Phytoremediation potential and growth response of pasture species used in the revegetation of coal mine substrates

by

Barbara Ernestine Schmidhuber

17284882

Dissertation submitted in fulfilment of the requirements for the degree

***Magister Scientiae* (MSc) Environmental Management**

Supervisor: Dr W.F. Truter

First submission: January 2023

Final submission: February 2024

Contents

List of Tables

List of Figures

Abbreviations

A	Plant Available Concentration
A%	Element Availability Percentage
ADF	Acid Detergent Fibre
ADP	Adenosine Diphosphate
AF	Accumulation Factor
AIP	Alien Invasive Plant Species
Al	Aluminium
Al_2O_3	Aluminium Oxide
AMD	Acid Mine Drainage
AMF	Arbuscular Mycorrhizal Fungi
APS	Adenosine 5'-Phosphosulphate
ARD	Acid Rock Drainage
As	Arsenic
ATP	Adenosine Triphosphate
B	Boron
Ba	Barium
BAI	Bioaccumulation Index
C	Carbon
Ca	Calcium
CARA	Conservation of Agricultural Resources Act (Act 43 of 1983)
Cc	Coefficient Of Curvature
Cd	Cadmium
CEC	Cation Exchange Capacity
CH_3SH	Methyl Mercaptan
Cl	Chloride
CLI	Contamination Load Index
Co	Cobalt
CoPC	Constituents of Potential Concern
COS	Carbonyl Sulphide
CS_2	Carbon Disulphide
Cu	Coefficient Of Uniformity
D	Grain Diameters
DEA	Department of Environmental Affairs
DMR	Department of Mineral Resources
EC	Electrical Conductivity

Fe	Iron
FTIR	Fourier Transform Infra-Red Spectroscopy
GGI	Grade of Growth Inhibition
H	Hydrogen
H_2S	Hydrogen Sulphide
H_2SO_4	Sulphuric acid
Hg	Mercury
HTER	Hyperaccumulation Threshold Exceedance Ratio
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
K	Potassium
KCl	Potassium chloride
L	Litre
LA-ICPMS	Laser Ablation-Inductively Coupled Plasma Mass Spectroscopy
Mg	Magnesium
MI	Mobility Index
Mn	Manganese
MPRDA	Minerals and Petroleum Resources Development Act (Act 28 of 2002)
N	Nitrogen
Na	Sodium
NDF	Neutral Detergent Fibre
NEMA	National Environmental Management Act (Act 107 of 1998)
NH_4	Ammonium
Ni	Nickel
NIRS	Near Infrared Reflectance Spectroscopy
NO_3	Nitrate
O	Oxygen
OC%	Organic Carbon Content
OM	Organic Matter
P	Phosphorus
PFI	Particle-Fluid Interaction
PO_4	Phosphate
PS	Phytosiderophores
PS II	Photosystem II
PSD	Particle Size Distribution
PTE	Potential Toxic Elements
RO	Reverse Osmosis

S	Sulphur
Se	Selenium
SiO_2	silicon Dioxide
SO_2	Sulphur Dioxide
SO_4	Sulphate
SOM	Soil Organic Matter
SPAD	Soil Plant Analysis Development
Sr	Strontium
SSV	Soil Screening Value
T	Total Concentration
TER	Threshold Exceedance Ratio
XRF	X-ray Fluorescence
Zn	Zinc

Glossary

(Adapted from van Deventer *et al*., 2021; Sparks, 2003; Hickey and King, 2000).

Terms	Definitions
Abiotic	Non-living components and compounds like the physical, geological, or chemical aspects of the environment.
Acid generation	Material containing sulphur or sulphide minerals generates acid when exposed to oxidising conditions and is mainly associated with mining activities due to the exposure of the acid-generating material to oxidising conditions.
Acid mine drainage (AMD)	A metal-rich water which is formed from the chemical weathering of rocks containing sulphur-bearing minerals with water. The solution is usually acidic and frequently comes from areas where ore or coal mining activities have exposed rocks containing pyrite.
Acidophilic, acidophilous plants	Plants that are adapted to or thrive in acid soil.
Affinity	The tendency of an atom to chemically react with another compound or atom to form a chemical compound
Anabolism.	Metabolic pathways that construct molecules from smaller units using energy.
Anthropogenic	Occurring as a result of or influenced by human activities.
Apoplastic movement	The route through the free spaces outside the plasma membrane within which material diffuses freely.
Bioaccumulation	The gradual accumulation of substances, such as trace elements or other toxins, in an organism.
Biomagnification	Substances like trace elements and toxins become concentrated at high trophic levels in the food chain due to bioaccumulation.
Biomass	The total mass of living animal and plant material produced per unit area or volume. This term is used interchangeably with phytomass to refer to the mass of plant material.
Biotic	Living components like biological organisms, animals or plants.
Catabolism	The opposite of anabolism and the breakdown of molecules into smaller units.
Chalcophile	A group of elements that have a strong affinity for transition metal-bearing minerals (refer to affinity)
Chlorophylls	Natural plant pigments which absorb sunlight and use the energy to photosynthesise to produce carbohydrates.
Chlorosis	A condition in plants due to a reduction in chlorophyll synthesis caused by nutrient deficiencies or other physiological disorders. Leaves of chlorotic plants range from light green through yellow to almost white.
Coal refuse	Coal waste, coal tailings, waste material, culm and/or the material left over from coal mining.
Exudates	Low molecular weight fluid that is emitted through the roots of plants to inhibit harmful microbes and promote growth.
Ferralitic soils	Similar to Fersialitic soils (refer to Fersialitic soils) but with a medium to low base saturation.
Fersialitic soils	Soils that are red as a result of iron oxidation and also imply well-

