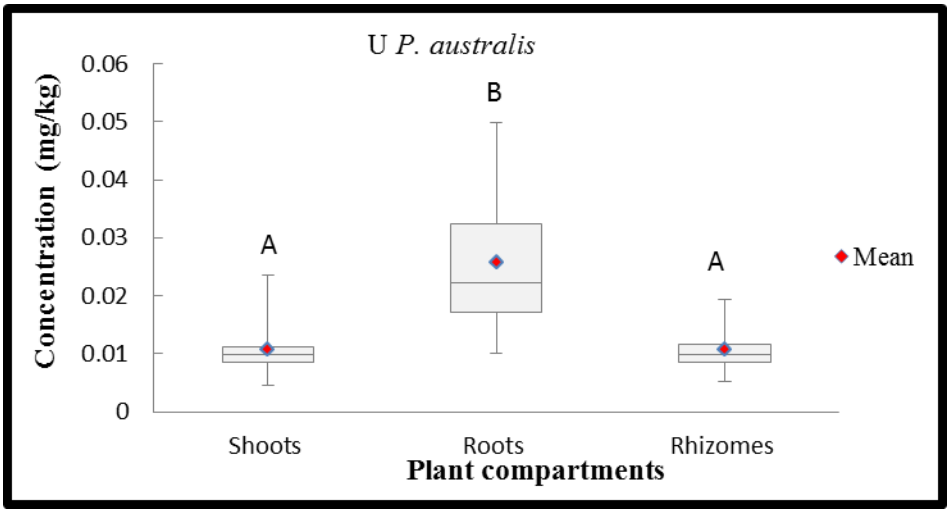


Figure 49: Concentration of U in *P. australis* compartments (N=20); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

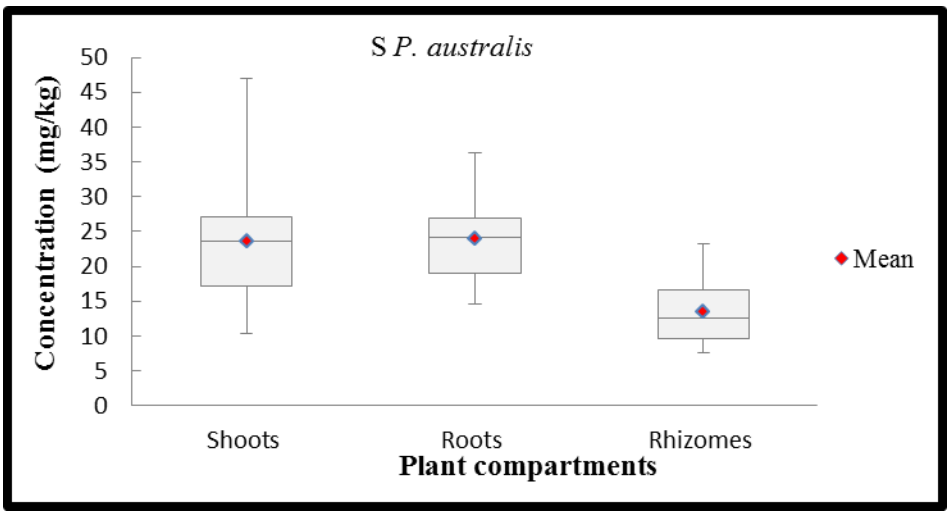


Figure 50: Concentration of S in *P. australis* compartments (N=20); there was no significant difference in mean concentration (P-value = 0.057); One Way ANOVA at $p \leq 0.05$.

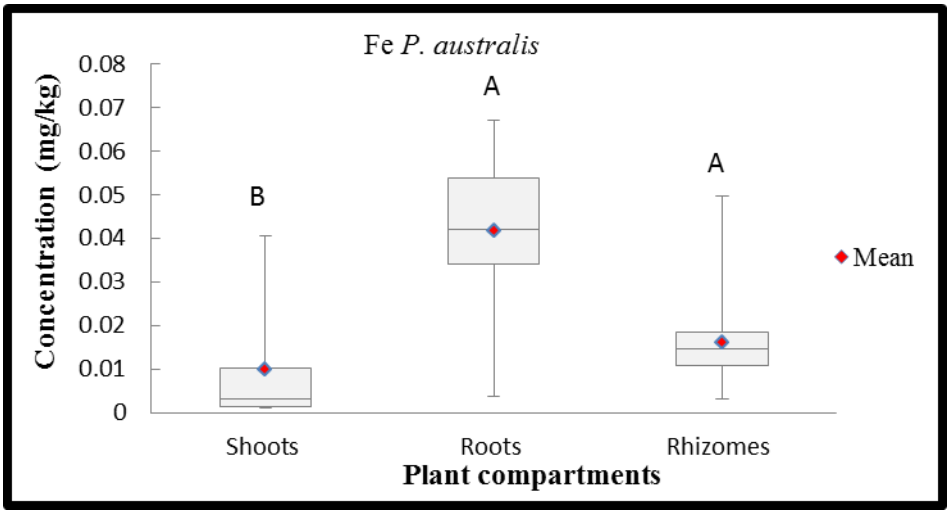


Figure 51: Concentration of Fe in *P australis* compartments (N=20); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

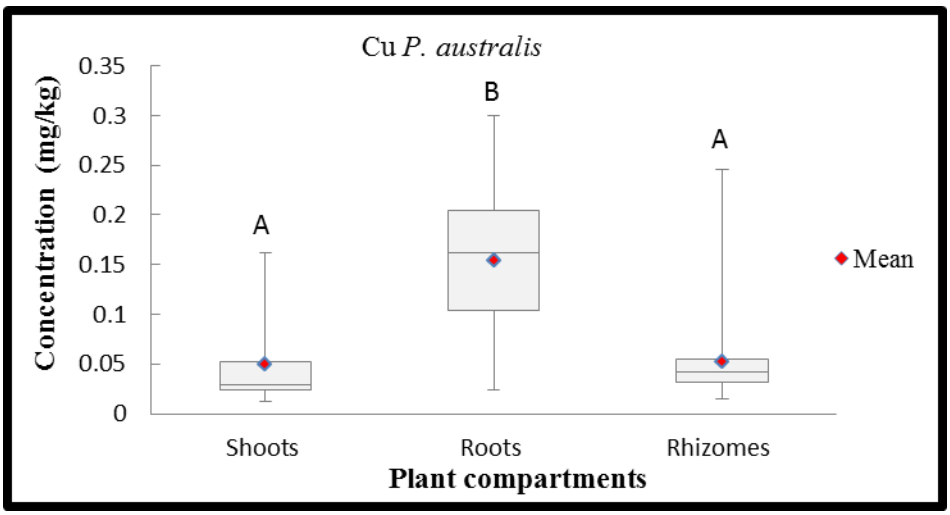


Figure 52: Concentration of Cu in *P. australis* compartments (N=20); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

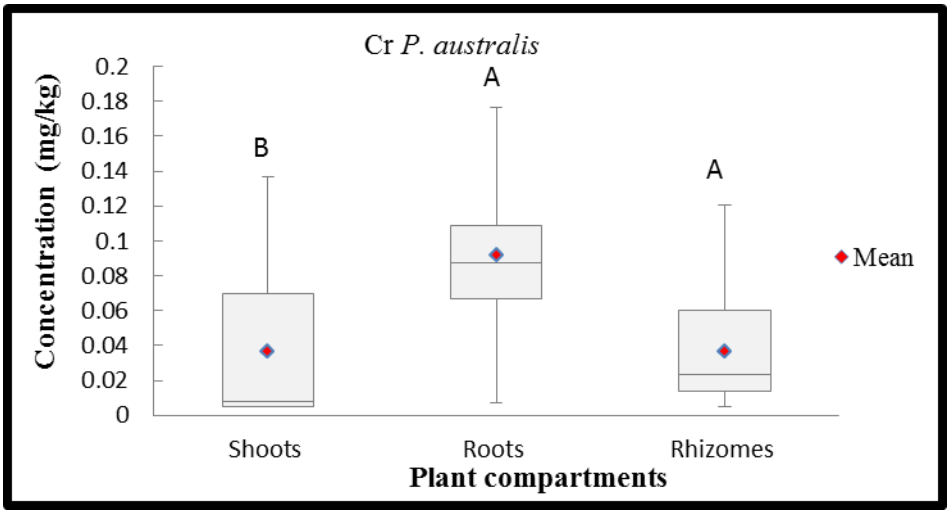


Figure 53: Concentration of Cr in *P. australis* compartments (N=20); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

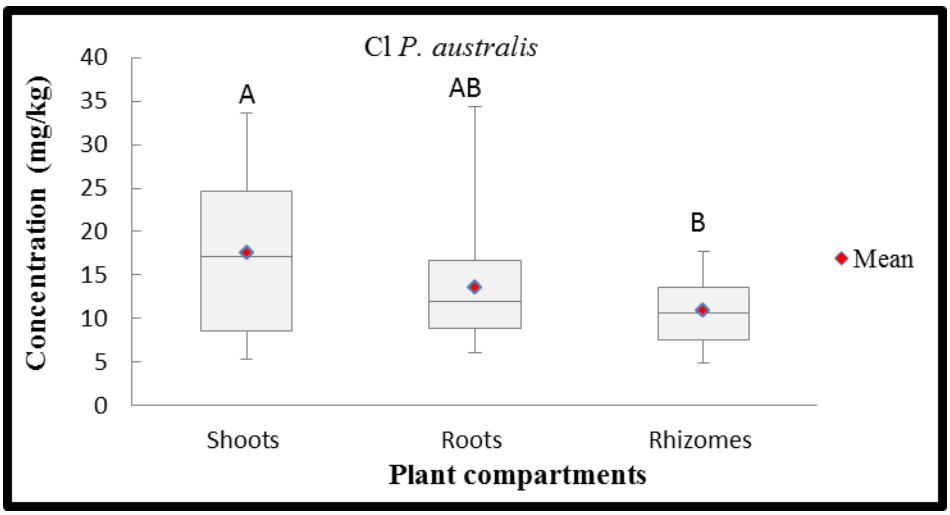


Figure 54: Concentration of Cl in *P. australis* compartments (N=20); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

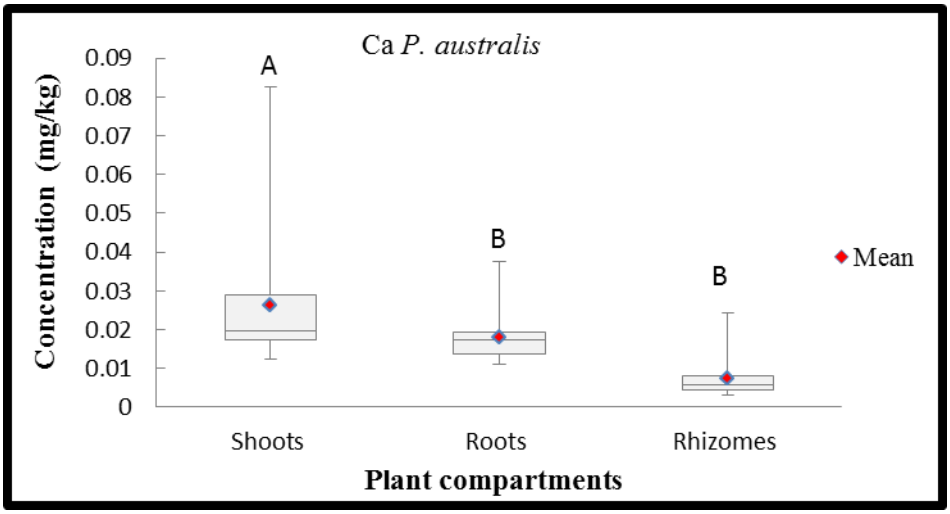


Figure 55: Concentration of Ca in *P. australis* compartments (N=20); those marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

4.5.2 Elemental weighted concentration in both plant species per paddock

This section presents the weighted overall concentration in both plants for each paddock, i.e. each boxplot represent 4 plants per individual paddock. Figures 56 to 63 present the weighted elemental concentrations of Zn, U, S, Fe, Cu, Cr, Cl and Ca respectively for individual plants of each species per paddock. There were no significant differences in weighted concentration between paddocks except for S in *P. australis* only (Table 8; Figure 58). Sulphur in *P. australis* showed no significant difference between

paddocks ww1, ww2 and ww4 but differences were significant between paddocks ww6,ww7 and all the other paddocks (Figure 58).

Although differences were not significant, for *P. australis* weighted concentrations in elements such as Zn, U, Fe, Cr and Cu were found to be higher in the plants from paddock ww4 while for S, Cl and Ca the highest weighted concentrations were found in paddock ww6. Similar observations were also found in *S. corymbosus;* the highest weighted concentrations in elements such as Zn, U, S, Cu, and Ca were found in paddock ww4, though patterns were mixed for other elements. The only expected result, assuming that pollutants enter the wetland through the uppermost paddocks i.e. paddock ww1 and ww2 respectively were found with higher Fe and Cr concentrations in *S. corymbosus* in paddock ww1.

Table 8: Weighted mean element concentrations showing no significant difference between paddocks for both plant species (except for S in *P. australis*); One Way ANOVA, at $p \leq 0.05$ in two plant species of the uppermost paddock

Elements	*S. corymbosus* P – value	*P. australis* P value
Zn	0.7449	0.1998
U	0.6257	0.7861
S	0.5473	0.0355
Fe	0.7189	0.9037
Cr	0.7893	0.7893
Cu	0.9902	0.9020
Cl	0.3261	0.5850
Ca	0.1152	0.5483

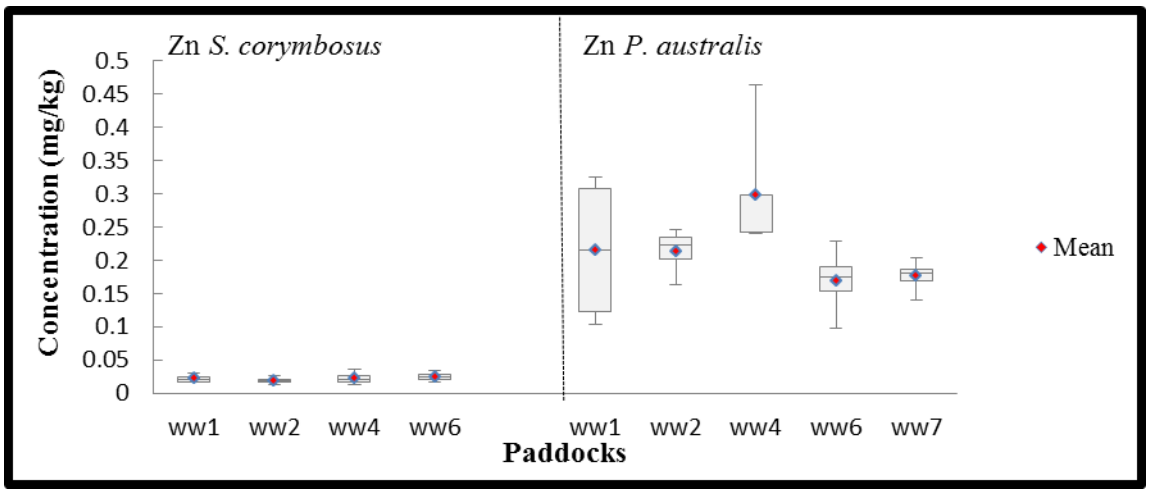


Figure 56: Weighted Zn concentration per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); weighted concentrations do not differ significantly between paddocks, One Way ANOVA, at $p \leq 0.05$.

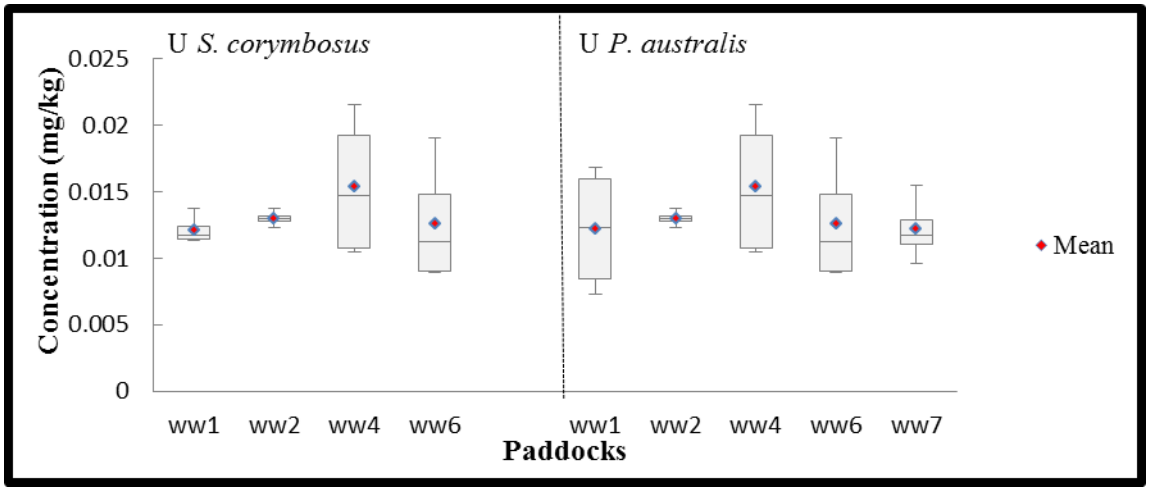


Figure 57: Weighted U concentration per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); weighted concentrations do not differ significantly between paddocks, One Way ANOVA, at $p \leq 0.05$.

