The use of water hyacinth mulch and sewage sludge in gold tailings to improve soil fertility and stability

By

Macdonald. T. Wanenge

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Declaration

I declare that this dissertation is my own work and that it contains no material previously published or written by another person or University, except where due reference is made in the text. It is being submitted for the Degree of Masters of Science at the University of Witwatersrand, Johannesburg.

Macdonald T. Wanenge

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Abstract

Gold tailings contained in Tailing Storage Facilities (TSFs) contain pyrite which on exposure to air and water becomes a source of acid mine drainage (AMD). AMD has high salinity, elevated levels of heavy metals and low pH, which presents serious threats to surface and groundwater systems. These characteristics in tailings present a hostile environment for plant establishment and growth (Witkowski and Weiersbye 1998a). Therefore, it was hypothesized that organic mulch sourced from sewage sludge and water hyacinth could improve tailings fertility on TSFs in the Highveld gold mines of South Africa. The aim of this study was to develop a greenhouse study to understand how four indigenous plants (*Asparagus laricinus* Burch. (Asparagaceae), *Eragrostis curvula* (Schrad.) Nees. (Poaceae), *Hyparrhenia hirta* (L.) Stapf (Poaceae) and *Sutherlandia frutescens* (L.) R.Br. (Fabaceae) naturally colonizing the Highveld gold TSFs would survive, grow and accumulate metals from tailings amended using different percentages of water hyacinth and/or sewage sludge, and the susceptibility of the amended tailings to metal leaching.

Tailings amended with WH: SS-1.0% proved to be the overall best amendment from the 19 treatments based on the variable tested (e.g. plant growth, plant metal uptake and metal leaching). Amending gold tailings with water hyacinth and/or sewage sludge improved seedling survival, plant survival and growth as compared to non-amended tailings. Tailings amended with dry water hyacinth (WH) created the most favourable plant growing conditions especially at 0.5% of amendment, while those amended only with sewage sludge (SS) presented the most challenging plant growth conditions for all four study species. Amending tailings with water hyacinth and/or sewage sludge showed no significant difference in tailings fertility. However, C (%) and total N decrease significantly after plant growth in all treatments. *Hyparrhenia* plants grown in tailings amended with WH: SS-1.0% accumulated significantly higher concentrations of Al, Cr, Ni and Zn, while those growing in tailings amended with WH-0.5% accumulated significantly lower concentration of Al, Co, Cr, Fe and Zn as compared to other treatments. Tailings amended with WH-1.0% leached significantly higher concentrations of Mn, while those amended with WH: SS-0.5% and WL-2.0% leached significantly higher concentrations of S as compared to other treatments. All four species accumulated significantly higher concentrations of Al, Co, Cr, Cu, Fe and Ni in the roots than the shoots, except for *A. laricinus* which accumulated significantly higher concentrations of S, Co, Cr, Mn, Ni and Zn in the shoots than the roots. *Sutherlandia frutescens* retained all the elements tested in its root biomass. Future field studies in the use of water hyacinth and sewage sludge as organic tailings amendments will be required to get a better understanding of these two potential tailings amendment treatment.

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Acronyms and abbreviations

ADEC	Australian Department of Environment and Conservation
AMD	Acid Mine Drainage
ANOVA	Analysis Of Variance
BCF	Bio-concentration Factor
BL	Benoni Lakes
CEC	Cation Exchange Capacity
EEPP	Ecological Engineering and Phytotechnology Programme
EPA	Environmental Protection Agency
ESP	Exchangeable Sodium Percentage
EU	European Union
HWSD	Harmonized World Soil Database
ICPOES	Induced-Coupled Plasma with Optical Emission Spectrophotometer
ITRC	Interstate Technology & Regulatory Council
RCF	Root Concentration Factor
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TF	Translocation Factor
TSF	Tailings Storage Facility
SS	Sewage sludge
USEPA	United States Environmental Protection Agency
VR	Vaal River
WH	Dry water hyacinth
WHO	World Health Organization
WH: SS	Dry water hyacinth and sewage sludge
WL	Fresh water hyacinth
WL: SS	Fresh water hyacinth and sewage sludge

Chapter 1. General Introduction

1.1. Introduction

Gold occurs in association with pyrite minerals in the Witwatersrand Basin, and therefore Tailings Storage Facilities (TSFs – also known as mine dumps) in the Highveld of South Africa contain pyrite minerals. On exposure to air, water and bacterial activity (sulphur-utilizing bacteria), pyrite in the tailings oxidizes, releasing sulphuric acid and becomes a source of acid mine drainage (AMD). AMD has high electrical conductivity or salinity (due to its sulphate concentration), elevated heavy metals and low pH, which present serious threats to both surface and groundwater systems. Using AMD tolerant indigenous trees for the hydraulic control of AMD, and other indigenous plants for rehabilitation of TSFs is considered an ecologically sound and sustainable initiative (Weiersbye *et al*. 2006). However, TSFs often present a hostile environment to plant establishment and growth due to high EC or salinity, low pH, low plant nutrient availability and low soil organic matter (Witkowski and Weiersbye 1998a; Witkowski and Weiersbye 1998b). Organic composts and mulches have been used world-wide to improve soil/tailings fertility in degraded soils and mine tailings (Okalebo *et al*. 2006). Two organic mulches that offer potential in improving tailings fertility in the Highveld mines are water hyacinth and sewage sludge. Apart from being abundant in the Highveld, several studies (Lal 1988; Palazzo and Reynolds 1991; Towers and Paterson 1997; Amoding *et al*. 1999; Wong 2003; Chiu *et al*. 2006; Okalebo *et al*. 2006) have shown that water hyacinth and sewage sludge have the potential to improve the fertility of degraded soils like tailings. Therefore, it was hypothesized that organic mulch sourced from sewage sludge and water hyacinth could improve tailing fertility on TSFs in the Highveld gold mines of South Africa. However, the ideal application rate of organic mulch and any associated improvement in plant growth and survival are still unanswered questions. Therefore, this study was designed to test if different plants survive, grow and accumulate metals from tailings amended with different percentages of water hyacinth and sewage sludge. In addition, this study tested the susceptibility to leaching of sulphur and heavy metals in amended tailings.

1.2. Acid Mine Drainage (AMD)

The process of acid rock drainage formation has always occurred naturally with very little environmental harm, but mining (in this case gold mining) has exacerbated the exposure of the sulphide bearing rock on the earth's surface and hence increased the production of AMD (Akcil and Koldas 2006). Therefore, mining is to a large extent responsible for the environmental problems associated with AMD. However, not all mining operations that expose sulphide bearing rock result in AMD. For example, AMD will not occur if the sulphide mineral is non-reactive, or if the rock contains sufficient base potential to neutralize the acid, or if appropriate AMD control measures are successfully implemented (Barton-Bridge and Robertson 1989).

Depending on the type of mining, gold mining sites can present several AMD sources which can be divided into primary and secondary sources (Akcil and Koldas 2006; Broughton and Robertson 1992). Some of the common primary sources of AMD on a gold mine site include TSFs, underground workings and waste rock dumps, while secondary sources of AMD can include ore stockpiles and TSF footprints (footprints in this regard denote contaminated soil occupying areas left behind after re-mining of the original TSF to reprocess and recover residual gold (Akcil and Koldas 2006; Broughton and Robertson 1992). Gold mine tailings and associated footprints cover an area of about 400 km^2 in the Witwatersrand Basin and pose serious threats to ground and surface water reserves, and tailings are also a common source of dust to surrounding communities (Weiersbye *et al*. 2006).

AMD is characterised by high concentrations of metals (e.g. copper and manganese), low pH and other toxic elements from tailings like uranium (U), arsenic (As), nickel (Ni) and zinc (Zn), which if allowed to enter the environment will contaminate soil, and surface and ground water systems (Akcil and Koldas 2006, Manungufala *et al*. 2005). Weiersbye and Witkowski (2003) observed that AMD polluted soils resulted in increased seedling abnormalities in the radicle and cotyledon growth during the germination of Acacia plants as compared to unpolluted soils.

Generally, if a mining operation results in the exposure of sulphide bearing rock and therefore AMD, control measures of either soil, or surface and groundwater have to be adhered to in order to protect the environment (Barton-Bridge and Robertson 1989). However, prior to 1991 there was very little legislation directed towards environmental impacts caused by mining, which has resulted in South Africa inheriting serious environmental liabilities caused by mining (Weiersbye *et al*. 2006).

1.2.1. Control of AMD

Control of AMD involves three basic methods which essentially involve the control of AMD generation, control of AMD migration and AMD treatment (Barton-Bridge and Robertson 1989; Akcil and Koldas 2006). These different AMD control methods and associated techniques are listed in Table 1-1. However, due to site-specificity the effectiveness and applicability of each AMD control method will vary. For example, a passive system like a wetland used to treat AMD will be effective in treating moderate AMD contamination with fairly low metal concentration and pH values. This is because wetlands have living organisms, facilitating complex interactions between terrestrial and aquatic systems. These living organisms grow and survive within a given metal concentration and pH range; exceeding this range could result in the organisms dying, hence reducing the effectiveness of AMD treatment.

Table 1-1 Three common methods used to control AMD on mine sites (modified from Barton-Bridge (1989) and Akcil and Koldas (2006))

Control of Acid Generations	Control of AMD Migration	Collection and Treatment of AMD
• Conditioning of tailings or waste rock to remove or exclude sulfide minerals • Covers and seals to exclude water • Covers and seals to exclude oxygen (including water cover) • Waste segregation and blending to control pH • Base additives to control pH	• Diversion of surface water flowing towards the polluted site • Controlled placement of waste to minimize infiltration • Interception of ground water	• Active systems- Chemical treatment plants • Passive systems- Treatment by wetlands

Although these methods have been successful in controlling AMD, the biggest challenge is that they are expensive and may be unsustainable (Linacre *et al*. 2005). For example, liming can be used to neutralise AMD and precipitate heavy metals as hydroxides (Akcil and Koldas 2006), but liming is very expensive. For example, the cheapest lime (e.g. dolomitic agricultural lime) costs between R150 to R300 per tonne (including transport costs). The amount of lime required to neutralize a cubic metre of AMD contaminated medium varies depending on the concentration and reactivity of AMD in the medium. Therefore, sustainable and cost effective control methods of AMD have to be developed (Sustainability in this regard relates to the wise use of natural, renewable resources such as vegetation that will be used to control AMD requiring minimal monitoring and maintenance). Sustainability becomes a crucial issue when considering that most mines in South Africa are fast approaching the end of their operational life span and some have closed (Limpitlaw *et al*. 2005). Therefore, appropriate sustainable control methods are required to control AMD on mines for the long term protection of the environment.

Apart from the production of AMD from TSFs, TSFs are also common sources of dust which is a nuisance to surrounding communities (Weiersbye *et al*. 2006). Inhaling dust from TSFs containing harmful elements and radioactive materials could lead to acute or chronic illnesses through cell mutations, cancer and respiratory disease (Manungufala *et al*. 2005). This could become a serious legal issue for mining companies, especially in South Africa where the health and safety of South African citizens is a basic human right under the Constitution of the Republic of South Africa (Act 108 of 1996) Chapter 2, section 24. Therefore, sustainable control methods for AMD should have both environmental and human health considerations.

One suite of control methods that has gained great interest in the treatment of contaminated soils and control of the migration of AMD through reducing erosion and containing pyrite movement through dust are phytotechnologies (Pulford and Dickinson 2006; Cunningham and Berti 1993). Phytotechnologies (e.g. different plant remediation strategies (ITRC 2009)) take advantage of a plant's ability to acquire nutrients from the soil, and from the movement of nutrients entering the plant as part of the plant's food, health, and regenerative requirements. Therefore, before addressing phytotechnologies it is instructive to look at plant nutrition, including different plant nutrients' sources and how these nutrients relate to heavy metals.

1.3. Plant nutrition

Plant nutrition can be defined as the study of the chemical elements that are necessary for plant growth. These chemical elements are often termed plant nutrients and divided into inorganic and organic nutrients. The most commonly used source of plant nutrients (e.g. nitrogen (N) and potassium (K)) come from inorganic fertilizers sourced mainly from man-made fertilizers especially for crop production in agriculture (Miller and Donahue 1990), while organic nutrients sourced from organic materials like plants and animals are a less common plant fertilizer in agriculture production. This is because inorganic fertilizers can be mass produced in a concentrated form and made in a way that they are bioavailable to the plant. Organic fertilizers on the other hand are required in larger quantities and take longer to liberate bioavailable nutrients to the plants since complex organic matter needs to be decomposed first by bacteria and soil fauna (Okalebo *et al*. 2006).

Although inorganic nutrients are commonly used in agriculture to increase plant productivity, Miller and Donahue (1990) identified the following plant nutritional benefits of using organic materials unlike inorganic nutrient sources:

- Organic matter increases soil cation exchange capacity (CEC). CEC is the capacity of a soil (e.g. often negatively charged) to attract positively charged ions (often nutrients) in the soil water matrix. By using organic matter, large cation exchange sites are created having the potential to increase soil CEC by 30-70 %. Therefore, soils with a high CEC have a higher capacity to attract positively charged nutrients moving in the soil-water matrix (Miller and Donahue 1990).
- Organic matter improves soil texture and to some extent soil fabric which consequently improves the aeration and water content of the soil. Soil texture is the proportion of sand, silt and clay in a soil, while soil structure addresses how these three are grouped or aggregated.
- Organic matter can act as a chelate, which helps sequester micronutrient metal ions increasing their availability to plants. This is done by organic compounds such as ligands that can bond to insoluble metals by more than one bond and form rings or cyclic structures resulting in a soluble chelate (e.g. soluble organic compound containing both the organic compound and metal).
- Organic matter is a carbon source for many micro-organisms in the soil. Micro-organisms play a vital role in the formation of soil and supply of nutrients to plants. Through the break-down of organic matter, bacteria help accelerate mineralisation, decomposition and nutrient turnover (Lavelle 1997), while other soil organisms like arthropods are the main decomposers and help aerate the soil.

- Lastly, organic matter can act as a buffer to salinity, acidity, alkalinity, heavy metal and metal toxicity.

Nevertheless, regardless of the source, plant nutrients are divided into essential and non-essential nutrients with the absence of the former resulting in the plant being unable to complete a normal life cycle or the development of severe abnormalities in plant growth, development and reproduction (Taiz and Zeiger 2006). On the contrary, non-essential nutrients (e.g. cadmium (Cd) and lead (Pb)) have no known physiological contribution to plant biology (Lasat 2002). However, the presence of non-essential nutrients in soils even at low concentrations could cause serious threats to the survival, growth and reproduction of plants (McCauley 2009).

Essential nutrients are further divided into macro- and micro- nutrients. Macro-nutrients are required in large quantities, while micro-nutrients are required in relatively smaller quantities.Table 1-2 lists these essential macro- and micro- nutrients and their associated concentrations common to most vascular plants. These nutrients are acquired either passively through the transpiration stream created by the difference in soil moisture potential between plant roots and leaves, or actively through the transport proteins associated with the root membrane (ITRC 2009).

Plant nutrient requirements operate within a given range (defined as the sufficiency range) upon which an increase or decrease could interfere with the plant's growth and/or health (Figure 1-1) (McCauley 2009). Therefore, although essential nutrients are important in improving plant productivity, they may become toxic at high concentrations. Plant nutrient toxicity varies with the type of plant and nutrient; generally in nature, macro-nutrients are less toxic because they are required in larger amounts by the plant unlike micro-nutrients or non- essential nutrients (Hopkins, 1995; McCauley 2009).

Table 1-2 Essential nutrients and associated dry mass concentrations common in most vascular plants (Modified from Raven *et al*. 1999)

Elements	Chemical symbol	Adequate concentration in dry tissue	
		mg/kg	%
Macronutrients			
Sulphur	S	1000	0.1
Phosphorus	P	2000	0.2
Magnesium	Mg	2000	0.2
Calcium	Ca	5000	0.5
Potassium	K	10000	1.0
Nitrogen	N	15000	1.5
Carbon	C	450000	45
Micronutrients			
Molybdenum	Mo	0.1	0.00001
Copper	Cu	6	0.0006
Zinc	Zn	20	0.002
Manganese	Mn	50	0.005
Boron	B	20	0.002
Iron	Fe	100	0.01
Chlorine	Cl	100	0.01

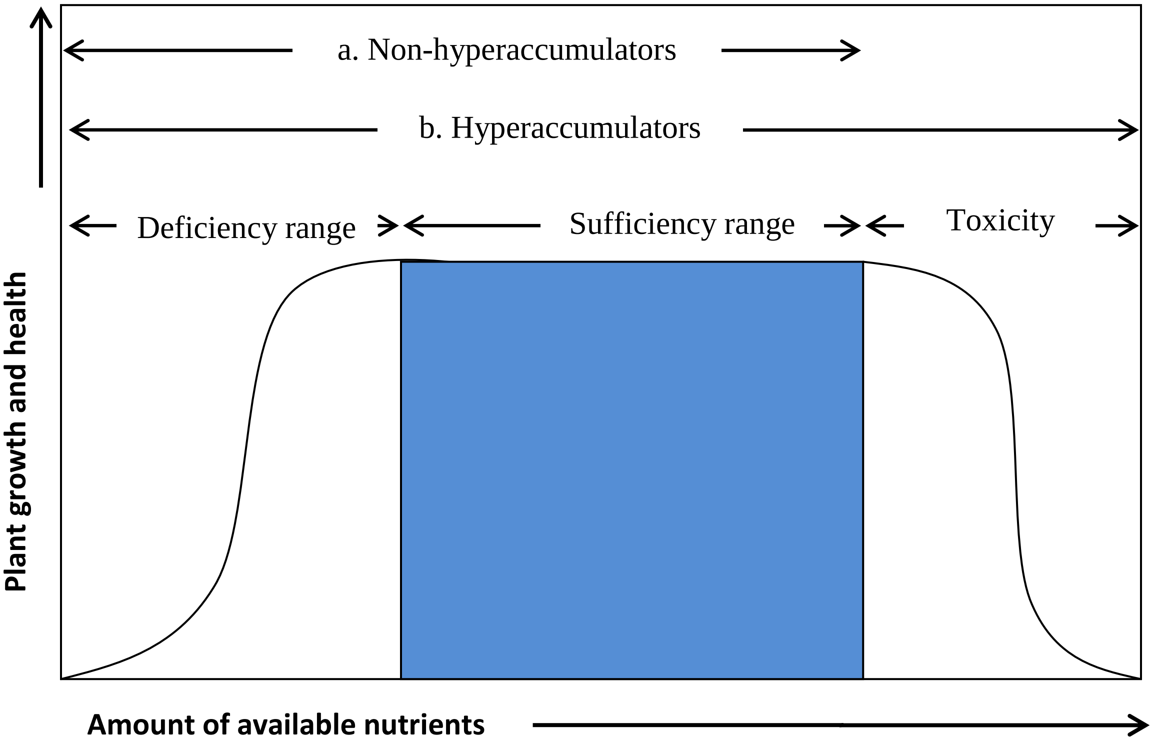


Figure 1-1 Relation between plant growth and health, and amount of available nutrients. Also showing the range of- a) non-hyperaccumulating plants and b) hyperaccumulating plants (Modified from McCauley 2009)

However, due to industrialisation macronutrients have in some cases reached toxic levels due to over application of fertilizer containing nitrogen (N), phosphorus (P) and potassium (K) in agriculture, and accumulation of essential and non- essential nutrients (e.g. including heavy metals like arsenic (As), cadmium (Cd) and lead (Pb)) due to agriculture and mining (McCauley 2009). The term ‘heavy metals’ in this regard is used to mean any element that is often used in industry and is usually toxic to humans, animals, and to aerobic and anaerobic processes, including arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn) (Duffus 2002).

Heavy metals are of some serious environmental concern because they persist indefinitely in the environment unlike organic contaminants (e.g. petroleum hydrocarbons, crude oil, chlorinated compounds, pesticides, and explosive compounds) that can be easily degraded by plants or other organisms (Garbisu and Alkorta 2001; ITRC 2009). For example, arsenic (As) at concentrations greater than 50 mg/kg.As.L^{-1} persisting in drinking water has been associated with the promotion of cancer of the bladder, lung, skin and prostate in humans (Peralta-Videa *et al*. 2009).

Environments containing heavy metals and characterized by low pH, high EC, low macronutrients availability and low soil organic matter, are often termed “contaminated environments” or “toxic environments” (Doumett *et al*. 2008; Mishra *et al*. 2009). These two terms will also be used interchangeably in this report to describe such environments.

1.4. Phytoremediation

Phytoremediation is defined as the use of plants and/or algae to remove or sequestrate pollutants and render the pollutant harmless to biological organisms (e.g. plants, and terrestrial and aquatic animals) (Pulford and Dickinson 2006). Fundamental to phytoremediation is the ability of plants to tolerate toxic environments. Plants can either:

- remove the metal contaminants from the environment and absorb or adsorb them in the harvestable plant biomass (phytoextraction) (Doty 2008);
- produce chemical compounds that immobilize metals in soils or roots, thus reducing metal mobility and bioavailability (phytostabilization) (Smit and Freeman 2006; Doumett *et al*. 2008);
- take-up water containing metal contaminants and release these contaminants during transpiration (phytovolatilization) (Yang *et al*. 2005); or

- degrade and/or metabolize organic contaminants (like those from the pesticide industry) within plant morphology (phytodegradation) (Yang *et al*. 2005; Smit and Freeman 2006).

Generally, phytodegradation and phytovolatilization focus on remediating organic contaminants (Smit and Freeman 2006; Doumett *et al*. 2008), while phytoextraction, and phytostabilization focus on remediating toxic mining (e.g. gold mine) environments associated with AMD and inorganic contaminants- including heavy metals (Yang *et al*. 2005). Since the main focus here is on the remediation of AMD and associated inorganic contaminants coming from gold mine TSFs, the discussion that follows below will be on phytoextraction and phytostabilization.

1.4.1. Phytoextraction

Phytoextraction involves the use of plants to remove inorganic contaminants, primarily metals from polluted soils (e.g. tailings and surrounding soils) through the plant roots along the transpiration stream (Lasat 2002; ITRC 2009). The success of phytoextraction to achieve phytoremediation goals will depend on several factors. These factors include the extent of soil contamination, bioavailability of metals, and the plant's ability to intercept, absorb and accumulate metals in its harvestable biomass (Lasat 2002; Doumett *et al*. 2008; ITRC 2009).

For most metals except mercury, uptake takes place from the aqueous phase through the roots. Therefore, if a metal is more strongly bonded to the soil than the plant's capacity to absorb it, the metal will not be bioavailable to the plant (Lasat 2002). As a general rule of thumb, bioavailable inorganic contaminants for plant uptake include arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), and zinc (Zn); moderately bioavailable metals include cobalt (Co), iron (Fe) and manganese (Mn), while chromium (Cr), lead (Pb) and uranium (U) are not very bioavailable (ITRC 2009). However, in acidic soils (e.g. tailings) metals are more bioavailable due to metals being displaced from the negatively charged soil particles by H^+ ion (Palazzo and Reynolds 1991).

Generally when grown in a medium containing elevated concentrations of heavy metals, the vast majority of plants can either absorb or adsorb large amounts of metals onto/into their roots, but translocate very little of this to their shoots (Macnair *et al*. 1999). However, some unique plants called "hyperaccumulators" are able to accumulate large amounts of metals into their shoots compared to roots. A metal hyperaccumulator has been defined by Doty (2008) as, "a plant that can concentrate the metals to a level of 0.1% for nickel, cobalt, copper and lead, 1% for zinc and

0.01% cadmium in its biomass", while ITRC (2009) defined a hyperaccumulator as a plant able to accumulate at least 0.1% (dry weight) of a specific metal. An example of a hyperaccumulator is South Africa's asteraceous plant, *Berkheya coddii* Roessler which is able to hyperaccumulate both Ni and Co (Keeling *et al*. 2003). Instead of a decrease in growth and health when grown in the toxicity range for most plants, a hyperaccumlator will maintain similar growth as it exhibited in the sufficiency range or even increase in growth (Figure 1-1).

Hyperaccumulators are generally discredited because of their slow growth rates; low harvestable biomass, shallow roots, and they often accumulate only one specific metal (Macnair *et al*. 1999; Lasat 2002; Doumett *et al*. 2008). These drawbacks have resulted in plants with a high above- and belowground biomass (e.g. trees and shrubs) which might not necessarily be hyperaccumultors being more favoured for phytoextraction to remediate contaminated soils (Rosselli *et al*. 2003; Hinton *et al*. 2005). The success of high biomass plants (e.g. trees and shrubs) in phytoextraction depends firstly on the ability of the plants to tolerate contaminated environments, quickly grow and produce large quantities of biomass, and regenerate in toxic environments (Doumett *et al*. 2008), secondly, by the relative ability of the plant to accumulate metals from the soil into the above harvestable biomass .

1.4.2. Phytostabilization

The main goal of phytostabilization is to stabilize contaminated areas from wind and water erosion, and reduce leaching of contaminants to ground and surface water (Mains *et al*. 2006; Padmavathiamma and Li 2007). Unlike other phytotechnologies like phytoextraction, phytostabilization is not intended to remove contaminants from a site, but rather immobilize contaminants in the soil through either the accumulation, adsorption or precipitation of contaminants within the root zone (Figure 1-2) (Padmavathiamma and Li 2007).