	drained soils and yellow as a result of iron reduction (Eutrophic soils)
Hyperaccumulation	The ability of plants to take up trace elements from the contaminated growth medium and accumulate them within the plant at a higher concentration level than in the contaminated growth medium.
Metabolites	The biochemical end product of plant metabolic activities.
Mine dumps	Waste stockpiles consisting of overburden and other mine or processing waste materials.
Necrosis	Death associated with discolouration and dehydration of plants.
Pasture species	Includes a wide array of tufted or creeping, temperate or tropical, perennial or annual grasses or legumes.
Phytoremediation	The use of plants and associated soil microbes to reduce the concentrations of trace elements and other toxins in a growth medium or solution.
Phytoextraction	The use of plants to remove contaminants from growth mediums.
Phytofiltration	The use of plants and associated microorganisms to remove contaminants from water.
Phytomining	Using vegetation extract minable elements from a growth medium as a mining method for economic purposes.
Pioneer species	The initial species that quickly colonise an area of disturbance.
Rehabilitation	Returning a disturbed land to a stable, non-polluting and self-sustaining condition, aligned with a specified land use.
Remediation	The mitigation or clean-up of pollution or contamination of water or soil using pre-determined methods.
Restoration	Artificially accelerate the processes of natural succession by returning the area to its former, undisturbed state.
Rhizosphere	The soil zone in the immediate vicinity of the plant root and includes the soil and microorganisms (bacteria, fungi).
Rhizobia	Bacteria that are established inside root nodules and fix nitrogen.
Siderophile	A group of elements that have a strong affinity for sulphide-bearing minerals (refer to affinity)
Speciation	Various chemical or physical forms of an element.
Soil amelioration	Improving the soil's physical and/ or chemical properties by applying lime, gypsum, fertilisers, compost or synthetic soil conditioners, amongst others.
Soil compaction	The increase in soil bulk density, and concurrently decreasing the soil porosity.
Soil Screening Values (SSV)	The threshold concentrations of elements found in the soil below which no adverse effects on the environment, including plants, animals and invertebrates, in terms of pollution are expected.
Soil structure	The combination or arrangement of soil particles with differing in size, shape, and grade.
Soil texture	The different size soil particles in the soil and refers to the proportion of sand, silt and clay particles.
Stress	A state where plant growth is inhibited due to non-ideal growth conditions.

Sustainable	The use of resources at a rate that does not lead to long-term decline does not disturb ecological integrity or processes and continues to meet the need of future generations.
Trace elements	Trace elements are present at levels <0.1%, and heavy metals can be defined as elements with densities of >5.0 g/cm^3 (Sparks, 2003).

Chapter 1 Conceptualisation of coal mines, rehabilitation constraints, phytoremediation and effects on plants

Schmidhuber[1], B.E and Truter, W.F[2]

[1]Centre for Environmental Studies, University of Pretoria

[2]Department of Plant and Soil Sciences, University of Pretoria

Abstract

South Africa is known for its abundant mineral resources, and coal is currently still the country's principal source of energy. The coal mining industry contributes significantly to the country's overall economic growth; however, mining poses significant and irreparable damage to the receiving environment. The fifth-largest coal deposit globally, comprising 19 coal fields, belongs to South Africa, with the majority located in the Highveld coalfields of the Mpumalanga province. The impacts of mining activities on the environment mostly become apparent only after mining ceases.

The importance of topsoil as a rehabilitation medium is vastly underestimated and is the main reason for ineffective topsoil management from pre-mining to eventual post-mining phases. Mine spoils and tailing facilities are prone to erosion and must be stabilised according to legislation to prevent pollution of the surroundings and potential financial liabilities. The primary aim of rehabilitation is to re-establish functional ecosystems. Vegetation cover is the most prevalent method used in South Africa to stabilise mined surfaces and tailings facilities due to plants' ability to stabilise and increase biodiversity for sustainability. The success of vegetation establishment is dependent on the soil's chemical, physical and biological characteristics. Therefore, it is evident that soil is the most essential component for successful rehabilitation.

The investigation will focus on the potential to use mine-impacted soil and coal spoils as a growth medium by quantifying the physical and chemical properties of the growth medium and the associated impacts on the growth performance between different pasture species. The use of plants to chemically and physically ameliorate the substrate to sustain vegetation cover in perpetuity will also be investigated.

Keywords: *Coal mining, Topsoil, Contaminants, Rehabilitation, Ameliorate, Vegetation*

1.1 Introduction

Anthropogenic activities such as mining activities pose a significant threat to soil's abiotic and biotic health. Due to mine activities, historically, environmental pollution has become an increasing concern worldwide. Some contaminants are considered lethal at low concentrations, while others can accumulate at higher trophic levels in a process known as bioaccumulation.

Mine refuse material, including spoils, tailings material and discard alike, in South Africa is tantamount to environmental contamination, erosion and is contingent on the material characteristics, making it strenuous to rehabilitate. Mine tailings and refuse materials are being considered as potential growth mediums, where pedogenesis has not yet occurred, for rehabilitation purposes because good quality topsoil is scarce, especially in the Highveld. It is challenging to establish vegetation on coal spoils or coal-contaminated soil mainly due to the acidity of the substrate that results from the chemical weathering and oxidation of sulphide-bearing minerals that are present to produce sulphuric acids. Plants as a remediation component have widely been investigated in the past few years.

Phytoremediation involves the uptake of constituents of potential concern (CoPCs) from soil or any growth medium through plant shoots or plant roots. The proportion of phytoremediation depends on plant biomass production; however, seeing that specific pasture species are commonly being used in rehabilitation practices in South Africa, the focus will be on pasture species that are presently being utilised. The emphasis on pasture species is due to grazing being the most popular land capability for coal mines in terms of rehabilitation and closure commitments.