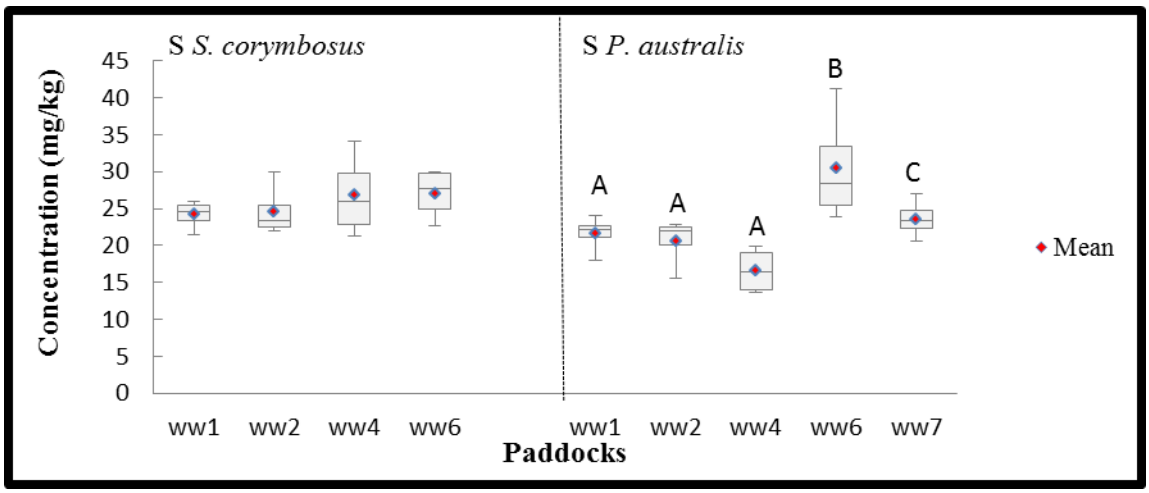


Figure 58: Weighted S concentration per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); *S. corymbosus* weighted concentrations do not differ significantly between paddocks, One Way ANOVA, at $p \leq 0.05$. *P. australis* paddocks marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

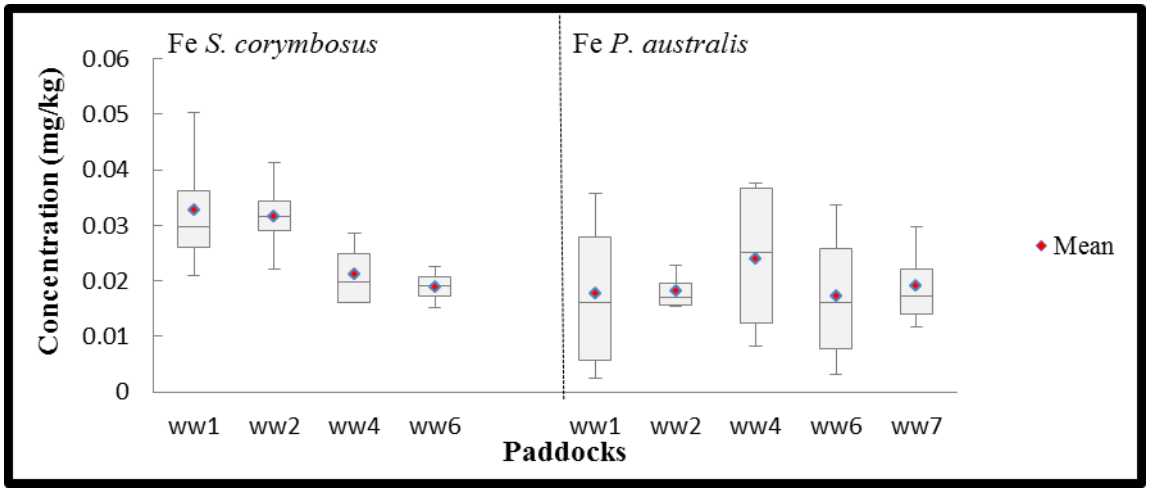


Figure 59: Weighted Fe concentration per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); weighted concentrations do not differ significantly between paddocks, One Way ANOVA, at $p \leq 0.05$.

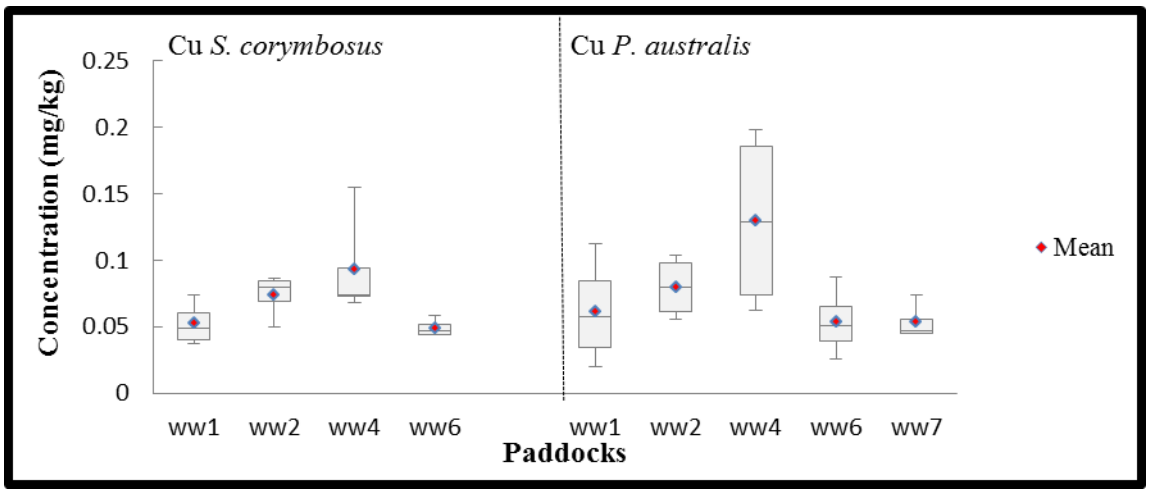


Figure 60: Weighted Cu concentration per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); weighted concentrations do not differ significantly between paddocks, One Way ANOVA, at $p \leq 0.05$.

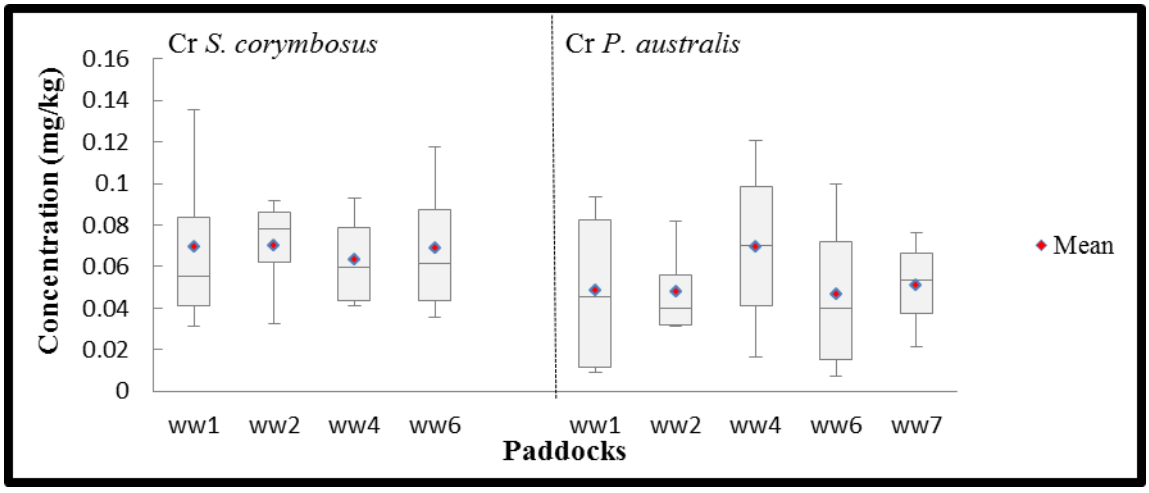


Figure 61: Weighted Cr concentration per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); weighted concentrations do not differ significantly between paddocks, One Way ANOVA, at $p \leq 0.05$.

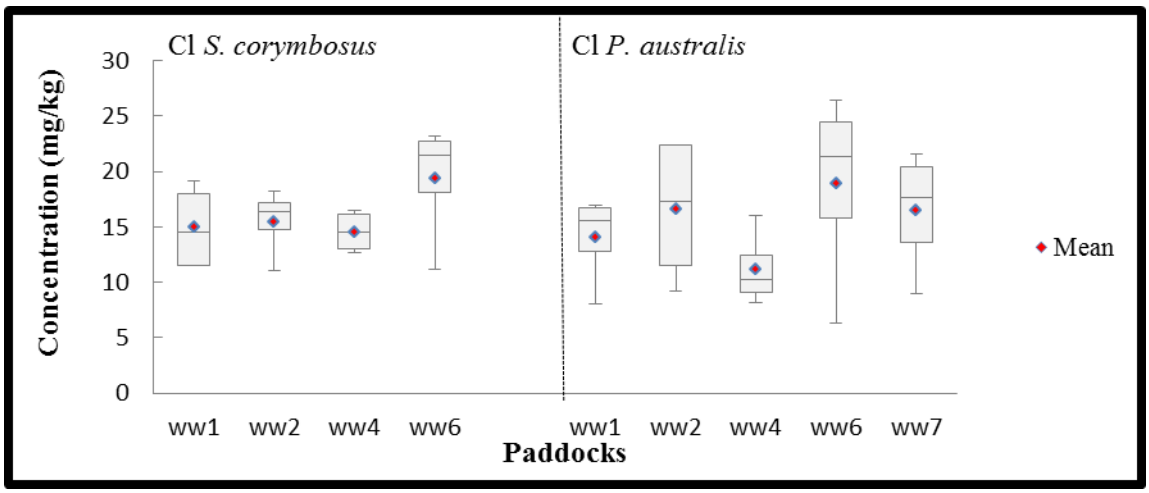


Figure 62: Weighted Cl concentration per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); weighted concentrations do not differ significantly between paddocks, One Way ANOVA, at $p \leq 0.05$.

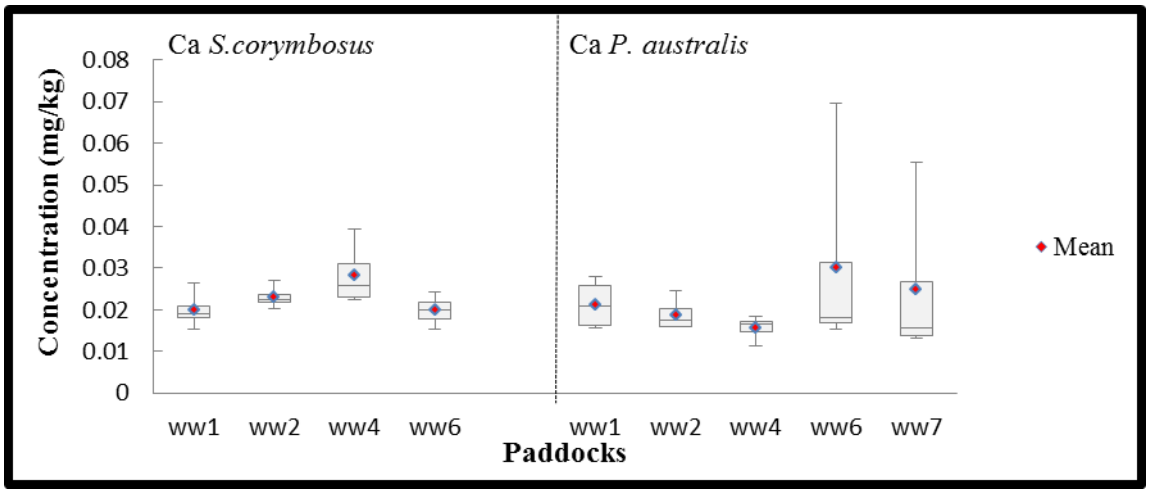


Figure 63: Weighted Ca concentration per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); weighted concentrations do not differ significantly between paddocks, One Way ANOVA, at $p \leq 0.05$.

4.5.3 Mean elemental mass accumulation in both plant species per paddocks

This section presents the mean elemental mass accumulation per paddock (Figures 64 to 71). Paddock ww4 showed the highest mass per plant of U, S, Fe, Cr, Cu, Cl and Ca for *S. corymbosus*, while in *P. australis*, paddock ww6 showed the highest mass per plant of U, S, Fe, Cl and Ca. There was no significant difference in Zn mean mass pool in both plants.

Assuming that pollutants enter the wetlands through the uppermost paddocks i.e. paddock ww1 and ww2 respectively only Zn in *P. australis* showed the highest mass per plant in paddock ww1. In general, the mass per plant of Zn, U, S, Fe, Cu, Cr, Cl and Ca showed no significant difference between paddocks except for S in *P. australis* only, One

Way ANOVA, at $p \leq 0.05$ (Table 9). As with concentrations, sulphur mass in *P. australis* showed no significant differences between paddocks ww1, ww2 and ww4 but differences were significant between paddocks ww6, ww7 and the rest (Figure 66).

Table 9: Mean element mass per plant showing no significant difference in both plant species, excluding S in *P. australis*; One Way ANOVA, at $p \leq 0.05$ in two plant species of the uppermost paddock

Elements	*S. corymbosus* P – value	*P. australis* P value
Zn	0.4123	0.8667
U	0.4124	0.3676
S	0.0573	0.0364
Fe	0.7937	0.8278
Cr	0.8037	0. 9980
Cu	0.0523	0.2985
Cl	0.1612	0.2440
Ca	0.0552	0.5858

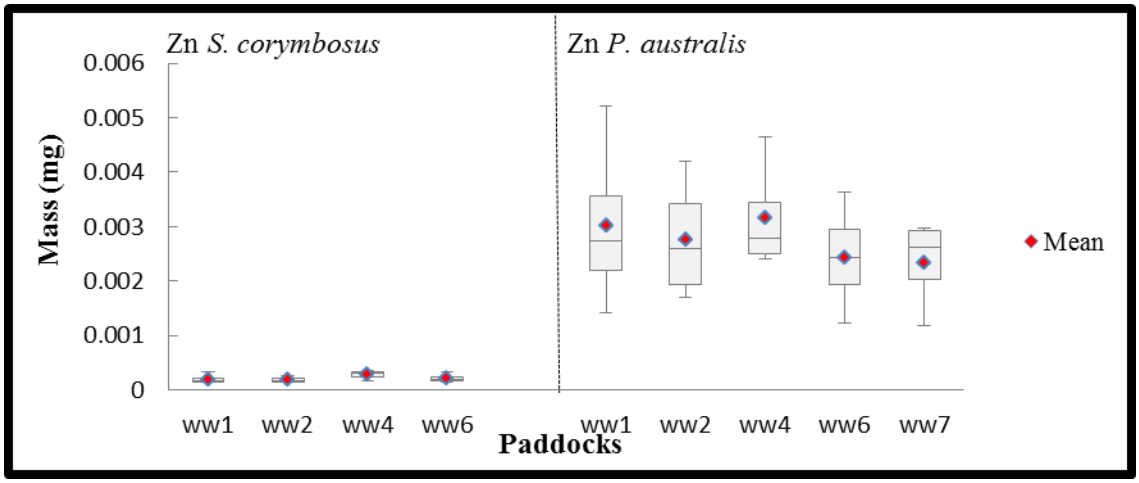


Figure 64: Variation in Zn mass accumulation per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); values do not differ significantly from each other, One Way ANOVA, at $p \leq 0.05$

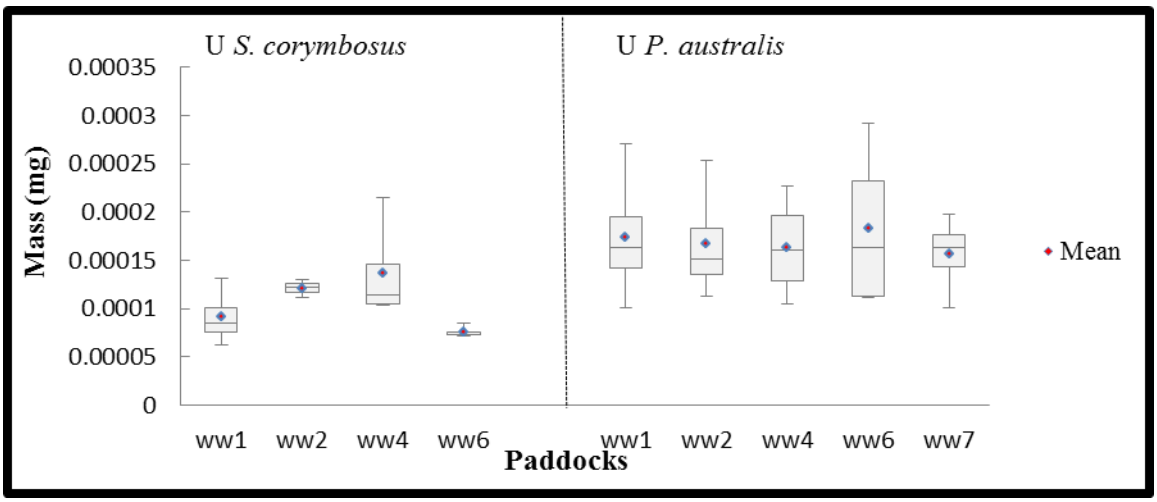


Figure 65:Variation in U mass accumulation per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); values do not differ significantly from each other, One Way ANOVA, at $p \leq 0.05$