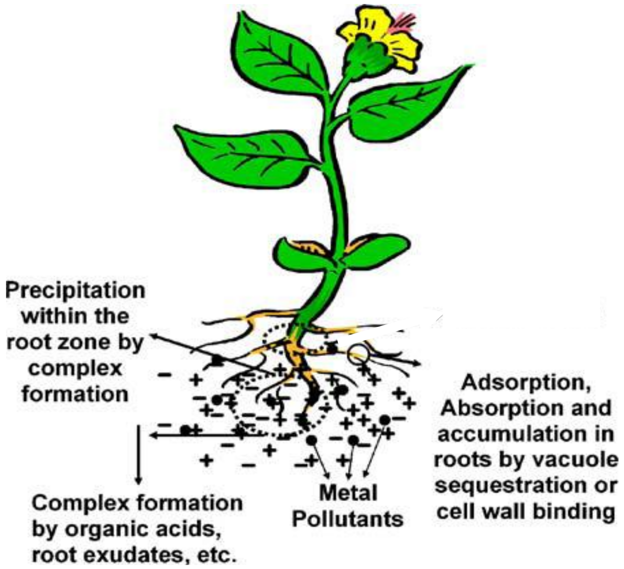


Figure 1-2 The mechanisms of phytostabilization by plants (Source: Padmavathiamma and Li 2007)

An individual plant however, can be used for both phytostabilization and phytoextraction; hence the characteristics of plants appropriate for phytostabilization are similar to those of phytoextraction. The only difference is that unlike phytoextraction which accumulates more of the contaminants into the shoot, phytostabilization either accumulates more contaminants into the roots or precipitates the contaminants in the rhizosphere (Padmavathiamma and Li 2007; Doumett *et al*. 2008). Generally plants with a high bio-concentration factor (BCF) and low translocation factor (TF) (e.g. the metal concentration ratio of the plant shoot to root) are the best candidates to use for phytostabilization (Padmavathiamma and Li 2007).

1.5. The Ecological Engineering and Phytotechnology Programme

Since late 2002 research has been conducted as part of the "Ecological Engineering and Phytotechnology Programme" initiated in 1996 between AngloGold Ashanti Ltd and the University of the Witwatersrand. The programme uses AMD tolerant, naturally colonizing indigenous plants for the hydraulic control and cleaning of AMD in groundwater, and rehabilitation of TSFs (Witkowski and Weiersbye 1998a; Witkowski and Weiersbye 1998b; Weiersbye *et al*. 2006). Using these naturally tolerant plants, the program aims at developing phytotechnologies that are ecologically sound and sustainable to reduce environmental impacts and liabilities caused by mining. The underlying assumption is that artificial ecosystems with a persistent plant cover can be created, which will contain AMD and associated pollution on and around each TSF, with minimal maintenance (Weiersbye and Witkowski 2002).

Some of the AMD tolerant species used are given in Weiersbye *et al*. (2006). However, this present study will focus on four species which include *Asparagus laricinus* Burch. (Asparagaceae), *Eragrostis curvula* (Schrad.) Nees. (Poaceae), *Hyparrhenia hirta* (L.) Stapf (Poaceae) and *Sutherlandia frutescens* (L.) R.Br. (Fabaceae). These species have been chosen because they are common natural colonisers of contaminated environments in the Highveld mines (Weiersbye *et al.* 2006) and they have a relatively fast growth rate which is fundamental for this project. For a description of these four species the reader is referred to chapter three.

1.5.1. Challenges faced by plants growing on tailings

Progress has been made in the Ecological Engineering and Phytotechnology Programme which includes, the identification of natural AMD tolerant plants growing on and around TSFs (Weiersbye *et al*. 2006), measuring their seed germination and viability (Weiersbye and Witkowski 2002; 2003) and recording the formation of local plant and bacteria ecotypes in AMD contaminated areas (Angus *et al*. 2005). However, the fertility of tailings still presents one of the greatest challenges to plant growth and establishment (Witkowski and Weiersbye 1998a; Witkowski and Weiersbye 1998b). These naturally tolerant plants are subject to soils which are essentially man-made habitats consisting of milled rock (which has not subsequently weathered) and ore with very little or no nutritive value for plant growth. Tailings have no topsoil, no soil organic matter (SOM) and often present very unstable surfaces (Wong 2003). The physio-chemical properties of tailings impede soil-forming processes and nutrient cycling, resulting in ‘soils’ which are often deficient in major nutrients like nitrogen and phosphorus (Wong 2003; Okalebo *et al*. 2006).

1.5.2. Potential solutions to growing plants in tailings

The success of phytoremediation centers on the ability of plants to colonize, grow, establish and regenerate in contaminated environments, whilst being able to stabilize or accumulate contaminants. The best candidate plants are those that are already naturally colonizing these contaminated environments. However, there is a need to manipulate tailings’ fertility to improve growing conditions for plants used in phytoremediation

Organic composts and mulches have been used world-wide to improve soil/tailings' fertility in degraded soils and mine tailings (Okalebo *et al*. 2006). Organic composts and mulches use the ability of bacteria and microorganism to breakdown organic matter (like plant biomass, animal waste and sewage sludge) releasing essential plant organic nutrients. Composts take relatively less time to release nutrients as compared to mulches. This is because composts contain organic matter already broken down unlike mulches which contain un-decomposed organic matter. However, preparing composts takes time, more labour and requires more area since an allowance has to be made to give time for organic matter to decompose, unlike mulches. Therefore, organic mulch from sewage sludge and water hyacinth could find potential use in the Highveld gold mines due to their ready availability and potential use as organic amendments to improve tailings fertility.

1.6. Fertility of tailings and contaminated soils

Soil fertility is defined as a complex of soil properties and processes enabling plant growth (Badalikova 2010). Results from a soil fertility test help to give basic information on the nutrient supplying capacity of the soil. Although several soil physical and chemical measurements can be used to evaluate soil fertility (Marschner 1995; Okalebo *et al*. 2006; Rajan *et al*. 2010), the following variables are commonly used, cation exchange capacity (CEC), soil organic carbon (SOC), pH, total nitrogen, exchangeable sodium percentage (ESP), and exchangeable cations (e.g. K, Ca and Mg).These parameters will also be used in this study as measures of tailings fertility.

Apart from being soil fertility indicators, CEC, ESP, SOC, pH, exchangeable cations and total N are directly or indirectly influenced by soil organic matter and/or soil clay content (Camberato 2001; Schumacher 2002; Deenik 2006; Milne 2009). Therefore, these indicators can be used to identify the contribution of water hyacinth and sewage sludge as amendments on gold mine tailings. A brief description of each of these soil fertility indicators is given below.

1.6.1. Cation exchange capacity (CEC), exchangeable cations and exchangeable sodium percentage (ESP)

Cation exchange capacity can be referred to as the quantity of negative charges in soil existing on the surface of clay and/or organic matter that gives the soil particles the ability to bind cations

(Camberato 2001). These negative charges on clay and/or organic matter attract positively charged cations which are often plant nutrients found on the soil's exchange sites. Although several cations (e.g. Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+} and Co^{2+}) can be found on the soil's exchange sites, Ca^{2+}, Mg^{2+} and K^{+} account for the highest percentage of the total exchangeable cations (Mikkelsen 2011). Therefore, since CEC measures the capacity of a soil to attract and store nutrients, it provides an indication of the reservoir of nutrients (Ca, Mg and K) in the soil together with other cations like NH_4^{+}, Zn^{2+} and Cu^{2+} (Hodges 2010). Cation exchange capacity also helps characterize the stability of the soil under conditions of erosion and leaching. A soil with a high CEC results in less nutrient leaching due to the high concentration of negative charges which attract the nutrients to the soil (Camberato 2001).

Related to the soil CEC is the soil exchangeable sodium percentage (ESP). Soil exchangeable sodium percentage is a measure of the sodium fraction adsorbed/bonded on the soil particles expressed as a percentage of CEC (see equation below) (Miller and Donahue 1990). Exchangeable sodium percentage is important in soil fertility because high concentrations of sodium in the soil will make soil basic (with pH values of 8.5 to 10.5) and impermeable to water. This is because small soil particles dispersed by sodium get entrapped in the soil pores and seal them (Miller and Donahue 1990). Therefore, ESP can be used as an indicator of plant moisture stress in plants receiving an adequate supply of water.

$$\text{ESP (\%)} = [\text{Exchangeable sodium ions/ Soil cation exchange capacity}] \times (100)$$

1.6.2. Soil organic carbon (SOC)

Soils contain carbon in either organic or inorganic form, with most carbon held in soil being in an organic form (Milne 2009). Soil organic carbon (SOC) refers to the organic carbon occurring in the soil as part of the soil organic matter (SOM). Soil organic matter refers to the organic constituent in the soil which includes dead flora and fauna and their products after decomposition, and soil microbial biomass (Schumacher 2002; Milne 2009). Soil organic carbon provides food for growing soil microorganism, and can be used as an indicator of a soil's degradation status (Rajan *et al*. 2010). Like soil CEC, SOC is strongly influenced by the soil clay content. This is because soils with high clay content can retain higher concentrations of organic matter (Milne 2009).

1.6.3. pH

Low soil pH can result in an increase in hydrogen, aluminium and manganese ions, which could become toxic to plants at high concentrations. A low pH soil increases the mobility of the Al^{3+} ions impeding nutrient availability in soil. Soil pH also influences the mobility of many metals like Cr, Se, Co, Pb, As, Ni and Cu and other heavy metals (Violante *et al*. 2010). Low pH in soil also reduces the availability of macronutrients like Mg, Ca and P, while reducing the mobility of Mo. Apart from these chemical interferences to plant nutrient uptake, low pH soils (e.g. < 5) also inhibit root growth and water uptake, which results in nutrient deficient and drought stress symptoms in affected plants (Marschner 1995).

Although pH affects root elongation, roots still play a crucial role in nutrient uptake in low pH, saline and high metal concentrated soils. Roots tend to develop a buffer effect and are able to take-up nutrients in these nutrient limiting environments. This is, however dependent on the plant species, soil type, soil structure and soil aeration.

1.6.4. Total nitrogen

During the break-down of organic matter, organic nutrients like ammonium and nitrates are released through a process called nitrogen mineralization. Nitrogen mineralization is a process where soil microorganisms convert organic nitrogen, during the decomposition of soil organic matter into plant useable inorganic nitrogen (e.g. ammonium [NH_4^+] and nitrate [NO_3^-]) (Deenik 2006). Nitrogen mineralization has an antagonistic relationship with N-immobilisation, instead of microorganisms breaking down organic N in N-mineralization, plants and microbes actually assimilate the NH_4^+ and NO_3^- and transform them into amino acids and proteins (e.g. organic N) through a process called N-immobilisation (Deenik 2006). The summation of inorganic N (nitrates, nitrite and ammonia) and organic N is the total nitrogen.

Both mineralization and immobilization are on-going processes in the soil and generally are in balance with one another. However, this balance can easily be disrupted by incorporating organic amendments into the soil, especially amendments with a high C: N ratio (Hodges 2010). This is because amendments with high C: N ratios are difficult to decompose because of their high carbon content which consequently results in reduced plant available N being released for plant uptake since most of the N will be assimilated by soil microorganisms (Hodges 2010). However, sewage sludge and most young and succulent plants have a fairly low C: N ratio and are therefore beneficial soil amendment candidates which improve fertility of low nutrient environments like tailings (van Scholl and Nieuwenhuis 2004).

1.7. Aims

- To test if *Eichhornia crassipes* (water hyacinth) can be used together with sewage sludge as a mulch to improve fertility and stability of tailings.
- To test if composting tailings with different water hyacinth and sewage sludge ratios improves the growth and survival of *Hyparrhenia hirta*, *Sutherlandia frutescens*, *Eragrostis curvula*, and *Asparagus laricinus* in tailings.

1.8. Objectives and key questions

a. To assess some tailings' fertility parameters (e.g. in terms of soils organic carbon, cation exchange capacity, exchangeable cations, exchangeable sodium percentage, total N and pH) in response to doses of dry water hyacinth (WH), liquidized fresh water hyacinth (WL), sewage sludge (SS), and combinations of water hyacinth and sewage sludge (WH: SS and WL: SS) as an organic mulch to improve tailings (T) fertility.
b. To assess plant growth (e.g. in terms of height) responses to doses of the amendments- T: WH, T: WL, T: SS, T: WH: SS and T: WL: SS, using *Sutherlandia frutescens, Eragrostis curvula, Hyparrhenia hirta* and *Asparagus laricinus*.
    - How do different plants respond to doses of water hyacinth and/or sewage sludge amended tailings?
c. To evaluate plant metal allocation to root and shoot, and whether the above test plants will take up metals while growing in the different tailings and organic mulches.
d. To assess tailings stability by evaluating whether sulphur and metals will leach from the amendments- T: WH, T: WL, T: SS, T: WH: SS or T: WL: SS into tailings.
    - Which tailings amendment results in metal leaching?

1.9. Dissertation Structure

This dissertation is divided into five discrete chapters. The first chapter introduces the project and supporting literature on how water hyacinth and sewage sludge could be used as organic tailings amendments. Chapter two addresses the elemental content of water hyacinth and sewage sludge and uses water hyacinth and sewage sludge total C and N concentrations to formulate tailings amendments. Chapter three looks at the survival and growth of the four phytoremediation study species in the tailings amendments created in chapter two. Chapter three examines the effects of water hyacinth and sewage sludge on tailings' fertility. Finally, chapter four discusses plant elemental allocation in root and shoot, and any metal accumulation of the four study species, and the stability (e.g. leaching) of tailings treatments. Chapter five draws conclusions from the preceding chapters and makes recommendations for future research on organic ameliorants on gold mine tailings.

Chapter 2. Elemental concentrations of water hyacinth and sewage sludge, and the potential of water hyacinth in phytoremediation

2.1. Introduction

The use of sewage sludge or water hyacinth as soil amendments is not a new approach, but has been used for several decades world-wide, especially in agriculture (Towers and Paterson 1997, Gashamura 2009). However, no study has been conducted which mixes these two organic amendments. Sewage sludge and water hyacinth are considered potentially valuable, inexpensive soil amendments which have the potential to provide nutrients such as N, P and K (Towers and Paterson 1997; Gunnarsson and Petersen 2007). However, sewage sludge can contain various toxic elements which have potential impacts on the environment (Towers and Paterson 1997). Although not documented as extensively as sewage sludge, water hyacinth also has the potential to contaminate soils when used as an amendment, especially if the water hyacinth grew in a medium containing toxic elements (Agunbiade *et al*. 2009). The aim of this chapter is to compare the elemental concentration of water hyacinth from two aquatic environments and that of sewage sludge, and use their carbon to nitrogen ratios (C:N) to formulate treatments in which *Hyparrhenia hirta*, *Sutherlandia frutescens*, *Eragrostis curvula*, and *Asparagus laricinus* would grow for six weeks. The chapter also discusses water hyacinth as a potential phytoremediation candidate for use in a closed cycle water remediation and application on tailings.

2.1.1. Sewage sludge as an organic soil amendment

The composition of sewage sludge varies considerably depending on the wastewater composition and the treatment processes used, but generally sewage sludge is composed of inorganic and organic materials, plant nutrients, numerous trace elements and organic chemicals and some pathogens (Stehouwer 1999). Sewage sludge also contains potentially toxic elements like chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), and zinc (Zn), manganese (Mn), aluminum (Al), iron (Fe), cobalt (Co), sodium (Na), and Gold (Au) which could become toxic to humans and animals when sewage sludge is used as an organic amendment. For example, Cd is toxic to nearly every system in the human body and could affect the liver and kidney since Cd competes with other minerals like Zn, Fe and Cu for binding sites (Raikwar *et al*. 2008). Therefore, regulations for land application of biosolids (e.g. sewage sludge) was established by the U.S. Environmental Protection Agency (EPA) in 1993 to avoid health risks by noting potential toxicities to animals or humans from land applications of municipal sewage sludge containing heavy metals (Kelley *et al*. 1984). In cognizance of this, the South Africa Sludge Guidelines (SASG) requires that sewage sludge samples be evaluated in terms of leachable and total extractable metals in sewage sludge. The Permissible Utilisation and Disposal of Sewage Sludge, Edition 1 (1997) give details of these guidelines (Snyman *et al*. 2004). In this document, sludge is classified into four groups (Types A, B, C, and D) depending on their risk in agricultural usage. The most restricted sewage sludge on agricultural soils is Type A, while Type D is the safest and has unrestricted use on agricultural soils if applied at a maximum application rate of 8t.ha^{-1}.yr^{-1}. Sewage sludge used in this study was classified as type D prior to the study. Type D sewage sludge is characterized as a low metal concentration, highly stable (e.g. low leaching), insignificant odour and fly nuisance potential sludge (Appendix 1).

2.1.2. Water hyacinth as an organic soil amendment and as a phytoremediation candidate

Boyd and Vickers (1971) investigated the elemental concentrations in water hyacinth from 17 different habitats including lakes, ponds and natural streams in Orlando Florida and observed that elemental concentrations of water hyacinth varied greatly from different sites (as shown by the large coefficient of variation- Table 2-1) with some specimen’s maximum elemental concentrations being several times higher than the minimum specimens’ elemental concentrations, for example iron and zinc in Table 2-1. Studies done by Boyd and Vickers (1971) are applicable to water hyacinth in South Africa since studies done by Zhang *et al*. (2010) have observed that there are no genetic differences between introduced populations of water hyacinth in Africa, and North America and Central America. The concentration of heavy metals/nutrients in water hyacinth is also influenced by the type and concentration of elements in the medium in which water hyacinth grew and exposure time in the medium (Soltan and Rashed 2003).

Generally water hyacinth roots accumulate more heavy metals than the shoots, while water hyacinth shoots accumulate more macronutrients than roots (Soltan and Rashed 2003; Mishra *et al*. 2009). Therefore, water hyacinth is a good candidate for phytoremediation. However, it should be noted that water hyacinth is recognized as the world’s worst aquatic weed and should never be intentionally introduced. Water hyacinth finds use mainly in remediating metal-contaminated aquatic environments through a phytotechnology called rhizofiltration (Rai 2010). Rhizofiltration is defined as, "the use of plants, both terrestrial and aquatic to absorb, concentrate and/or precipitate heavy metals from polluted aqueous sources in their roots" (Jadia and Fulekar 2009). Although rhizofiltration is mainly effective in remediating metals like lead (Pb), cadmium (Cd), copper (Cu), nickel (Ni), zinc (Zn) and chromium (Cr) (Jadia and Fulekar 2009), water hyacinth has also been successfully used in absorbing / adsorbing other metals including arsenic (As), manganese (Mn), and iron (Fe) in the roots (Agunbiade *et al*. 2009). Water hyacinth shoots can also be used in the phytoextraction of elevated macronutrients (e.g. sulphur (S), phosphorus (P), magnesium (Mg), calcium (Ca), potassium (K), and nitrogen (N)) (Mishra *et al* 2009).

Table 2-1 Elemental concentrations of water hyacinth from 17 different habitats. Modified from (Boyd and Vickers 1971)

Element	Min (mg/kg)	Mean +SE (mg/kg)	Max (mg/kg)	Coefficient of variation (%)
Nitrogen	1330	2390 ± 13	3330	22.68
Phosphorus	140	540 ± 50	800	38.3
Sulfur	370	480 ± 20	630	13.76
Calcium	660	1350 ± 120	2100	37.95
Magnesium	200	550 ± 40	880	31.38
Potassium	1600	4450 ± 340	6700	31.98
Sodium	170	410 ± 50	750	49.79
Iron	522	3420 ± 824	14440	99.22
Manganese	87	270 ± 25	400	38.47
Zinc	25	67 ± 11	209	68.39
Copper	7	15 ± 5	100	144.19
Boron	15	20 ± 1	25	19.22
Molybdenum	2	12 ± 3	40	89.47

Identifying if water hyacinth accumulates macronutrients in the shoots, or micronutrients and heavy metals in roots, can be done by elemental analysis. However, to determine if the elements are being absorbed or precipitated in water hyacinth roots (e.g. rhizofiltration), water hyacinth roots need to be washed with both distilled water and a dilute acid (e.g. acetic) prior to elemental analysis. Dilute acid breaks bonds between adsorbed elements and the roots, while distilled water only removes debris and is often too weak to break such bonds. Therefore, differences in elemental concentrations of water hyacinth roots washed with distilled water and those washed with dilute acid should give an indication of whether elements are absorbed or precipitated by water hyacinth.

2.2. Methods and Materials

2.2.1. Study site

Water hyacinth was collected from two sites. These included a tributary of the Vaal River (VR) called the Schoonspruit, as the river passes through AngloGold Ashanti- Vaal River mine operation, Shaft 10 (S 26.96907, E 26.64441), and from Benoni Lakes (BL) (S 26° 11' 0.4" E 28° 19' 44.0) which is located in the eastern region of the Gauteng Province of South Africa. Sewage sludge (e.g. Type D) was collected from Mponeng sewage treatment plant (Plant 8) at AngloGold Ashanti Ltd- West Wits mining operation, while tailings were collected from Sulphur Pay Dam at AngloGold Ashanti-Vaal River mine operation.

2.2.2. Sample preparation

Water hyacinth from VR and BL was collected at different times of the year, because of logistical constraints. Water hyacinth from VR was randomly collected from the Vaal River in March 2010. The water hyacinth was then spread across a plastic sheet, sun dried and crushed using a maize sheller powered by a tractor. Water hyacinth from BL was collected in July 2010. The fresh water hyacinth was initially chopped into small pieces with a knife, before blending it into a liquid paste using a Kenwood (750W) hand blender. Therefore, water hyacinth from VR constituted the dried water hyacinth and was stored in two 50 kg bags, while water hyacinth from BL constituted fresh water hyacinth and was stored in a cold room (2 ^{o}C) at Witwatersrand University in two 20 *l* plastic containers.

Water hyacinth plant samples measuring approximately 200g were randomly collected at the same time as a bulk collection from both VR (N= 8) and BL (N= 10). The samples were divided into root and shoot subsamples and washed using either dilute acetic acid (pH 3.5 - 4.0) or distilled water. The dilution of acetic was made by mixing distilled water and concentrated acetic acid, stirring and monitoring the pH till it reached pH 3.5/4.0, therefore no actual dilution rates were given. Dilute acetic acid involved washing plant parts with distilled water during the first and last washes, while the other two intermediate washes used dilute acetic acid. The distilled water wash included washing plant parts four times in distilled water. After either of the washes, the representative plant subsamples were freeze dried for six day using a Labconco freeze drying system from the School of Chemistry at the University of the Witwatersrand. The following sequences of freeze drying were used, 0^{o}C for 12 hours, -10^{o}C for 24 hours, -40^{o}C for 48 hours, -10^{o}C for 24 hours, and 20^{o}C for 36 hours. The dry root and shoot subsamples were then milled using a mortar and pestle before being sent back to the School of Chemistry for a carbon, nitrogen and sulphur (CNS) analysis and a full elemental analysis (e.g. C, N, P, K, S, Mn, Mg, Al, Ca, Fe, Co, Cu, Cr, Ni, Hg, Na, Zn and Au) using Induced-Coupled Plasma with Optical Emission Spectrophotometer (ICP-OES).

Eight sewage sludge samples and four tailing samples were randomly collected from Mponeng sewage treatment plant and Sulphur Pay Dam, respectively. The samples were crushed and sieved, followed by freeze-drying, then send to the School of Chemistry for a CNS analysis and a full elemental analysis, as described above.

2.2.3. Sample analysis

Prior to CNS and full elemental analysis, water hyacinth from VR (N= 16) and BL (N=20), sewage sludge (N= 8) and tailings (N= 4) samples were digested in a microwave digester (Anton Paar Multiwave 3000) using different mixtures of concentrated acids. Water hyacinth (0.1 g) was mixed with HNO_3 (8 ml) and H_2O_2 (2 ml), while sewage sludge (0.1 g) and tailings (0.1 g) were each mixed with HNO_3 (2 ml), HCl (6 ml) and HF (1 ml) before microwave digestion (Pulford and Dickinson 2006).

A CHNS Autoanalyser -932 (manufactured by Leco Corporation, St Joseph, USA) was used to measure C, N and S concentrations in water hyacinth and sewage sludge, while an ICP-OES-Genesis Fee (manufactured by Spectro Analytical Instruments, GubH and Co.) was used to measure total elements (e.g. C, N, P, K, S, Mn, Mg, Al, Ca, Fe, Co, Cu, Cr, Ni, Hg, Na, Zn and Au) in water hyacinth, sewage sludge and tailings. An Orchard Leaves Standard Reference Material (National Bureau of Standards Certificate of Analysis- SRM 1571, Washington, D.C. 20234) was used to verify the accuracy of elemental determination for water hyacinth, while a certified reference soil material [(NCS DC 73315 (GBW 07305): Stream sediment) from China National Analysis Center for Iron and Steel 2004] was used to verify elemental determination for sewage sludge and tailings.

2.2.4. Development of tailings amendments

Five different tailings amendments were formulated based on the ratios of water hyacinth and sewage sludge in tailings and replicated four times. These included tailings mixed with- sewage sludge (SS), dry water hyacinth (WH), fresh water hyacinth (WL), WH: SS, WL: SS. Tailings with no amendment constituted the control. Four different percentages of amendments were applied to tailings based on the C: N ratios (e.g. dry mass) of water hyacinth and sewage sludge, making the total treatments 21- including control (Table 2-2). Elemental concentrations in each treatment were then calculated based on water hyacinth, sewage sludge and tailings elemental concentrations (e.g. dry mass).