1.2 Literature Review

Coal is presently Southern Africa's primary energy source and ranks seventh on the global scale, according to the Statistical Review of World Energy (British Petroleum Company, 2021), in terms of coal consumption and accounts for 17,8% of the world's total consumption.

1.2.1 Geological overview

The Karoo Supergroup is relevant to all South Africa's coalfields. It is known for its distinctive plant assemblages, terrestrial vertebrate fossils, thick glacial deposits, flood basalts and associated dolerite sills and dykes. The Karoo Supergroup strata has a thickness of roughly 12 km and encompasses an area of 700 00 km^2 (Pone *et al*., 2007; Hancox and Götz, 2014). The basement rocks from west to east include metavolcanic, metasedimentary, and dolomitic rocks of the Transvaal Supergroup, metavolcanic and meta-sedimentary rock from the Waterberg Group and Bushveld Igneous Complex (Figure 1-1) (Hancox and Götz, 2014; Johnson *et al.,* 2006).

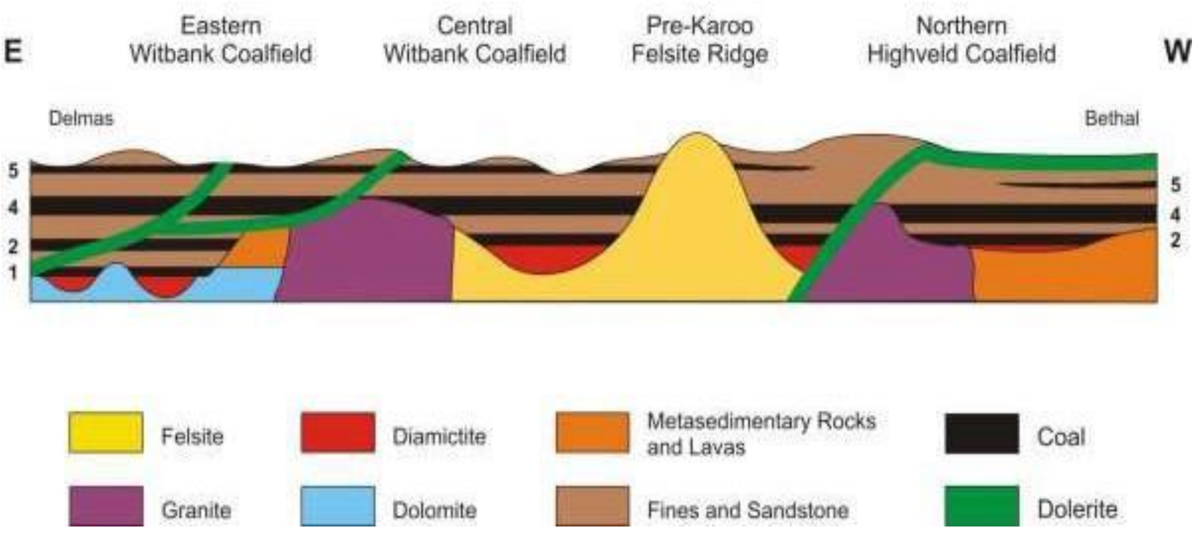


Figure 1-1: East-West cross sections of the coalfields (Hancox and Götz, 2014).

The Karoo Supergroup is partitioned into four foremost lithostratigraphic units, which from the bottom upwards are the Dwyka, Ecca, Beaufort and Stormberg (Molteno, Elliot and Clarens formations) groups. (Johnson *et al*., 2006). The Permian Ecca Group comprises 16 formations, and apart from the extensive formations like the Whitehill Formation and Prince Albert, the other individual formations are grouped into three geographical areas (southern, north-eastern, and north-western). The Vryheid Formation is associated with the north-eastern section of the Ecca Group and has the bulk of South Africa's economically extracted coal. The Vryheid Formation predominantly consists of sandstone, siltstone, and shale (Johnson *et al.,* 2006; Hancox and Götz, 2014).

The coalfields of South Africa are contained within the Main Karoo Basin and its sub-basins. There are 19 coalfields in South Africa, based on origin, formation, geographic consideration, and distribution (Hancox and Götz, 2014; Johnson *et al.,* 2006).

Most coal mined in South Africa comes from only roughly half of the 19 coalfields. The locations of the 19 coalfields in South Africa are depicted in Figure 1-2.