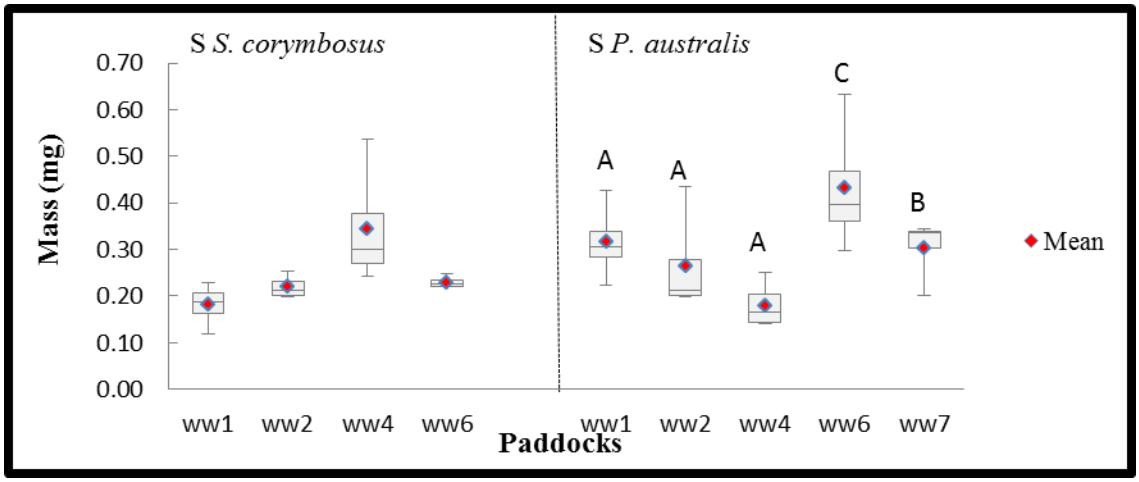


Figure 66: Variation in S mass accumulation per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4);S. corymbosus values do not differ significantly from each other, and *P. australis* paddocks marked with same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

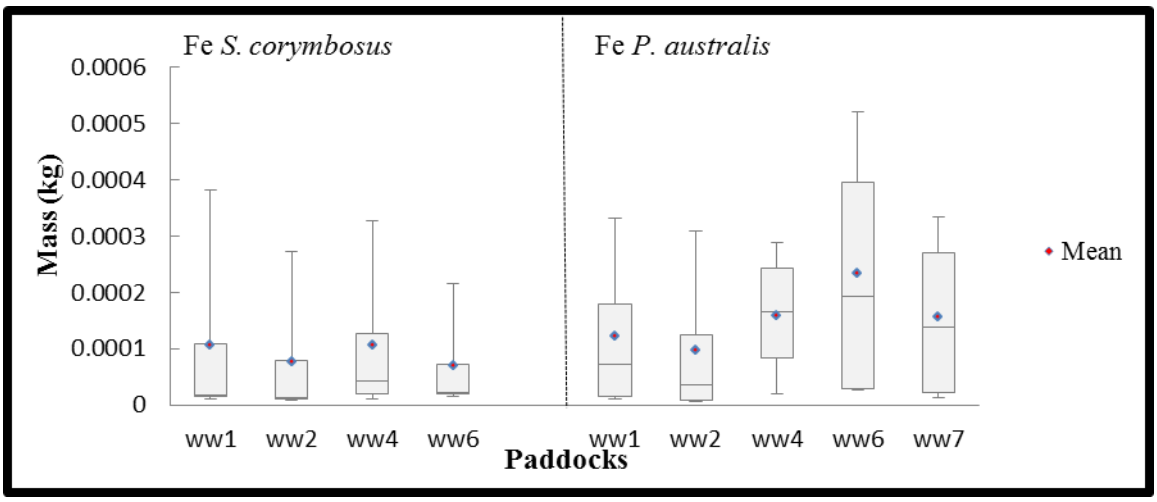


Figure 67: Variation in Fe mass accumulation per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); values do not differ significantly from each other, One Way ANOVA, at $p \leq 0.05$.

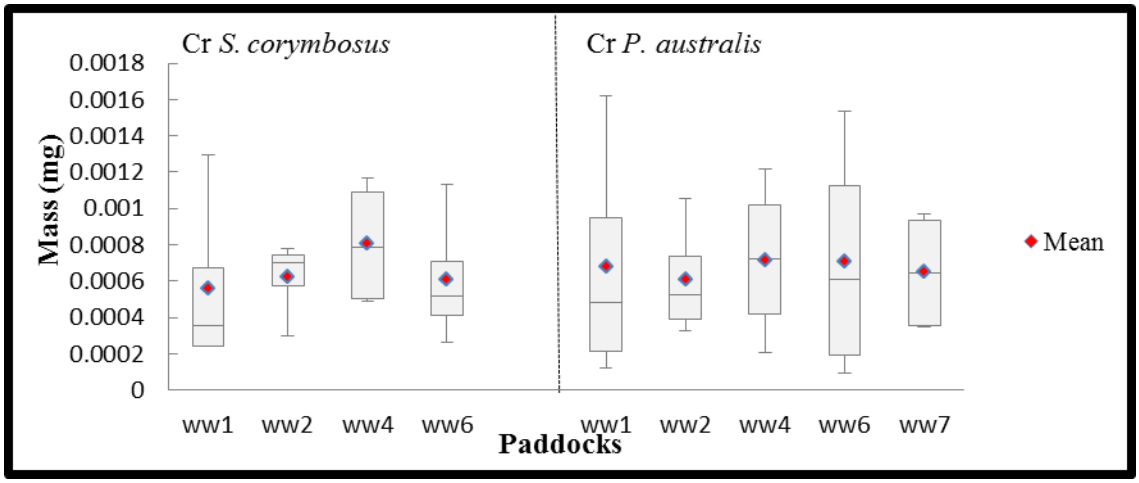


Figure 68: Variation in Cr mass accumulation per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); values do not differ significantly from each other, One Way ANOVA, at $p \leq 0.05$.

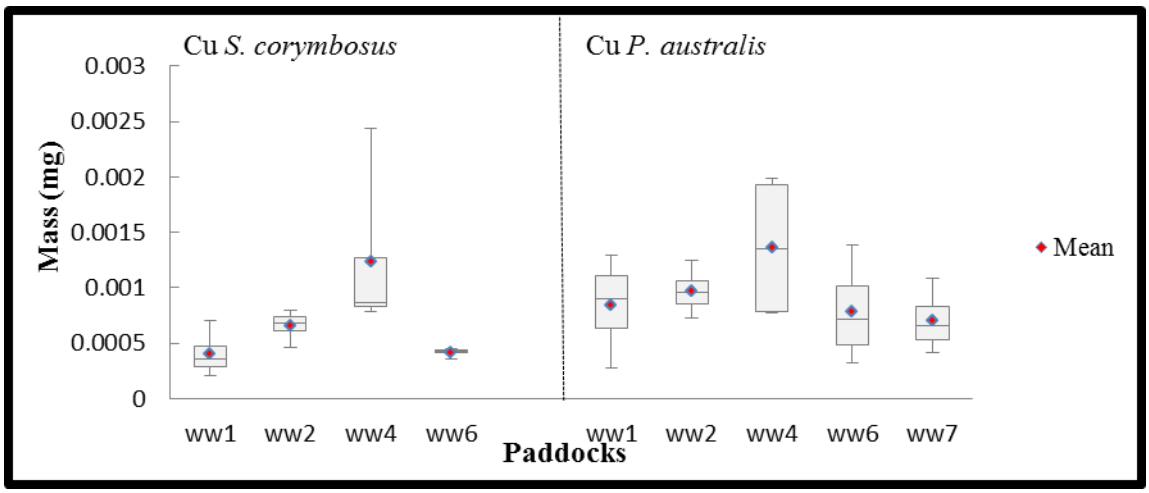


Figure 69: Variation in Cu mass accumulation per plant per paddock of *S. corymbosus* (N=4) and *P. australis* (N=4); values do not differ significantly from each, One Way ANOVA, at $p \leq 0.05$.

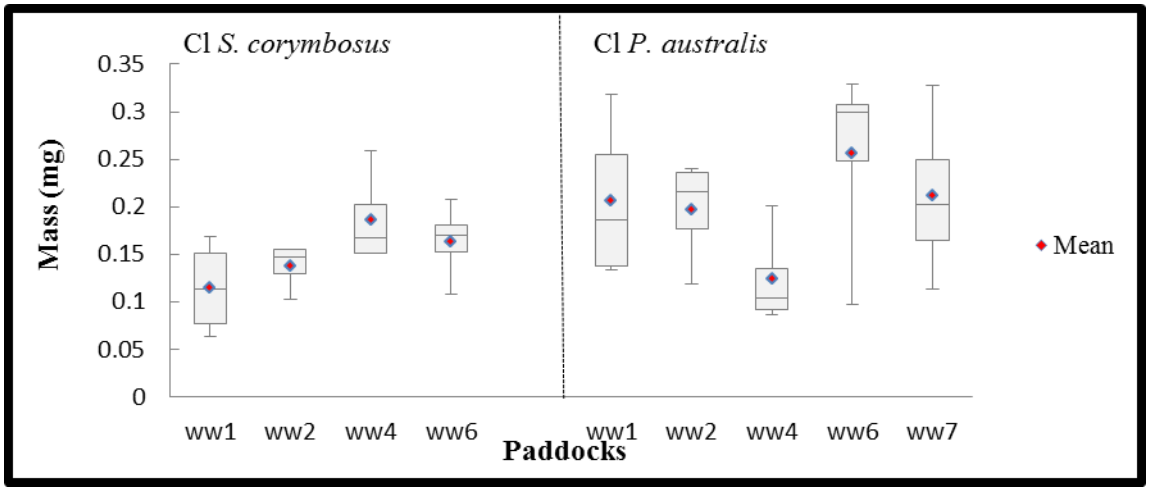


Figure 70: Variation in Cl mass accumulation per plant per paddock of *S. corymbosus* (N=4) and *P australis* (N=4); values do not differ significantly from each, One Way ANOVA, at $p \leq 0.05$.

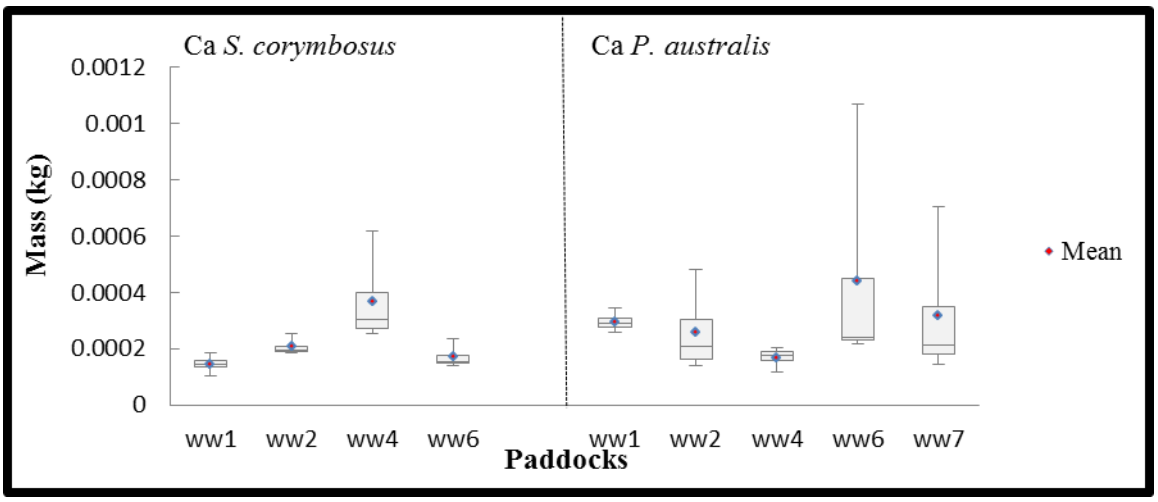


Figure 71: Variation in Ca mass accumulation per plant per paddock of *S. corymbosus* (N=4) and *P australis* (N=4); values do not differ significantly from each, One Way ANOVA, at $p \leq 0.05$.

4.5.4 Average plant compartment mass for all individual plants within the plots in both plant species

This section presents the average plant compartment pollutant mass within a paddock for all individual *P. australis* and *S. corymbosus* plants in paddock ww1 only. Although this was done for all paddocks, the mass percentile charts for the other elements can be found in Appendix F. Figures 72-79 in this section illustrate the average mass in percentiles of Zn, U, S, Fe, Cu, Cr, Cl, and Ca.

Copper and Zn mass percentages were higher in roots of *P. australis* than in any other compartments for either species (Figure 72 and 77). For Cu and Zn the next most important compartments were rhizomes and shoots of *P. australis* and in both cases, all *S. corymbosus* plant compartments had smaller masses than in *P. australis*. From the elements tested in both plants, the overall mass was higher in *S. corymbosus* for Fe only with a higher mass percentage in the roots than in the shoots and rhizome, (Figure 75).

Uranium and S showed the highest mass percentages in shoots of *P. australis*. The second and third highest masses of U were found in the roots of *P. australis* and shoots of *S. corymbosus* respectively (Figure 72). The second and third highest masses of S were found in shoots of *S. corymbosus* and the roots of *P. australis* respectively (Figure 74).

Chromium was the only element with equal highest mass percentages in the shoots and roots of *P. australis* (Figure 76). The third and fourth highest Cr mass percentages were found in the shoots and roots of *S. corymbosus* respectively.

Chlorine and Ca are presented in figures 78-79 respectively; in both by far the highest mass percentages were found in the shoots of *P. australis*. The second highest mass percentages in both Cl and Cl were in the shoots of *S. corymbosus*. In general, *P. australis* accumulated more mass than *S. corymbosus*, with the shoots accumulating more mass of U, S, Cl and Ca but the roots accumulating more Zn, Fe and Cu.

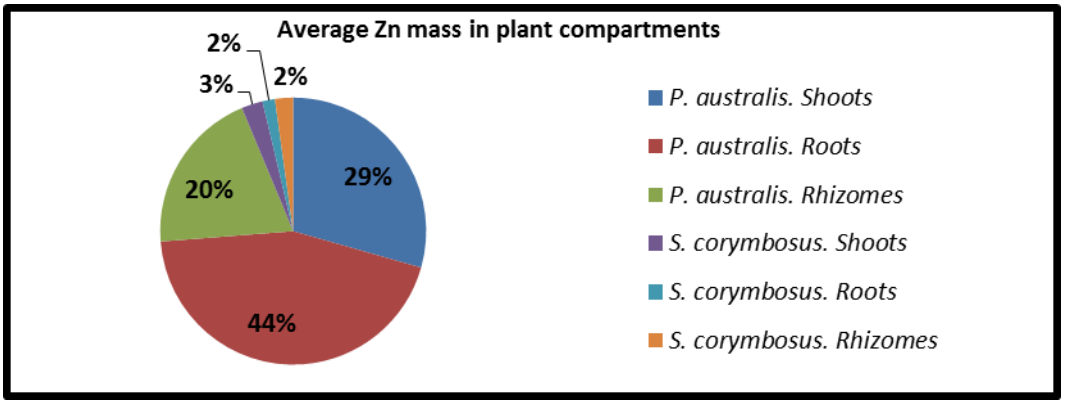


Figure 72: Average Zn mass (%) in plant compartments for all individual plants within the plots of *P. australis* (N=3) and *S. corymbosus* (N=3).

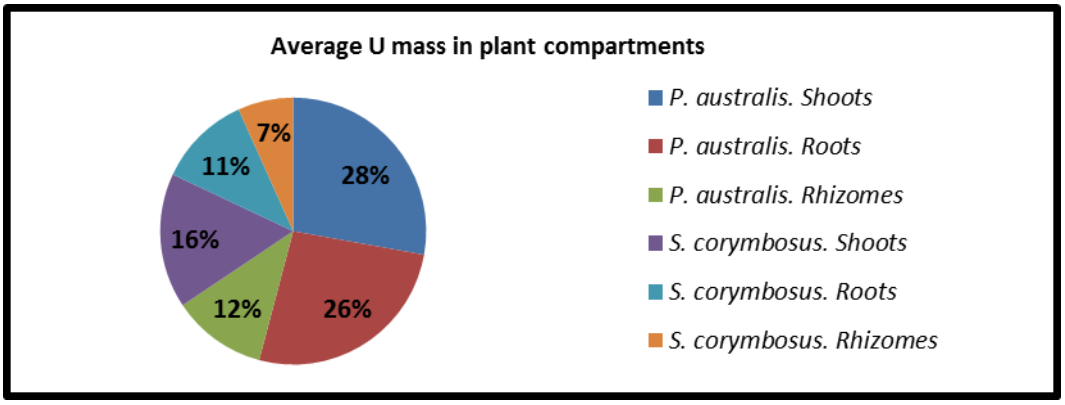


Figure 73: Average U mass (%) in plant compartments for all individual plants within the plots of *P. australis* (N=3) and *S. corymbosus* (N=3).