Table 2-2 Set-up of tailings treatments

Amendments	**Percentages**
Tailings (T)	0%
Dried water hyacinth (WH)	WH- 0.5%
	WH-1.0%
	WH-2.0%
	WH-4.0%
Sewage sludge (SS)	SS- 0.5%
	SS-1.0%
	SS-2.0%
	SS-4.0%
Fresh water hyacinth (WL)	WL-0.5%
	WL-1.0%
	WL-2.0%
	WL-4.0%
WH:SS	WH:SS- 0.5%
	WH:SS-1.0%
	WH:SS-2.0%
	WH:SS-4.0%
WL:SS	WL:SS 0.5%
	WL:SS-1.0%
	WL:SS-2.0%
	WL:SS-4.0%

2.3. Statistical analysis

Data were analyzed using Statistica (Version 6). Firstly, water hyacinth elemental data was pooled based on the different sites (e.g. Vaal River and Benoni Lakes) and compared using a student's *t*-test. Pooled water hyacinth data from the two sites were also used to compare elemental concentrations of water hyacinth against sewage sludge using a factorial ANOVA, and C/N ratios of water hyacinth against sewage sludge, using a One-Way ANOVA. Secondly, water hyacinth data were separated into sites, plant parts and type of washes and compared using 3-Way ANOVA, to get an indication of elements accumulated by water hyacinth plant parts and to see if water hyacinth is either absorbing or adsorbing elements (e.g. phytoremediation). All factorial ANOVA's were followed by a Post hoc Tukey tests.

2.4. Results

2.4.1. Concentrations of elements in water hyacinth from Vaal River and Benoni Lakes

A student's *t*- test showed that water hyacinth collected from Vaal River (VR) had significantly higher macronutrient concentrations (C, N, P and Mg) as compared to water hyacinth from Benoni Lakes (BL) which had significantly higher micronutrient and heavy metal concentrations (Mn, Fe, Na and Au) (Table 2-3). Gold (Au) and sodium (Na) were only detected in water hyacinth collected from BL, while zinc (Zn) and mercury (Hg) were not detected in water hyacinth from either sites (Table 2-3).

Water hyacinth shoots showed significantly higher concentrations of macronutrients in both VR (N, P, K, Ca and Mg) and BL (K, Ca, P and Mg) than roots, while water hyacinth roots showed significantly higher concentrations of micronutrients and heavy metals in VR (Al, Co, Cu, Fe and Ni) and BL (Al, Co, Cr, Cu, Fe, Ni, and Au) than shoots (Figure 2-1). Sulphur was the only macronutrient that was significantly higher in water hyacinth roots than shoots at both sites, while Co was only detected in water hyacinth roots and none in shoots from both sites (Figure 2-1).

Table 2-3 Mean elemental concentrations of water hyacinth (whole plant) (mg/kg- dry mass) from the Vaal River (N= 16) and Benoni Lakes (N= 20). Values with a star (*) within rows are significant; *t*- test at $p < 0.05$

Element (mg/kg)	Benoni Lakes	Vaal River
<u>Macronutrients</u>		
Carbon	345300 ± 1069	381575 ± 6017*
Nitrogen	18928 ± 1528	40996 ± 3344*
Phosphorus	4073 ± 572.9	7635 ± 879.6*
Potassium	32284 ± 5087*	29734 ± 4326
Sulphur	4294 ± 710.2	5422 ± 465.4
Calcium	9303 ± 1310	9661 ± 798.4
Magnesium	3579 ± 534.5	6330 ± 614.0*
<u>Micronutrients</u>		
Manganese	6452 ± 2337*	1789 ± 513.6
Iron	8846 ± 2760*	2499 ± 609.4
Copper	16.88 ± 1.795	21.50 ± 7.277
Zinc	-	-
Nickel	43.50 ± 10.98	31.19 ± 4.622
<u>Non- essential</u>		
Cobalt	20.55 ± 7.463	13.38 ± 3.726
Chromium	29.05 ± 5.834	22.69 ± 1.645
Mercury	-	-
Aluminum	2565 ± 973.0	1734 ± 400.8
Sodium	1781 ± 274.6*	-
Gold	187.7 ± 37.04*	-

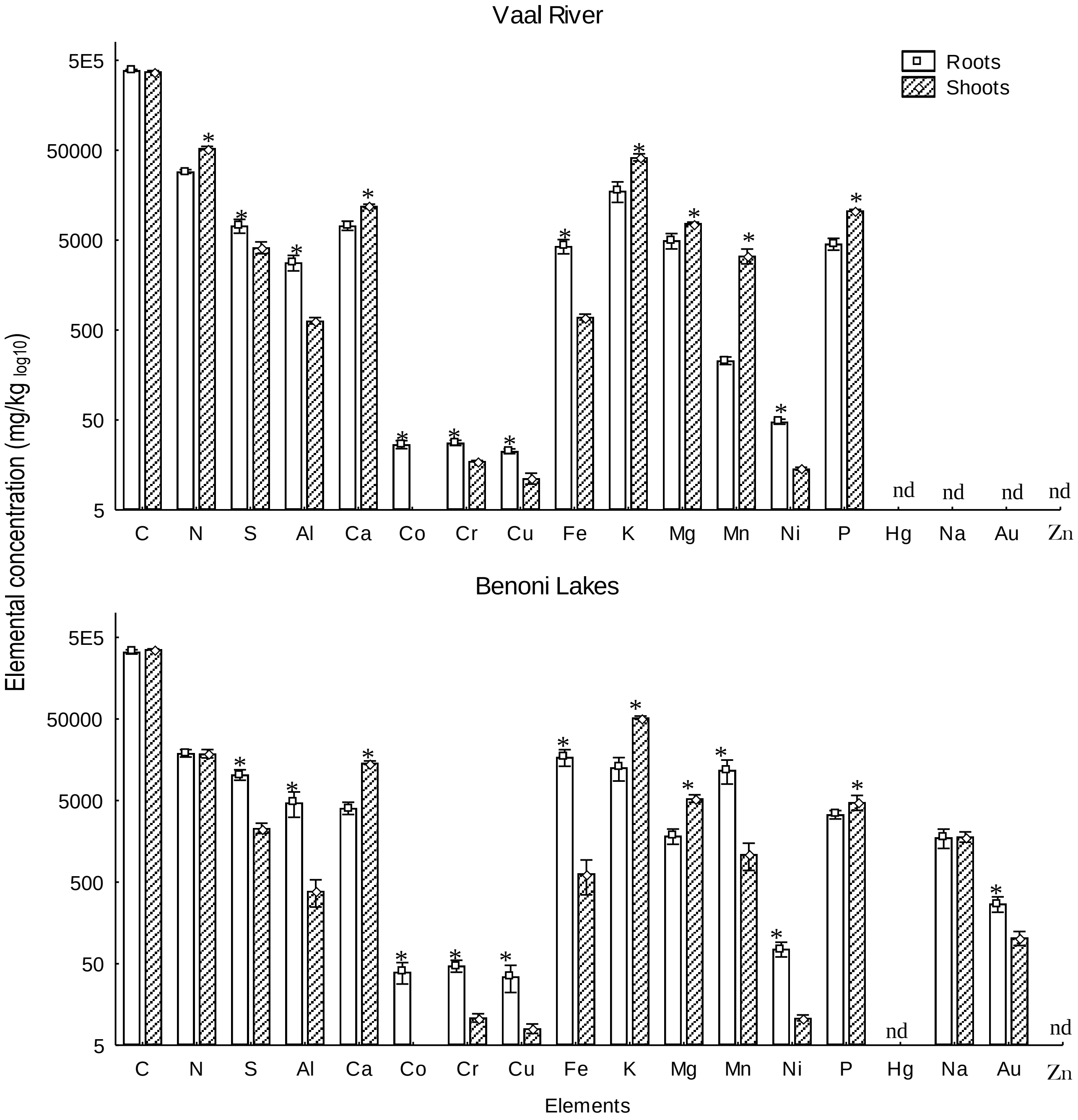


Figure 2-1 Mean elemental concentrations (mg/kg $_{\log10}$ - dry mass) of water hyacinth roots and shoots from Vaal River (N= 16) and Benoni Lakes (N= 20) irrespective of the type of wash used. * denotes significant difference in elemental pairs; 2-Way ANOVA at $p < 0.05$

From the 17 elements tested in water hyacinth, S and Mg were the only elements that showed significant differences in water hyacinth plant parts after the dilute acid and distilled water wash. Water hyacinth roots collected from both VR and BL showed a significant decrease in S concentrations after being washed with dilute acetic acid instead of distilled water (Figure 2-2).

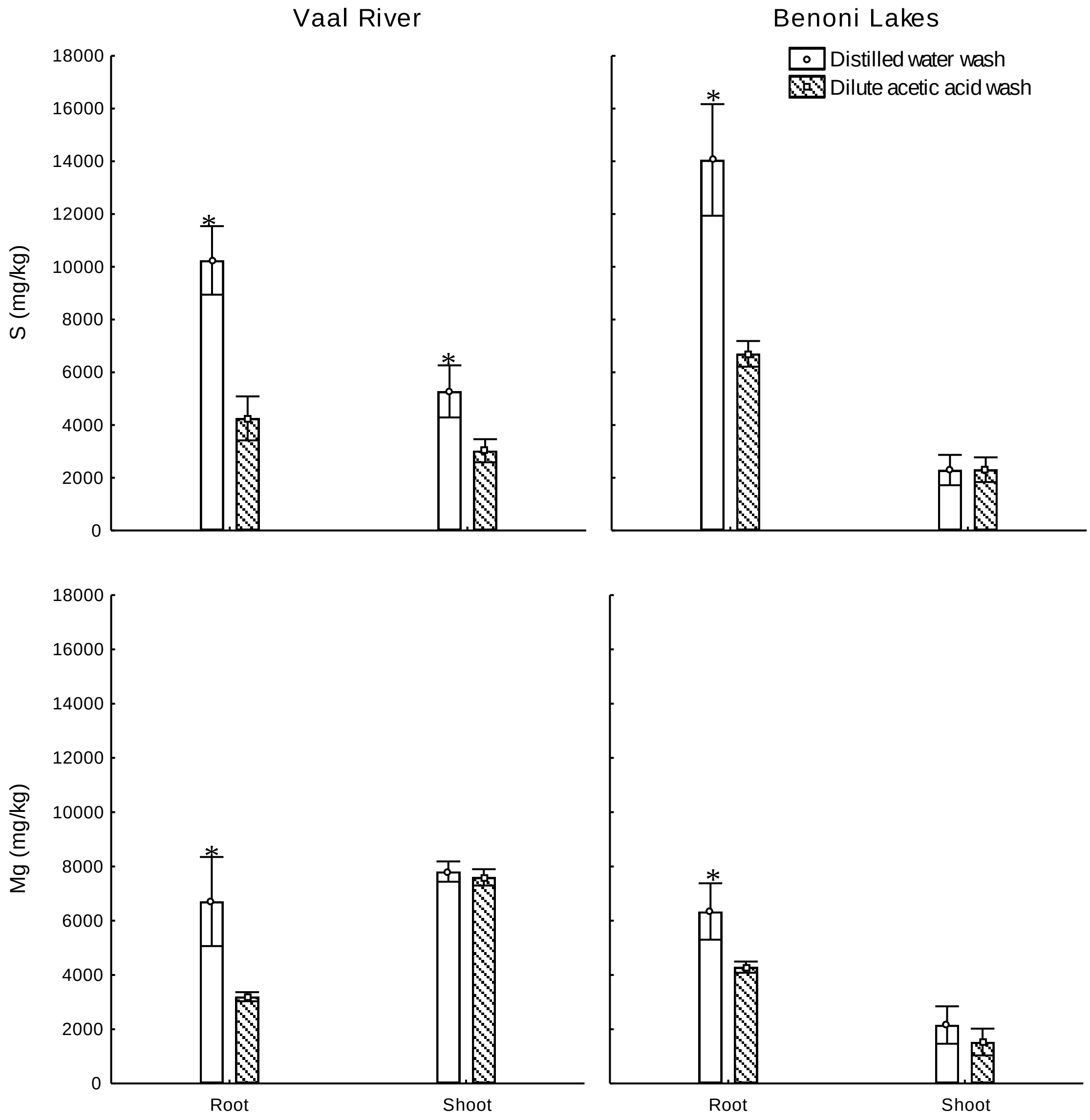


Figure 2-2 Mean sulphur and magnesium concentrations (mg/kg- dry mass) of water hyacinth roots and shoots collected from Vaal River (N= 16) and Benoni Lakes (N=20). * denotes significant difference in elemental concentrations of water hyacinth roots or shoots washed with either distilled water or dilute acetic acid; 3-Way ANOVA at $p < 0.05$

Water hyacinth shoots from VR also showed a similar decrease in S after being washed in dilute acetic acid instead of distilled water (Figure 2-2). Water hyacinth roots from both VR and BL showed a significant decrease in Mg concentration after being washed with dilute acetic acid (Figure 2-2).

2.4.2. Comparison of elements in water hyacinth and sewage sludge

Water hyacinth from both VR and BL showed significantly higher C concentrations than sewage sludge. However, sewage sludge had significantly higher N than water hyacinth from BL (Table 2-4). Water hyacinth from BL had a significantly higher C: N ratio compared to either water hyacinth from VR, or sewage sludge (Table 2-4). No significant differences were observed between sewage sludge and water hyacinth S concentrations from either site (Table 2-4).

Table 2-4 Mean carbon and nitrogen concentrations (dry mass %), and C/N ratios of water hyacinth (whole plant) collected from Vaal River (N= 16) and Benoni Lakes (N= 20), and sewage sludge collected from Vaal River (N= 8). Values with different letters within columns are significantly different; One-Way ANOVA at $p < 0.05$

	C (%)	N (%)	S (%)	C:N
Water hyacinth- Vaal River	38.16 ± 0.61^{a}	3.85 ± 0.42^{a}	0.57 ± 0.08	9.92 ± 1.43^{b}
Water hyacinth- Benoni Lakes	34.53 ± 1.07^{a}	1.89 ± 0.15^{b}	0.63 ± 0.12	18.24 ± 7.01^{a}
Sewage sludge- Vaal River	24.51 ± 1.25^{b}	3.53 ± 0.25^{a}	0.71 ± 0.07	7.18 ± 0.59^{b}

Water hyacinth K concentrations were significantly higher in both VR and BL, as compared to sewage sludge, while Mg concentrations of water hyacinth from VR were significantly higher than both water hyacinth collected from BL and sewage sludge (Figure 2-3). Manganese concentrations of water hyacinth from BL were significantly higher than those of water hyacinth from VR and sewage sludge. Sodium was only observed in water hyacinth from BL, while sewage sludge showed significantly higher concentrations of Al, Ca, Co, Cr, Cu, P, Ni and Zn as compared to water hyacinth from either site. Zinc was only detected in sewage sludge, while Cu concentrations in sewage sludge were almost 18 times higher than those in water hyacinth from either site (Figure 2-3).

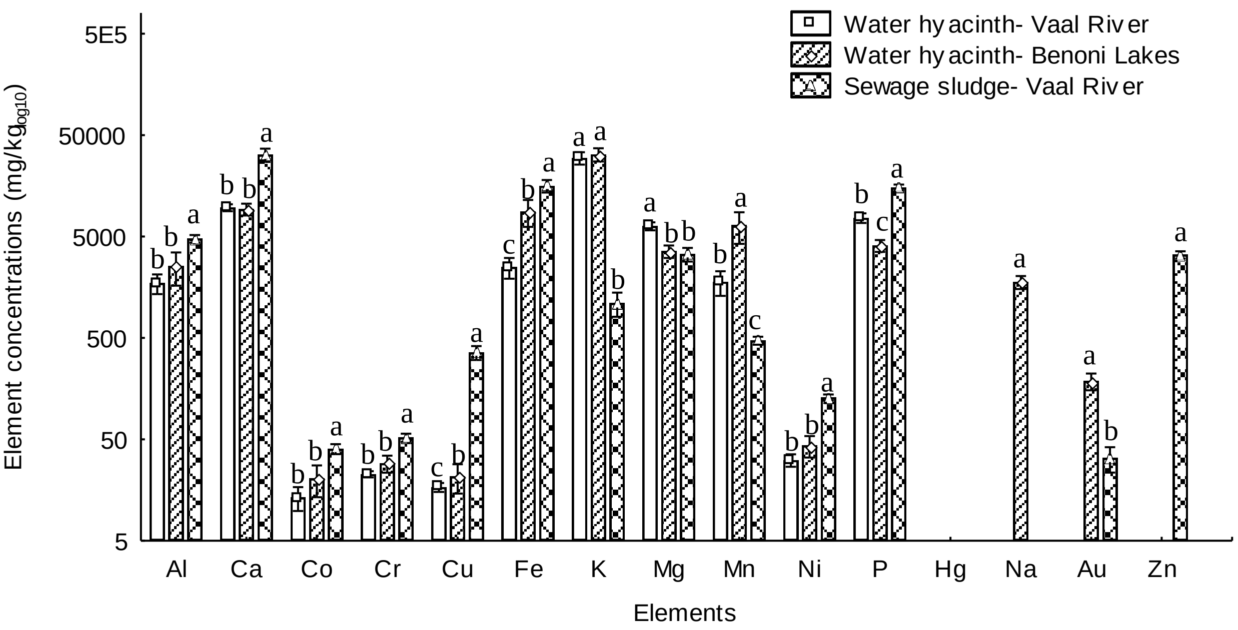


Figure 2-3 Mean elemental concentrations (mg/kg $_{log10}$-dry mass) of water hyacinth (whole plant) collected from Vaal River (N= 16) and Benoni Lakes (N= 20), and sewage sludge collected from Vaal River (N= 8). Different letters denote significant difference in elemental concentrations between water hyacinth (whole plant) from VR, BL and sewage sludge from VR; One-Way ANOVA at $p < 0.05$

2.4.3. Concentration of elements in tailings

Tailings contained the highest nutrient and heavy metal concentrations when mixed with sewage sludge at the four application percentages as compared to tailings mixed with either fresh or dry water hyacinth. Tailings amended with 4% of SS had the highest nutrient and metal concentrations for all the treatments in the study. This was followed by tailings amended with 4.0 % of either WH: SS or WL: SS. Tailings amended with 0.5 % of either WH or WL contained the lowest concentrations of nutrients and heavy metals (Table 2-5). Since iron (Fe) was high in both tailings and sewage sludge, an increase in the percentage of water hyacinth resulted in a decrease in Fe concentrations for tailings amended with either dry (WH) or fresh (WL) water hyacinth (Table 2-5).

Table 2-5 Mean elemental concentrations (mg/kg-dry mass) of tailings (T) amended at different percentages of dried water hyacinth (WH), fresh water hyacinth (WL), sewage sludge (SS), WL: SS and WH: SS

Treatments (dry mass)	(%)	Al	Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Ni	P	Hg	Na	Au	Zn
T (control)	0	0	0	11	21	19	9620	0	1080	438	33	89	0	0	0	48
WH	0.5	11	47	11	21	19	9602	156	1099	458	33	117	1	5	1	48
WH	1	22	95	11	21	19	9584	312	1117	477	33	145	2	10	1	47
WH	2	44	189	11	21	19	9548	623	1154	517	33	200	5	20	2	47
WH	4	88	378	11	21	19	9476	1246	1229	596	33	312	10	40	4	46
SS	0.5	44	183	11	21	21	9670	6	1091	438	33	165	0	0	0	64
SS	1	87	365	11	22	23	9719	11	1103	438	34	241	0	0	0	81
SS	2	174	730	11	22	26	9819	22	1125	439	35	392	0	0	1	113
SS	4	348	1460	12	22	33	10018	44	1171	439	37	696	0	0	1	178
WH: SS	0.5	27	115	11	21	20	9636	81	1095	448	33	141	1	2	0	56
WH: SS	1	55	230	11	21	21	9652	161	1110	458	33	193	1	5	1	64
WH: SS	2	109	460	11	22	23	9684	323	1140	478	34	296	2	10	1	80
WH: SS	4	218	919	11	22	26	9747	645	1200	517	35	504	5	20	3	112
WL	0.5	11	47	11	21	19	9602	156	1099	458	33	117	1	5	1	48
WL	1	22	95	11	21	19	9584	312	1117	477	33	145	2	10	1	47
WL	2	44	189	11	21	19	9548	623	1154	517	33	200	5	20	2	47
WL	4	88	378	11	21	19	9476	1246	1229	596	33	312	10	40	4	46
WL: SS	0.5	27	115	11	21	20	9636	81	1095	448	33	141	1	2	0	56
WL: SS	1	55	230	11	21	21	9652	161	1110	458	33	193	1	5	1	64
WL: SS	2	109	460	11	22	23	9684	323	1140	478	34	296	2	10	1	80
WL: SS	4	218	919	11	22	26	9747	645	1200	517	35	504	5	20	3	112

2.5. Discussion

2.5.1. Elemental concentrations of water hyacinth from the Vaal River and Benoni Lakes sites

Despite being grown under different climatic, topographical regions and water-bodies, most of the elements in water hyacinth (whole plant) from both VR (P, S, Mg and Fe) and BL (N, P and S) were in a similar range to those recorded by Boyd and Vickers (1971) (Table 2-3 and Table 2-4). However, water hyacinth from VR and BL had higher concentrations of macronutrients (N, P and Mg), and micronutrients (Fe and Mn), respectively as compared to water hyacinth concentrations recorded by Boyd and Vickers (1971) on similar elements.

Water hyacinth from VR was obtained in an area surrounded by mining and agricultural activities. The land adjacent to the VR site is in close proximity to agricultural activities which are often a potential source of elevated concentrations of N, P, K and Mg in the form of fertilizers used in crop production. The possible pathways for these nutrients to enter the river may include runoff or flooding. Flooding is the most probable pathway since the Vaal River has been associated with a series of floods over the past two years and flooding is often associated with flushing of nutrients from agricultural land bordering rivers (Poff 2002). The water hyacinth samples collected for this study were collected two weeks after a flooding event in the Vaal River which could explain the high N, P and Mg concentrations in water hyacinth from VR as compared to BL.

On the other hand, water hyacinth from BL was collected from an urban area in close proximity to a highway and a shopping mall (e.g. Lakeside Mall). Manganese is often used as an indicator of urban effluent (Gonzalez *et al*. 1989). Therefore, the significantly higher concentrations of Mn in water hyacinth from BL as compared to that from VR may be coming from urban effluent entering the lake from various human activities. The Blesbokspruit information sheet (1998) records old mine workings, sewage works and urban development surrounding the lake as possible human activities increasing the pollution of the BL site. On the other hand, the high Fe in water hyacinth from BL might have entered the lake in the form of suspended Fe particles especially as the rainwater passes through concrete pavements (Tutu *et al*. 2008) or the highway. Rain water has been observed to undergo chemical changes when it passes through concrete (e.g.

increase in pH due to lime in concrete) which facilitate Fe oxidation (Tutu *et al*. 2008). Although the VR is also subjected to anthropogenic activities like mining and urban development, the area in which water hyacinth was collected had limited influence from such activities as compared to Benoni Lakes. This could explain the variations in elements recorded in water hyacinth from the two sites, such site variations in elemental concentrations of water hyacinth were also observed by Boyd and Vickers (1971) in Orlando Florida.

Tutu *et al*. (2008) observed that lakes in the Witwatersrand Basin are often less polluted than the rivers or streams especially in summer due to surface run-off and unpolluted groundwater discharging which acts to dilute the water in lakes. Water hyacinth from BL was collected in the dry season which is often associated with high nutrient loading in the lake due to minimum nutrient dilution because of reduced run-off and groundwater recharge (Tutu *et al*. 2008) which could be another explanation for the high K, Mn and Fe in water hyacinth from BL as compared to that from VR. Although water hyacinth from VR was collected from a river (e.g. often associated with high nutrient loading), it was collected in summer which is a period associated with the dilution of the river by un-polluted groundwater discharge (Tutu *et al*. 2008).

2.5.1.1. Water hyacinth as a phytoremediation candidate

As expected, water hyacinth shoots accumulated significantly higher concentrations of macronutrients (e.g. VR- N, P, K, Mn, Ca and Mg, and BL- K, S, Ca, Mn and Mg) than the roots, while water hyacinth roots accumulated significantly higher concentrations of micronutrients and heavy metals (VR- Al, Cr, Cu, Fe, Co and Ni, and BL- Al, Co, Cr, Cu, Fe, Ni, Na and Au) than shoots (Figure 2-1). These findings are supported by Gonzalez *et al*. 1989; Vesk *et al*. 1999; Soltan and Rashed 2003; Mishra *et al*. 2009, who observed that water hyacinth accumulates higher macronutrients in the shoots as part of the plant's nutritional requirements, and higher micronutrients and metals in the roots than the shoots.