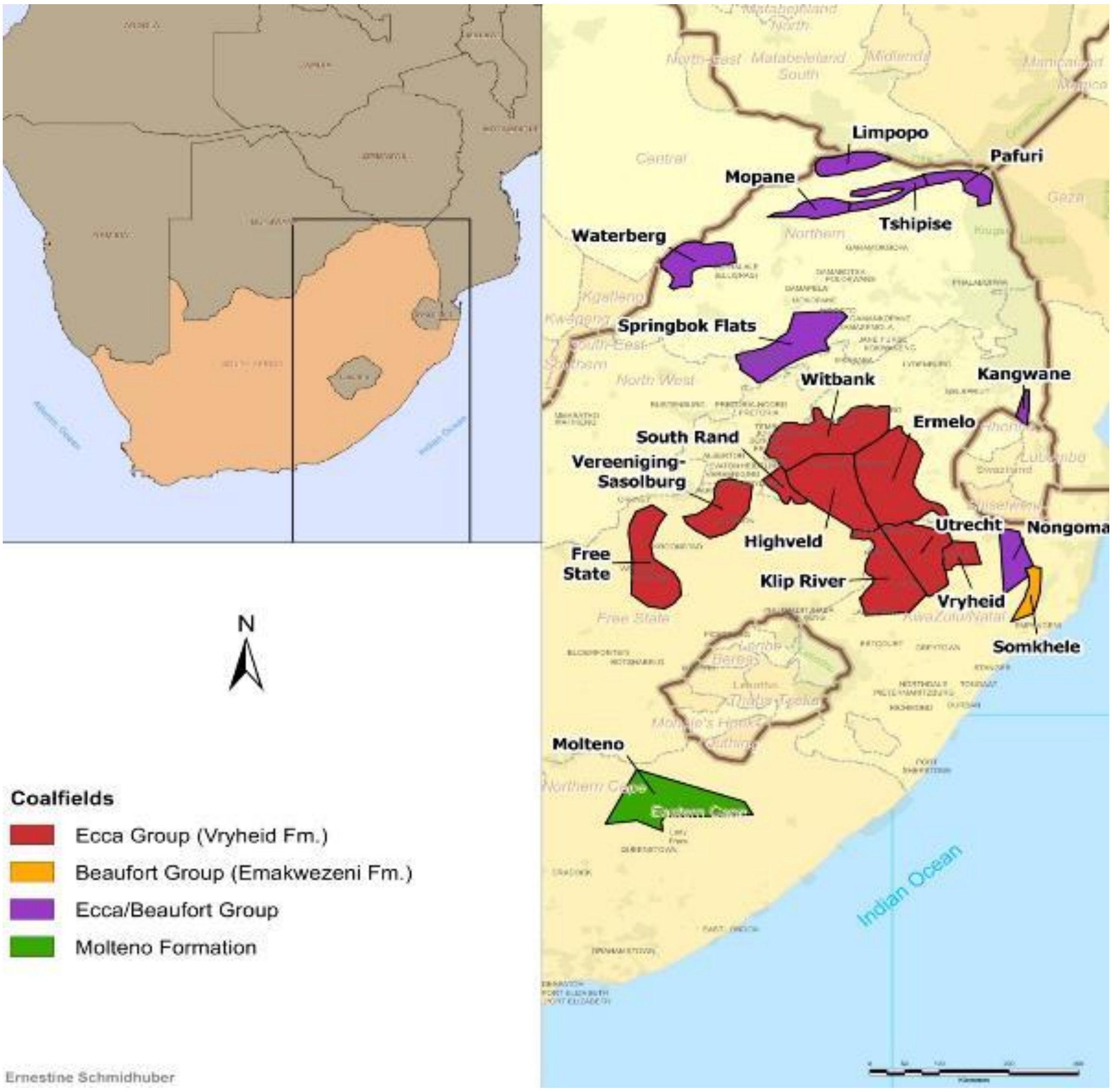


Figure 1-2: Coalfields of South Africa (*adapted from* Hancox and Götz, 2014)

Within the various coalfields, sedimentary cyclic variability is typical within various coalfields, whereas allocyclic variability is the exception and allows for the correlation of genetically related sequences (Johnson *et al.,* 2006). Most of the coal-bearing successions have dolerite intrusions, which may cause devolatilisation and combustion of the coal, create structural instabilities and related seam correlation problems, and difficulties during mining (Hancox and Götz, 2014: Johnson *et al*., 2006).

The Witbank coalfields is situated within the Karoo Supergroup of the Main Karoo Basin. The Karoo Supergroup of the Main Karoo Basin is the most continuous and developed sub-Saharan basin (Johnson *et al*., 2006).

The Witbank coalfields are mature in exploration and exploitation and are situated in the northern part of the Main Karoo Basin, covering an area of more than 568 000 ha. The north boundary of the coalfield consists of pre-Karoo basement rocks, and the southern border in the central section of the basin is the sub-outcrop against a ridge known as the Smithfield Ridge (Hancox and Götz, 2014). The north-south cross-section of the coalfields is presented in Figure 1-3.

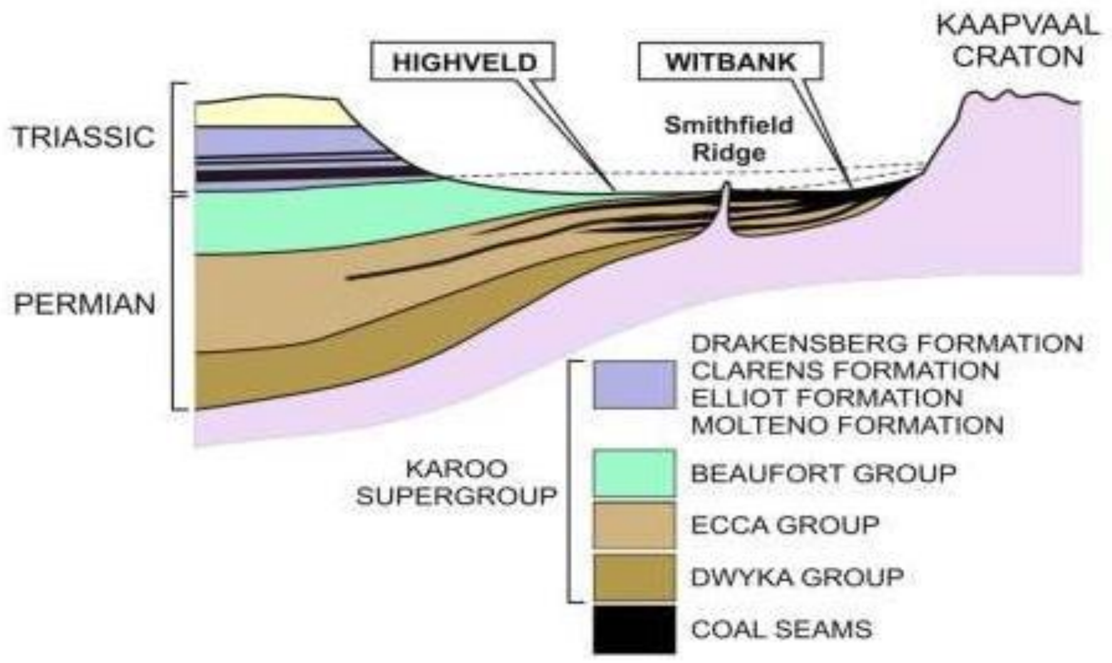


Figure 1-3: North-south cross-section of the Witbank and Highveld coalfields separated by the Smithfield Ridge (Hancox and Götz, 2014).