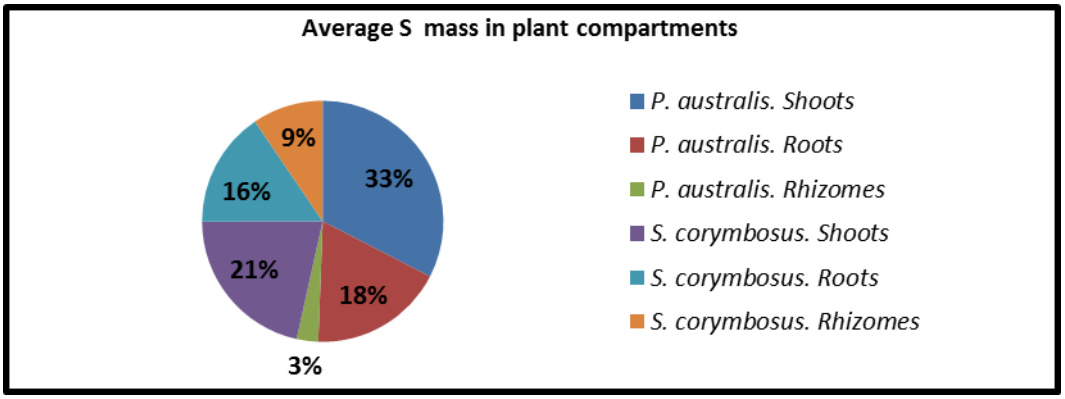


Figure 74: Average S mass (%) in plant compartments for all individual plants within the plots of *P. australis* (N=3) and *S. corymbosus* (N=3).

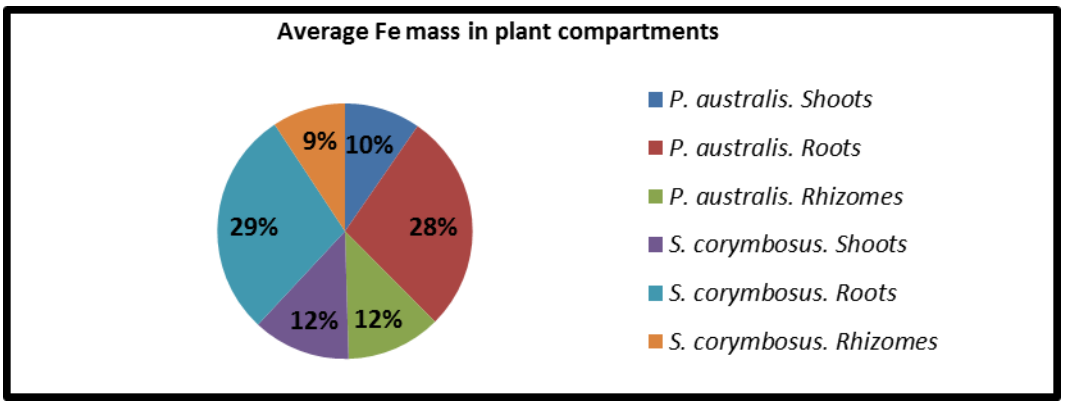


Figure 75: Average Fe mass (%) in plant compartments for all individual plants within the plots of *P. australis* (N=3) and *S. corymbosus* (N=3).

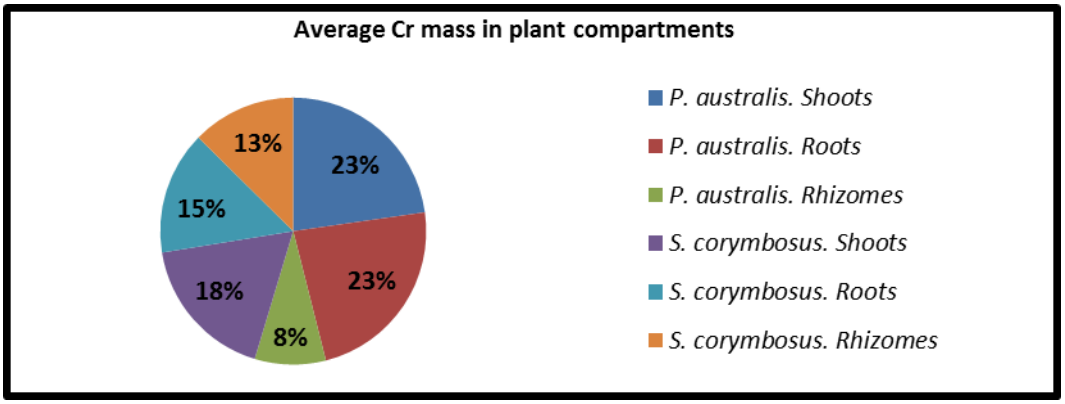


Figure 76: Average Cr mass (%) in plant compartments for all individual plants within the plots of *P. australis* (N=3) and *S. corymbosus* (N=3).

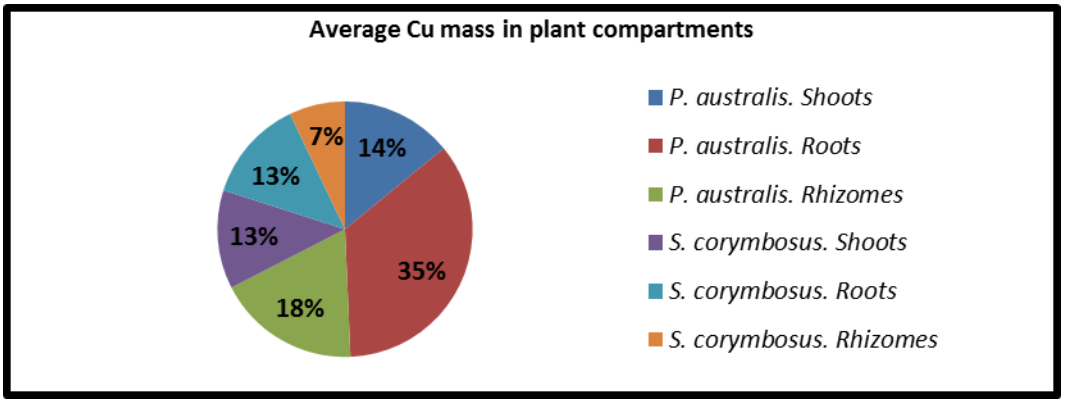


Figure 77: Average Cu mass (%) in plant compartments for all individual plants within the plots of *P. australis* (N=3) and *S. corymbosus* (N=3).

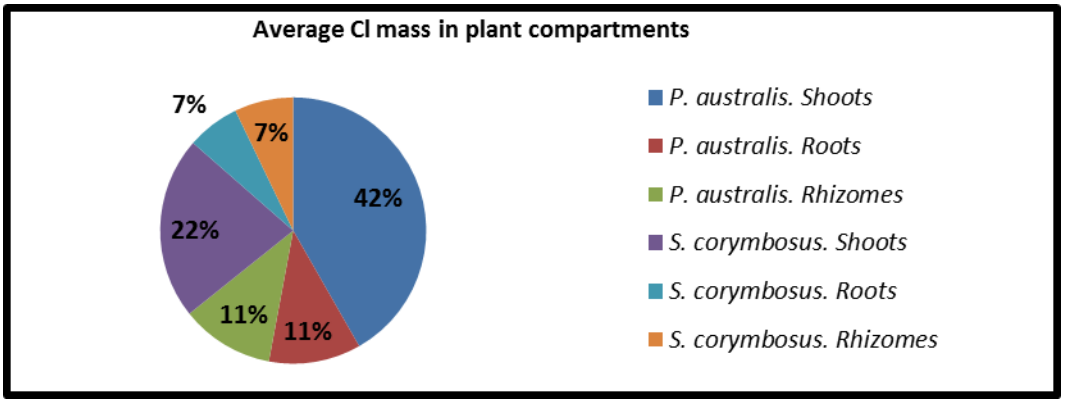


Figure 78: Average Cl mass (%) in plant compartments for all individual plants within the plots of *P. australis* (N=3) and *S. corymbosus* (N=3).

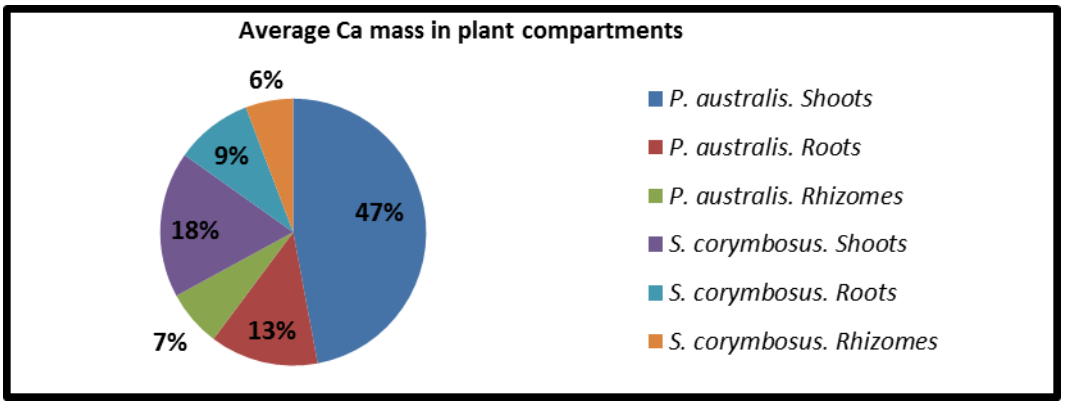


Figure 79: Average Ca mass (%) in plant compartments for all individual plants within the plots of *P. australis* (N=3) and *S. corymbosus* (N=3).

4.6 Sediment element concentrations and mass

The followings sections present the elemental mean concentration of sediment cores by interval depths across the uppermost wetland paddocks and the elemental mean mass pool accumulation of sediment cores. The weighted elemental concentration calculation by paddocks can be found in Appendix C2. As found in the previous section on plants, it should also be noted that Na and Hg were below detection limits in sediment samples as well.

4.6.1 Mean elemental concentrations in sediment cores by depth

This section presents the mean elemental concentration of sediment cores by interval depth across the uppermost wetland paddocks (Figures 80-87). Figures 82, 83, 86 and 87,

present the elemental concentration of S, Fe, Cl and Ca respectively with depth. These elements significantly increased with sediment profile depth; in general, it was found that surface sediment concentrations were significantly lower than sub sediment concentrations.

Zn and U concentration showed an apparent decrease with depth in sediment cores but there was no significant difference observed between middle (2-10cm) and lower (10-30cm) intervals (Figure 80 and Figure 81). Cu concentration showed an apparent increase with depth in sediment cores but there was no significant difference observed differences between middle (2-10cm) and lower (10-30cm) intervals (Figure 84).

Chromium statistics showed that there was no significant difference observed in element concentrations in the sediment profile of the studied paddocks (P-value = 0.081) at ANOVA p ≤0.05 (Figure 85).

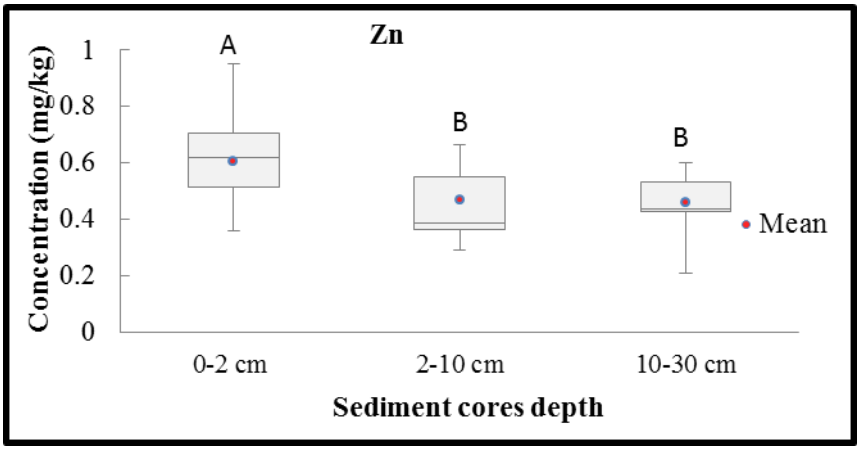


Figure 80 : Elemental concentration of Zn in sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. Depths marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at p ≤ 0.05.

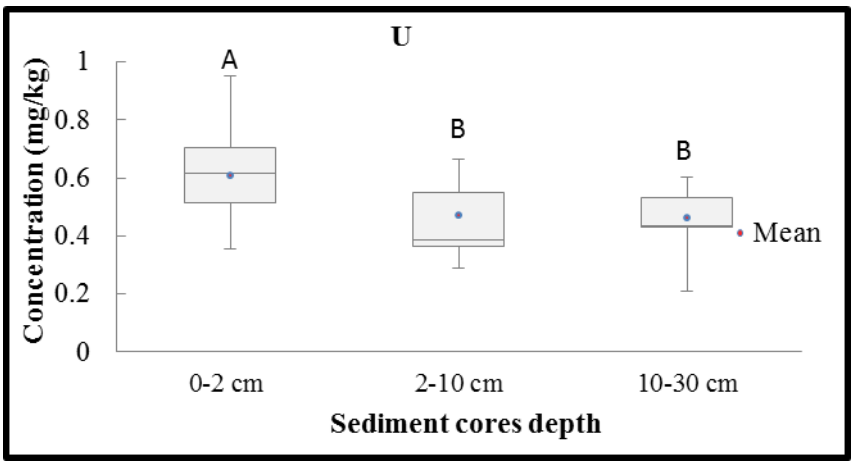


Figure 81: Elemental concentration of U in sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. Depths marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

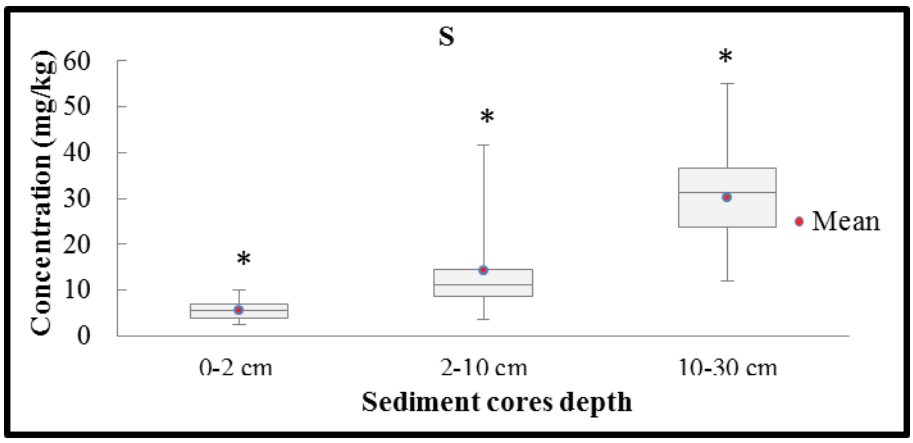


Figure 82 : Elemental concentrations of S in sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. * denotes significant difference in elemental pairwise comparisons; One Way ANOVA, at $p \leq 0.05$.

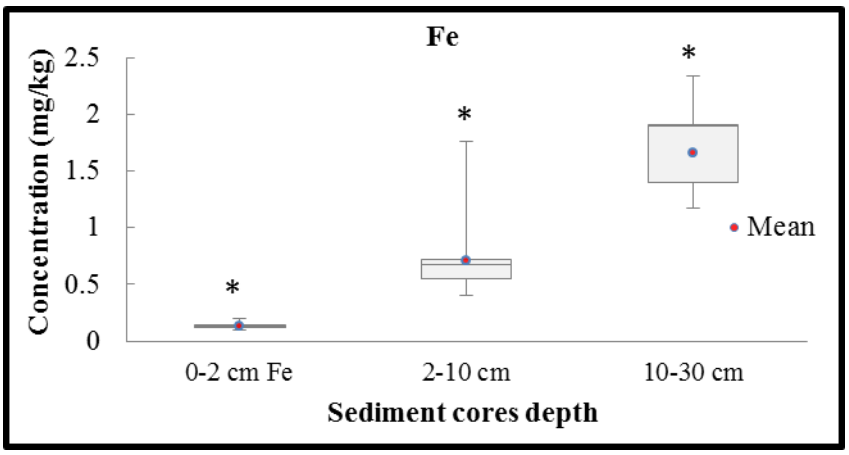


Figure 83 : Elemental concentration of Fe in sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. * denotes significant difference in elemental pairwise comparisons; One Way ANOVA, at $p \leq 0.05$.

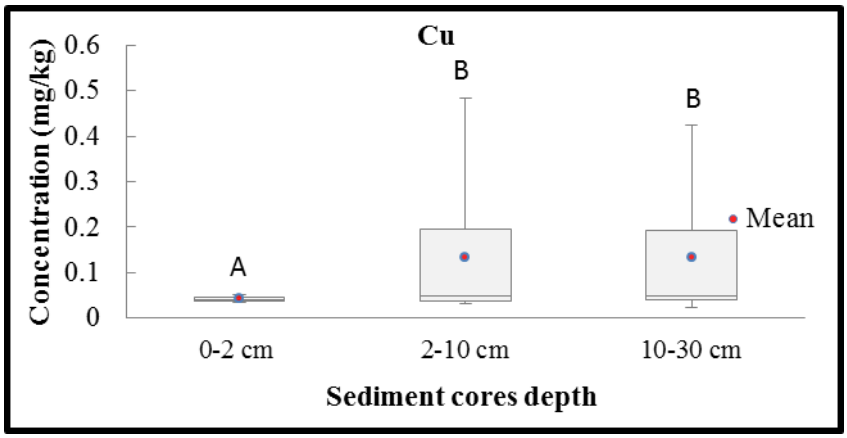


Figure 84: Elemental concentrations of Cu in sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. Depths marked with the same letter do not differ significantly from each other after pairwise comparison; One Way ANOVA, at $p \leq 0.05$.