The N and Mg concentrations in water hyacinth shoots from BL were consistent with concentrations recorded in most vascular plant; however, P, K, and Ca in water hyacinth shoots from both sites and N in water hyacinth from VR were almost double and four times higher than those recorded in most vascular plant species, respectively (Raven *et al*. 1999). Concentrations of Mn in water hyacinth shoots from both sites (VR- 3350 mg/kg and BL- 11807 mg/kg) were almost three and ten times higher than the Mn concentrations recorded for water hyacinth shoots by Soltan and Rashed (2003) (e.g. 1485 ± 110 mg/kg) in the Nile River. Nitrogen and manganese are essential macro- and micronutrients, respectively (Raven *et al*. 1999) and these nutrients can be accumulated at high concentrations (e.g. especially in plant shoots (Soltan and Rashed 2003)) depending on their concentrations in the medium in which the plant is growing (Hopkins 1995). Therefore, elevated concentrations of N in water hyacinth shoots from VR could indicate that the Vaal River is polluted by high concentrations of N. Cilliers *et al*. (1996) identify agricultural activities, industrial and mining enterprises, informal settlements and urban areas in Gauteng as the responsible culprits for the pollution of the Vaal. On the other hand, the high Mn in water hyacinth shoots from BL could indicate urban effluent contamination in the Benoni Lakes coming from the highway, sewage works or the adjacent Lakeside Mall, as highlighted earlier.

Although Co, Cu, Cr, Fe, and Ni concentrations in water hyacinth roots were significantly higher than shoots from either site, these concentrations were lower than those observed for water hyacinth roots in the Nile River (Egypt) by Soltan and Rashed (2003). This could be due to differences in study sites, seasons and the chemical nature of the habitats (Boyd and Vickers 1971; Soltan and Rashed 2003).

No differences were observed in water hyacinth root and shoot elemental concentrations after the distilled water and dilute acid wash, except for S and Mg from both sites. Water hyacinth roots from both VR and BL showed a significant decrease in S and Mg after being washed with dilute acetic acid instead of distilled water, while water hyacinth shoots from VR showed a decrease in S concentration after being acid washed (Figure 2-2). This suggests that water hyacinth roots from either site are precipitating, and not absorbing, S and Mg compounds on the root surface. This could indicate that water hyacinth is growing in a dolomitic environment in a water body that is heavily impacted by AMD, since dolomitic environments often contain high concentrations of Ca and Mg (Vandeginste and John 2012), and AMD is associated with high sulphur and metal concentrations (Akcil and Koldas 2006). However, water hyacinth roots did not adsorb Ca; this could be because Ca is less mobile than Mg (Miller and Donahue 1990; Uchida 2000). Another reason could be that Ca was accumulated more in the aboveground biomass of water hyacinth since the nutrient is required at almost double the concentration of Mg (Raven *et al*. 1999).

2.5.2. Elemental concentrations of water hyacinth and sewage sludge

Considering only the macronutrient contents and eventual decomposition of water hyacinth and sewage sludge in a mulch, sewage sludge (SS) seemed to be a better nutrient amendment than water hyacinth since SS showed a higher P concentration, a higher N (e.g. than water hyacinth from BL (Figure 2-3)), and a lower C: N ratio than water hyacinth (Table 2-4). However, sewage sludge showed higher metal concentrations (Al, Ca, Cr, Cu, Fe, Ni, and Zn) than water hyacinth although these metal concentrations were lower than those recommended as maximum allowable in Type D sewage sludge under the Permissible Utilisation and Disposal of Sewage Sludge, Edition 1 (1997).

Potential sources of these high heavy metal concentrations in sewage sludge (as compared to water hyacinth) could be coming from the mine and the mine residences, since industrial and municipal wastes are often characterized by high concentrations of heavy metals (Burnison *et al*. 2003). Although heavy metal concentrations in sewage sludge are below permissible limits (e.g. Type D) and can therefore be used to amend tailings, frequent application could elevate heavy metals (McGrath and Lane 1989) in tailings.

Tailings amended with sewage sludge (SS) contained the highest nutrient and heavy metal concentrations at the four application percentages as compared to tailings amended with water hyacinth at similar percentages (Table 2-5). Therefore, if sewage sludge is to be used as a tailings amendment, treatments containing lower percentages of sewage sludge (e.g. 0.5% of SS, or 0.5% and 1.0% of either WH: SS and WL: SS) (Table 2-5) would be most preferred since they contain lower concentrations of heavy metal (e.g. Cr, Cu, Hg, Ni and Zn) as compared to tailings amended with higher percentages of SS.

2.6. Conclusion

Since water hyacinth is accumulating high concentrations of P, K, Mn and Ca from both the VR and BL, and N from the VR in amounts greater than those recorded in most vascular plants, water hyacinth has the potential of providing an ecological service of removing these elements from water-bodies, whilst also acting as a good organic amendment to tailings. Sewage sludge contains higher concentrations of N and P, a low C: N ratio and relatively lower metal concentrations than those recommended for Type D sewage sludge which also makes it a good candidate organic amendment to tailings. However, the ideal application of these two amendments and the form in which water hyacinth should be applied (dry or fresh) to improve tailings conditions for plant survival and growth is unknown. These issues will be addressed in the next chapter.

Chapter 3. Effects of water hyacinth and/or sewage sludge treatments on seedling emergence, plant survival and growth, and tailings fertility

3.1. Introduction

Tailing storage facilities (TSFs) are essentially man-made habitats of crushed rock characterized by very low soil organic matter (SOM), high macronutrients (e.g. Ca, Mg and Na), high micronutrients (e.g. Sr, Pb, As, Ni and Cr), high salinity, and low pH (Witkowski and Weiersbye 1998 a). These characteristics present a hostile environment for plant establishment and growth (Witkowski and Weiersbye 1998a; Witkowski and Weiersbye 1998b). Although both indigenous and exotic plant species do colonize gold tailings in the Witwatersrand Basin (Weiersbye *et al*. 2006), the regeneration of these naturally tolerant species has been associated with high seed abnormalities and reduced germination (Weiersbye and Witkowski 2003). Water hyacinth and sewage sludge could potentially be used to amend tailings and improve growth conditions for these species (Chapter 2). However, the optimal amount of amendment that would result in improved plant survival and growth is unknown. This chapter attempted to identify which tailings amendment from those formulated in chapter two, improved tailings' fertility whilst enhancing the survival and growth of four indigenous plants naturally colonizing gold tailings in the Witwatersrand Basin over a six week growth period. These indigenous plants include *Hyparrhenia hirta* (L.) Stapf (Poaceae), *Sutherlandia frutescens* (L.) R.Br. (Fabaceae), *Eragrostis curvula* (Schrad.) Nees. (Poaceae), and *Asparagus laricinus* Burch. (Asparagaceae). These four species were chosen to grow in tailings treatments based on their tolerant growth in tailings, inherent fast growth rate and ability to accumulate metals in their above and below ground biomass (Weiersbye *et al*. 2006; Padmavathiamma and Li 2007).

3.1.1. Organic and inorganic fertilizers

Inorganic fertilizers have been used to improve soil productivity in commercial agriculture for decades (Wilson 2000). However, inorganic fertilizers have a short-term effect on the soil's nutrient status unlike organic fertilizers which have a lasting effect on the soil's physical, chemical and nutrient status (Senesi 1989; van Scholl and Nieuwenhuis 2004). Although various sources of organic fertilizers exist, world-wide, this study focuses only on water hyacinth and sewage sludge as organic amendments to improve the fertility of gold mine tailings. This is because these two amendments are abundant in the Highveld mines and create serious management and disposal challenges. Using them as tailings amendments could present a feasible management solution.

3.1.2. Sewage sludge as a soil amendment

Sewage sludge is considered an inexpensive and effective soil amendment which has the potential of providing large amounts of nutrients such as N, P and K. Sewage sludge has the capacity to improve the fabric and water holding capacity of the soil creating more favourable conditions for the growth of plant roots and improving plants drought tolerance (Pathak *et al*. 2009). Therefore, amending tailings with sewage sludge could improve tailings fertility and consequently plant growing conditions in contaminated soil environments (Palazzo and Reynolds 1991; Wong 2003; Chiu *et al*. 2006).

Sewage sludge however, has often been restricted in its use on agricultural soils because it often contains high concentrations of heavy metals, pathogenic bacteria and other toxic compounds (Pathak *et al*. 2009). The concentrations of heavy metals in sewage sludge vary from 0.5% to 6.0 % on a dry mass basis (Pathak *et al.* 2009). Repeated application of heavy metal contaminated sewage sludge to soils or tailings may increase the concentrations of heavy metals in the soil, consequently resulting in the leaching of these metals into surface and groundwater systems (Pathak *et al*. 2009). Therefore, an optimal application of sewage sludge to soils or tailings is required which will benefit both soil and plants by creating a better nutrient reserve while not interfering with surface and groundwater systems. Although the European legislation stipulates a maximum application rate of $10t.ha^{-1}$ of sewage sludge in agricultural soils (Ashworth and Alloway 2004), there is no standard rate for sewage sludge application since the quality (e.g. nutrients, toxic metal and pathogens) of sewage sludge and soil characteristics (e.g. pH, texture and moisture content) vary. The quality of sewage sludge will vary based on the source of sludge (e.g. household or industrial), its level of treatment (primary, secondary, or tertiary) and the intended land-use (agriculture or mined land). For example, in South Africa, Type D sewage sludge (i.e. considered to be the safest sewage sludge to use on agricultural soils) should be applied at a maximum rate of $8t.ha^{-1}.yr^{-1}$ on condition the soil has a pH above 6.5 (Appendix 1).

Applying sewage sludge alone to soil has been associated with reduced decomposition efficiency due to the limited porosity in sewage sludge (Wong *et al*. 2011). Wong *et al*. (2011) however observed that mixing fresh or dry sewage sludge with other organic amendments like horse stable straw improved the decomposition rate by over 50%. This could be because horse stable straw improved the porosity of the sewage sludge which consequently increased the aeration and movement of bacteria in the organic matter, hence increasing bacterial activity and the decomposition rate.

3.1.3. Water hyacinth as a soil amendment

Water hyacinth [*Eichhornia crassipes* (Mart. and Zucc.) Solms] is a perennial, mat-forming aquatic plant found mainly in sub-tropical and tropical regions (Penfound and Earle 1948). Water hyacinth infests the Vaal River as the river passes through AngloGold Ashanti (Ltd) property and has been identified as a noxious weed in the Vaal River since the early 1960's (Cilliers *et al*. 1996). Manual, mechanical, herbicide application and biological control have been used to combat water hyacinth in the Vaal system (Sharp 2009). However, mechanical control has proved labour intensive and herbicidal control too expensive, while biological control is relatively slow compared to the previous two control methods (Gunnarsson and Petersen 2007; Sharp 2009; Byrne *et al*. 2009). Therefore, an integrated water hyacinth management program which incorporates mechanical, herbicide and biological control has been seen as the best method of controlling water hyacinth in the Vaal system (Sharp 2009; Byrne *et al*. 2009). A typical integrated approach could involve herbicidal and/or mechanical methods controlling 95 % of the water hyacinth infestation, while leaving the other 5 % of water hyacinth infestation for biological control (Sharp 2009). Water hyacinth controlled through mechanical harvesting could find use as a mulch to amend tailings.

Water hyacinth has been used as a mulch to improve plant production (Gunnarsson and Petersen 2007; Gashamura 2009), such as in the tea estates of India (Gopal 1987). Studies done in Gahororo, Eastern Rwanda showed that green compost made from water hyacinth has the ability to raise soil pH, organic matter, N, P, and soil cation exchange capacity (CEC) (Gashamura 2009). Water hyacinth contains higher levels of major nutrients than compost prepared from cattle manure (Okalebo *et al*. 2006). Water hyacinth also acts as an insulator, protecting the soil surface from temperature extremes, and stores moisture (Wong 2003). The decomposition of water hyacinth could contribute to the improvement of chemical, physical and biological tailings properties hence creating better conditions for plant growth and survival (Lal 1988; Amoding *et al*. 1999; Okalebo *et al*. 2006).

The state and quantity in which water hyacinth is incorporated into the soil will influence how effectively water hyacinth will improve soil fertility. Okalebo *et al*. (2006) and Gashamura (2009) observed that fresh water hyacinth showed better soil improvement than dry water hyacinth, however dried water hyacinth is often used because of lower transport and labour costs. Gashamura (2009) applied both fresh and dry water hyacinth to soils at application rates ranging between 150 N.kg/ha and 450 N.kg/ha during a study to identify the effects of water hyacinth on soil fertility and maize performance in Rwanda. The study revealed that amending soils with 0.1% of fresh water hyacinth (i.e. 150N.kg/ha- equivalent to 150kgs of nitrogen per hectare) resulted in the best maize yields and most improved soil fertility among the treatments tested.

The way in which water hyacinth is incorporated into the soil or tailings is also important, Amoding *et al*. (1999) observed that incorporating water hyacinth into the soil increased cabbage yield, unlike adding it as a surface mulch. On the other hand, Adesina *et al*. (2011) observed that although mixing water hyacinth (WH) with mineral fertilizer (MF) improved plant growth as compared to plants growing in non-fertilized soils, applying water hyacinth alone to soil at higher application rates created the best plant improvement among the treatments tested. This was attributed to the high biological activity of the soils combined with the high nutrient (macro and micro) potential of water hyacinth.

3.2. Methods and Materials

A greenhouse experiment to test the effects of growing *Hyparrhenia hirta*, *Sutherlandia frutescens*, *Eragrostis curvula*, and *Asparagus laricinus* in tailings amended with water hyacinth and/or sewage sludge was conducted at the University of the Witwatersrand. *Eragrostis curvula* and *S. frutescens* were grown from seed, while *A. laricinus* was grown from seedlings and *H. hirta* from tussocks, each based on its regenerative mechanism. Watering was controlled to prevent leaching from the tailings.

3.2.1. Species descriptions

3.2.1.1. *Hyparrhenia hirta*

Hyparrhenia hirta (L.) Stapf (Poaceae) (Figure 3-1) is commonly known as thatch grass. The plant is found in all provinces of South Africa except the north-western Free State (Roberts 1973). Growing up to 120 cm, *H. hirta* is a successful coloniser of bare soils and can be used to re-vegetate eroded slopes and poor soils, including polluted infertile soils (Roberts 1973; Witkowski and Weiersbye 1998b). *Hyparrhenia hirta* naturally colonizes mine tailings by tolerating high metal concentrations in the rhizosphere, with low metal concentrations translocated into the shoot making it a good candidate for stabilizing mine tailings (Padmavathiamma and Li 2007).

3.2.1.2. *Sutherlandia frutescens*

Sutherlandia frutescens (L.) R.Br (Figure 3-1) (Fabaceae) is distributed across Lesotho, southern Namibia, south-eastern Botswana and South Africa (van Wyk and Albrecht 2008). The plant is an attractive small, soft wooded leguminous shrublet (Fig 3.1b), growing between 0.2 m – 2.5 m in height (van Wyk and Albrecht 2008). *Sutherlandia frutescens* is a popular species in traditional medicine and is believed to cure illnesses including diabetes, indigestion and colds (van Wyk and Albrecht 2008).

3.2.1.3. *Eragrostis curvula*

Eragrostis curvula (Schrad.) Nees. (Poaceae) (Figure 3-1) is known as weeping love grass in South Africa or African love grass in Australia (Roberts 1973). *Eragrostis curvula* is native to Botswana, Kenya, Mozambique, Namibia, Swaziland, Tanzania, Zambia and Zimbabwe (Guertin 2003) and all provinces of South Africa, execpt the drier western district (Roberts 1973). *Eragrostis curvula* is a perennial, warm season pasture grass adapted to poor soils which can tolerate acid growing conditions (Guertin 2003; Weiersbye *et al*. 2006). The grass has fibrous roots and can grow to a height of between 30- 160 cm (Roberts 1973; Guertin 2003). *Eragrostis curvula* reproduces through seed production, with temperature and pH influencing germination (Guertin 2003). Under field conditions germination will occur within 7 days, with a success rate of between 83 -88 %, mainly in substrate of moderate to alkaline pH (e.g. 4.0- 11.5) (Guertin 2003). A soil pH below 3 will result in no germination of *E. curvula* (Guertin 2003).

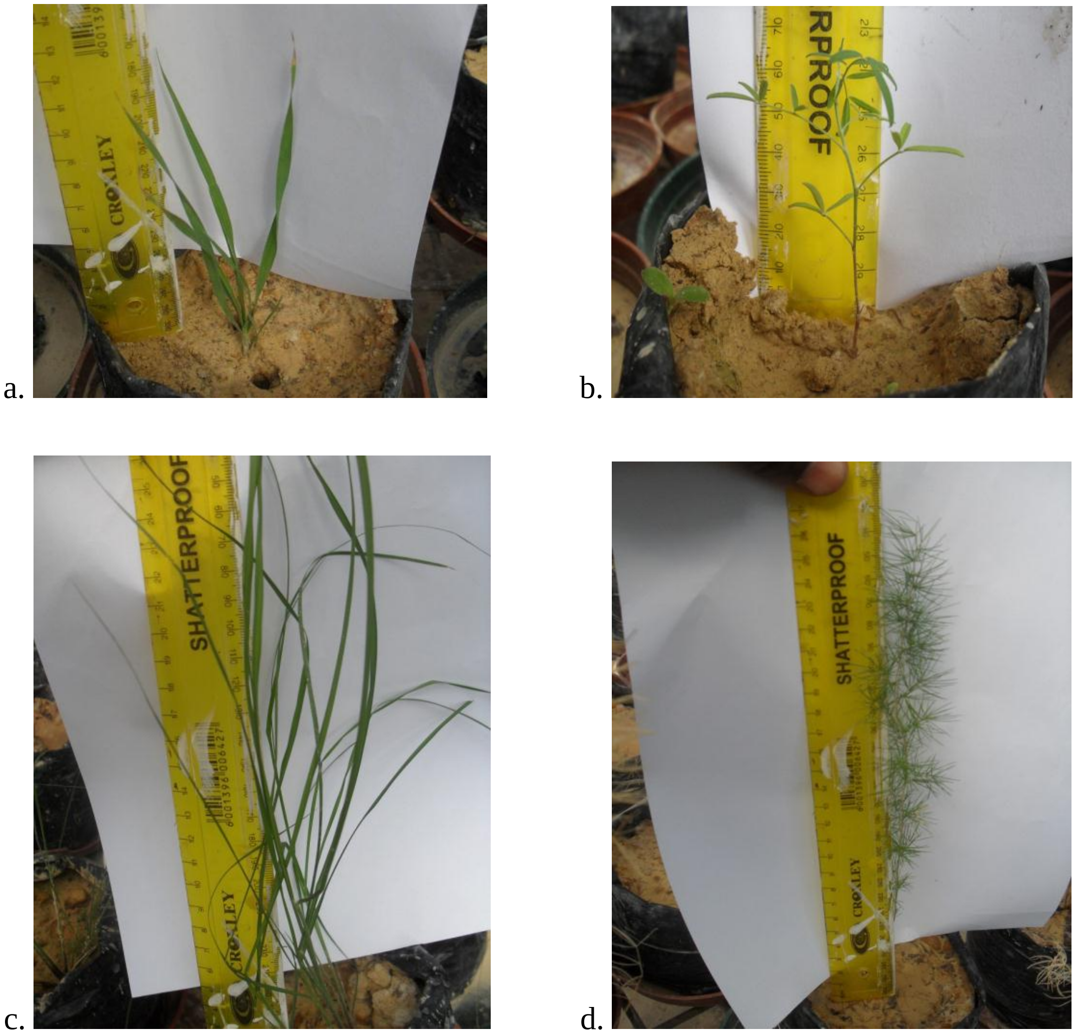


Figure 3-1 Tolerant natural colonizers of mine tailings in the Witwatersrand Basin- a. *Hyparrhenia hirta*, b. *Sutherlandia frutescens*, c. *Eragrostis curvula*, and d. *Asparagus laricinus*

3.2.1.4. Asparagus laricinus

Asparagus larcinus Burch. (Asparagaceae) (Figure 3-1) is a spiny shrub that can grow between 1- 3 m in height (Malcomber and Demissew 1993). It is distributed through Angola, Botswana, Namibia, South Africa, Zambia and Zimbabwe. Apart from growing on and around gold tailing dams in the Witwatersrand Basin, *A. laricinus* has also shown high seed production and viability in polluted soils (Witkowski and Weiersbye 1998b; Weiersbye *et al*. 2006).

3.2.2. Experimental Design

Hyparrhenia hirta, *Sutherlandia frutescens*, *Eragrostis curvula*, and *Asparagus laricinus* were each grown in 21 tailings treatments (Table 2-2). The treatments were laid out as randomized complete block design with four replicates each, making the total number of plants grown for the experiment 336. However, before planting could commence, starter fertilizer NPK (3:1:5) (38%) and double superphosphate (1:2:0) (33%) were both dissolved into water at a rate of 3.2 g/litre. The fertilizer/water mixture was used to water each treatment bag (including the control) at 60% of the treatment's field capacity (field capacity is explained in the following section).

3.2.3. Planting

The four study species were prepared differently prior to the growth experiment due to the different regenerative mechanisms exhibited by the plants. *Eragrostis curvula* and *Sutherlandia frutescens* were grown from seed, while *Asparagus laricinus* and *Hyparrhenia hirta* were grown from tubers and cuttings respectively, due to unavailability of seeds. Since *E. curvula* and *S. frutescens* were grown from seed, a seedling emergence experiment was designed to measure the time and percentage of seedlings that emerged from seeds subjected to tailings amended with water hyacinth and/or sewage sludge.

3.2.3.1. Hyparrhenia hirta

Hyparrhenia hirta was grown from tussocks of mature *H. hirta* plants growing on TSFs near City Deep, south of Johannesburg. The tussocks were planted in "insert" trays containing ordinary potting soil and allowed to grow for 5 weeks before being transplanted into each of the 84 treatment bags.

3.2.3.2. Sutherlandia frutescens

Sutherlandia frutescens seeds were first treated by heating them in warm water (approximately 50^{o}C) for 30 seconds to break dormancy (Weiersbye and Witkowski 2003). Ten *Sutherlandia* seeds were sown in each of the 84 treatment bags containing amended tailings and observed for seedling emergence over a 20 day period. However, *Sutherlandia* seedlings from the emergence experiment could not be used in the plant growth experiment because they rapidly died after emerging. Therefore, a second batch of treated *Sutherlandia* seeds was prepared and sown in a 250 ml seed tray containing ordinary potting soil. The seeds were watered and kept moist until they emerged. After emergence (at approximately 5 cm in height), seedlings were transferred to insert trays and allowed to grow for 7 weeks before being transplanted into treatment bags.

Before transplanting the *Sutherlandia* seedlings in treatment bags, the seedlings were inoculated with mycorrhizae to increase their chances of survival in amended tailings. Seedling inoculation involved collecting soils under mature *Sutherlandia* plants and *Sutherlandia* root nodules from contaminated sites of AngloGold Ashanti (Ltd). Soil and crushed *Sutherlandia* root nodules were each mixed with distilled water at a ratio of 1:10 and 1:4, respectively. Five millilitres of each of the two mixtures were then administered into each insert containing the 7 week old *Sutherlandia* seedlings. Two weeks after inoculation, *Sutherlandia frutescens* seedlings were found to be infested and were then transplanted into 84 treatment bags the following day.

3.2.3.3. Eragrostis curvula

Eragrostis seeds were sown directly into each of the 84 treatment bags. In each treatment bag, 20 *Eragrostis* seeds were sown and recorded for seedling emergence over a 20 day period (i.e. the seedling emergence experiment). *Eragrostis* seedlings that emerged and survived after the seedling emergence experiment were allowed to continue growing and constituted the plant growth experiment.

3.2.3.4. Asparagus laricinus

Asparagus laricinus seedlings (~ 1.5 months old) obtained from Vaal River nursery were planted into 84 treatment bags and used for the growth experiment.

3.2.4. Transplanting procedure

To avoid contaminating the treatments with either ordinary potting soil or dead root biomass, seedlings with a large root biomass, like *Asparagus* and *Hyparrhenia*, were washed using tap water, while excess soil on *Sutherlandia* roots was removed by gently tapping seedling roots on the hand to avoid breaking the fragile roots and attached root nodules. Treatment bags containing seedlings and tailings mixed with sewage sludge and or water hyacinth were then weighed to calculate the amount of water required to irrigate each treatment bag to field capacity.

3.2.5. Field capacity measurements

Field capacity measurements for the amended tailings were carried out to avoid leaching of nutrients from the treatment bags during irrigation. This was done by weighing the dry masses of the four replicate subsamples of each treatment bag using an electronic balance (± 0.01g).

Approximately 10 ml of water was initially added to each treatment and 5-10 ml there after depending on how easily the water infiltrated each treatment. Adding water to the treatment bags was halted as soon as water started draining out at the base. The volume of water added to each treatment was recorded (A) and the volume that drained (B). Field capacity was taken as the difference between these water volumes (e.g. A-B). Irrigation was maintained at 75 % of field capacity in all the treatments during the 6 week growth experiment by maintaining a constant weight of each treatment bag.

Tailings often form hard crusts when constantly watered, which often reduces aeration for roots and microorganisms (Weiersbye *per comm*). To avoid low oxygen levels in treatments at least two small holes (i.e. the diameter of a pencil and approximately 5cm deep) were dug on opposite sides of each plant.