The ridge is a generally east-west curving hemispherical-shaped ridge consisting of pre-Karoo granites, felsites, and the Bushveld Igneous Complex diabase, extending for roughly 60 km. The southern boundary is indiscriminately apparent to the east and west of the central portion. The profound eastern and western boundaries are characterised by the coal-bearing sedimentary succession against the pre-Karoo basement (Pone *et al*., 2007; Johnson *et al*., 2006).

1.2.2 Impacts of coal mines and rehabilitation constraints

The most deleterious effects of coal mining activities on the receiving environment result from the mobilisation of contaminants, like trace elements associated with the mine waste material or the previously unexposed parent material and the formation of acid mine drainage (AMD).

The contaminants get transported by seepage and leaching to surface water resources and groundwater resources, consequently polluting and diminishing water quality (Welsh *et al*., 2007; Grimshaw, 2007). The unpredictability of mine waste material leads to erosion, ultimately resulting in contaminated surface runoff, soil contamination, air pollution and water pollution (Grimshaw, 2007).

Coal mine waste tends to be acidic by nature due to the oxidation of associated sulphide-bearing minerals. The acidity of the mine waste results in the increased solubility of trace elements, rendering it bioavailable for vegetation. The improved bioavailability expedites bioaccumulation to toxic levels and the decline in vegetation cover (Wu *et al*., 2011).

Section 28 of the National Environmental Management Act (NEMA) (Act 107 of 1998) states that measures must be implemented to reduce and rectify pollution or degradation of the environment for which the cause could not have been otherwise prevented (RSA, 1998). Section 43 of the Mineral and Petroleum Resources Development Act, 2002 (MPRDA) (Act 28 of 2002) states that a mine closure certificate is only issued if the mine complies with the responsibly and requirements of protecting the environment (RSA, 2002).

According to Hattingh (2019) and The Australian Government *et al*., 2016), three overarching rehabilitation objectives need to be attained, namely:

- The area should be sustainable and have perpetual stability in terms of the soil, waste and water;
- The rehabilitation should increase ecosystem capacity and ecosystem functions;
- The area should be non-polluting and should not threaten the receiving environment.

Rehabilitation attempts to restore degraded environments, functions and services of the ecosystem to stable and sustainable states, similar to the natural or historical reference environments (Palmer *et al*., 2007). The Society of Ecological Restoration (SER) defines a restored environment as an ecosystem with "*sufficient biotic and abiotic resources to continue its development, sustaining itself structurally and functionally, and demonstrating resilience to normal ranges of environmental stress*" (SER, 2004).

The success of the rehabilitation of a mine refuse dump is measured by the criteria that it should be safe, stable, and non-polluting. Rehabilitation is required when areas have been subjected to pollution or degradation to stabilise the waste material or prevent erosion and environmental pollution. Rehabilitation appertains to disturbed sites with no pre-existing vegetation establishment (Egan *et al*., 2011; Kellner, 2015). After that, plant species are introduced to stabilise the growth medium after diligent landscape practices and soil amelioration (Egan *et al*., 2011). Rehabilitation improves and prevents the degradation of natural capital, like water, air, and soil, due to pollution.

The graph below (Figure 1-4) represents the relationship between restoration and rehabilitation. Restoration is the process of restoring an area to its former self, while rehabilitation refers to restoring a degraded site to an alternative land use (Egan *et al.,* 2011; Kellner, 2015; Bradshaw, 1998). Rehabilitation attempts to reimpose degraded ecosystem functions and ecosystem services to a functional and stable state, not like a pre-existing ecosystem, but where it is self-sustaining (Egan *et al.,* 2011; Haagner, 2008).

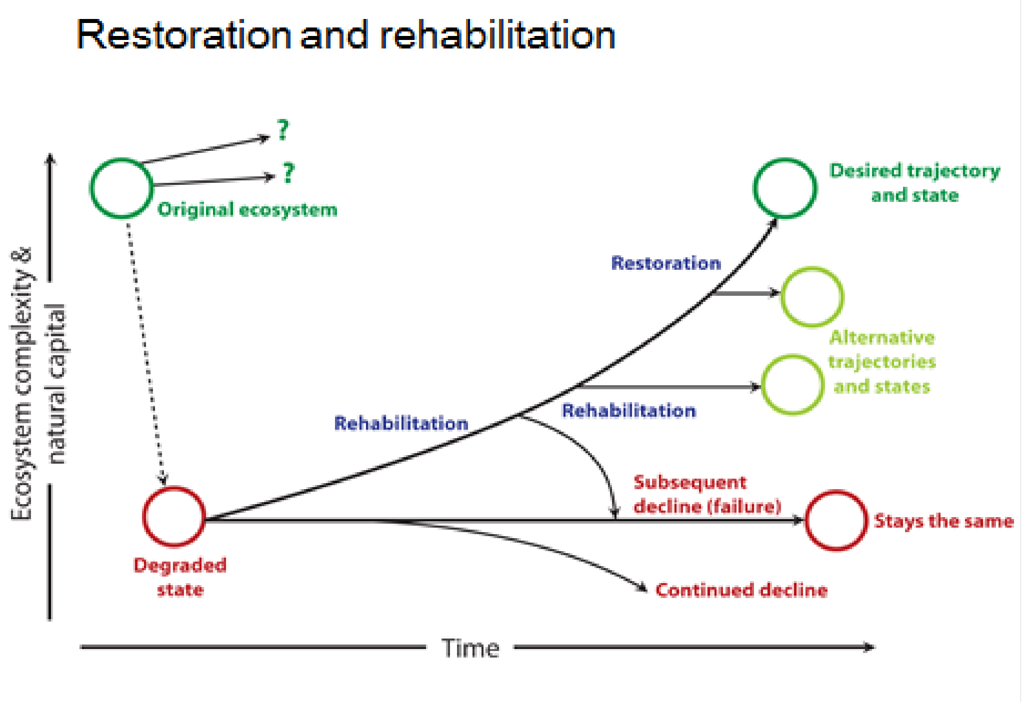


Figure 1-4: Relationship between rehabilitation and restoration (Kellner, 2015).