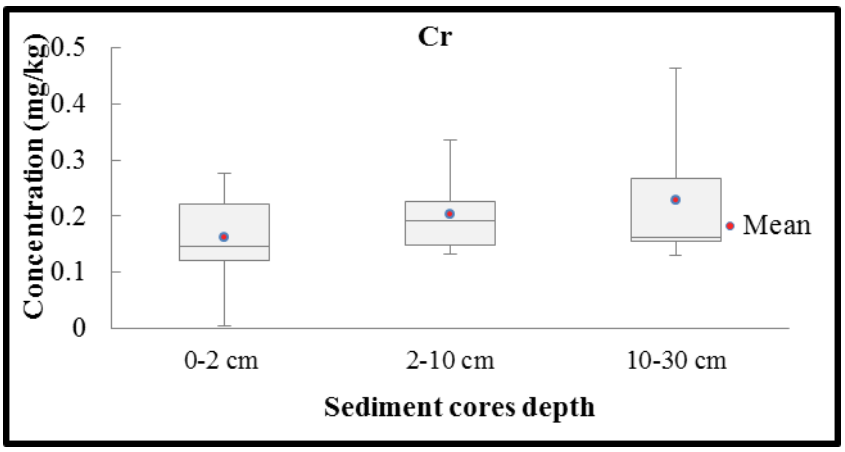


Figure 85: Elemental concentrations of Cr in sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. There was no significant difference in elemental concentrations (P value = 0.081), One Way ANOVA at $p \leq 0.05$.

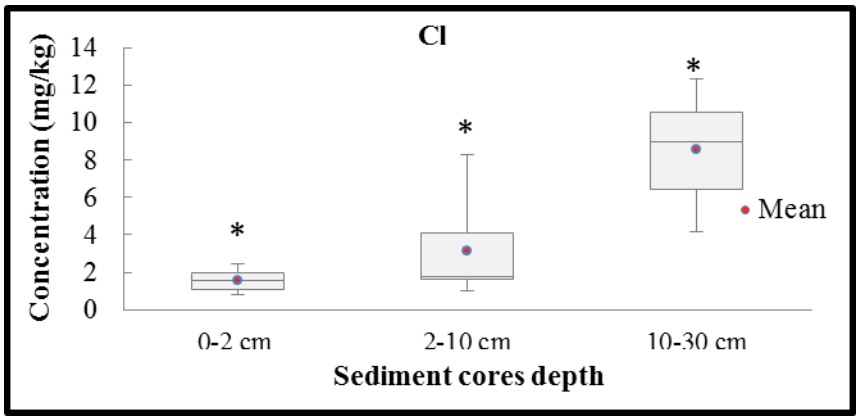


Figure 86: Elemental concentrations of Cl in sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. * denotes significant difference in elemental pairwise comparisons; One Way ANOVA, at $p \leq 0.05$.

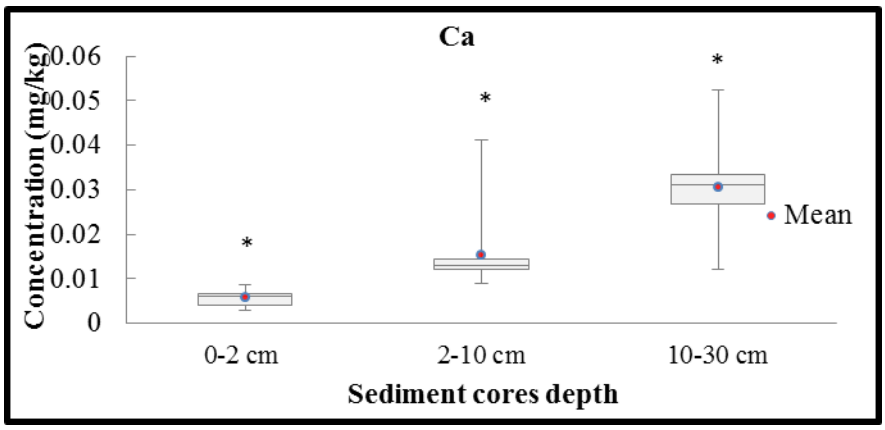


Figure 87: Elemental concentrations of Ca in sediment core interval depths 0-2 cm (N=15), 2-10 cm (N=15) and 10-30 cm (N=15) across the uppermost paddocks in the Varkenslaagte canal. * denotes significant difference in elemental pairwise comparisons; One Way ANOVA, at $p \leq 0.05$.

4.6.2 Elemental mean mass pool accumulation in the whole sediment cores per paddocks

This section present the elemental mean mass pool accumulation of the whole core pool per individual paddocks. Figures 88 to 95, excluding Figure 90, present the elemental mean mass accumulation of Zn, U, Fe, Cu, Cr, Cl and Ca respectively. The statistical test showed that there was no significant difference observed in elemental mean mass pool accumulation of the aforementioned elements by paddock in uppermost paddocks at ANOVA $p \leq 0.05$ (Table 10). Generally, no trends or patterns were observed in these elements across the uppermost wetland paddocks.

Figures 90, present the S elemental mean mass accumulation, it was found that S in ww6 was significant higher than in all paddocks and there was no significant difference between paddock ww1, ww4 and ww7 but paddock ww1 and ww7 were significantly higher than paddock ww2.

Table 10: The elements showing no significant difference in mean mass in sediment cores per paddock, One Way ANOVA, at $p \leq 0.05$

Elements	P – values
Zn	0.0536
U	0.3775
Fe	0.2447
Cu	0.1556
Cr	0.4232
Cl	0.0533
Ca	0.1014

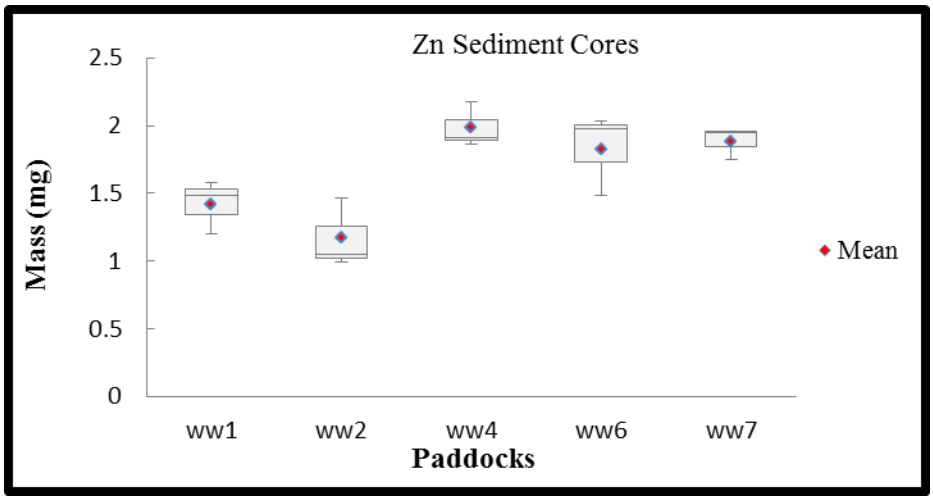


Figure 88: Mean elemental mass pool accumulation of Zn per whole sediment core (N=3) within each paddock, in the Varkenslaagte canal. There was no significant difference in elemental mass between paddocks (P-value =0.0536); One Way ANOVA at $p \leq 0.05$

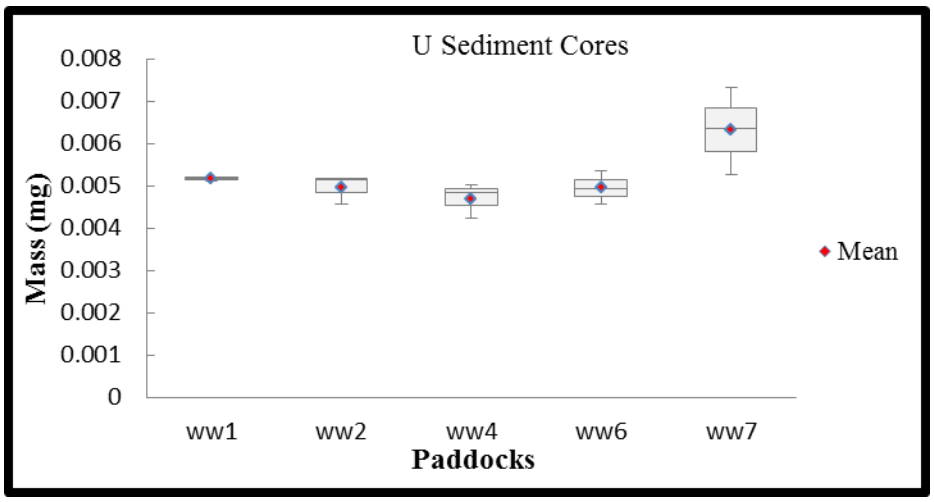


Figure 89: Mean elemental mass pool accumulation of U per whole sediment core (N=3) within each paddock, in the Varkenslaagte canal. There was no significant difference in elemental mass between paddocks (P-value =0.3775); One Way ANOVA at $p \leq 0.05$

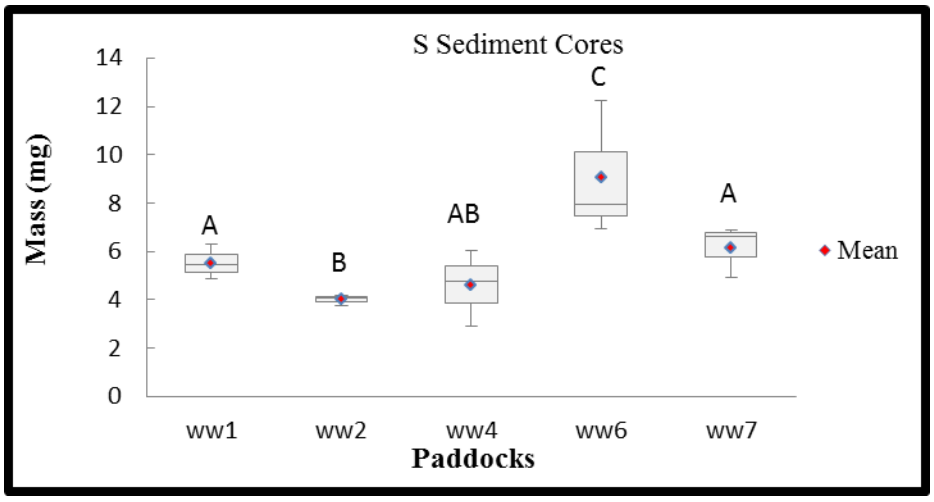


Figure 90: Mean elemental mass pool accumulation of S per whole sediment core (N=3) within each paddock, in the Varkenslaagte canal. Paddocks marked with a different letter showed significant differences in elemental mass pool size; One Way ANOVA at $p \leq 0.05$.

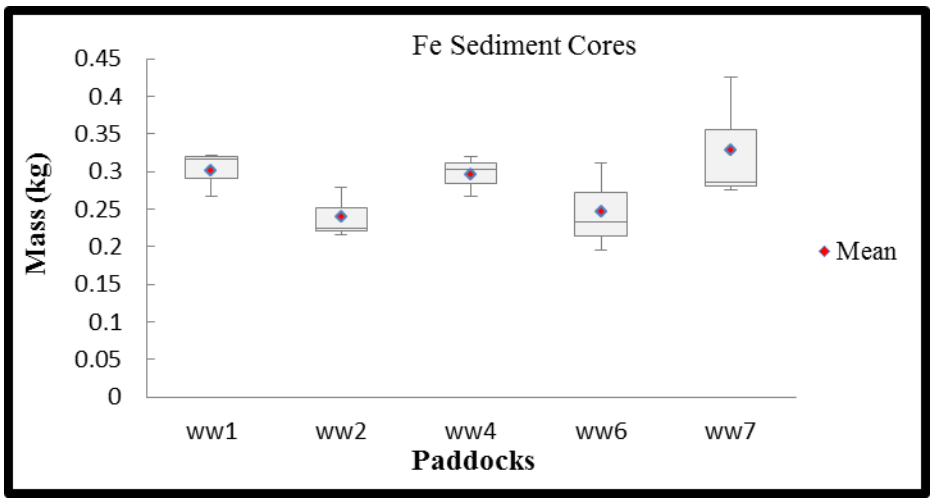


Figure 91: Mean elemental mass pool accumulation of Fe per whole sediment core (N=3) within each paddock, in the Varkenslaagte canal. There was no significant difference in elemental mass pool size (P-value =0.2447); One Way ANOVA at $p \leq 0.05$.

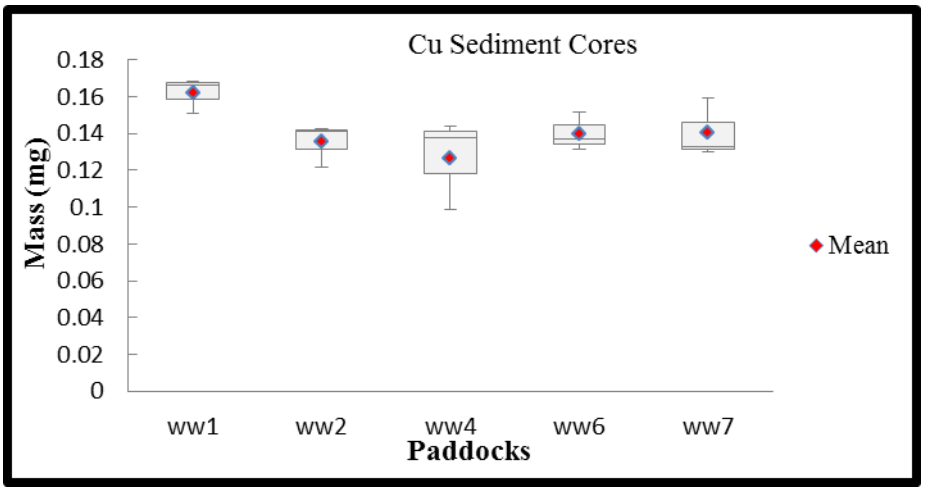


Figure 92: Mean elemental mass pool accumulation of Cu per whole sediment core (N=3) within each paddock, in the Varkenslaagte canal. There was no significant difference in elemental mass pool size (P-value =0.1556); One Way ANOVA at $p \leq 0.05$.

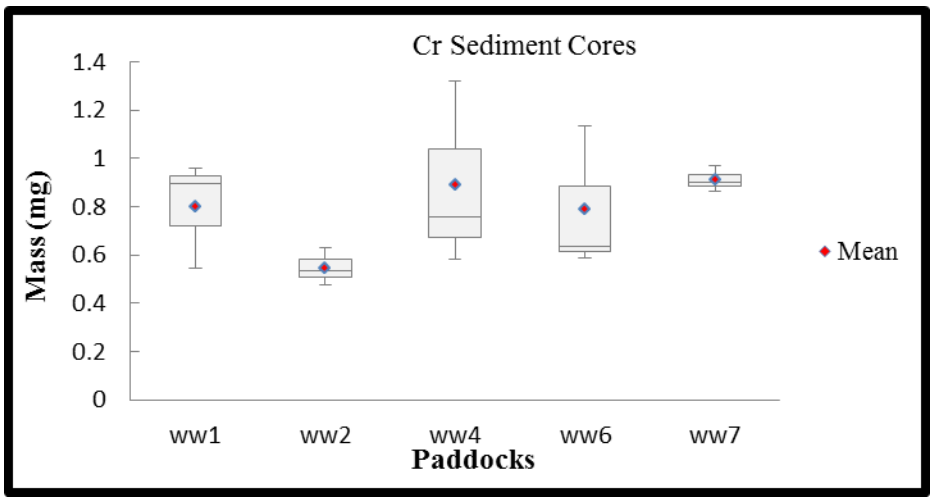


Figure 93: Mean elemental mass pool accumulation of Cr per whole sediment core (N=3) within each paddock, in the Varkenslaagte canal. There was no significant difference in elemental mass pool size (P-value =0.4232); One Way ANOVA at p ≤ 0.05.

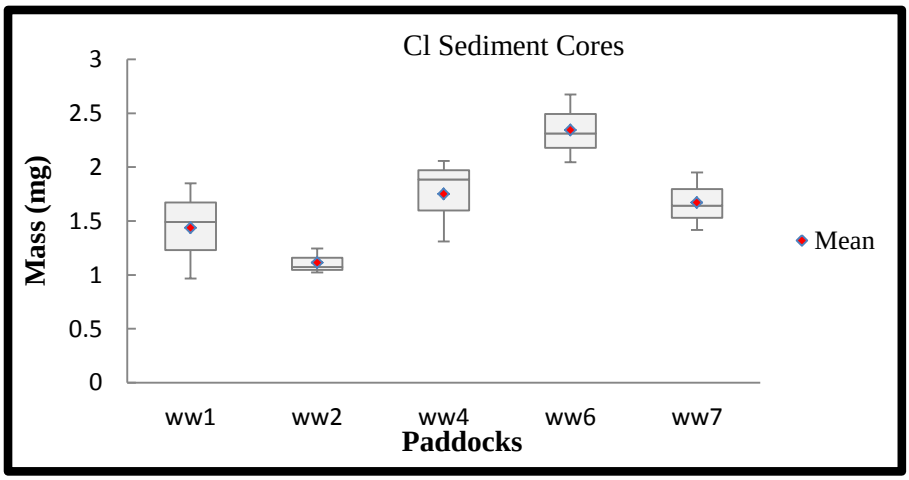


Figure 94: Mean elemental mass pool accumulation of Cl per whole sediment core (N=3) within each paddock, in the Varkenslaagte canal. There was no significant difference in elemental mass pool size (P-value =0.0533); One Way ANOVA at p ≤ 0.05.