3.2.6. Monitoring and recording

Seedling emergence was monitoring by counting the number of seeds that had an elongation of the embryonic axis (Bewley 1997). The number of seedlings that emerged divided by the total number of seeds sown multiplied by 100 gave the germination percentage. Plant survival and growth were monitored at 10-day intervals by visual observation and by measuring plant height (using either a 30 cm ruler or a one meter measuring tape), respectively for 6 weeks.

3.2.7. Tailings fertility analysis

Fertility of tailings was measured in each treatment before (e.g. after amending tailings with either water hyacinth or sewage sludge) and after growing the four study species. A sub-sample of approximately 100 g was collected from each treatment before the plant growth experiment (N= 21), and two replicate sub-samples in treatments containing survived plants (N= 64) after the plant growth experiment. The following fertility variables were measured:

- Exchangeable sodium percentage (ESP) and cation exchange capacity (CEC) were derived from extractable cations (e.g. K^+, Mg^{2+}, Ca^{2+}, and Na^+ - using the ammonium acetate method (Lawrence 1998)), using the following formulae:

$CEC= \sum [K^{+}{}_{(meq.100g\text{-}1)} + Mg^{2+}{}_{(meq.100g\text{-}1)} + Ca^{2+}{}_{(meq.100g\text{-}1)} + \text{Exchangeable acidity}_{(meq.100g\text{-}1)}]$

$ESP = [Na^{+} (_{meq.100g\text{-}1})]. [CEC (_{meq.100g\text{-}1})]^{-1}$

- Soil organic carbon (SOC) (Walkley and Black method as described by Jackson (1973)),
- Total nitrogen (Kjeldahl nitrogen determination method (Rhee 2001)
- Measuring pH (one part sample to two parts 1*M* KCl (Manson and Roberts 2000))

Information from the Harmonized World Soil Database (HWSD version 1.1) (Appendix 2) - database that classifies soils around the world into different categories based on different soil fertility parameters- was used during the discussion to compare soil fertility variables obtained in this study with those recorded globally.

3.3. Statistical analysis

Seedling emergence of *Eragrostis curvula* and *Sutherlandia frutescens* were compared against treatments using a One-Way ANOVA. Plant growth and survival of *Hyparrhenia hirta*, *Sutherlandia frutescens, Eragrostis curvula* and *Asparagus laricinus* were compared amongst treatments and amongst species using a 2-Way ANOVA followed by a Post hoc Tukey test. A 2 –Way ANOVA was used to compare tailings' fertility (e.g. pH, cation exchange capacity, exchangeable sodium percentage, exchangeable cations and total nitrogen) before and after plant growth, and between treatments.

3.4. Results

3.4.1. Seedling emergence of *Sutherlandia frutescens* and *Eragrostis curvula*

Seedling emergence was generally low, with both *S. frutescens* and *E. curvula* showing seedling emergence less than 20 % for all treatments (Figure 3-2). Tailings amended with WH: SS- 1.0%, WL- 4.0%, WL: SS-0.5% and WL: SS- 4.0% had the highest seedling emergence for *Sutherlandia* seeds unlike other treatments, while tailings amended with WH- 0.5%, WH- 2.0%, and WL: SS- 1.0% had the best seedling emergence for *E. curvula* (Figure 3-2). Tailings amended with WL: SS- 0.5% showed similar seedling emergence for both *E. curvula* and *S. frutescens*. *Eragrostis curvula* seedlings only emerged in tailings amended with sewage sludge at 4.0% of SS and 2.0% of WH: SS.

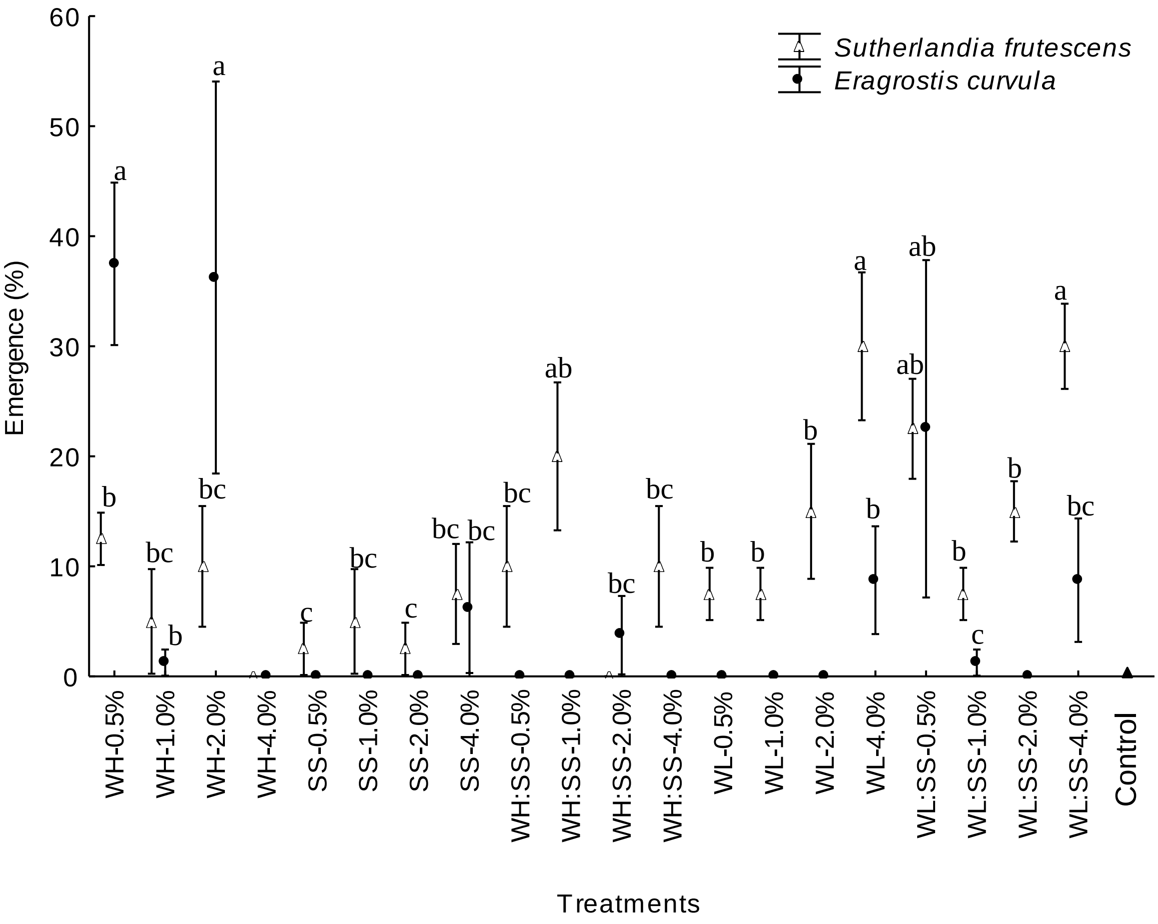


Figure 3-2 Mean seedling emergence of *Eragrostis curvula* seeds (N=1680) and *Sutherlandia frutescens* seeds (N=840) sown in 21 tailings treatments for 20 days. Different letters within a species are significantly different, One Way ANOVA at $p < 0.05$

3.4.2. Plant survival and growth

A total of 92 plants from the 320 planted, survived to the end of the six week growth period. *Hyparrhenia hirta* showed the best survival in all treatments among the four study species, followed by *Asparagus* which showed a significant decrease in plant survival during the second week in most treatments, then become constant till the end of the experiment (Table 3-1). *Eragrostis curvula* and *Sutherlandia frutescens* showed the worst survival among the four study species (Table 3-1). Unlike the other three study species, *Asparagus* re-sprouted from ‘dead’ stems, resulting in fluctuations in plant survival during the six week growth period (Table 3-1).

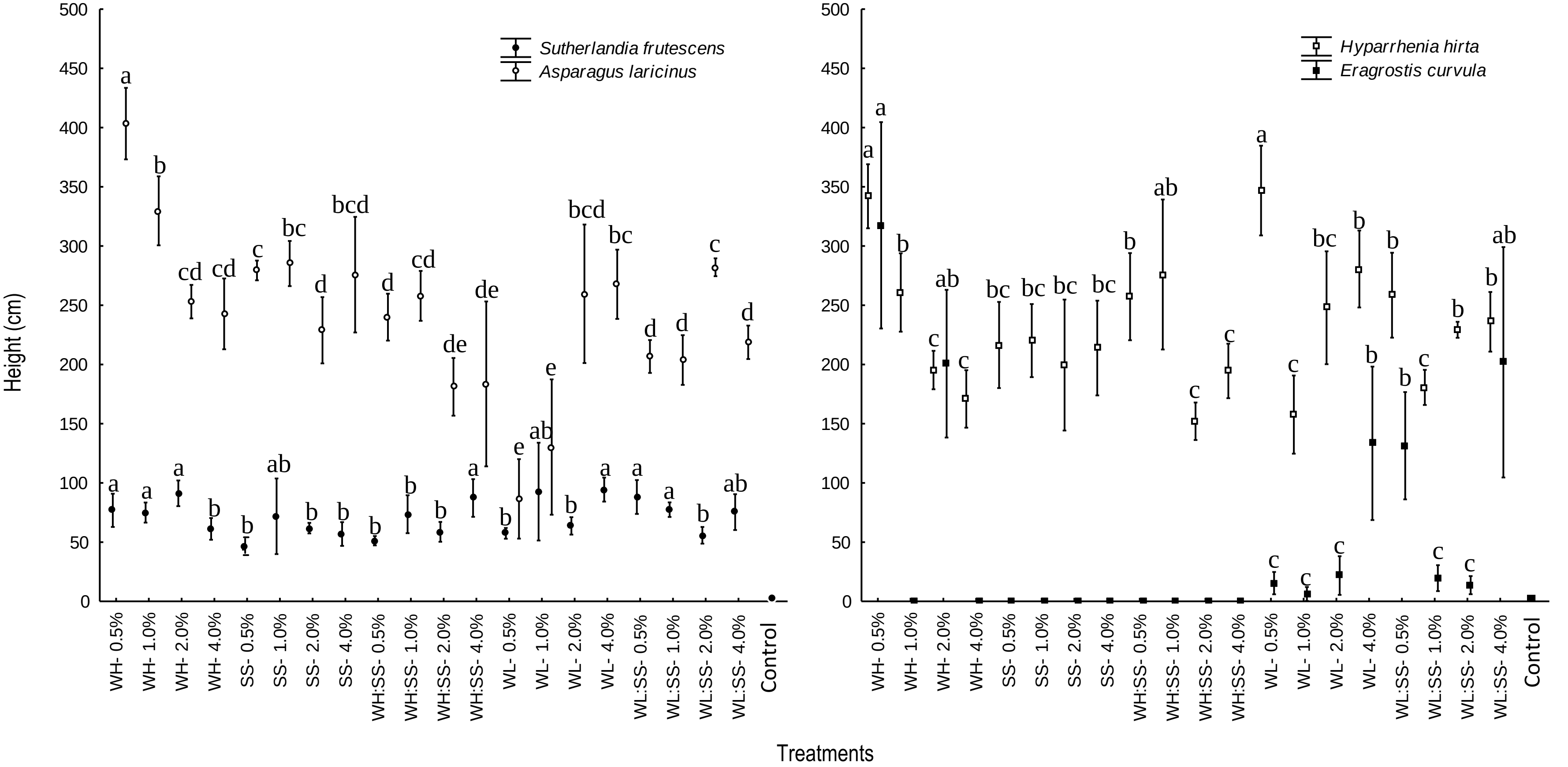


Figure 3-3 Mean heights of *Hyparrhenia hirta*, *Sutherlandia frutescens*, *Eragrostis curvula* and *Asparagus laricinus* grown in 21 tailings treatments over six weeks. Values with different letters within a species are significantly different, 2- Way ANOVA at $p < 0.05$.

Plants (e.g. *Hyparrhenia*, *Sutherlandia* and *Asparagus*) died, after growing in non-amended tailings (control) for duration of one week (Table 3-1).

The four study species grew differently in the 21 tailings treatments, with no plants growing in the control (e.g. tailings alone) (Figure 3-3). Generally all species seemed to grow well in tailings amended with WH, expect for *S. frutescens* which showed low growth in all treatments The tallest *Hyparrhenia*, *Eragrostis* and *Asparagus* plants among all the treatments grew in tailings amended with WH-0.5%, except for *Hyparrheni*a which was also tall in tailings amended with WL-0.5% (Figure 3-3). *Hyparrhenia hirta* and *Asparagus laricinus* showed a significant reduction in height with increase in WH amendment percentage (Figure 3-3).

Asparagus laricinus showed an increase in height with an increase in the percentage of WL (Figure 3-3). No significant changed in plant growth were observed in any of the four plant species when grown in tailings amended with different percentages of sewage sludge (SS) (Figure 3-3). Mixing SS with either WH or WL did not show a definite improvement in the growth of *Hyparrhenia*, *Sutherlandia* and *Asparagus*, while *Eragrostis curvula* did not grow in tailings amended with either SS or WH: SS (Figure 3-3).

3.4.3. Tailings fertility

No significant differences were observed in carbon percentage, total nitrogen, cation exchange capacity, exchangeable sodium percentage, total nitrogen, pH and exchangeable cations in the treatments (Table 3-2). However, C (%) and total N were significantly lower after plant growth (Table 3-2).

Table 3-1 Mean plant survival percentage (%) of *Hyparrhenia hirta*, *Sutherlandia frutescens*, *Eragrostis curvula* and *Asparagus laricinus* during a six week growth period. Increase in plant survial due to resprouting of *Asparagus laricinus* denoted by ^

	Hyparrhenia hirta						*Sutherlandia frutescens*						*Eragrostis curvula*						*Asparagus laricinus*					
Weeks	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
WH 0.5%	100	100	100	100	100	100	100	100	0	0	0	0	100	100	100	100	100	100	100	75	75	100^	75	50
WH 1%	75	100	100	100	100	100	100	50	0	0	0	0	0	0	0	0	0	0	100	75	75	75	50	50
WH 2%	100	100	100	100	100	100	100	75	0	0	0	0	100	75	75	75	75	75	100	50	75^	75	75	75
WH 4%	100	75	75	75	75	75	100	0	0	0	0	0	0	0	0	0	0	0	100	75	50	50	50	25
SS 0.5%	100	100	100	75	75	75	100	50	25	25	25	0	0	0	0	0	0	0	100	75	75	50	50	25
SS 1%	100	100	100	75	75	75	75	25	0	0	0	0	0	0	0	0	0	0	100	75	50	25	25	25
SS 2%	100	100	75	75	75	75	100	25	0	0	0	0	0	0	0	0	0	0	100	50	50	50	25	25
SS 4%	100	100	100	75	75	25	100	50	0	0	0	0	0	0	0	0	0	0	100	75	75	75	25	0
WH:SS 0.5%	100	100	100	100	100	100	100	75	0	0	0	0	0	0	0	0	0	0	100	25	25	25	0	25^
WH:SS 1%	100	100	75	75	75	75	100	50	0	0	0	0	0	0	0	0	0	0	100	75	75	100^	25	25
WH:SS 2%	100	75	25	25	25	25	100	50	0	0	0	0	0	0	0	0	0	0	100	50	50	50	50	25
WH:SS 4%	100	100	100	75	75	75	100	75	50	25	0	0	0	0	0	0	0	0	75	75	75	75	50	25
WL 0.5%	100	100	100	100	100	100	100	0	0	0	0	0	50	25	0	0	0	0	100	50	50	50	75^	25
WL 1%	100	75	75	75	75	75	75	50	50	50	50	50	25	0	0	0	0	0	100	75	50	75^	25	25
WL 2%	100	75	50	50	50	50	100	0	0	0	0	0	50	25	25	25	25	25	100	100	100	100	50	25
WL 4%	100	100	100	100	100	100	100	100	50	25	25	25	75	75	75	50	50	50	100	100	100	100	75	100^
WL:SS 0.5%	100	100	100	75	50	25	100	50	0	0	0	0	75	75	75	75	25	25	100	100	50	50	75^	75
WL:SS 1%	100	75	25	25	0	0	100	100	50	25	25	25	50	25	0	0	0	0	100	50	50	50	25	25
WL:SS 2%	100	100	100	100	100	100	100	50	25	25	0	0	50	0	0	0	0	0	100	25	50^	25	50^	25
WL:SS 4%	100	100	100	75	75	75	100	100	75	25	25	25	100	100	75	75	25	25	100	50	50	50	75^	75
Control	100	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	100	25	0	0	0	0

Table 3-2 Tailings fertility (C %, CEC, ESP, total N, pH and exchangeable cations) in tailings amended with water hyacinth and/or sewage sludge before and after *Hyparrhenia hirta*, *Sutherlandia frutescens*, *Eragrostis curvula* and *Asparagus laricinus* had grown in the treatments for six weeks. Values with a * within columns are significantly different, 2- Way ANOVA at $p<0.05$**. Note: (-) denoted no samples analysed**

Treatments (% dry mass)	C (%)	CEC (meq.100g^{-1})	ESP (%)	Total N (%)	pH	Exchangeable cations (meq.100g^{-1})				
						K^+	Na^+	Ca^{2+}	Mg^{2+}	Ca: Mg
Before plant growth	2.52*	51.54	0.63	0.73*	3.6	0.35	0.55	62.28	5.27	12
After plant growth										
WH 0.5%	0.11	61.9	0.83	0.015	3.6	0.54	0.32	50.27	3.20	16
WH 1%	0.11	61.88	0.88	0.017	3.5	0.47	0.52	53.07	4.02	13
WH 2%	0.17	65.61	0.77	0.017	3.8	0.45	0.74	56.87	4.78	12
WH 4%	0.30	78.73	1.24	0.033	3.9	0.25	0.53	63.00	9.67	7
SS 0.5%	0.07	46.18	0.82	0.021	3.5	0.33	0.66	66.40	5.52	12
SS 1%	0.09	72.38	1.05	0.019	3.5	0.28	0.88	66.78	7.91	8
SS 2%	0.11	71.08	1.55	0.029	3.5	0.21	0.74	67.90	6.70	10
SS 4%	0.17	75.13	0.76	0.015	3.7	0.15	0.72	59.70	6.99	9
WH:SS 0.5%	0.08	59.38	0.96	0.017	3.6	0.26	0.79	69.22	6.29	11
WH:SS 1%	0.12	56.67	1.05	0.025	3.5	0.16	0.67	50.16	6.42	8
WH:SS 2.0%	-	-	-	-	-	-	-	-	-	-
WH:SS 4%	0.21	66.1	0.61	0.017	3.9	0.69	0.85	56.21	6.34	9
WL 0.5%	0.08	65.02	0.74	0.030	3.7	0.43	0.27	66.15	2.47	27
WL 1%	0.09	43.29	1.55	0.026	3.7	0.28	0.28	70.61	3.65	19
WL 2%	0.12	49.01	0.93	0.018	3.6	0.25	0.33	55.50	3.57	16
WL 4%	0.13	55.76	0.65	0.016	4	0.80	0.44	48.06	2.40	20
WL:SS 0.5%	0.10	50.74	0.73	0.041	3.6	0.08	0.35	55.62	2.57	22
WL:SS 1.0%	-	-	-	-	-	-	--	-	-	-
WL:SS 2%	0.14	57.88	0.81	0.014	3.5	0.40	0.16	36.33	2.53	14
WL:SS 4%	0.18	73.85	1.08	0.021	3.7	0.72	0.43	37.89	3.75	10
Control	-	-	-	-	-	-	-	-	-	-

3.5. Discussion

3.5.1. Effects of sewage sludge and/or water hyacinth treatments on seedling emergence, and plant survival and growth

As concluded in chapter two, tailings amended with both sewage sludge and water hyacinth has the potential to improve tailings fertility. However, the plant growth results presented do not show a consistent pattern in seedling emergence, plant survival, plant growth and tailings fertility in the different treatments, except for tailings amended with dry water hyacinth (WH). Generally the three study species except *Sutherlandia frutescens* showed that tailings amended with dry water hyacinth (WH) created the most favourable plant growing conditions (e.g. plant growth (Figure 3-3) and survival (Table 3-1)) out of the five amendments (e.g. WH, WL, SS, WL: SS and WH: SS). Tailings amended with 0.5% of WH seemed to create the most favourable plant growing conditions for plants growing in tailings amended with WH, since the three study species except *S. frutescens* exhibited their greatest heights at that dry water hyacinth content (Figure 3-3). Adding more WH to tailings (e.g. 1.0%, 2.0% and 4.0%) however, resulted in a significant decrease in plant height especially for *Hyparrhenia hirta* and *Asparagus laricinus* (Figure 3-3). *Eragrostis curvula* also showed some decrease in plant height with increase in WH percent although not as clear and significantly different as the two previous species (Figure 3-3).

The second most favourable tailings amendment (e.g. without mixing sewage sludge) was fresh water hyacinth (WL). Seedling emergence and plant height increased with increase in the percentage of WL. This was clearly shown in seedling emergence of *S. frutescens* (Figure 3-2), and in plant survival and height of *A. laricinus* (Table 3-1 and Figure 3-3). The best percentage of WL amendment tested in tailings was 4.0 %, because seedling emergence of *S. frutescens* and *E. curvula* (Figure 3-2), plant survival for all four species (Table 3-1), and plant height of *E. curvula* and *A. laricinus* (Figure 3-3) were highest at this percentage of WL amendment.

The gradual decline in plant height (e.g. less growth) with increase in the percentage of WH amendment, or increase in plant height with increase in the percentage of WL can be attributed to the state in which the amendments were applied to tailings. Both dry and fresh water hyacinth were incorporated into tailings as un-decomposed and crushed organic matter (OM). Fresh water hyacinth had a higher surface area since it was further blended after crushing. Amending tailings with un-decomposed OM is often associated with more adverse than beneficial effects on plant growth, because it disrupts the physical, chemical and biological soil properties. This is because natural soils have a relatively higher OM and bacteria load which creates a buffer when un-decomposed OM is added (Senesi 1989), unlike man-made soils like tailings which contain very little or no OM/ bacteria (Witkowski and Weiersbye 1998a; Wong 2003). When large amounts of un-decomposed OM are added to tailings there is a reduction in plant available N since most of the N will be assimilated by bacteria (e.g. N immobilisation) to assist in decomposition (Hodges 2010), consequently resulting in a reduction in plant available N for plant uptake. However, adding low doses of un-decomposed OM or relatively higher doses of OM characterised by a high surface area (e.g. WL) has been observed to have less detrimental effects in plant growth than un-decomposed OM, since less energy is require for decomposition, hence most of the N in OM will be released as plant available N (Senesi 1989) which could explain plant response to these two amendments. Similar response have also been highlighted by Miller and Donahue (1990), who observed that bacteria and other soil microorganisms excrete a selection of enzyme which breakdown OM to release nutrients like nitrogen and carbon. The released nutrients act as a food source for the bacteria to grow and reproduce. However, if large amounts of un-decomposed OM exist as compared to the bacterial populations, most of the nutrients produce will be assimilated by the bacteria for growth and reproduction until the system had reached an equilibrium between OM and bacteria.

Although tailings amended only with sewage sludge (SS) presented the most challenging treatment for plant growth and survival conditions for all four plant species, they were better than tailings alone. From the sewage sludge C and N analysis in chapter 2, sewage sludge showed a low C: N ratio than water hyacinth (Table 2-4) which is associated with the improvement of tailings fertility and quicker liberation of nutrients (Hodge 2010). However, tailings amended with sewage sludge had low seedling emergence of *E. curvula* and *S. frutescens* (Figure 3-2), and no variations in the height of *H. hirta* were observed with increase in the percentage of sewage sludge (Figure 3-3 and Table 3-1). This could be because some of the metals contained in sewage sludge were liberated into solution. Although sewage sludge showed lower metal concentrations (Al, Cr, Cu, Ni and Zn) than those recommended for Type D sewage sludge under the Permissible Utilisation and Disposal of Sewage Sludge, Edition 1 (1997), one of the requirements for the use of Type D sewage sludge in soils is that the soil pH should be above 6.5. Soils with a pH below 6.5 will result in metals present in sewage sludge becoming bioavailable (Cornu *et al*., 2001). However, all tailing treatments including those containing sewage sludge had a pH below 4 (Table 3-2), but since sewage sludge also had higher metal concentrations than water hyacinth (Figure 2-3), this could have resulted in a higher concentration of metals becoming soluble in tailings amended with SS as compared to those amended with water hyacinth alone. Generally an increase in the solubility of metals like Al, Cr, Ni and Zn would impede plant nutrient uptake because these metals will start competing with essential nutrients for exchange sites, which would result in reduced plant nutrient uptake and consequently interfere with the survival and growth of the plant (McCauley 2009). For example, the Al^{3+} ion has been observed to be among the metals that become more available in tailings with a decrease in pH and has been seen to interfere with P-accumulation during early plant growth (Weiersbye and Witkowski 2003) which could explain the low seedling emergence, and plant survival and growth of plants growing in tailings amended with sewage sludge.

Tailings amended with mixed sewage sludge and either dry or fresh water hyacinth gave different plant survival and growth responses for each of the four study species. *Sutherlandia frutescens* and *E. curvula* showed better growth and survival in tailings amended with WL: SS. Applying 4.0 % of WL: SS amendment gave the highest seedling emergence for *S. frutescens* (Figure 3-2), and the tallest *E. curvula* plants growing in WL: SS among the mixed amendments (e.g. water hyacinth and sewage sludge) (Figure 3-3). On the other hand, tailings amended with mixtures of WH and SS showed better growth of *A. laricinus* and *H. hirta* especially at 1.0 % of WH: SS amendment. Therefore amended tailings with either WL: SS - 4.0% or WH: SS- 1.0% created better mixtures of water hyacinth (both dry and fresh) and sewage sludge.