Successful rehabilitation encompasses the following objectives (van Deventer *et al*., 2008; Mendez and Maier, 2008):

- surface stability,
- resistance to degradation or pollution;

- restoration of ecosystem functionality;
- practical and implementable sustainable post-closure land use, and
- a prolonged succession of plant communities.

The Guidelines for Environmental Protection in South Africa states that a combination of chemical-based ameliorants and vegetation establishment is the most effective technique of rehabilitation or remediation (Chamber of Mines South Africa, 1979). Using vegetation during rehabilitation contributes to achieving a self-sustainable ecosystem and provides other crucial advantages. Vegetation aids in recuperating autogenic processes like ecosystem functions and services (SER, 2004). Vegetation also supports carbon sequestration and ground-water hydrological processes and optimises the desired end-land use (Lange *et al*., 2012). Vegetation establishment contributes to surface stability, minimises erosion, reduces or prevents leaching and dust generation, and contributes to the aesthetic value of a disturbed area (Fourie, 2007; Chamber of Mines of South Africa, 1979; Lange *et al.,* 2012).

Numerous factors, like land use, biophysical factors, and plant physiological factors, can influence the selection of appropriate plant species for rehabilitation (Wescott, 2004). Plant selection also depends on environmental factors like chemical properties of the growth medium, temperature ranges, microbial activity, and slope stability (Wescott, 2004; Wu *et al*., 2011; Tordoff *et al*., 2000).

Indigenous vegetation, for waste stabilisation, especially in semi-arid to arid regions, is established with ease and outperforms conventional seed mixes as they are adapted to specific climatic conditions (Mentis, 1999; Mendez and Maier, 2008). The use of non-invasive, native species also adheres to the Conservation of Agricultural Resources Act (CARA) (Act 43 of 1983), which prohibits the colonisation and spreading of species listed as alien invasive plant (AIP) species (RSA, 1983).

Ideally, using indigenous species for mine rehabilitation advocates the establishment of flourishing vegetation. However, growth mediums vary due to differences in underlying geological, climatic conditions, weathering, and processing methods of waste material. Prevalent characteristics of coal mine waste materials include poorly graded particles, high salinity, elevated concentrations of trace elements and acid-generating potentials (McCarthy, 2011; Weiersbye, 2007).

Acid mine drainage, also known as acid rock drainage (ARD), is the most profound issue related to coal mines, and the following section elaborates on the chemical reactions from the parent material to the formation of the acid, as well as the associated analysis to quantify the acid generation potential.

Apart from AMD formation, sedimentation of surface water resources resulting from wind and water erosion is another primary concern in the coal mining industry, particularly after mining, when rehabilitation or capping of the waste was unsuccessful. Post-closure AMD generation will require treatment, which poses financial implications for mining entities; therefore, adequate rehabilitation is pertinent to prevent future long-term seepage of AMD.

Coal mines also contribute to air pollution, and Mpumalanga was declared an air quality priority area due to the elevated pollutants like sulphur dioxide (SO_2), particulate matter (PM) (PM 10 and PM 2.5) and nitrogen oxides (NO_x's) (Munnik, 2010). Spontaneous combustion releases toxic compounds like methane, benzenes, carbon dioxide and –monoxide, which also contribute to the deteriorated air quality within the vicinity of the mines (Munnik, 2010).

Standard coal mine rehabilitation involves the deposition of a topsoil layer of at least 300 mm thick, the addition of fertiliser, and the seeding of mixtures of annual and perennial grass species (Cowan *et al*., 2016). The establishment of vegetation on coal mining waste like discard or spoils is challenging due to the hostile conditions like a lack of macro-nutrients, for example, nitrogen and phosphorus, high temperatures, growth medium acidity and elevated concentrations of trace elements (Cowan *et al*., 2016). Remediation strategies conventionally relied on creating soil micro-environments to facilitate vegetation growth. Topsoil provides an ideal environment for germination and vegetation establishment; however, it does not degrade the underlying carbonaceous material and only masks the potential problem. Coal spoil rehabilitation is also considered challenging due to the amount of topsoil required for successful vegetation establishment. Coal discard material can pollute soils if not managed adequately, reducing soil fertility and overall soil quality. In low pH soils, the excess hydrogen ions immobilise the protein framework which limits the uptake of macro-nutrients (Myburgh, 2017).

Current practices involve the placement of topsoil on top of discard and mining spoil material to establish adequate conditions for seed germination; however, it might not lead to the degradation or remediation of the underlying material. Chemical, physical and microbial characteristics of topsoil are altered by topsoil stripping, stockpiling, and soil replacement techniques. Factors like stripping depth, the timing of stripping and equipment use, and topsoil stockpile height and storage period, among others, deteriorate the soils' nutrient status, microbial activities, water retention rate, and soil texture and structure, to name a few. Typical problems associated with topsoil used for rehabilitation are tabulated in Table 1-1.