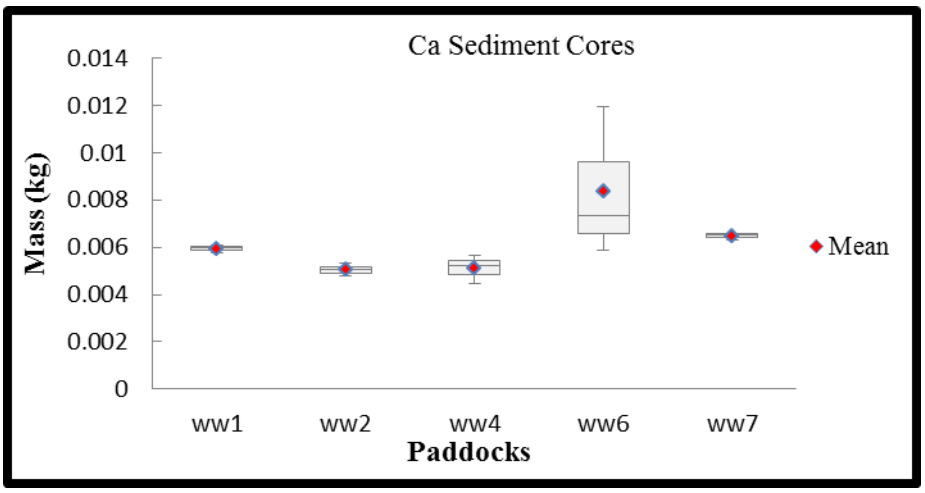


Figure 95: Mean elemental mass pool accumulation of Ca per sediment core (N=3) within each paddock, in the Varkenslaagte canal. There was no significant difference in elemental mass pool size (P-value =0.1014); One Way ANOVA at p ≤ 0.05.

4.7 Water element concentrations

4.7.1 Elements in water samples

In this section, non-essential elements (Na and aluminium), macronutrient elements (phosphorus, S, Ca and magnesium) together with micronutrients (Fe) that were analyzed in water samples are illustrated in boxplots showing the range, mean, and the median. The elements are compared to the water sample data that were analyzed by Omo-Okoro (2015). The element raw data are found in Appendix D2. Standard deviation in these elements is relatively low and indicates that representative samples of these element concentration samples may be representative of concentrations in the wetland paddocks.

The mean element concentration comparison is presented in Table 11. Phosphorous has been excluded from the table and graph presentations, as this element was not detected in any of the paddocks in both water samples for this study and Omo-Okoro (2015). Aluminum was also not detected in water samples for this study as compared to Omo-Okoro(2015). Water samples from this experimental study together with the water samples from Omo-Okoro(2015) were statistically analyzed and their elemental concentrations were compared with each other in Table 11.

Table 11 : Comparison of mean element concentrations found in stream water samples of the uppermost paddocks with data of Omo-Okoro (2015).

	Paddocks					
Elements	ww1	ww2	ww4	ww6	ww7	
S (mg/l)	705.43	678.87	1049.73	942.4	918.63	
Mg (mg/l)	192.97	195.73	285.73	321.67	300.33	
Na (mg/l)	181.43	174.6	280.67	232.93	227.53	
Ca (mg/l)	163.33	158.47	143.68	176.6	183.53	
Fe (mg/l)	0.03	0.04	0.02	0.08	0.09	
Omo-Okoro, 2015	Paddocks					
Element	ww1	ww4	ww7	ww10	ww13	ww15
S (mg/l)	780.6	804.67	1080.37	702.43	848.2	1180.83
Mg (mg/l)	197.9	143.9	221.17	128.63	208.83	310.6
Na (mg/l)	271.73	240.27	319.5	226.9	181.47	210.53
Ca (mg/l)	227.03	185.13	260.87	189.27	210.93	274.5
Al (mg/l)	0.12	0.03	0.09	0.12	0.11	0.11
Fe (mg/l)	0.08	0.04	0.1	0.08	0.02	0.06

In general, the water samples from this study had Fe, Ca, and Na concentrations that were below those found by Omo-Okoro (2015). Omo-Okoro (2015), samples exhibited a slightly higher mean S concentration in paddocks ww1 and ww7 as compared to concentrations found in this study. Water samples from this study displayed a higher mean S concentration in paddock ww4 and higher Mg concentrations in paddocks ww4 and ww7 when compared to the respective concentrations that were found in water samples from Omo-Okoro (2015).

The water samples from Omo-Okoro,(2015) in paddock ww15 contained the highest S mean concentrations. Although Fe concentrations of Omo-Okoro (2015) water samples were higher, the concentration values were within a very similar range and only the maximum value exhibited in paddock ww7 exceeded the target for water quality range of 0-0.1 (DWAF, 1996).

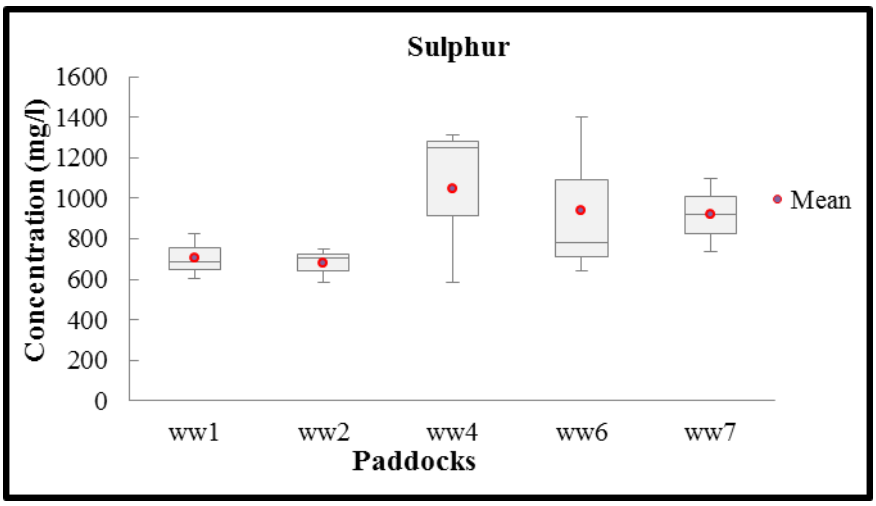


Figure 96: Mean S concentration in stream water samples of the uppermost wetland paddocks in the Varkenslaagte canal (N=3).

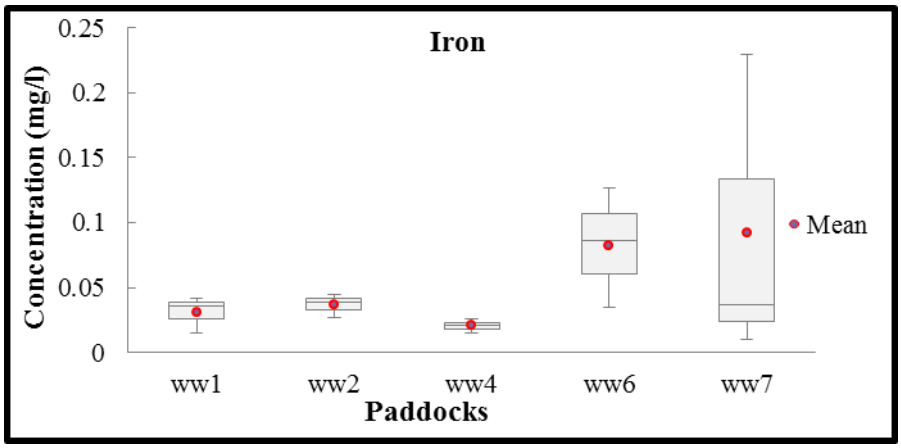


Figure 97: Mean Fe concentration in stream water samples of the uppermost wetland paddocks in the Varkenslaagte canal (N=3).

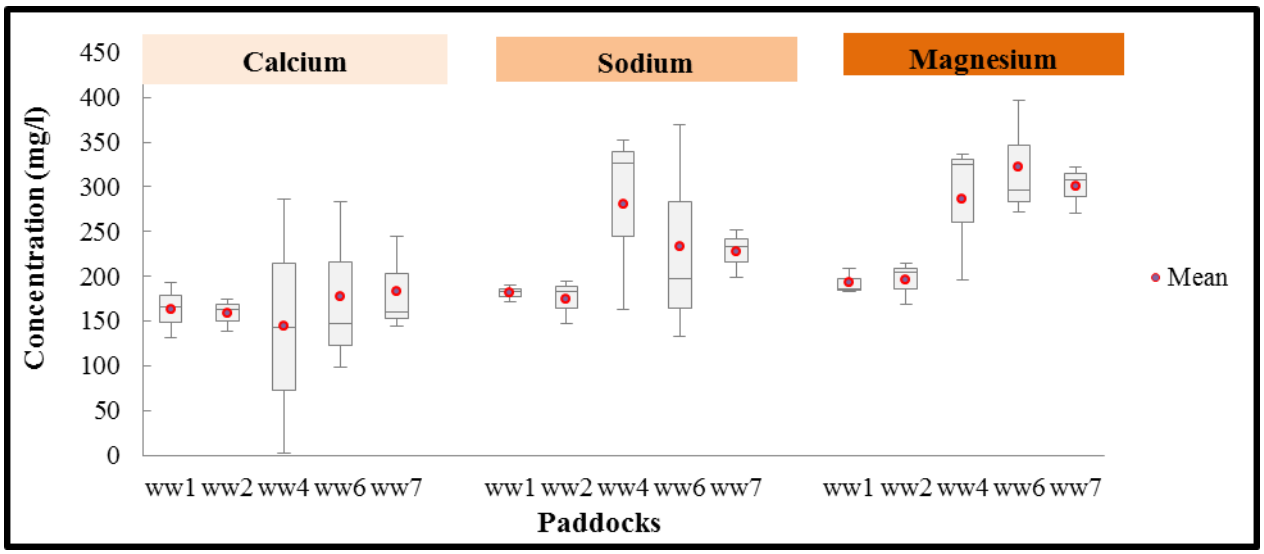


Figure 98: Mean elements concentrations in stream water samples of the uppermost wetland paddocks in the Varkenslaagte canal (N=3).

Aluminum and phosphorous from the stream water samples were not detected in any of the paddocks. Sulphur concentrations are far above the recommended limit for moderate human consumption of 0-200 mg/l of South African Water Quality Guidelines (DWAF, 1996). The mean concentration values lies between 678.87 and 1049.73 mg/l (Table 15). Sulphur exhibited its maximum value in paddock ww6 while the minimum value was displayed in paddock ww4 (Figure 96). Although the lowest minimum value was displayed in paddock ww4, the highest mean concentration was also recorded in paddock ww4. There is no trend or pattern observed in S concentration in the stream water from the wetland paddocks.

The concentrations of Fe in the water samples from paddocks ww1 to ww4 were within the acceptable Fe target water quality range of 0.001 -0.05 mg/l in the South African Water Quality Guidelines for domestic use (DWAF, 1996). There is a slight increase in Fe concentrations in paddocks ww6 and ww7 displaying greater maximum values which are way above the Fe target of the South African Water Quality Guideline (Figure 97). Sodium concentration is far above the guideline for moderate human consumption of 0-100 mg/l in the South African Water Quality Guidelines (DWAF, 1996). The concentration values lie between 193.0 and 300.3mg/l and the highest mean and maximum concentrations were found in paddocks ww4 and ww6 (Figure 98) while the lowest value was also found in paddock 6.

The concentration of magnesium increased below paddock ww2 and slightly dropped in paddock ww6. The concentration increased to 280.7 mg/l in paddock ww7, which is far

above the acceptable South African water quality range 0- 30 mg/l (DWAF, 1996). South African water quality guidelines for Ca concentration range from 0-32 mg/l (DWAF, 1996). The concentration of Ca in the paddocks ranged from 143.7-183.5 mg/l. A slight drop in Ca was displayed in paddock ww4. Calcium exhibited its minimum value (0.001) in paddock ww4 and maximum values in paddocks ww4 and ww6 respectively. There was no trend or patterns observed in S, Ca and Mg in water downstream from the uppermost wetland paddocks.

4.8 The average mass allocation in plants and sediments by paddocks

This section of results presents the calculated mass pool of macronutrients, micronutrients, and non-essential elements and quantifies their distribution in the wetland compartments.

Table 12 : The average mass allocation in sediments per paddock

	Averaged Mass Pool Size in the whole paddock							
Paddocks	Fe (kg)	Ca (kg)	Cl (g)	Cr (g)	Cu (g)	Zn (g)	U (g)	S (g)
ww1	2069.17	41.00	9.85	5.48	1.11	9.75	0.035	37.87
ww2	1647.86	35.00	7.63	3.75	0.93	8.02	0.034	27.40
ww4	2033.67	35.00	12.01	6.09	0.87	13.60	0.032	31.38
ww6	1693.44	57.00	16.07	5.40	0.96	12.55	0.034	62.04
ww7	2256.36	44.00	12.45	6.25	0.96	12.91	0.043	42.06

Table 12 above provides the average mass element pool of sediments in each wetland paddock. The average mass accumulation of Ca, S, and Cl were found to be highest in paddock ww6 while Fe, Cr and U were highest in paddock ww7. In general, paddock ww7 was found to have the second highest elemental mass pools. Copper and Zn were found to be highest in paddocks ww1 and ww3 respectively.

The lowest mass pools for most elements were found in paddocks ww2 while the bottommost paddocks (ww6 and ww7) showed most of the highest mass pools.

Table 13: The average mass pool allocation in *S. corymbosus* per paddock

	Averaged element mass (mg) across all plant plots within a paddock								
Paddocks	Total number of plant plots in a paddock	S (mg)	Cl (mg)	Ca (kg)	Cu (mg)	Cr (mg)	Fe (kg)	Zn (mg)	U (mg)
ww1	150	27.12	17.18	0.02181	0.0608	0.0842	0.0358	0.0609	0.0137
ww2	150	65.77	41.33	0.06181	0.1970	0.1862	0.0851	0.1970	0.0364
ww4	300	172.78	92.80	0.18463	0.6182	0.4032	0.1345	0.6182	0.0682
ww6	500	34.45	24.49	0.02564	0.0625	0.0912	0.0245	0.0625	0.0114

Table 14 : The average mass pool allocation in *P. australis* per paddock

	Averaged element mass across all plant plots within a paddock								
Paddocks	Total number of plant plots in a paddock	S (mg)	Cl (mg)	Ca (kg)	Cu (mg)	Cr (mg)	Fe (kg)	Zn (mg)	U (Mg)
ww1	150	47.35	30.90	0.04444	0.1261	0.1016	0.0351	0.4536	0.0261
ww2	150	75.62	55.33	0.07554	0.2748	0.1741	0.0646	0.7518	0.0467
ww4	300	90.08	61.74	0.08428	0.6813	0.3591	0.1253	1.5813	0.0817
ww6	500	64.80	38.44	0.06614	0.1179	0.0914	0.0392	0.3653	0.0274
ww7	150	45.65	31.73	0.04554	0.1056	0.0120	0.0377	0.3518	0.0234

Table 13 and 14 above provides the elemental mass average pool size per plant plots of *P. australis* and *S. corymbosus* species per wetland paddock (i.e. how many mg of dry mass did *P. australis* and *S. corymbosus* accumulate in each paddock).

The average accumulation of S and Cl elements were found to be highest in paddock ww4 in both species with S accumulated more by *S. corymbosus* and Cl by *P. australis*. In paddock ww4, S average mass pool accumulation of 90.08 mg in *P. australis* was lower than the 172.78 mg in *S. corymbosus* while the Cl average mass pool size of 92.80 mg in *S. corymbosus* was higher than 61.74 mg in *P. australis*. Paddock ww2 appeared to be the second paddocks to accumulate higher S in both species with *P. australis* accumulating more mass in both elements than in *S. corymbosus.*

The lowest element mass pool accumulated by the two plants was U with the lowest accumulation in paddock ww1 in *P. australis* and lowest in paddock ww7 in *S. corymbosus*.

In general, the average mass accumulation of Fe, Ca, Cr, Cu, Zn, U elements were found to be higher in paddocks ww4 and paddock ww2 was found to be the second highest in both species (Table 13 and Table 14). Paddock ww1 was found to have lowest average mass accumulation of the abovementioned elements as compared to paddock ww6 in both species. Paddock ww7 did not have *S. corymbosus* during this study.

4.9 Plant compartment comparisons

Table 15: *S corymbosus* **compartment comparison**

S corymbosus P. australis compartment concentrations			
Element	Shoots	Roots	Rhizomes
Zn	L	L	H
U	L	H	L
Sulphur	H	L	L
Fe	L	H	M
Cu	L	H	M
Cr	L	H	H
Cl	H	M	L
Ca	H	M	L

Table 15 above provides summary of *S. corymbosus* compartment concentrations and it should be noted that L refers to Low, M to Medium and H to High, this format will be used frequently in the following tables below. Zinc and Cr were found to be highest in rhizomes and S, Cl and Ca were highest in shoots while U, Fe, Cu, Cr were highest in roots.