Several factors however favour the use of WH: SS- 1.0% as a tailings amendment as opposed to WL: SS- 4.0%. Firstly, amending tailings with 4.0 % of WL: SS would mean more water hyacinth and sewage sludge material would be required since the same plant growth conditions could be created by tailings amended by WH: SS- 1.0 %. Although water hyacinth and sewage sludge are abundant in the Highveld gold mines as highlighted earlier, these two resources are finite. This becomes a serious concern especially if we consider that approximately 400 km^2 of the Witwatersrand is covered with either gold tailings or footprints requiring rehabilitation (Weiersbye *et al*. 2006). Secondly, WH: SS requires only sun drying and crushing before incorporating into tailings, while WL: SS requires a more complicated and costly preparation process of crushing, blending, transportation and storing before incorporating into tailings

(Okalebo *et al*. 2006). Finally, chapter 2 showed that water hyacinth and sewage sludge contained heavy metals. From the results shown in Table 2-5, amending tailings with WL: SS- 4.0 % would mean adding more heavy metal to tailings than using WH: SS- 1.0 %, hence resulting in more metal additions to the soil.

3.5.2. Effects of treatments on tailings fertility

3.5.2.1. Carbon percentage and total N

No defined pattern was observed in fertility (C%, CEC, total N, exchangeable cations and pH) between treatments and between species. Carbon percentage (C %) and total N were the only parameters that showed a significant decrease after plant growth in all treatments (Table 3-2). Tailings treatments went from being extremely high in carbon before the experiment to being low in carbon (e.g. organic matter), based on the Harmonized World Soil Database (HWSD version 1.1).

The tailings fertility readings were recorded at two different intervals. Firstly, 2 weeks after mixing organic amendments (e.g. water hyacinth and/or sewage sludge) to tailings (referred in Table 3-2 as ‘before plant growth’) and 12 weeks after this initial reading. A total of 12 weeks accounted for both the time allowed for tailings to ‘age’ and the six week growth experiment. The initial fertility reading probably did not allow sufficient time for the organic matter to breakdown, since the breakdown of OM reduces C% while releasing nutrients like nitrogen (Hodge 2010). However, total N also showed a decrease after plant growth, and this could mean that a significant amount of N released by the organic amendments (e.g. water hyacinth and/or sewage sludge) was accumulated by the plants, since the plants were growing in nutrient limiting environments (Witkowski and Weiersbye 1998b).

3.5.2.2. Cation exchange capacity (CEC)

Cation exchange capacity results in all treatments (before and after plant growth) ranged between 35meq.100g^{-1} to 85meq.100g^{-1} (Table 3-2). Soils having CEC values above 20 meq.100g^{-1} are known to be well drained, requiring less frequent liming and fertilizer, and exhibiting low leaching potential (Hodge 2010). Soils exhibiting such CEC values are ranked as very high in CEC for most soils around the world based on the HWSD version 1.1 (Appendix 2). These high

CEC values might have been attributed to tailings (e.g. acting as clay) and the addition of organic matter (e.g. water hyacinth and sewage sludge), since OM and clay have been observed to increase CEC (Camberato 2001). Although tailings are considered essentially as fine-crushed rock (Witkowski and Weiersbye 1998a), tailings often exhibit characteristics similar to natural clay (e.g. water retention and high field capacity) resulting in several authors (Mislevy *et al*.1988; Krester *et al*. 1997) referring to tailings as "clay tailings". Therefore, the increase in CEC after plant growth could be attributed to the time allowed for OM to decompose, which together with tailings could have increased negative charges (e.g. CEC) hence increasing CEC. An increased negative charge allows more nutrients to be attached to the soil and hence reduce the chances of nutrients leaching or erosion (Camberato 2001), which consequently allows for better plant growing conditions.

3.5.2.3. Exchangeable sodium percentage (ESP)

Exchangeable sodium percentage (ESP) values were below 2 % before (ranging between 0% and 1.7 %) and after (ranging between 0.4% and 1.7 %) plant growth in all treatments (Table 3-2). Although there is a 0.4 % increase in ESP after plant growth, no significant difference was observed in ESP before and after plant growth. All treatments recorded an ESP below 6% after plant growth which resembles a soil extremely low in sodium (HWSD version 1.1-Appendix 2). Soils low in sodium are referred to as non-sodic soils (Isbell 1996) and characterised by being permeable to water and well aerated (Miller and Donahue 1990), allowing for good plant survival and growth. These findings further confirm the characteristic of the tailings treatments deduced above based on the CEC values.

3.5.2.4. pH

Soil pH was mainly measured to determine the effects produced by adding organic amendments specifically sewage sludge to tailings, since adding sewage sludge to soil often lowers the soil's pH (Forsberg and Ledin 2006). Generally all the treatments showed low pH values (ranging between 3.4 and 4.0) (Figure 3-2 and Table 3-2). These pH values are considered extremely low and not ideal for plant growth (van Scholl and Nieuwenhuis 2004; HWSD version 1.1). Such low pH values are common in gold mine tailings in the Witwatersrand Basin of South Africa (Aucamp and van Schalkwyk 2003). These low pH values could have been caused by the oxidation of un-oxidised tailings underlying oxidised tailings during sample collection. When fresh tailings are deposited on a TSF after gold processing, they oxidised often forming a hard-crust which covers the un-oxidised tailings. If this layer is removed the un-oxidised layer reacts with air and water, lowering the pH. However, Forsberg and Ledin (2006) also noted that adding sewage sludge to soils often lowers the pH of the soils. Although no significant differences were observed between water hyacinth and sewage sludge amended soils, tailings amended with SS had relatively lower pH values than those amended with water hyacinth (Table 3-2). As highlighted earlier, low pH soils liberate toxic metals which might interfere with plant survival and growth.

3.6. Conclusion

Amending tailings with low percentages (WH-0.5%) of dry water hyacinth and high percentages (WL-4.0%) of fresh water hyacinth created best seedling emergence, plant survival and growth conditions among all the treatments tested, while tailings amended with sewage sludge had the worst. Tailings amended with WL: SS - 4.0% or WH: SS- 1.0% created the best mixtures of water hyacinth (both dry and fresh) and sewage sludge. Although both treatments (e.g. WL: SS - 4.0% and WH: SS- 1.0%) exhibit low pH values, several factors discourage the use of WL: SS- 4.0% as opposed to WH: SS- 1.0%, among these factors is the potential for more metals leaching out of tailings amended with WL: SS- 4.0% as compared to tailings amended with WH: SS- 1.0% due to high concentrations of metals being contained in the latter. The next chapter attempts to compare metal leaching in the different treatments and also looks at the four study species as phytoremediation candidates.

Chapter 4. Plant heavy metal allocation and leaching from different tailings amendments

4.1. Introduction

Mixing sewage sludge and water hyacinth has the capacity to create better growing conditions for phytoremediation species such as *Eragrostis curvula*, *Sutherlandia frutescens*, *Asparagus laricinus* and *Hyparrhenia hirta* growing on tailings (chapter 3). However, these amendments may also contain heavy metals (chapter 2). If these heavy metals are not retained in the tailings, or contained by the plants, either by adsorption on to the plant roots or absorption into above and below ground plant biomass, the heavy metals could leach. The leaching of heavy metals into soils amended with sewage sludge has been recorded in several studies (Fytianos and Charantoni 1998; Kelly *et al*. 1999; Cornu *et al*. 2001; Almendro-Candel *et al*. 2007; Andres and Francisco 2008). The leached metals could either end up in surface water during runoff or erosion, groundwater as the contaminated water seeps into the soil, plant tissue during plant nutrient uptake in contaminated soils, or in human or animal tissue after consuming plants or drinking water contaminated with heavy metals. However, little or no research has been done on the leaching of heavy metals in soils amended with water hyacinth. This chapter looks at heavy metal leaching from tailings amended with water hyacinth and sewage sludge, and examines how much of the heavy metals are retained in amended tailings or accumulated by *Eragrostis curvula*, *Sutherlandia frutescens*, *Asparagus laricinus* and *Hyparrhenia hirta* over a six week growing period.

4.1.1. Phytoremediation

As highlighted in chapter 1, the term “heavy metal” is used to mean any element that is often used in industry and is toxic to humans, animals, and to aerobic and anaerobic processes, including copper (Cu), cobalt (Co), chromium (Cr), manganese (Mn), nickel (Ni), iron (Fe), zinc (Zn), aluminum (Al), arsenic (As) and mercury (Hg) (Duffus 2002). Raikwar *et al*. (2008) classify the first 7 heavy metals listed above as micronutrients but at elevated concentrations they become toxic, while Al is classified as less toxic or non-essential, and the last two highly toxic. Gold (Au), sulphur (S) and uranium (U) have also been included in the heavy metals classification of this report since tailings in the study came from a gold mining region were elements like Au and U are likely to be found at elevated concentrations due to mining and gold processing (Weiersbye *et al*. 2006) and S from pyrite in tailings (Akcil and Koldas 2005).

Phytoremediation is seen as an ecologically sound, cheaper and sustainable remediation method to treat heavy metal contaminated soil, surface water and shallow groundwater, and control the migration of AMD (Cunningham and Berti 1993; Rosselli *et al*. 2003; Smit and Freeman 2006). Two phytotechnologies identified in remediating inorganic contaminants are phytoextraction and phytostabilization. Phytoextraction reduces the concentrations of metal contaminants in a contaminated medium (e.g. soil or water) by translocating the contaminants into the plant's harvestable above-ground biomass, while phytostabilization reduces the mobility of contaminants and prevents migration to groundwater or air (Padmavathiamma and Li 2007).

The potential of plants to be used either for phytoextraction or phytostabilization are often evaluated based on the concentration of heavy metals in the plant tissue and the translocation factor (TF) (Lorestani *et al*. 2011). The translocation factor indicates the plant's ability to transport metals from the roots to the shoots and is calculated as:

$$TF = [\text{Metal}]_{\text{Shoot}} / [\text{Metal}]_{\text{Root}}$$ (Lorestani *et al*. 2011).

Plants with a translocation factor greater than one (TF> 1) have potential use in phytoextraction, while those with a translocation factor less than one (TF< 1) have potential in phytostabilization (Lorestani *et al*. 2011). Translocation factor can further be used to identify heavy metal hyperaccumulators (e.g. plants that are able to accumulate very high concentrations of metals in the plant's aboveground biomass). The term hyperaccumulator was first introduced by Brooks *et al*. (1977) to describe the high capacity of certain plants to absorb elements from the soil into their aboveground biomass. If a plant has a TF >1 for a particular metal, then it has the potential to hyperaccumulate that particular metal (Lorestani *et al*. 2011). However, not all plants that phytoextract metals (e.g. TF> 1) can be labelled as hyperaccumulators. As a rule of thumb, a metal hyperaccumulating plant should concentrate > 1000 mg/kg of Cu, Co, Cr, Ni, or Pb, or > 10000 mg/kg of Mn or Zn in the harvestable biomass (e.g. dry matter basis) (Baker and Brooks 1989).

The TF can also be used in assessing if a plant is a good candidate for the phytostabilization of tailings and has no unforeseen environmental risk like the ingestion of contaminated plant leaves by animals. This is because, unlike plant nutrients that can be accumulated and utilized by the plant, heavy metals will remain in the plant's biomass (e.g. leaves, bark, or wood) and if ingested by the animal could compromise the health, reproduction and survival of the animals. For example high levels of mercury affects the coordination of movement, causes visual aberration and decline in awareness in livestock (Raikwar *et al*. 2008).

Several species have been reported by Weiersbye *et al*. (2006) to colonize and grow on and around gold TSFs in the Highveld mines of South Africa including *Sutherlandia frutescens*, *Eragrostis curvula*, *Hyparrhenia hirta* and *Asparagus laricinus*. However, little has been recorded on the metal accumulation of these species on and around TSFs in the Highveld except for *Hyparrhenia hirta* which is the most studied of these four species. Conessa *et al*. (2007) observed that *H. hirta* is a good candidate in the phytostabilization of Pb, Cu and Zn.

4.1.2. Heavy metals leaching from sewage sludge and water hyacinth amended tailings

Sewage sludge and water hyacinth contain both nutrients and heavy metals. Upon adding sewage sludge and water hyacinth to soil, they improve the chemical, physical and biological characteristics of the soils (Pathak *et al*. 2009; Gashamura 2009). However, continuous application of these amendments (especially sewage sludge) to soils has been observed to elevate heavy metal concentrations in the soil resulting in these metals eventually leaching from the soils to surface and groundwater systems (McGrath and Lane 1989; Pathak *et al*. 2009). However, the magnitude of the metal leaching in soils will depend on several factors which include the composition of the sludge, soil characteristics, and the ability of the plants to accumulate heavy metals (Kelley *et al*. 1984).

Although any element can become toxic at elevated concentrations, the most common potentially toxic elements in sewage sludge, are listed in the Australian Department of Environment and Conservation (ADEC 2010) report (Appendix 3), and include As, Co, Cr, Cu, Hg, Mn and Ni. One of the aims of this study is to assess the potential of heavy metals to leach from amended tailings; therefore As, Co, Cr, Cu, Hg, Mn and Ni will be measured. Sulphur will also be included in the heavy metal list above, since S is found at high concentrations in tailings and is often associated with the pyrite rock that reacts with oxygen and water to form Acid Mine Drainage (AMD) (Akcil and Koldas 2005).

4.1.3. The European Community Bureau of Reference (BCR) 3- step sequential extraction procedure

The BCR program, under the European Commission developed a standard procedure for determining extractable/exchangeable heavy metals in soils amended with sewage sludge called the BCR 3-step sequential extraction. Exchangeable metals are metals attached on the soil sediment and can easily become soluble and therefore leach, after a change of the ionic composition of the water/soil has occurred, for example additions of weak acid like acetic acid to soil (Kashem *et al*. 2007). The BCR 3-step sequential extraction procedure centers on the use of three different acids (e.g. acetic acid [0.11 mol.L^{-1}], hydroxylammonium chloride [0.1 mol.L^{-1}], and ammonium acetate [1.0 mol.L^{-1}]), used at different steps- step 1, step 2 and step 3, to break tight bonds between the soil-element continuum, resulting in the liberation of exchangeable, reducible and oxidisable metals, respectively (Rauret *et al*. 2000).

Since this study aims at looking at the leaching potential of heavy metals in tailings amended with sewage sludge and/ or water hyacinth, the exchangeable (leachable) heavy metal phase (step 1) of the BCR will be done. This will be done by calculating the leachable fraction (exchangeable percenatge) of heavy metals in the amended tailings using the following relationship:

$$\text{Exchangeable percentage (\%)} = ([\text{Extractable element}].\ [\text{Total element}]^{-1}) \times 100$$

(Aucamp and van Schalkwyk 2003)

Where:

- Extractable elements refers to the elemental concentrations of the leachate left after adding dilute acetic acid [0.11 mol.L^{-1}] to tailings treatments (mg/L) as determined by ICP-OES
- Total element concentration refers to the total elemental concentrations of tailings treatment sample (mg/kg) as determined by ICP-OES

4.2. Methods and Materials

4.2.1. Experimental design

4.2.1.1. Plant samples

A total of 92 plants from the four study species survived to the end of the six week growth period (Table 3-1). However, samples (e.g. for plant and tailings metal analysis) were only taken from treatments containing at least two surviving plants per species, resulting in 52 plant samples for the study. These plants were weighed and divided into roots and shoot (N= 104). The root/shoot samples were washed using distilled water, freeze dried, crushed, microwave digested, followed by a heavy metal (e.g. Al, As, Au, Co, Cr, Cu, Fe, Hg, Mn, Ni, U and Zn) and S analysis using ICP-OES following the same procedure as in Chapter 2 for water hyacinth. Since *A. laricinus*, *E. curvula* and *S. frutescens* had small sample sizes, the effect of the tailings treatments on plant metal accumulation was only carried out with *H. hirta*

4.2.1.2. Tailings samples

A subsample of 10 g of homogenized tailings was collected from each of the 52 treatments (Table 3-1) and divided into two equal batches. The first batch (N= 52) was used for the BCR extraction- explained below, while the other batch (N= 52) was freeze dried, crushed, microwave digested, and analysed for heavy metals (e.g. As, Co, Cr, Cu, Hg, Mn and Ni) and S using ICP-OES following the same procedure for as tailings in Chapter 2. Three replicate samples of Certified Reference Material [NCS DC 73315 (GBW07305), Stream sediment, China National Analysis Centre for Iron and Steel 2004] where included during the BCR extraction as reference checks for the extraction procedure.

4.2.2. BCR sequential extraction

4.2.2.1. Preparation

The following apparatus- 40 ml plastic urine bottles, 1 L graduated plastic bottles, 50 ml- blue lidded centrifuge tubes, 250 and 500 ml beakers, 25 ml pipette, 25 ml and 1 L measuring cylinder were sterilized in an acid bath containing 4 mol. L^{-1} nitric acid solution for 24 hours prior to the BCR to remove any metal traces.

A subsample of 5 g from each of the 52 homogenized tailings samples was freeze dried, followed by sieving using a 1.4mm sieve (1.4 Clear Edge Test Sieve). Dilute acetic acid solution (denoted Solution A [0.11 mol.L^{-1}]) was then mixed with each of these 52 tailings subsamples as shown below.

4.2.2.2. BCR extraction (STEP 1)

The 52 tailings subsamples were randomly divided into three batches (~17 samples per batch) with each batch containing a CRM.

The BCR extraction procedure was carried out as follows (Rauret *et al*. 2000):

- 1 ± 0.001 g of tailings was weighed (Precisa 92SM-202A electronic balance) into a centrifuge tube.
- 40 ml of Solution A was added to the centrifuge tube and closed
- Centrifuge tubes containing the tailings and Solution A mixture were put on a shaker over night at a speed of 150 rpm for 16 hours.
- After shaking, the tubes were put on a rotor (Thermo Scientific- Sorvall- RC 6+ centrifuge) and centrifuged for 20 minutes at 3000g.
- The supernatant solution was removed from the centrifuge tubes and stored in the refrigerator at 4 ^{o}C in 40 ml plastic urine bottles,
- The 52 leachate samples were analysed for As, Co, Cr, Cu, Hg, Mn, Ni and S using ICP-OES (Chapter 2).

4.3. Statistical analysis

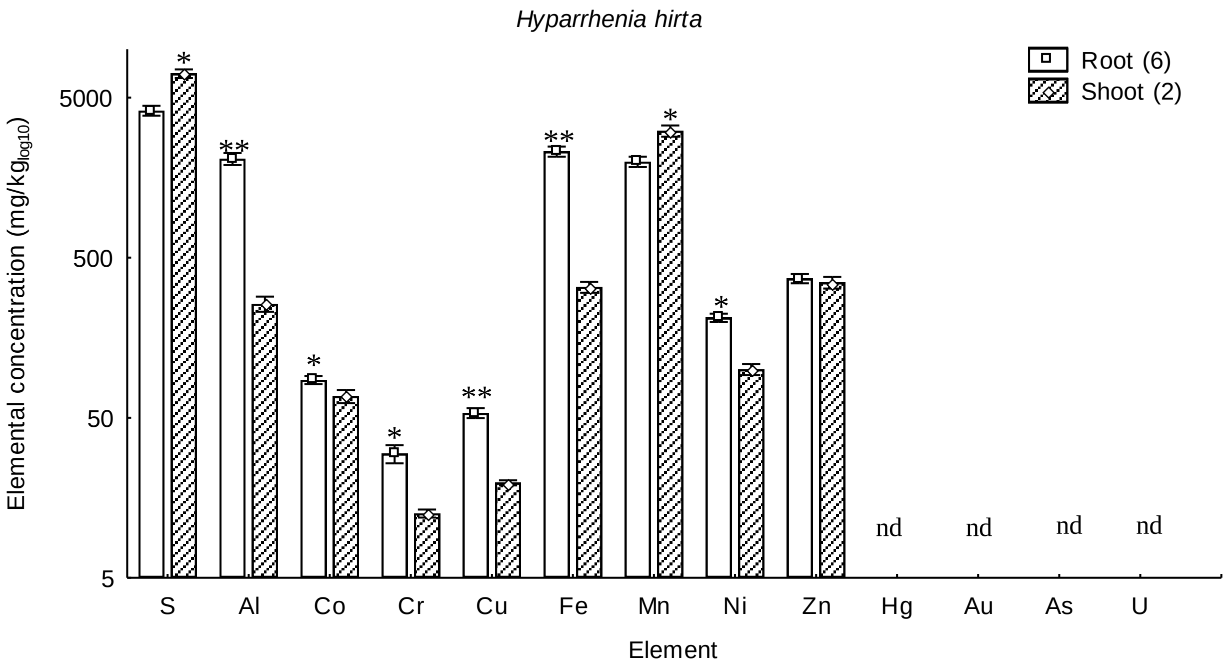
Elemental data (i.e. pooled treatments) were analyzed using a student's *t*- test to compare heavy metal accumulation in plant root and shoot of each of the four study species. These data were also used to compare the translocation factor (TF) between the species per element using a One-Way ANOVA. To get an indication of the effects of tailings treatments on plant metal accumulation, elemental data for *Hyparrhenia hirta* were compared between the treatments using a One- Way ANOVA, since the other three species had insufficient sample sizes for such a comparison (Table 4.1). Finally, to assess the effects of tailings treatments on metal leaching, metals in the leachate were compared between the different tailings treatments using a One-Way ANOVA. All ANOVAs were followed by a post-hoc Tukey tests using Statistica version 6.

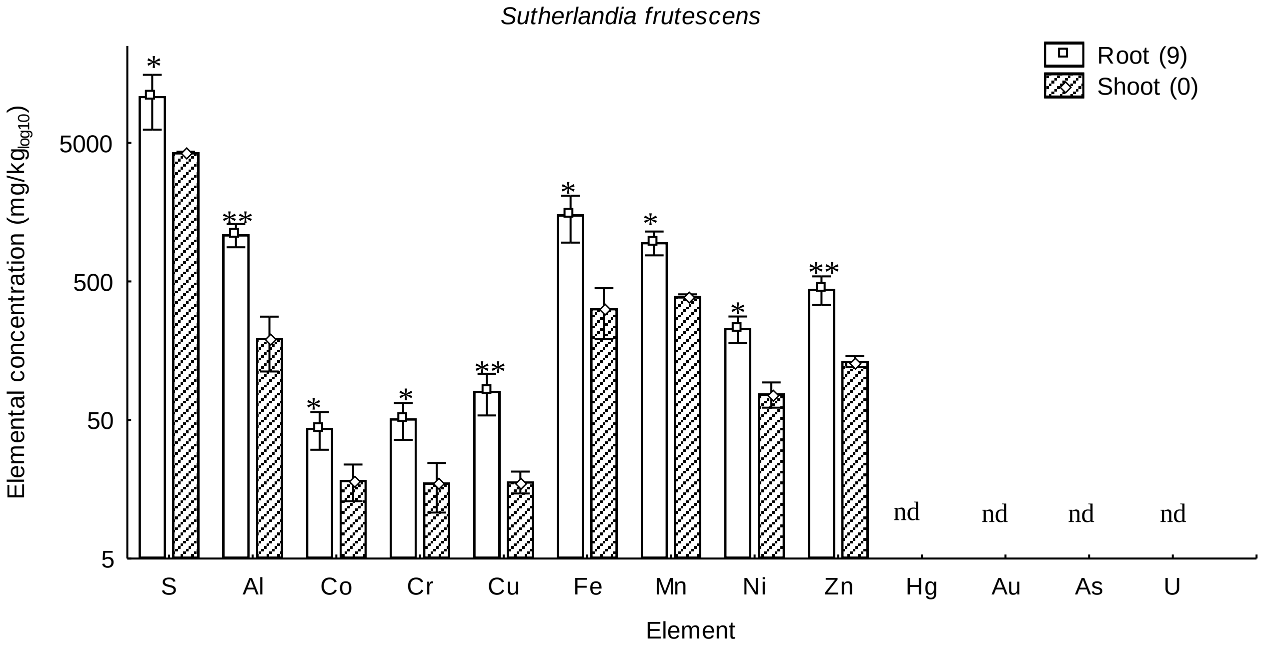
4.4. Results

4.4.1. Plant heavy metal allocation by the study species

Significantly higher concentrations of Al, Co, Cr, Cu, Fe and Ni were found in the plant's roots than in the shoots for all three species, except *A. laricinus* which allocated more S, Co, Cr, Mn, Ni and Zn in the shoots than the roots (Figure 4-1) and had a TF > 1 for these metals (Figure 4-2).

Sutherlandia frustescens and *E. curvula* allocated significantly higher concentrations of S in their roots than shoots, while *H. hirta* and *A. laricinus* allocated significantly higher concentrations of S in their shoots than roots (Figure 4-1). *Asparagus laricinus* allocated almost double the concentrations of Co in the shoots as compared to the roots (Figure 4-1). Significantly higher concentrations of Mn were allocated in the shoots of all three species than the roots, except *S. frutescens* which allocated higher concentrations of the heavy metals (e.g. 8 heavy metals) in the roots (Figure 4-1) resulting in *S. frutescens* showing a TF< 1 for these heavy metals (Figure 4-2). Although only *A. laricinus* showed a significantly higher concentration of Zn in the shoots than *E. curvula* and *H. hirta*, all three species had a TF >1 for Zn and Mn (Figure 4-2). Mercury, arsenic, gold and uranium were not detected in any of the samples studied (Figure 4-1).