Table 1-1: Typical problems experienced with topsoil in rehabilitation or revegetation projects (adapted form Harmse and Hattingh, 1985; Fanourakis, 1991; Hattingh and van Deventer, 2004)

Problems experienced	**Occurrence**
Hard setting	The hard setting is a common problem relating to topsoil in the mining industry, especially in the highveld coal mine region, where the area is dominated by ferralitic and fersialitic (Oxidic) soils. It is less common on steep slopes due to erosion's continuous removal of hard setting material. Hard setting inhibits root penetration and ultimately reduces vegetation establishment.
Dispersion	Dispersion mainly occurs in sodic soils, making them particularly vulnerable to erosion through water. In soil where the colloidal fraction is saturated with sodium ions (sodic soils like prismacutanic and pedocunatic or duplex soils), soil conglomerates disintegrate rapidly into fine particles and wash away.
Swelling and stability	This is seen in smectitic (shrink-swell) clays (Vertisols) that are self-mulching, resulting in soil loss by erosion.
Low infiltration capacity	Low infiltration capacity is a characteristic of compacted soils, soils with increased clay content, and steeper slopes. Low infiltration capacities result in inadequate vegetation establishment.
Non-cohesiveness *(low soil strength)*	The soil's structure tends to alter and degrade once stripped and stockpiled. Low soil strength can cause wind- and water erosion if not properly covered.
Slaking	Slaking involves the breakdown of a soil lump into reduced fragments upon wetting. It is caused when clay expands upon wetting and the trapped air bursts out. Soil organic matter serves as a binding agent; it can reduce the rate of wetting and slaking. Soils of the central vegetable-growing districts are prone to slaking and pose a significant problem on steep slopes.
Water erosion *(not due to swelling, low infiltration, dispersion, slaking or lack of strength)*	Poor vegetation cover and a slow rate of vegetation establishment can cause severe water erosion and, ultimately, sheet flow in rehabilitated areas.
Wind erosion	Poor vegetation cover and slow rate of establishment as well as wind amplification on steep slopes and lack of soil structure.
Water-holding	Poor water retention rates and low water-holding capacity are due

Problems experienced	Occurrence
capacity and water retention	to particular particle size distribution (sandy) and lack of soil structure and organic matter.
Change in geochemical composition with time	Aluminium toxicity after oxidation of subsoil horizons, e.g., G horizons and plinthic horizons.
Low or no microbial enzymatic activities	Overall, microbial activity (Dehydrogenase) is a good marker of soil health. Establishing alkaline and acid phosphatase is essential to determine if phosphorus is plant-available. B-glucosidase measures cellulose breakdown (organic matter breakdown), and Urease measures hydrolysis of urea, all of which are important for vegetation establishment.

According to the industry, it is often not financially and practically feasible to strip the exact soil horizons prior to mining and stockpile the horizons separately for replacement in the original sequence of areas being rehabilitated. This has the effect of "diluting" the soil's organic material, seed bank and nutrient status.

Once mining has been concluded, spoils are landscaped to a free-draining environment that is followed by topsoil placement, application of soil ameliorants like lime and fertiliser, based on soil analysis before the area is seeded (Hattingh *et al*., 2019).

Stripping or replacing soil with a high moisture content leads to compaction, one of the most fundamental problems encountered during the rehabilitation phase. Consequently, these soils have lower infiltration rates, reduced water holding capacity, lower soil aggregate stability, and a greater ability to avoid root extension, inhibiting plant growth (Davies *et al*., 1995). Soil is predominantly stripped year-round as it is not feasible for mines or contractors to only strip during the winter months when the soil has low moisture content. Seasonal stripping also has financial implications due to delayed mining and reduced production rates.

Surfaces, where topsoil has been replaced are smoothed with graders, leading to compaction, where after the areas get ripped with tractors and not by dozers, limiting the maximum depth of tillage and resulting in compacted layers just below the 400 mm mark. Compaction on rehabilitated areas is further exacerbated by settling under gravity, increasing the bulk density as the percentage of macro pores reduces (Nortcliff, 2001). The increased density affects nutrient availability, denitrification resulting from anaerobic conditions (Davies *et al*., 1995). As a result of the changes, the soils become less favourable for soil organisms, reducing the growth and sustainability of vegetation (Nortcliff, 2001). The subsurface compaction also results in limited root development, a limiting factor for effective long-term vegetation establishment.

The availability of topsoil for rehabilitation purposes constitutes another problem, and rehabilitation will have to focus on transforming coal waste into a viable growth medium and successfully establishing vegetation on spoils or discard. Data from trials at a coal mine in Mpumalanga implies that *in-situ* organic manufacturing of an organic-rich soil was manufactured when coal, acting as the carbon donor, is degraded by *Arbuscular mycorrhizal fungi* (AMF) and *Cynodon dactylon.* The study concluded that it took six years to fully transform coal discard to a depth of 650 mm (Cowan *et al*., 2016). Large-scale rehabilitation of coal discard must focus on practical applications that can be applied without topsoil to ensure sustainable rehabilitation (Cowan *et al*., 2016).

During a recent study (Akinyeye *et al*., 2016), techniques like X-ray Fluorescence (XRF), Fourier Transform Infra-Red Spectroscopy (FTIR) and Laser Ablation-Inductively Coupled Plasma Mass Spectroscopy (LA-ICPMS) were used to determine the chemical composition of coal found in South Africa. Based on the results of the XRF analysis, high concentrations of silicon dioxide (SiO_2), and aluminium oxide (Al_2O_3) were evident. Concentrations greater than 100 $mg{\cdot}kg^{-1}$ of barium (Ba), arsenic (As) and strontium (Sr) were observed in the coal (Akinyeye *et al*., 2016). The sulphur (S), nitrogen (N), and phosphorus (P) concentrations in South African are reliant on maceral composition (carbon [C], hydrogen [H] and nitrogen composition) or coal grade.