Table 16: *P. australis* **compartment comparison**

P. australis compartment concentrations			
Elements	Shoots	Roots	Rhizomes
Zn	L	H	M
U	L	H	L
Sulphur	-	-	-
Fe	L	H	H
Cu	L	H	L
Cr	L	H	H
Cl	H	M	L
Ca	H	L	L
Zn	L	H	M

Table 16 above provides summary of *P. australis* compartment concentrations. Zinc, U, Fe, Cu, and Cr were highest in roots while Cl, and Ca were highest in shoots, Fe was

highest in roots but not significantly different from the rhizomes. Sulphur in plant compartments was not significantly different.

Table 17: *P. australis* **and** *S. corymbosus* **mass**

	P. australis				*S. corymbosus*			
Element	Shoots	Roots	Rhizomes	Total (%)	Shoots	Roots	Rhizomes	Total (%)
Zn	29	44	20	93	3	2	2	7
U	28	26	12	66	16	11	7	34
Sulphur	33	18	3	54	21	16	9	46
Fe	10	28	12	50	12	29	9	50
Cu	14	35	18	67	13	13	7	33
Cr	23	23	8	54	18	15	13	46
Cl	42	11	11	64	22	7	7	36
Ca	47	13	7	67	18	9	6	33

Table 17 above provides summary of *P. australis* and *S. corymbosus* elemental masses. Sulphur, Fe, Cr had a similar elemental masses between species, and U, Cu, Cl, and Ca were double the total mass in *P australis* while Zn had a total mass of 93% in *P. australis* as well. In general, *P. australis* had the highest total masses as compared to *S. corymbosus.*

4.10 Sediment depth interval concentrations comparison

Table 18: Sediment Concentrations by depth interval

	Sediment Concentrations by depth interval		
Element	0-2cm	2-10cm	10-30cm
Zn	H	L	L
U	H	L	L
Sulphur	L	M	H
Fe	L	M	H
Cu	L	H	H
Cr	-	-	-
Cl	L	M	H
Ca	L	M	H

Table 18 above provides summary of sediment elemental concentrations by depth interval. Zinc and U were highest in depth interval of 0-2cm while sulphur, Fe, Cu, Cl, and Cl were highest in depth interval of 10-30cm, although Cu was not significantly different

from depth interval 0-2 cm. There was no significant difference in depth interval concentration of Cr.

4.11 Sediment masses comparison per paddock

Table 19: Sediment masses comparison per paddocks

	Sediment masses per paddocks				
Element	ww1	ww2	ww4	ww6	ww7
Zn	-	-	-	-	-
U	-	-	-	-	-
Sulphur	M	L	M	H	M
Fe	-	-	-	-	-
Cu	-	-	-	-	-
Cr	-	-	-	-	-
Cl	-	-	-	-	-
Ca	-	-	-	-	-

Table 19 above provides summary of the sediment elemental masses per paddock. In all the test elements, there was no significant difference between paddocks except for S . Sulphur mass was highest in paddock ww6 and lowest in paddock ww2.

5 CHAPTER FIVE

5.1 Discussion

This section discusses the results and limitations of the data collected and analysed from key environmental compartments. The first section discusses the *in situ* water measurements and the last two sections discuss the results analysed from plant, sediment and water samples.

5.2 *In situ* test strips and YSI water measurements

This section briefly discusses the results obtained from *in situ* water samples collected during summer in December 2013 in comparison to winter data (May and July 2013) collected by Joubert (2013). The water samples were collected from five of the seven uppermost paddocks while the *in situ* measurements were undertaken in all of the 15 paddocks located along the Varkenslaagte canal. The statistical table of the *in situ* probe and test strip data can be found in Appendix E.

5.2.1 Dissolved oxygen (DO)

The low concentration of dissolved oxygen could be caused by the presence of oxidizable organic matter in both this study and the study by Joubert (2013); according to Wetzel (1983), algae may also cause the consumption of oxygen. It was noted that wetland paddocks during the study had massive surface algal mats. There was no trend or pattern observed in DO downstream along the Varkenslaagte canal.

5.2.2 pH

The pH decline from 7.48 to a minimum of 5.82 observed in the bottommost paddocks in this study could be attributed to seepage water entering the paddocks from the side canal banks and such water may decrease the pH values. However, this study found higher pH values in the uppermost paddocks than the winter surveys of Joubert (2013). The rainfall received during the summer season might have increased the pH values because the lower pH values from additional data by Joubert (2013) has been attributed to the lower flow rate and this has been observed during sampling in the winter season when seepage from the side banks was at the lowest rates. The net effect of rainfall will depend on the relative importance of dilution of acidic seepage versus possible surface runoff mobilizing pollutants. The distribution of pH values shows lower values during the winter season (i.e. May and July 2013) and increases during the summer seasons (November and December 2013) when the study area receives more rainfall. The additional data by Joubert

(2013) obtained during the winter seasons, showed a decrease in pH whereby seepage from the side banks may have contributed to the low pH observed between paddocks ww3 and ww7. From this study there was no clear linear pattern observed along the canal except for a slight decrease in pH values through most of the wetland observed during the winter season.

5.2.3 pH and pH Fix (0-14PT) comparison

The YSI probe and test strip pH measurements were compared to assess the potential to reduce *in situ* analytical costs. The test strips provide "categorical" data, and the probes provide "continuous" data. The pH-Fix test strips yielded poor results, indicating lower pH-Fix values than the YSI probe data in paddocks ww5, ww8, ww12, Mangaan Drive and paddock ww13 after the bridge away from the tailing contaminants source (Figure 10). However, there seems to be a tendency of agreement between the pH-Fix test strips and pH probe values at paddocks ww1 to ww4, ww6 to ww7, ww9 to ww11 and ww13 to ww16 (Figure 26). As explained previously the pH values were found to be in the range of 5.82 to 7.48 and pH-Fix values were in the range of 4 and 8.

The lowest pH values were found in paddocks ww13 and ww16. During the study period, no extreme mean pH values below 4 were recorded. In both methods there was no linear pattern observed along the canal.

5.2.4 Electrical Conductivity (EC)

This study found no clear pattern in EC through the wetland paddocks, but very high values in the range 4000-6000 µS/cm were found in most paddocks. An extreme high value for EC was observed in paddock ww2, while much lower values were found at Mangaan Drive and in the lowest paddock ww18.

Generally, this study found higher summertime EC values than the data collected by Joubert (2013) in autumn and winter (Figure 27). While no definitive explanation may be offered, is possible that the high EC values from this study may be attributed to increased wet season flushing of AMD entering laterally as seepage through the contaminated soil profile (Naicker *et al.,* 2003). Surface runoff flushing of accumulated surface salts during the wet season is also a possible influence, although the relative importance of this process against the opposing effect of dilution by higher rainfall cannot be established with existing data.

5.2.5 Redox Potential

The Eh measurements showed strong seasonal variation, with lower values in winter compared to summer readings (Figure 28). This may be as the result of water rainfall washing AMD into the canal or alternatively, an indication of AMD seepage from the side banks during summer seasons. However, this is not conclusive as the predictions of AMD rely upon long-term assessments.

5.2.6 Nitrate probe and Nitrate test strips (10-500 mg/l)

The test strips provide "categorical" data, and the probes provide "continuous" data. The nitrate test strips provide "categorical" data and yield high results compared with the nitrate probe data, suggesting higher nitrate concentrations of up to 25 mg/l in some of the lower paddocks. The nitrate probe data indicate much lower concentrations than the test strip data (Figure 29 and 30). However, the test strips provided categorical values of 0, 10 or 25mg/l while the YSI probe indicated values lower than 1 mg/l. The lack of common patterns in both datasets gives little confidence in either method for this study site. Further testing of both methods against an established laboratory method would be required to adequately assess their suitability for AMD affected sites such as the Varkenslaagte Canal.

5.2.7 Nitrite (NO_2^-) Concentration

The nitrite concentration showed peaks in paddocks ww7 and ww12 but otherwise indicated zero values down to paddock 10, with increased values thereafter (Figure 31). According to the South African Water Quality Guideline DWAF (1996a) on nitrite concentration for domestic water use, a nitrite concentration of 20 mg/l is regarded as the safest limit for babies so the concentrations in this study did not exceed the acceptable level of nitrite concentration in drinking water. However, it should be noted that the categorical test strip data indicated only 0, 1 or 5mg/l for the study sites.

5.2.8 Sulphate (SO_4^{2-})

For sulphate using the test strips in water, the highest values of 1600 mg/l (above the target water quality range) were observed at the uppermost paddocks ww1 to ww7 and in paddocks ww9, ww12 and ww14, ww16 (Figure 32). There is an apparent general decrease below paddock ww7 and especially downstream from Mangaan Drive, but for all paddocks the sulphate concentration in general is far above the target water quality range (0-200 mg/l) according to (DWAF, 1996).

The general decreases in sulphate suggest that downstream transport in surface water is reduced through the wetland system.

5.2.9 Sulfite (SO_3^{2-})

The only non-zero concentration peaks were found at paddocks ww14, 17 and 18 towards the bottom of the wetlands (Figure 33). The categorical intervals of 0, 10 or 25 mg/l may be too large to provide useful data on spatial patterns in the paddocks.

5.2.10 H_2O Hardness

The test strips provide "categorical" data, but all concentrations were outside the detection range of 25 mg/l throughout the Varkenslaagte canal from the upper paddock to the bottom paddock (ww1 to ww18). Hence, the test strips are of no value for analysis in this system.

5.2.11 Test strips utility

Based on these results, the test strips are not considered be useful indicators of water quality parameters in the Varkenslaagte canal, except possibly for sulphate where an apparent declining downstream trend was shown. In general, the test strip showed poor results, for nitrite, nitrate, sulfite H_2O hardness and pH suggesting little utility in this extreme contaminant plume-receiving environment.

5.3 Sediments analysis of pH, Eh, and EC by depth and paddocks

The aim of this study was to assess and quantify the mass pool size of contaminants source (macronutrients, micronutrients, non-essential trace elements) within and between a subset of paddocks from various compartments including sediments, aboveground biomass (shoots, stems and leaves), and belowground biomass (roots and rhizomes).

The mean values of pH by paddocks ranged from 5.7 to 7.2 with the lowest means in paddocks ww6 and ww7 (Figure 35). There was no significant difference in pH values by interval depth in the sediment (Figure 38). There were no extreme pH values lower than 5 or greater than 8 recorded (Figure 35). Soil pH lower than 5 affects uptake of essential nutrients and water (Marschner, 1995). Low pH in soil also reduces the availability of macronutrients *inter alia* Mg, Ca and P. The sediment pH showed a significantly decreasing linear pattern downstream in the uppermost paddocks, with unexpected lower values observed in paddocks ww6 and ww7 away from the known upstream pollution source in the TFSs (Figure 35).

As described previously the bottom paddocks were fairly dry as compared to the uppermost paddock, so it is possible that the water that has evaporated from these paddocks left concentrated pollutants resulting in lower pH values away from the tailing

pollutant source. Alternatively, lateral inputs of AMD may have impacted the lower paddocks. Soil pH behavior affects the mobility of many metals *inter alia* Cu, Cr and Co, and other heavy metals (Violante *et al*., 2010).

According to Ritter *et al.* (2002), processes of oxidation-reduction influence the environmental chemistry of metals. The Eh similar to pH showed no significant variation with depth (Figure 37). The Eh mean values in sediment cores ranged from 248 to 379 mV. Element mobility in sediments is affected by changes in pH and Eh (Morgan and Stumm, 1996). There was no specific pattern observed in Eh values along the paddocks in the Varkenslaagte canal (Figure 34) but generally, the lowest values were in paddock ww1 and highest in the downstream paddocks ww6 and ww7. The Eh values in sediment cores were much higher in the sediments than *in situ* stream water measurements.

Sediment EC values showed a significant decreasing trend with interval depth but there were no significant differences in EC values between paddocks of the Varkenslaagte canal (Figure 36 and 39). The average values of EC were in the range of 432.3 µS/cm to 637.0 µS/cm, similar to the higher EC *in situ* stream water readings. According to Sheoran and Sheoran (2006), Navarro *et al.* (2008) and Galvan *et al.* (2012), water characterized by AMD has high salinity (and high electrical conductivity). According to a previous study done by Lusilao (2012), the Varkenslaagte drainage area is well known to be extremely contaminated. In this study, the sediment cores from the canal as well as surface waters yielded high EC values.

5.4 Elemental concentrations and mass pool allocation in plants

As described previously, analysis in this study was undertaken for a large number of trace and metal elements. However only few macronutrients, micronutrients, and non-essential elements that have significant importance to AMD in the West Wits Mining Operation (based on previous studies) were selected and statistically tested (i.e. Zn, U, S, Fe, Cu, Cr, Cl and Ca). This section discusses the elemental concentrations and mass pool accumulation in the key environmental compartments of the selected five uppermost engineered wetland paddocks, i.e. sediments, water and biomass (live dominant vegetation – shoots (leaves and stems), fine roots and rhizomes).

Herein is a description and discussion of the uptake and distribution of metals in the whole plant, plant compartments and plant element distribution by paddocks of the two species i.e. *P. australis and S. corymbosus* over the summer season (December 2013). The sampling in summer only was due to financial constraints. Generally, from the elements

tested in the whole plant in both species, it was found that S, Fe, Cr had a similar elemental masses between species, while elements such as U, Cu, Cl, and Ca were double in their total masses in *P australis* compared with *S. corymbosus*. In plants, 93% of Zn mass was found *P. australis* and only 7% was found in *S. corymbosus* (Table 17). In general, *P. australis* had the highest total masses as compared to *S. corymbosus.*

The behaviour (fate of metal) has been consistent with patterns observed elsewhere in the literature for instance according to Marschner (1995*)* streams serve as a transport medium, the soil as a reservoir of heavy metals and the plants take heavy metals up as micronutrients such as Cu, Zn, Fe and others. This suggests that the two plants (*P. australis* and *S. corymbosus*) could be taking up significant amounts of these micronutrients from the paddocks. The study by Windhama *et al.* (2001) found that macrophyte plant species grow seasonally, whereby greater concentrations of heavy metals such as Zn, Cu and others are found in summer. In this study, it was not possible to compare the accumulation of heavy metals in different seasons, but both plant species showed accumulation of heavy metals. In terms of general mass accumulation, U accumulated the lowest average mass pool of 0.0134 mg and S accumulated the highest average mass pool of 127.78 mg in *S. corymbosus* while *P. australis* accumulated the lowest 0.0261 mg and highest 90.08 mg mass of U and S respectively per paddock.

The results showed that for the two species, metal mass up-take was consistent between the wetland paddocks in concentrations of metals in the whole plant only, i.e. there were no significant differences in metal uptake between paddocks for any element, except for S. However, the reason why *P. australis* and *S. corymbosus* accumulate elements such as Fe, Cr, Ca, Cu and Zn while resisting Hg and Na (which were below detection limits) in the Varkenslaagte canal should be examined in a further study.

P. australis and *S. corymbosus* plant compartments (i.e. shoots, roots and rhizomes) showed similar trace metal concentration patterns in terms of above versus below ground compartments across the uppermost wetland paddocks of the study area. Concentrations of Zn, U, Fe, Cu and Cr in belowground biomass of both species were greater than in the aboveground biomass while Cl and Ca were greater in the aboveground biomass (shoots) in both species. Sulphur concentrations presented key differences between these two species; S in *S. corymbosus* had greater concentrations in the aboveground biomass than the belowground, while in *P. australis* S did not show significant differences between plant compartments.

Patterns of mass allocation were rather different to patterns in concentration, due to the relative biomasses of the different compartments for both species. *P. australis* and *S. corymbosus* plant compartments (i.e. shoots, roots and rhizomes) showed similar metal mass allocation patterns in these compartments across the uppermost wetland paddocks of the study area. For S, Cr, Cl, U and Ca the proportion of mass accumulation of these elements in the aboveground biomass was higher than the belowground in both species. Similar allocation patterns are seen in the majority of wetland plants, which retain higher amounts of metals in their shoots than in roots and rhizomes.

The observations of element mass allocation in this study are relatively consistent with the patterns observed by Windhama (2001), who found that metal mass accumulation was consistently higher in the shoots than the roots. According to Du Laing *et al.* (2009) generally individual leaves acquire greater concentrations of metals over their life span. Weis and Weis (2004) reported that only 4–20% of the aboveground metals in reed plants were found in leaf tissue because stem biomass production is much higher. However, contrary to this, in *P. australis* the roots system accumulated more mass of Zn, Fe and Cu than the shoots. The lowest mass pool accumulation of most elements was in the rhizomes of both *P. australis* and S. *corymbosus* with the exception of Fe and Cu which had the lowest pools in shoots in *P. australis*. Presumably, most of the uptake from this study is through roots. However, it is possible that some metal uptake can also occur through different mechanisms (i.e. through the stoma) and possibly through other points of entry in the plant.

While specific differences in aboveground allocation may not influence overall wetland metal fluxes, plants may promote metal bioavailability by moving more metals into aboveground biomass (Windhama, 2001). In this study it was found that *S. corymbosus* compartment concentrations of Zn and Cr were found to be highest in rhizomes and S, Cl and Ca were highest in shoots while U, Fe, Cu, Cr were highest in roots. *P australis* compartment concentrations of Zn, U, Fe, Cu, and Cr were highest in roots while Cl, and Ca were highest in shoots. Iron elemental concentration was highest in roots but not significantly different from the rhizomes. Sulphur in plant compartments was not significantly different.