Hyparrhenia hirta


Sutherlandia frutescens

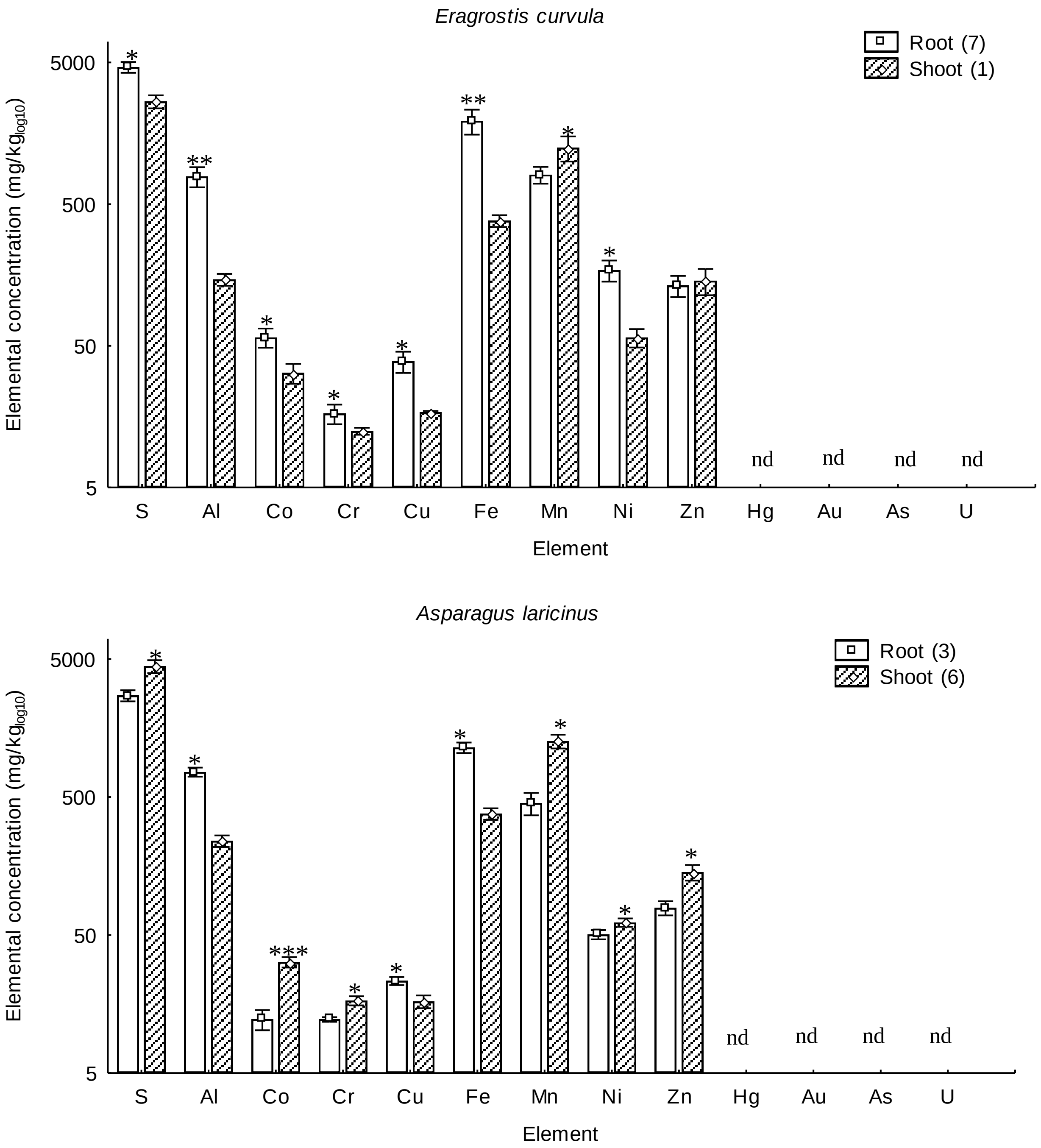


Figure 4-1 Heavy metal concentrations (mg/kg $_{log10}$- dry mass) of *Hyparrhenia hirta* (N= 32), *Sutherlandia frutescens* (N= 2), *Eragrostis curvula* (N= 5) and *Asparagus laricinus* (N= 12) in the roots and shoots after growing in 21 tailings treatments for six weeks. Stars (*) used to denote a significantly higher metal concentrations between the root and the shoot after a *t*-test at * $p < 0.05$, ** $p < 0.01$. **Note: nd= not detected**

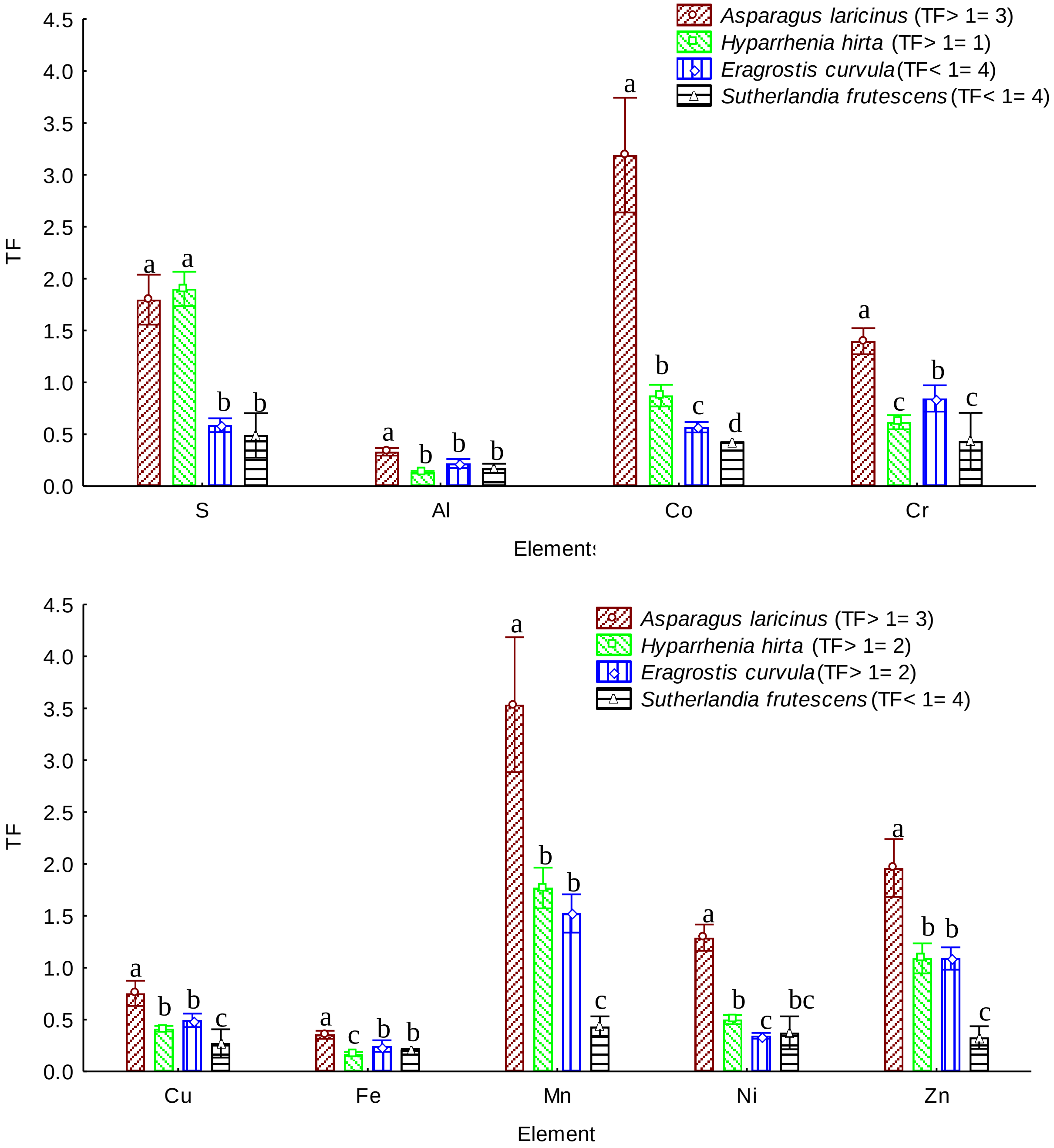


Figure 4-2 Translocation factor (Whole plant) for *Hyparrhenia hirta* (N= 32), *Sutherlandia frutescens* (N= 2), *Eragrostis curvula* (N= 6) and *Asparagus laricinus* (N= 12) growing in different tailings treatments. Different letters used to show significant differences in TF between species per element after a One Way ANOVA, at $p < 0.05$.

4.4.2. Effect of tailings treatments on *Hyparrhenia hirta* metal allocation

Hyparrhenia hirta accumulated significantly higher concentrations of metals (S, Al, Co, Cr, Fe, Mn, Ni and Zn) when grown in tailings amended with WH: SS- 1.0 % as opposed to other tailings amendments, except for tailings amended with WH- 1.0% (S, Al, Cr, Ni and Zn), WH-

4.0% (S, Co, Mn, Ni and Zn), SS- 0.5% (S, Al, Fe, Ni and Zn), SS- 1.0% (S and Zn), WH: SS- 0.5% (Zn), WL- 4.0% (S, Al and Zn) and WL: SS-4.0% (S, AL, Fe and Zn) (Table 4-1). *Hyparrhenia* accumulated the lowest concentrations of S, Al, Co, Cr, Mn, Ni and Zn in tailings amended with WH- 0.5%. *Hyparrhenia hirta* showed no significant difference in Cu when grown in the different tailings treatments (Table 4-1).

4.4.3. Leaching of metals in tailings treatments

The elements that leached in most tailings treatments were S and Mn (Table 4-2). Sulphur leached significantly in tailings amended with WH: SS- 0.5% and WL-2.0 %, while Mn leached significantly in tailings amended with WH- 1.0%, compared to all the other treatments (Table 4-2). Although tailings amended with WL- 1.0% had significantly higher concentrations of Mn as compared to other treatments, most of this Mn was retained (i.e. not leached) in the treatments. Tailings amended with WH- 2.0%, SS- 1.0%, WL: SS- 2.0% and WL: SS- 4.0% also

Table 4-1 Metals concentrations (mg/kg- dry mass) in *Hyparrhenia hirta* (N= 32) in different tailings amendments. Values with different letters within columns denoted significant differences in metal concentrations between the treatments, after a One- Way ANOVA, at $p < 0.05$.

Treatments	Al	Co	Cr	Cu	Fe	Mn	Ni	Zn	S
WH 0.5%	1020^{b}	70^{c}	19^{c}	61	1754^{b}	2264^{c}	158^{c}	434^{b}	7495^{b}
WH 1%	3087^{a}	156^{b}	66^{a}	92	2770	5390^{b}	385^{a}	880^{a}	12494^{a}
WH 2%	1562^{b}	141^{b}	44^{b}	67	2533	4120b^{c}	357^{a}	525^{b}	8624
WH 4%	2137	271^{a}	61^{ab}	95	2971	7404^{a}	457^{a}	820^{a}	11602^{a}
SS 0.5%	3635^{a}	160^{ab}	59^{ab}	75	3945^{a}	5634^{b}	364^{a}	761^{a}	12031^{a}
SS 1%	2532	154^{b}	30^{b}	74	2724	5688^{b}	290^{b}	713^{a}	11664^{a}
SS 2%	2241	135^{b}	40^{b}	69	2578	4592^{b}	299^{b}	671^{a}	9659
SS 4%									
WH:SS 0.5%	2276	163^{ab}	26^{c}	65	2441	5336^{b}	283^{b}	720^{a}	10965
WH:SS 1%	4534^{a}	210^{a}	104^{a}	96	3773^{a}	8260^{a}	486^{a}	1184^{a}	13156^{a}
WH:SS 2.0%									
WH:SS 4%	1336^{b}	114^{bc}	32^{b}	54	1357^{b}	4314^{bc}	233^{b}	559^{b}	9869
WL 0.5%	2527	97^{c}	28^{b}	53	2965^{a}	2753^{c}	214^{c}	682^{a}	8873^{b}
WL 1%	2527	182^{a}	51^{b}	91	2625	6679^{a}	351^{a}	694^{a}	13008^{a}
WL 2%	1389^{b}	164^{ab}	20^{c}	59	1390^{b}	5032^{b}	251^{b}	450^{b}	9817
WL 4%	2792^{a}	132^{b}	36^{b}	80	2527	4544^{b}	280^{b}	721^{a}	11125^{a}
WL:SS 0.5%									
WL:SS 1.0%									
WL:SS 2%	2308	154^{b}	19^{c}	63	2457	5332^{b}	261^{b}	608^{b}	9843
WL:SS 4%	3151^{a}	164^{ab}	45^{b}	76	4067^{a}	3964^{bc}	306^{b}	1097^{a}	12926^{a}

Table 4-2 Metal concentrations in tailings (e.g. before leaching) (T) (N= 67), and the exchangeable metal percentage (E) (N= 67) (e.g. after leaching) of 20 tailings treatments. Values with different letters within column denoted significant differences in metal concentrations between the treatments, after a One- Way ANOVA, at $p < 0.05$.

Treatments	As		Co		Cr		Cu		Hg		Mn		Ni		S	
	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	L
	(mg/kg)	(%)	(mg/kg)	(%)	(mg/kg)	(%)	(mg/kg)	(%)	(mg/kg)	(%)	(mg/kg)	(%)	(mg/kg)	(%)	(mg/kg)	(%)
WH 0.5%	369	0	0	0	86	0.02	26	0.29	-	-	131	5.39^{b}	0	0	1074^{c}	16
WH 1%	362	0	0	0	91^{a}	0.02	27	0.3	-	-	133	41.63^{a}	0	0	1409	15
WH 2%	312	0	0	0	70	0.03	23	0.35	-	-	142	8.29^{b}	0	3.57	1926^{a}	10^{b}
WH 4%	359	0	1	0	77	0.03	28	0.38	-	-	152^{a}	21.11	1	3.79	1458	14
SS 0.5%	324	0	1	0	88	0.04	23	0.69	-	-	131	18.59	1	0	1215	19
SS 1%	259	0	1	0	52	0.07	18	1	-	-	136	19.29	1	6.94	2054^{a}	10^{b}
SS 2%	395	0	1	0	105^{a}	0.03	32	0.53	-	-	165^{a}	15.56	1	0	1660^{b}	16
SS 4%	158	0	4	15.11^{a}	66	0.03	23	0.68	-	-	148	15.62	1	5.27	1276	15
WH:SS 0.5%	213	0	0	0	42^{b}	0.06	17	0.66	-	-	105	11.74	1	3.82	1146^{c}	25^{a}
WH:SS 1%	324	0	1	0	99	0.03	29	0.48	-	-	147	19.76	1	0	1653	11^{b}
WH:SS 2%	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
WH:SS 4%	385	0	0	0	84	0.02	30	0.37	-	-	173^{a}	8.95^{b}	1	0	1416	17
WL 0.5%	284	0	0	0	72	0.02	25	0.57	-	-	164^{a}	6.44^{b}	0	2.66	793^{c}	20
WL 1%	441	0	5	3.22	80	0.02	32	0.38	-	-	231^{a}	2.47^{b}	0	1.07	1227	19
WL 2%	306	0	6	5.5	57	0.04	24	0.95	-	-	165^{a}	9.26^{b}	1	1.91	786^{c}	26^{a}
WL 4%	372	0	3	6.18	73	0.02	24	0.39	-	-	155^{a}	5.45^{b}	0	1.76	910^{c}	17
WL:SS 0.5%	309	0	0	0	72	0.05	23	0.44	-	-	143	7.26^{b}	0	2.33	1707^{b}	11^{b}
WL:SS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
WL:SS 2%	352	0	0	0	48^{b}	0.03	15	0.42	-	-	61^{b}	9.02^{b}	0	2.26	2215^{a}	12^{b}
WL:SS 4%	407	0	0	0	61	0.02	24	0.31	-	-	86^{b}	5.86^{b}	0	2.52	2901^{a}	13^{b}

retained S, although these treatments had significantly higher S concentrations when compared to other treatments (Table 4-2). Tailings amended with SS-4.0% leached significantly higher concentrations of Co among all the treatments (Table 4-2). Although As exhibited the highest metal concentration (e.g. total As) in all treatments for all the metals tested, no leaching of this metal was observed in any of the treatments (Table 4-2). No total Hg or extractable Hg was recorded in all treatments (Table 4-2).

4.5. Discussion

4.5.1. Phytoremediation by the four study species

Plants require a balance between the uptake of essential metal ions to maintain growth, development and health whilst protecting sensitive cellular activity and structures from excessive levels of essential and non-essential metals (Garbisu and Alkorta 2001). Generally the concentrations of most elements tested in *Eragrostis curvula*, *Sutherlandia frutescens, Asparagus laricinus* and *Hyparrhenia hirta* were in a similar range to the concentrations recorded in most vascular plants by Raven *et al*. (1999), except for Cu, Fe, Mn and Zn. Copper, Fe, Mn and Zn were almost 30 times higher in the study species than the concentrations recorded by Raven *et al*. (1999). Copper and Fe were allocated more in the plant roots of all the study species as compared to the shoots, while Mn was allocated more in the plant shoots of *H. hirta*, *E. curvula* and *A. laricinus* than in the plant roots (Figure 4-1). Apart from Mn, *A. laricinus* also allocated significantly higher concentrations of Zn in the shoots than the roots (Figure 4-1).

Copper and Fe are plant micronutrients and these elements (together with Mn and Zn) were found at higher concentrations in all tailings treatments (Table 2-5) as compared to the plant’s nutrient requirements (Table 1-2). This suggests that the four study plants could be taking up luxury amounts of Cu and Fe, while *H. hirta*, *E. curvula* and *A. laricinus* could be taking up luxury amounts of Mn into their shoots. This response by plants to take up luxury 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 mediums containing excess nutrients or metals through phytoremediation (Cunningham and Berti 1993). However, plants’ strategies to take up luxury amounts of elements vary depending on the type of elements, ability of the plant to intercept, absorb and accumulate the element into the shoots, and the interaction between different elements in the soil-root interface (Lasat 2002).

Asparagus laricinus was the only plant among the four species that allocated higher concentrations of Zn into the plant shoots as compared to the plant roots. This could mean that the other three species are less tolerant to allocating high concentrations of Zn in their shoots but prefer allocating Zn into or onto the roots. However, due to budget constraints, allocation of Zn either into or onto the root biomass could not be deduced (e.g. as was done for water hyacinth in chapter 2).

4.5.2. Effects of tailings amendments on *H. hirta* metal accumulation

It is important to note that the effects of tailings treatments on plant metal accumulation could only be done using *H. hirta* because the other three species had limited samples for such an analysis. Although tailings amended with WH-0.5% created the best plant growth conditions for *H. hirta* (chapter 3), this treatment had the lowest concentrations of elements accumulated by *H. hirta*, while *H. hirta* plants growing in tailings amended with WH-4.0%, WL: SS- 4.0%, and WL- 4.0% had significantly higher concentrations of Co, and Fe as compared to plants growing in other treatments, respectively. The concentrations of heavy metals taken up by *H. hirta* in tailings amended with either WH- 0.5%, or WH-4.0%, WL: SS- 4.0%, and WL- 4.0% could be attributed to the metal concentrations in the treatments in which the plants grew (Table 2-5). Plants have been observed to increase the uptake of different elements with increase in the concentration of the elements in the growth medium (McCauley 2009). Both the physical and chemical state of the growth medium (e.g. tailings fertility - pH, CEC, ESP) have also been observed to influence plant elemental uptake (Chaney 1988). However no significant differences were observed in tailings fertility among the treatments (Table 3-2) to suggest tailings fertility as a factor to influence plant elemental uptake.

Plant growth results in chapter 3 showed that plant height reduced significantly in plants grown in WH: SS- 2.0% as compared to WH: SS- 1.0% especially for *H. hirta* due to different nutrient concentrations in the treatments (Figure 3-3). On the other hand, the plant metal allocation results showed that *H. hirta* grown in tailings amended with WH: SS- 1.0% contained the highest concentrations of Al, Cr, N and Zn as compared either WH: SS- 0.5%, WH: SS- 4.0% or the other tailings treatments (Table 4-1). These results could be explained by McCauley (2009), who showed that, at a certain nutrient threshold (denoted sufficiency range-Figure 1-1) a plant is able to take up available nutrients (e.g. essential and non-essential) in the growth medium whilst maintaining a healthy and gradual growth. However, if the sufficiency range is either reduced (e.g. amending tailings with WH: SS- 0.5%) or exceeded (e.g. amending tailings with WH: SS- 2.0% and WH: SS- 4.0%) the plants growth (Figure 3-3), metal uptake (Table 4-1) and health will be compromised. Therefore, amending tailings with WH: SS- 1.0% could be the sufficiency range for tailings amended with WH: SS.

4.5.3. Metal leaching

Sulphur and Mn are plant macro- and micro- nutrients, respectively (Raven *et al*. 1999). These two elements are often found at high concentrations in areas associated with high anthropological activities like mining, agriculture and municipal workings like sewage treatment plants (Gonzalez *et al*. 1989; Cilliers *et al*. 1996; Akcil and Koldas 2005). In Chapter 2, Mn was observed to be significantly higher in water hyacinth obtained from both the Vaal River and Benoni Lakes as compared to sewage sludge (Figure 2-3), while a significant concentration of S was adsorbed on water hyacinth roots (Figure 2-2).

In this section S and Mn were observed to have the highest potential to leach among all the elements tested in the different tailings treatments. The highest concentrations of S, and Mn were leached in tailings amended with WH: SS- 0.5%, WL- 2.0%, and WH- 1.0%, respectively. Tailings amended with WL-1.0% retained a significant percentage of Mn as compared to the concentration of Mn in the tailings treatment, while tailings amended with WH- 2.0%, SS-1.0%, WL: SS- 2.0% and WL: SS- 4.0% retained a significant percentage of S relative to the S concentration in the tailings treatments. Tailings treatments in which S and Mn were leached were among the treatments that exhibited the lowest plant growth in all the four species, while tailings treatments in which S and Mn were retained were among the treatments that experienced high plant growths (chapter 3). These results suggest that poor plant growth reduces plant elemental uptake and increases the chances of leaching in tailings.

No clear relationship could be observed between tailings treatments and how the treatments interact with plant S and Mn accumulation, and S and Mn leaching. Figure 4-3 and Figure 4-4 shows some of the relationship between the concentrations of Mn and S leached in tailings and those accumulated by *H. hirta* in the different tailings treatments. However, the relationships in the models were not statistically significant.

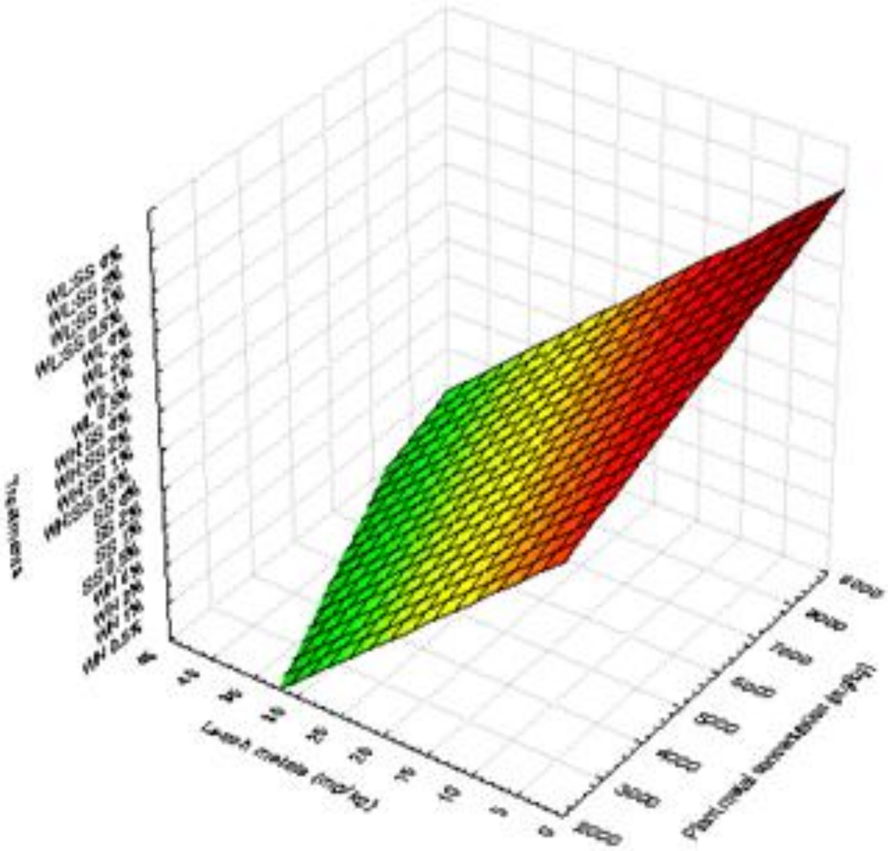


Figure 4-3: Response surface graphs for Mn accumulated by plants and that leached into the tailings treatments. *Equation*: Treatment = 97.6981 + 0.0008* plant metal concentration (mg/kg) + 0.2682* leach metal concentration (mg/kg)), $p = 0.0879$.