The statistical analysis revealed that in vegetation there was no significant difference in mass pool accumulation of all the elements tested along the paddocks except for S in *P. australis.* Sulphur was significantly highest in paddock ww6 and lowest in paddock ww4 (Figure 64). An initial assumption was that pollutants enter the wetland paddocks from the uppermost paddock i.e. paddocks ww1 and ww2 respectively, then discharge these

pollutants from the system in the two bottommost paddocks ww6 and ww7 respectively following a sequential chain down the paddocks. In general, the mass pool size accumulation of Zn, U, S, Fe, Cu, Cr, Cl and Ca showed no signal of linear reductions away from the pollutants source of entry, along the paddock wetland chain. This may suggest that there could be seepage of contaminant plumes from the New North slimes dams with lateral inputs into some of the paddocks. Alternatively, spatial heterogeneity in the distribution of sediment contaminants following initial construction of the paddocks may be driving patterns in the vegetation. Further longer-term studies would be needed to resolve these questions.

The comparison between paddocks of weighted elemental concentrations in each of the four sampled plants per paddock from the Varkenslaagte canal showed no significant difference in all weighted concentration except for S in *P. australis* only. Sulphur in *P. australis* showed an apparent increasing pattern down the chain of wetland paddocks but there were no significant differences between paddocks ww1, ww2 and ww4. However, ww6 and ww7 were significantly higher than the upstream paddocks. Paddocks ww6 and ww7 along the paddock chain are situated away from the assumed pollution entry point of the Varkenslaagte wetland rehabilitation system.

TSFs are the most obvious source of metals in the environment and often release metals in soluble form (Dallas and Day, 2004, Washington *et al.*, 2010). In this study, it is assumed that the sources of elements entering the Varkenslaagte wetland rehabilitation system are from the Old North Complex TSFs and importantly, based on the data collected, also lateral seepage entering the system at its western boundary into the middle wetland paddocks more precisely in paddock ww6 from the New North TSFs.

From the elements selected for this study, the results showed that U was detected at the lowest concentration values in the wetland paddocks vegetation compartment although Na and Hg were below detection limits. Ma (2005) suggested that accumulation of heavy metals by plants would be useful for monitoring the heavy metals associated with stream flows. This response by plants to take up significant amounts of either essential or non-essential elements in a medium containing elevated elements is among the fundamental mechanisms in which plants can be used to remediate contaminated substrates containing excess nutrients or metals through phytoremediation (Cunningham and Berti, 1993).

According to Lusilao *et al.* (2012), the Varkenslaagte drainage area is extremely contaminated. The wetland paddocks were engineered in 2011 to assess their potential as a

remediation measure and pollution control, and this study provides baseline data, which show no clear linear patterns in the vegetation along the chain of paddocks. Elemental concentration and thus mass accumulation within a plant structure may increase as a plant grows and is it possible that with time as the wetland system stabilises, a linear signal may become apparent. However, this is dependent on the effects of lateral seepage into the system.

Under certain environmental conditions, metals may accumulate to toxic proportions and can cause ecological damage (Mokaya *et al.*, 2004). According to Davies and Day (1998), pollutants may enter water bodies in a way that can be said to be non-point source such as seepage, and runoff. These processes may explain why often paddocks ww6 and ww7 had the highest element concentrations and mass pool accumulation in sediments. The higher mass pool accumulation and element concentrations can be linked to the lateral seepage entering the system at its western boundary into the wetland paddocks from the New North TSFs or a possible seepage of contamination plumes from the Old North Complex TSFs.

5.5 Core sediments element mass accumulation

As mentioned previously in sediment samples Na as well as Hg was below detection limits. For the elements Zn, U, S, Fe, Cu, Cr, Cl, and Ca, there was no consistent pattern in average mass pool in sediments in each wetland paddock (Table 12). For most elements the greatest total masses in sediments were found in the downstream paddocks (i.e. ww6 and ww7). However, Fe and Zn showed the greatest sediment mass pools in ww1, and ww4 respectively. Copper Cr, and U were highest in paddock ww7 while Ca, S, and Cl were in paddock ww6. Analysis of mean mass per sediment core (n=3 per paddock) found that the only significant differences between paddocks were for S, which was greatest in paddock ww6 and lowest in paddock ww2.

It was found that for most elements (i.e. Fe, Ca, Cl, Cu, and S) concentration, and hence mass pool accumulation (assuming a uniform bulk density) increased with depth and exhibited greatest accumulation in the deepest interval 10-30 cm. However, both Zn and U showed the opposite pattern, with highest concentrations in the surface sediment which decreased with depth.

Except for the changing concentrations with depth, no general trends or patterns were observed in the trace and metal concentrations in the sediment, with no clear pattern along the chain of wetland paddocks. There was no linear pattern of declining element

concentrations down the wetland paddock chain system, possibly due to the evidence of lateral seepage inputs into paddocks ww6 and ww7.

Generally, the bottommost paddocks (ww6 and ww7) had higher mass pools in sediments as compared to the lowest accumulation in the in the uppermost paddocks (ww1 and ww2). As mentioned previously a possible pollution source of these elements is the New North TSFs or a possible seepage from the Old North Complex TSFs.

According to Frost and Sullivan (2011), AMD associated with gold mining activities often contains radionuclides and heavy metals that are not only associated with surface water pollution, but also responsible for degradation of soil quality. The highly elevated concentrations of elements within the cores such as Fe, S, Ca, Cl and Cu are predominately associated with the West Wits Gold Mining activities. As mentioned earlier, the gold-bearing reefs contain different types of minerals such as native gold, U oxides and sulphide minerals with pyrite being the most abundant (Naicker et al., 2003). According to the results of the present study, there are few significant differences in the mass distribution of the elements analysed between paddocks, which is assumed to reflect either the heterogeneity in the underlying sediments following construction of the wetlands, or lateral inputs into the system as seepage from other TSFs.

5.6 Water analysis

The sulphate concentration is far above the moderate human consumption guideline of 0-200 mg/l of South African Water Quality Guidelines (DWAF, 1996). Concentrations lie between 2000 and 3000 mg/l from which the concentration had maximum peaks in paddock ww2 and ww7. A slight decrease was noted in paddock ww4 after which it increased. Sodium concentration is also far above the moderate human consumption guideline of 0-100 mg/l of South African Water Quality Guidelines (DWAF, 1996). Concentrations ranged between 193.0 and 300.3mg/l from which the concentration had maximum peaks in paddock ww2 (which is in close proximity to the assumed pollution source) and paddock ww7 (which far away from the assumed pollution source).

The concentration of both Mg and Na increased sharply from paddock ww4 to ww7, which may indicate lateral seepage continuously downstream from paddock ww4. The concentration of Mg exceeded 300 mg/l in the downstream paddocks, which is far above the acceptable South African water quality range of 0- 30 mg/l (DWAF, 1996). There was no clear trend in Ca, but the mean concentration of Ca ranged from 143.7-183.5 mg/l while

South African Water quality guidelines for Ca concentration range from 0-32 mg/l (DWAF, 1996).

Iron target water quality range is from 0-0.1 mg/l (DWAF, 1996). The concentration of Fe in paddocks ww1 to ww4 was within the acceptable limits, but a slight increase in the concentrations was noted from paddock ww6 and ww7, which exceeded the DWAF limit.

The earlier study of Omo-Okoro (2013) allows for a seasonal comparison of water chemistry in paddocks ww1, ww4 and ww7 that were sampled in both studies. In general, the summer water samples from this study had Fe and Ca concentrations that were below the concentrations found in the study of Omo-Okoro (2015), which were sampled in winter. Omo-Okoro's (2015) water samples study also exhibited higher S, Na and Mg mean concentrations in paddock ww1 and paddock ww7 of the system. However, paddock ww4 showed higher concentrations during summer (this study) for S, Mg and Na.

The water samples from Omo-Okoro (2015) in paddock ww15, which is far away from the 7 uppermost paddocks, contained higher mean S concentrations than any in this study.

5.7 Links between water, plants and sediments

Few clear relationships could be observed between element concentrations and how concentrations in water or sediment influence accumulation in the vegetation species. Of the elements tested, only S showed significant differences in concentrations in plants between paddocks, with the highest concentrations and mass in the downstream paddocks ww6 and ww7. These paddocks also had the greatest masses of S in sediments, and water concentrations were also highest in paddocks ww4, ww6 and ww7. Hence, high concentrations of S in all media tested were found in the downstream paddocks. Sodium in vegetation and sediments was found to be below detection limits. However, Na concentration in water samples had high mean concentration values, generally greater than Ca and comparable to Mg. The presence of elevated Ca and Mg in plants but not of Na suggested that there is preferential uptake of certain elements, and some species exclude certain metals from their structure while others may accumulate them.

Generally the two plants species studied are well known for their ability to uptake and accumulate various elements and have shown great potential for the remediation of AMD in past studies by Goldblatt and Manning (2000), Torresdey (2007), Ye *et al.* (1998), Butler *et al.* (2008) and Dye and Weiersbye (2010). Based on these results and reviewed

literature a concise conclusion was drawn that the use of these two plant species does exhibit great potential for AMD remediation and Varkenslaagte canal clean-up. However, it was emphasized in this section that there was no linear signal of reducing pollutants concentrations as yet observed along the chain of wetland paddocks. Although this may occur over time as the wetland matures, according to Nyquist and Greger (2009), this lack of linear pattern does not mean that the wetlands are not successfully ameliorating the AMD export from the system. Another important process is the control of the flow of water, which is something widely utilized in the control of AMD migration as water is the main transport medium for trace and metal elements (Akcil and Koldas, 2006).

Likewise, the proportion of the average plant elements allocation across the wetland paddocks was orders of magnitude below the allocation in the sediments – but this does not mean that the wetlands do not assist in reducing the transport of these elements out of the system. There were differences in the mass accumulation between species and also in the above versus belowground pools, with implications for management of the system.

The plant species were sampled from identical sites within the same geographical area and characterised by the same climate, soil genesis and amount of rainfall, but there was a difference observed between *P. australis and S. corymbosus* in terms of the average mass pool size. Although both plants indeed take up mass from sediments, whereas some plants obtains nutrients from both water and sediments and some plants from the water (Du Laing *et al.,* 2009). Overall, *P. australis* responded positively in mass pool size accumulation and exhibited greater element mass pools as compared to *S. corymbosus*. Due to the varying characteristics of the two plants species, differences in the elemental concentration and mass pool accumulation were expected.

P. australis due to its hyperaccumulation capabilities, was more effective in the uptake of the elements thus improving the overall water quality of the wetland as well. As stated earlier in the literature review, plant species with plenty of adventitious roots have the ability to extract water, reducing flow rates and contamination (Dye and Weiersbye, 2010).

Similar inverse relationships were observed for Zn, U, S, Fe, Cr, Cu, Cl and Ca between transfers from the total mass pool in the sediments to the plant species. These elements showed a major response in all the sediment paddocks thus increasing the capacity of elements mass pool in the plant. According to Naiker *et al.* (2002), a problem associated with AMD is the contamination by metals and metalloids such as Fe, Mn, Ni,

Co, Cu, Zn, Al, Cr, As, and others. These elements could potentially have fate as following fates, be taken up by plants and be bio-accumulated, leach into the groundwater or enter surface water bodies and aquatic water systems. This study has proved that the two macrophyte plant species of *S. corymbosus* and *P. australis* are effective in the phytoremediation process in Varkenslaagte canal, these plants have significantly taken up amounts of the selected elements.

The mass pool sequence accumulation in the plant compartment was often as follows in most elements; roots> shoots> rhizomes in both species but with some notable exceptions being Ca and Cl. This suggests that while the elements are extracted by the roots, reducing flow rate and contaminant transport along the chain of wetland paddocks, much gets trapped in the roots, and some translocates into the leaves and a small portion is stored in the rhizomes.

6 CHAPTER SIX

6.1 Conclusion

The study found that the metal mass pool accumulation in the Varkenslaagte canal indicates variable capture within and between a subset of paddocks from various compartments including sediments, aboveground biomass (shoots –stems and leaves), and belowground biomass (roots and rhizomes).

The collection, analysis and comparison of sediment samples and vegetation revealed that U, Cu, S, Fe, Cr, Cl, and Ca are elements of interest and of significant importance to West Wits AMD as these elements showed indications of progressive capture by selected key environmental compartments within the selected paddocks. The ANOVA results indicated that the concentration of the major metals in the Varkenslaagte canal showed significant differences in plants compartments. For *P. australis* and *S. corymbosus* the mass pool accumulation sequence and quantity of pollutant pool in both plant compartments was often as follows: roots > shoots > rhizomes in most selected elements in both species except for element such as Ca and Cl which tended to accumulate more in the shoots.

The study revealed that element mass accumulation in sediments of the above-mentioned elements significantly increased with depth, except for Zn and U, which suggested a possible capture of mobile elements in the paddocks by vertical leaching into the sediment profile. Alternatively, flushing of these elements from surface sediments may occur by surface exchange and leaching in surface runoff.

The major mass pool accumulation for all the elements of interest with significant importance to AMD in the West Wits Mining Operation was via sediment in the wetlands paddocks. Furthermore, Tables 12 to 14 clearly indicate and suggest successful mass pool capture from the sediment in both plants species. The elemental concentrations from water as well suggest that there might be a progressive capture of contaminants.

The study found that the lowest mass pool accumulation of the abovementioned elements was found in uppermost paddocks. Generally, the study showed that the bottommost paddocks (ww6 and ww7) had significantly higher mass pools as compared to the lowest mass pool accumulation in the uppermost paddocks (ww1 and ww2). The ANOVA analysis indicated that the mass pool size accumulation of Zn, U, S, Fe, Cu, Cr, Cl and Ca respectively showed no signal of reducing linear pollutants away from the pollutants source of entry along the paddock wetland chain, assuming that pollutants enter the wetland paddock chain from the uppermost paddocks.

The test strips are considered not be useful indicator of water quality parameters in the Varkenslaagte canal. This attempt for using the test strips method was to assess if the test strips can be a convenient and cost effective way to estimate the amount of nitrite, nitrate, sulfite H_2O and pH and other water quality parameter. The test strips yielded poor results for this extremely contaminated plume receiving environment.

6.2 Recommendations

T. capensis is a common macrophyte that is widespread in the West Wits artificial wetlands, and it is recommended that the extent to which this species can mediate export of metal mass needs to be investigated. Further studies are required on the additional key environmental compartments in order to determine the extent to which metals are significantly taken up (progressive capture) in the wetland paddocks.

Although algal biomass at the study site is insignificant by comparison with vegetation, this component (surface algal mats) has been included as a key environmental compartment for assessment in many studies as it is known that some algae are cable of removing heavy metals. The presence of functional groups such as carboxyl, sulfate, amino and hydroxyl groups in the algal cell wall makes it an ideal biosorbent for heavy metal removal. Further studies may include algal mats as a key environmental compartment in the Varkenslaagte canal.

It is also recommended that in winter the macrophytes are reasonably dry, thus experimental burning could be undertaken. This could be done to assess whether burning may result in the elemental mass loss or redistribution by determining the distribution and pool of macronutrients, micronutrients and non-essential elements within the remaining specified environmental compartments of the engineered wetland paddocks that is sediments, algal mats, water as well as export into other catchments of ashed biomass.

However, one significant key risk associated with burning experiments is the loss of habitat and biodiversity, so the extent of such an experiment would have to be carefully controlled

Finally, there is a clear need to continue longer term monitoring to assess the establishment of a steady-state between waters, vegetation and sediments within the wetland chain and determine whether linear patterns in pollutant retention become a feature in future.

6.3 The study research limitations

The following section briefly explains study area limitations. The research study initially was supposed to evaluate the ability or the use of engineered wetlands to remediate AMD and to treat already formed AMD, with special emphasis on the role of the wetland vegetation (i.e. *P. australis* and *S. corymbosus*) in the remediation process. The study was slightly amended based on financial constraints to investigate the mass pool allocation and concentration of macronutrients, micronutrients, non-essential trace elements within and between a subset of paddock compartments including sediments, and biomass (aboveground biomass shoots i.e. leaves and stems, and belowground i.e. rhizomes and roots). This was done to upscale the elemental concentrations and mass pool size investigation to the whole paddocks, however these samples were not collected on the same day but they were collected in the same summer season.

6.3.1 Bulk density calculation limitations

The core sediments and vegetation were sampled on the 20 December 2013 but were subjected to laboratory analysis in June and July 2014. During this sampling period, the wetland paddocks were wet with wetland biomass extremely fresh and green. The third field sampling was undertaken on the 29 June 2015 to collect sediment samples that were used to estimate bulk density assuming a constant bulk density down the core in the whole paddock to 30cm, since this information had not been collected from previous samples. These sediment cores were extremely dry during this winter period as compared to the summer sediment samples of 23 December 2013. During this sampling season, the two uppermost paddocks were fairly wet and the two bottom paddocks fairly dry.

6.3.2 Vegetation samples biomass calculation limitations

During this study, AngloGold Ashanti Ltd S.A and EEPP did not have records of the number of individually planted plants per paddock. The photographs taken during site visits and dated Google earth images were used to calculate the total number of plants of each species per paddock as they were planted systematically in rows within the paddocks. The total estimation of the number of plants per paddock was undertaken to upscale the vegetation biomass of individual plants to the whole paddock.

6.4 References

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6.5 APPENDIX

Please see next page for appendix