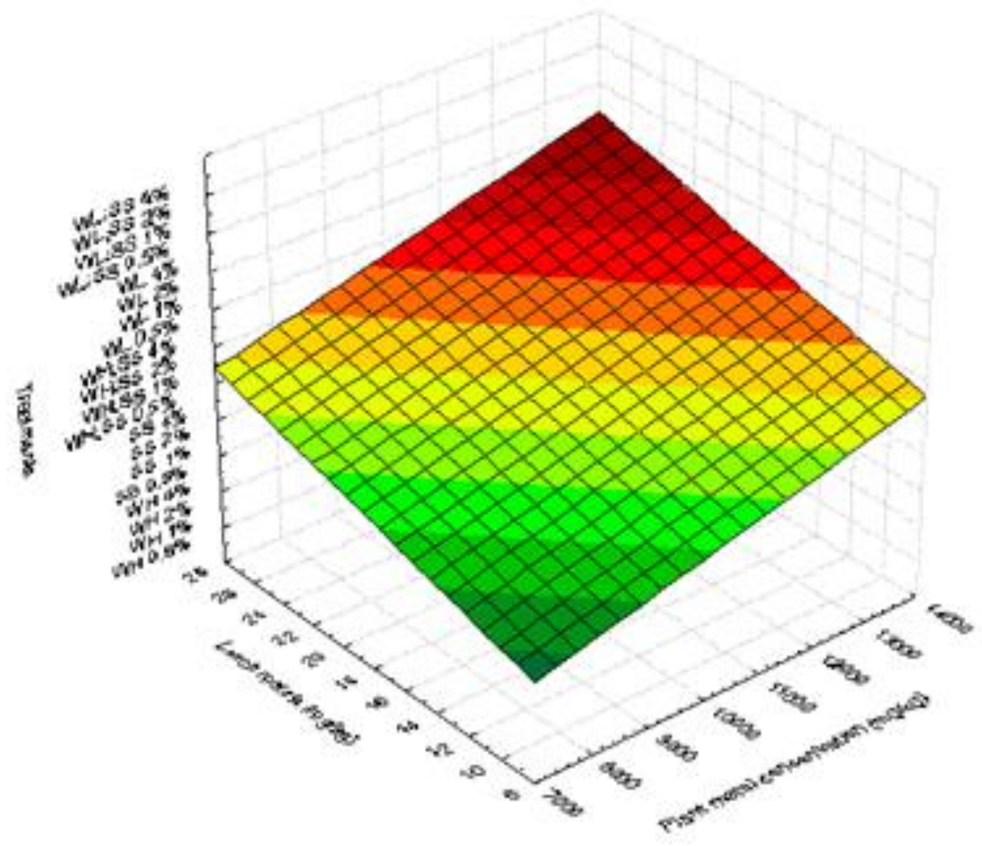


Figure 4-3: Response surface graphs for S accumulated by plants, and that leached into the tailings treatments. Equation: Treatment = 110.046 +0.001* plant metal concentration (mg/kg) – 0.4067 leach metal concentration (mg/kg)), *p* = 0.3799.

Total arsenic levels in all tailing treatments exceeded both the ecological and health levels based on the Australian Department of Environment and Conservation (ADEC 2010) (Appendix 3). However, no potential threats were posed on either the environment and humans since none of the As was leachable (Table 4-2). Total Co, Cr, Cu, Mn and Ni were lower than the permissible levels by ADEC (2010).

Chapter 5. Conclusion

The rehabilitation of gold TSFs, especially to reduce dust emissions and seepage, and potential exposure of surface and ground water to AMD requires urgent attention in South Africa, notably now that most gold mines are fast approaching their closure. Using indigenous plants to control and remediate heavy metals and salts associated with AMD is seen as an ecologically acceptable and inexpensive approach; however plant growth conditions on mine tailings are harsh. In an attempt to improve plant growth conditions on gold mine tailings, this report aimed to demonstrate the effectiveness of using water hyacinth and sewage sludge as tailings amendments, and to find the optimal application of amendment to tailings. Five amendments (dry water hyacinth (WH), sewage sludge (SS), fresh water hyacinth (WL), WH: SS and WL: SS), each applied at four different percentages (e.g. 0.5%, 1.0%, 2.0% and 4.0%) and a control was assessed for their ability to improve:

- tailings fertility
- tailings stability to metal leaching

This report also aimed to evaluate how these amended tailings influence:

- seedling emergence, and plant survival and growth
- plant S and metal accumulation

The tailings amendments were objectively compared using seedling emergence percentage, plant survival and plant height, plant elemental accumulation, and a range of soil measurements (pH, cation exchange capacity, exchangeable sodium percentage, soil organic carbon, exchangeable cations and total nitrogen). The 1st -step of the BCR- sequential extraction was used to compare metal leaching in each treatment.

5.1. Key Findings

The key findings of this study are summarized in the table below. Although all treatments significantly improved the growth conditions for plant growth compared to tailings alone. Tailings amended with WH- 0.5% created the most favourable conditions (e.g. for seedling survival, plant establishment and growth) for most plants tested, while WH: SS -1.0% and WL: SS-4.0% created the best water hyacinth and sewage sludge mixtures. Considering seedling emergence, plant survival and growth, plant metal allocation and leaching, tailings amended with WH: SS- 1.0% provided the most favorable results. Tailings amended with WH: SS-1.0% had several advantages as compared to tailings amended with WL: SS-4.0% or the other 18 amendments used in the study. These advantages include:

- use of both water hyacinth and sewage sludge to amend tailings,
- low application rate,
- minimised addition of heavy metals (e.g. from sewage sludge and water hyacinth) onto tailings during mulching,
- easy to prepare and mix, unlike mixing sewage sludge and fresh water hyacinth,

This study has deduced that amending tailings with WH: SS- 1.0% could create the best plant growth condition and take up high concentrations of elements whilst reducing environmental risk like leaching. However, further studies may still be required. These may include:

- a field comparison of water hyacinth and sewage sludge on TSFs following the experimental design and procedure used in this study,
- long-term effects (e.g. fertility, leaching and plant metal accumulation) of water hyacinth and sewage sludge amendments on tailings,
- using high biomass plants like shrubs and trees on water hyacinth and/or sewage sludge amended tailings instead of using low biomass plants like grasses,
- measuring the decomposition rate of water hyacinth when applied to TSFs at different slope positions and slope angles,
- viability of water hyacinth seeds on tailings,
- More site-specific studies on different gold TSFs around the Witwatersrand Basin, and
- Conduct a more detailed fertility analysis, especially for N-mineralization, cation exchange capacity (CEC) and exchangeable sodium percentage (ESP) before and after plant growth in tailings amended with water hyacinth and/or sewage sludge. This study was only able to conduct a total N and cation (i.e. K^+, Mg^{2+}, Na^+, and Ca^{2+}) concentration analysis due to the challenges faced with analyzing tailings. Generally, most laboratories do not analyse tailings material because they fear that tailings material will detrimentally

affect the performance of their equipment, since the equipment is mainly used to measure agricultural soils. This limited the methods used for fertility analysis. The initial 21 tailings mixtures (before the growth experiment) were analysed for N-mineralization, total N, cation concentrations, CEC and ESP at Cedara in KwaZulu Natal. However, the laboratory refused to analyse anymore samples containing tailings. The Agricultural Research Commission (ARC) in Pretoria agreed to analyse the fertility of the final tailings mixture (after plant growth). However, the ARC laboratories only analysed total N and cation concentrations. Therefore, only total N, and CEC and ESP (derived from cation concentrations) were used to compare tailings fertility before and after plant growth.

These future studies will result in a more informed decision being made on the use of water hyacinth and sewage sludge on tailings, especially considering that both these organic amendments are a serious environmental concern.

Summary of Results

Treatments	**(%)**	**Seedling emergence**	**Plant survival and growth**	**Tailings fertility**	**Plant metal uptake**	**Metal leaching**
T (control)	0					
WH	0.5	Significantly higher seedling emergence of *E. curvula* (i.e. together with seedling from WH-2.0%), as compared to other treatments	Grew some of the tallest *Eragrostis*, *Asparagus* and *Hyparrhenia*	no signficant difference among treatments	*H. hirta* accumulated lowest concentrations of all the elements tested (except Cu) as compared to other treatments	
WH	1					Highest concentrations of Mn leaching among all treatments
WH	2	Significantly higher seedling emergence of *E. curvula* (i.e. together with seedling from WH-0.5%), as compared to other treatments				
WH	4				*H. hirta* accumuluted the highest concentrations of Co in this treatment as compared to other treatments	
SS	0.5					
SS	1					
SS	2					
SS	4	The only SS treatment in which *E. curvula* seedlings emerged				Highest concentrations of Co leaching among all treatments
WH: SS	0.5					
WH: SS	1		better growth for *H. hirta* and *A. laricinus* as compared to other mixed amendments		*H. hirta* accumuluted the highest concentrations of Al, Cr, Cu, Mn, Ni, Zn and S in this treatment as compared to other treatments	
WH: SS	2					
WH: SS	4					
WL	0.5		grew some of the tallest *Hyparrhenia*		*H. hirta* accumulated the lowest concentrations of Co, Cu, Mn and Ni in this treatment, as compared to other treatments	
WL	1					Highest concentrations of Mn in treatment, but had the lowest leach of Mn among all the treatments
WL	2					
WL	4	highest seedling emergence for *S. frutescens* (i.e. together with plants growing in WL: SS-4.0%), as compared to other treatments				
WL: SS	0.5					
WL: SS	1					
WL: SS	2					
WL: SS	4	highest seedling emergence for *S, frutescens* (i.e. together with plants growing in WL-4.0%), as compared to other treatments				Highest concentrations of S in treatment, but had the lowest leach of S among all the treatments

Chapter 6. References

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Legislation

Constitution of the Republic of South Africa (Act 108 of 1996)

Appendix 1: Classification of sewage sludge: Permissible Utilisation and Disposal of Sewage sludge (Edition 1), August 1997

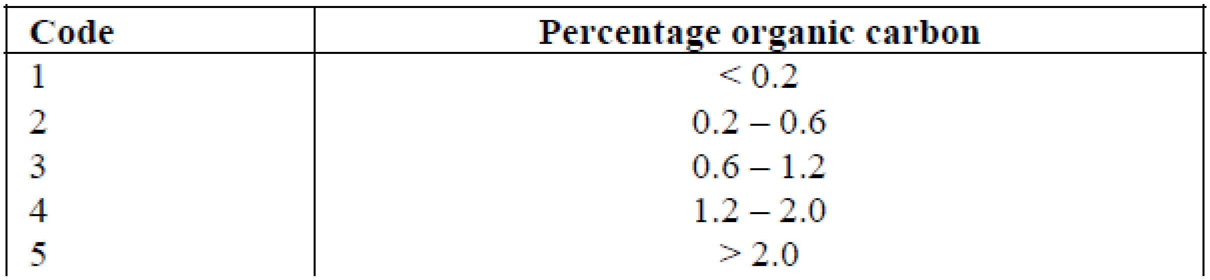
CLASSIFICATION OF SEWAGE SLUDGE TO BE USED OR DISPOSED OF ON LAND

TYPE OF SEWAGE SLUDGE	ORIGIN/TREATMENT (EXAMPLES)	CHARACTERISTICS-QUALITY OF SEWAGE SLUDGE
TYPE A SLUDGE	Raw sludge Cold digested sludge Septic tank sludge Oxidation pond sludge	•Usually unstable and can cause odour nuisances and fly-breeding •Contains pathogenic organisms •Variable metal and inorganic content
TYPE B SLUDGE	Anaerobic digested sludge (heated digester) Surplus activated sludge Humus tank sludge	•Fully or partially stabilised – should not cause significant odour nuisance or fly-breeding •Contains pathogenic organisms •Variable metal and inorganic content
TYPE C SLUDGE	Pasteurised sludge Heat-treated sludge Lime-stabilised sludge Composted sludge Irradiated sludge	•Certified to comply with the following quality requirement: (If not certified this sludge is considered a TYPE B SLUDGE) - Stabilised - should not cause odour nuisances or fly-breeding - Contains no viable Ascaris ova per 10g dry sludge -Maximum 0 Salmonella organisms per 10g dry sludge - Maximum 1000 Faecal coliform per 10g dry sludge, immediately after treatment (disinfection/sterilisation)
TYPE D SLUDGE A sludge product produced for unrestricted use on land with or without addition of plant nutrients or other materials **This product must be registered in terns of Act 36 of 1947 if used for agricultura/horticultural activities**	Pasteurised sludge Heat-treated sludge Lime-stabilised sludge Composted sludge Irradiated sludge	Certified to comply with the following quality requirements - Stabilised - should not cause odour nuisances or fly-breeding - Contains no viable Ascarls ova per 10g dry sludge -Maximum 0 Salmonella organisms per 10g dry sludge -Maximum 1000 Faecal coliform per 10g dry sludge,immediately after treatment (disinfection/sterilisation) •Maximum metal and inorganic content in mg/kg dry sludge Cadmium 15,7 Cobalt 100 Chromium (Cr^{3+}) 1750 Copper 50,5 Mercury 10 Molybdenum 25 Nickel 200 Lead 50,5 Zinc 353,5 Arsenic 15 Selenium 15 Boron 80 Fluoride 400 • User must be informed about the moisture and N P K content • User must be warned that not more than 8t/ha (or 10 kg sq.m)(dry sludge may be applied to soil and the pH of the soil should preferably higher than 6.5.

Appendix 2: Evaluation of soil parameters based on the Harmonized World Soil Database (HWSD), Version 1.1, 2009.

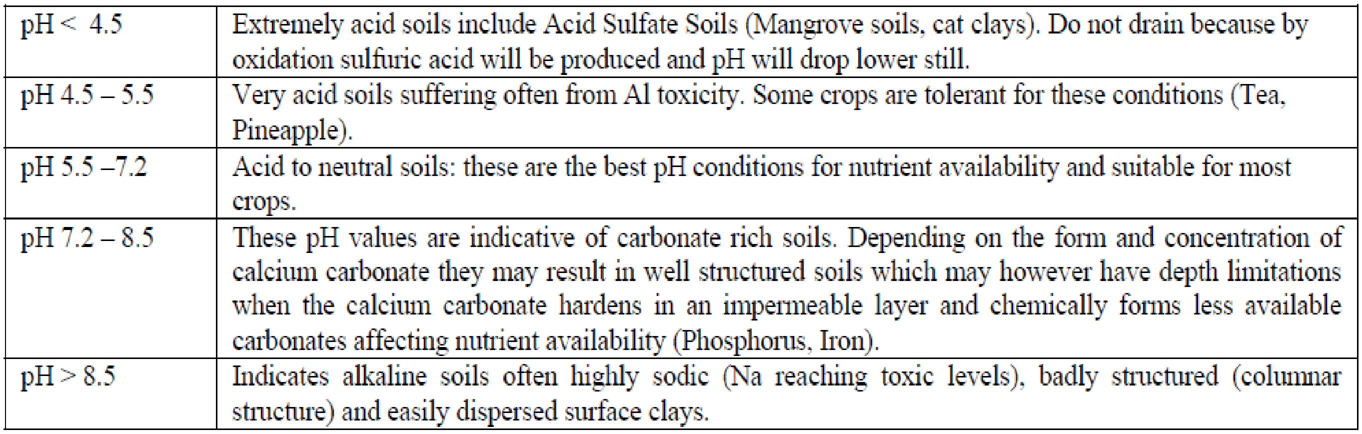
1. Soil organic carbon

Soil with percentage organic carbon <0.2% are considered very poor in organic carbon, while those >2.0% are considered very high in carbon



Code	Percentage organic carbon
1	< 0.2
2	0.2 – 0.6
3	0.6 – 1.2
4	1.2 – 2.0
5	> 2.0

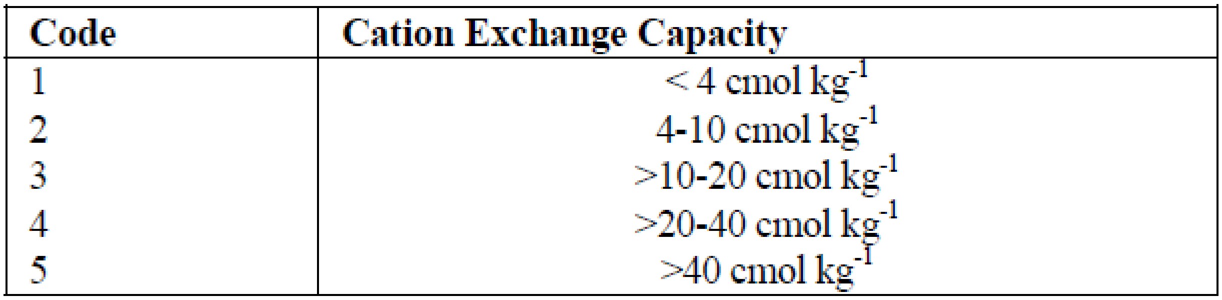
2. pH



pH < 4.5	Extremely acid soils include Acid Sulfate Soils (Mangrove soils, cat clays). Do not drain because by oxidation sulfuric acid will be produced and pH will drop lower still.
pH 4.5 – 5.5	Very acid soils suffering often from Al toxicity. Some crops are tolerant for these conditions (Tea, Pineapple).
pH 5.5 –7.2	Acid to neutral soils: these are the best pH conditions for nutrient availability and suitable for most crops.
pH 7.2 – 8.5	These pH values are indicative of carbonate rich soils. Depending on the form and concentration of calcium carbonate they may result in well structured soils which may however have depth limitations when the calcium carbonate hardens in an impermeable layer and chemically forms less available carbonates affecting nutrient availability (Phosphorus, Iron).
pH > 8.5	Indicates alkaline soils often highly sodic (Na reaching toxic levels), badly structured (columnar structure) and easily dispersed surface clays.

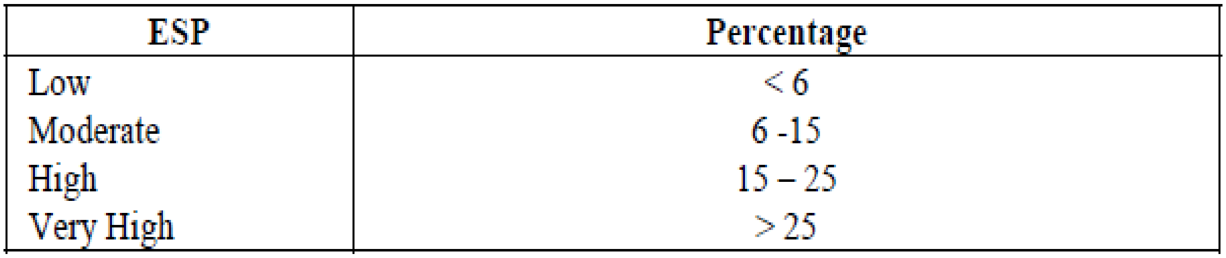
3. Cation exchange capacity (CEC)

Cation exchange capacity values above 10 cmol kg^{-1} are considered satisfactory for most crops



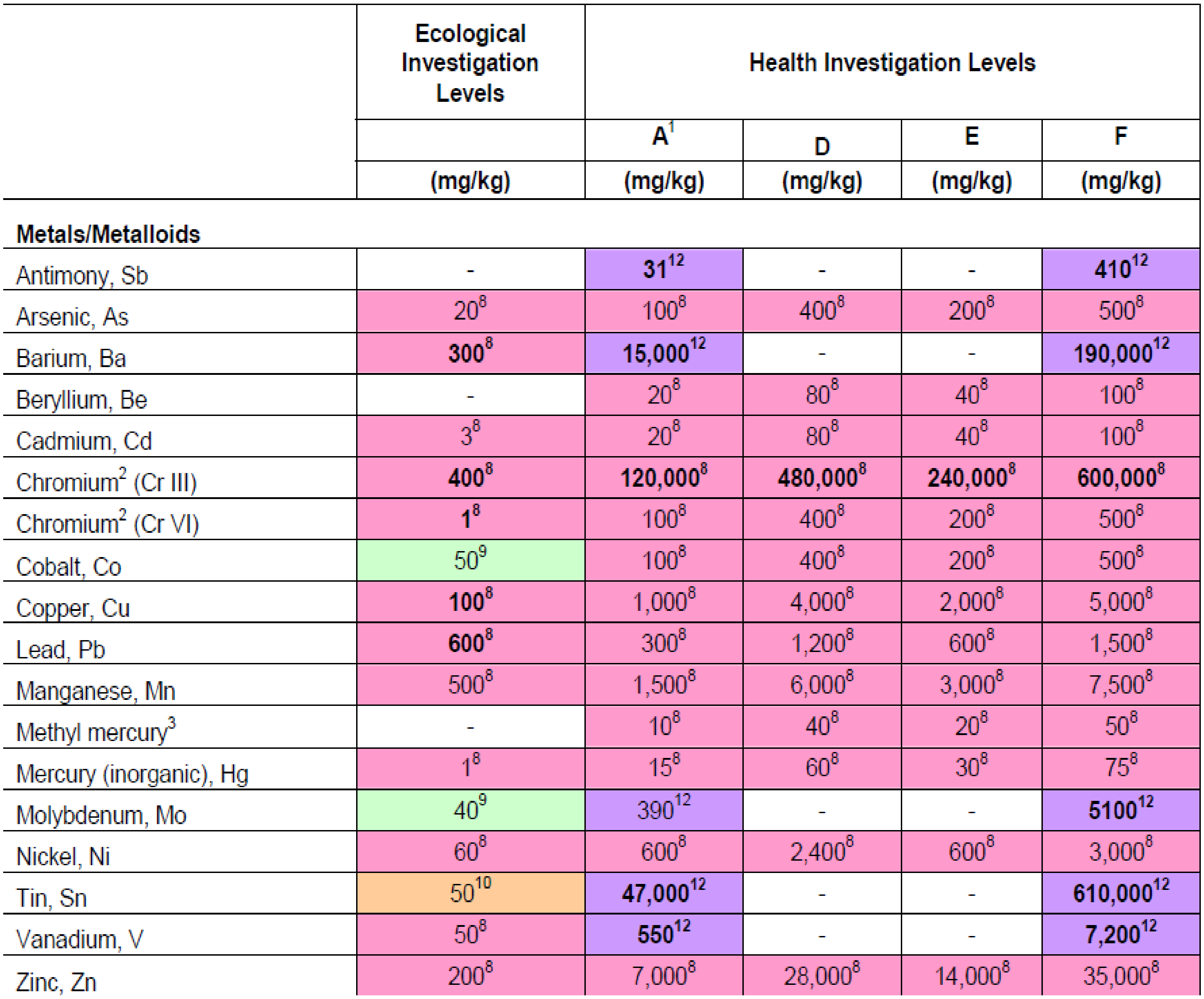
Code	Cation Exchange Capacity
1	< 4 cmol kg^{-1}
2	4-10 cmol kg^{-1}
3	>10-20 cmol kg^{-1}
4	>20-40 cmol kg^{-1}
5	>40 cmol kg^{-1}

4. Exchangeable sodium percentage (ESP)



ESP	Percentage
Low	< 6
Moderate	6 -15
High	15 – 25
Very High	> 25

Appendix 3 Assessment levels for metals in soil (ADEC 2010)



	Ecological Investigation Levels	**Health Investigation Levels**			
		A[1]	**D**	**E**	**F**
	(mg/kg)	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**
Metals/Metalloids					
Antimony, Sb	-	**31[12]**	-	-	**410[12]**
Arsenic, As	20[8]	100[8]	400[8]	200[8]	500[8]
Barium, Ba	**300[8]**	**15,000[12]**	-	-	**190,000[12]**
Beryllium, Be	-	20[8]	80[8]	40[8]	100[8]
Cadmium, Cd	3[8]	20[8]	80[8]	40[8]	100[8]
Chromium[2] (Cr III)	**400[8]**	**120,000[8]**	**480,000[8]**	**240,000[8]**	**600,000[8]**
Chromium[2] (Cr VI)	**1[8]**	100[8]	400[8]	200[8]	500[8]
Cobalt, Co	50[9]	100[8]	400[8]	200[8]	500[8]
Copper, Cu	**100[8]**	1,000[8]	4,000[8]	2,000[8]	5,000[8]
Lead, Pb	**600[8]**	300[8]	1,200[8]	600[8]	1,500[8]
Manganese, Mn	500[8]	1,500[8]	6,000[8]	3,000[8]	7,500[8]
Methyl mercury[3]	-	10[8]	40[8]	20[8]	50[8]
Mercury (inorganic), Hg	1[8]	15[8]	60[8]	30[8]	75[8]
Molybdenum, Mo	40[9]	390[12]	-	-	**5100[12]**
Nickel, Ni	60[8]	600[8]	2,400[8]	600[8]	3,000[8]
Tin, Sn	50[10]	**47,000[12]**	-	-	**610,000[12]**
Vanadium, V	50[8]	**550[12]**	-	-	**7,200[12]**
Zinc, Zn	200[8]	7,000[8]	28,000[8]	14,000[8]	35,000[8]