As mentioned above, the most ruinous minerals associated with coal mines are sulphides, mainly pyrite, which oxidise and form AMD. Mining generally releases pollutants into the environment, previously primarily immobile in the undisturbed ore body. Changes in soil characteristics include increased acidity, nutrient deficiencies or imbalances and overall changes in vegetation cover if present.

Environmental contaminants, trace elements, or CoPC can affect the soil biomass, soil fertility, crop yields, and expedites bioaccumulation in food chains (Gratão *et al*., 2005).

Constituents of potential concern remain in the environment for prolonged periods and can accumulate to such an extent that they can be rendered harmful to biotic species.

1.2.3 Common rehabilitation practices

The MPRDA (Act 28 of 2002) promulgated the "polluter pays" principle and necessitates mines to develop an Environmental Management Plan (EMP) that includes adequate financial provision and commitments in terms of rehabilitation through a closure plan (RSA, 2002). This act states that the mining right holder is responsible for all environmental liability like pollution, degraded ecosystems, treatment of extraneous water, and compliance with the conditions of the environmental-related authorisations. For a mine to be exempted from long-term responsibilities, the mine must apply and be granted a closure certificate, which is only issued if the mine can demonstrate that the historical activities have no environmental impacts. According to closure legislation, coal mining companies are obligated to rehabilitate or relinquish mined areas into a viable state with alternative uses and mitigate or ultimately prevent future environmental pollution (Limpitlaw and Briel, 2015; Weaver *et al*., 2019).

The Minerals Council of South Africa, previously known as the Chamber of Mines, published a rehabilitation guideline to encourage adequate rehabilitation measures, with a specific focus on opencast coal mines (Tanner, 2007). The Land Rehabilitation Guideline for Surface Mines, initially compiled in 1981 and updated in 2007, was subsequently updated again in 2019. The guideline was developed to provide a consolidated standard for good practices from the planning phase to the implementation phase of rehabilitation. Another objective was to provide technically sound and practical implementation measures regarding land rehabilitation.

The *Land Rehabilitation Guideline for Surface Coal Mines* explains the approach to mine rehabilitation in four stages: planning, execution, monitoring, and adaptive land management (Hattingh *et al*., 2019).

Successful and sustainable rehabilitation remains problematic regardless of the progressive improvement of rehabilitation and closure guidelines and regulatory requirements. Due to financial or knowledge constraints, inadequate rehabilitation attempts largely contribute to environmental impacts, like water, air, and soil pollution. The challenges encountered during rehabilitation need to be evaluated to successfully overcome all the constraints associated with the rehabilitation practices of coal mines. According to Mentis (1999), a principal rehabilitation paradigm establishes pastures instead of trees or annual crops.

Grass has a higher root turnover, resulting in the incorporation of organic material in the re-developing soil profile that restores normal soil functions (Mentis, 1999). Previously, lucerne was included in the seed mix, owning to the fact that the plant symbiotically fixates nitrogen to reduce costs associated with nitrogenous fertilisers.

Species that are recurrently used for the revegetation of mined areas include but are not limited to *Chloris gayana, Eragrostis tef, Eragrostis curvula, Digitaria eriantha, Cenchrus cilliaris, Cynodon dactylon* and *Medicago sativa* (Mentis, 1999). The seeding rates depend on the land-use requirements and are a combination of different species. Re-seeding activities are mostly required, and different reseeding methodologies are utilised, depending on the site-specific requirements and practical implications (Tordoff *et al*., 2000). This includes broadcast and row re-seeding activities using no-tillage implements or by hand.

The use of vegetation in mine rehabilitation is advantageous due to the following functions it provides (van Deventer *et al*., 2008; Haagner, 2008; Balková *et al*., 2021):

1. Vegetation decreases dust generation through stabilisation, and in some cases, dust pollution is terminated in degraded areas.
2. Plants have aesthetic value and reduce the visual impact of disturbed areas.
3. Vegetation intercepts surface run-off and reduces flow acceleration at the soil-plant interface known as basal cover and areas susceptible to surface crusting.
4. Vegetation stabilises surface areas, and wind or water erosion will erode little to no topsoil or subsoil.
5. Certain species can absorb trace elements and other toxins through phytoextraction, rendering a previously harmful environment harmless.
6. As soon as vegetation is established, the rest of the ecological cycle could recover faster by supporting insects, birdlife, and small animals.
7. The vegetation established can be used as a source of income and post-closure to the surrounding community (thatch grass, grazing) and can provide an alternative post-closure land use.
8. Vegetation improves the soil or growth medium's structure, which enhances other properties like permeability and water-holding capacity.
9. Vegetation can lower the water table (some trees utilise larger quantities of water than pasture species, like the blue gum tree (*Eucalyptus globulus*).
10. Vegetation with deep roots transports nutrients from the soil to the surface through brush-packing.

Many factors cause vegetation regression during the restoration or rehabilitation of coal mines. The primary reason for the deterioration of revegetated areas is the absence of aftercare, especially frequent defoliation, mowing, grazing or burning.

Vegetation in areas undergoing restoration deteriorates due to alien plant invasions competing for available nutrients and water (Meyer *et al.*, 2003). The soil can also undergo deterioration like salinisation through salt precipitation and acidification through the capillary rise of the acid water, also known as AMD, emanating from the underlying discard or spoils. During restoration, the fertility of the soil can become depleted and result in unhealthy vegetation or in severe cases, bare soil (Meyer *et al.*, 2003). Plants need certain macro- and micronutrients for optimal functionality.

1.2.4 Macronutrients

For sustainable growth, plants require a wide assortment of essential and non-essential nutrients. Some elements are termed essential if they form part of the plant's structure or metabolic activities (Hopkins and Hürner, 2008).

Essential elements are categorised into two groups based on the relative abundance that is required by vegetation, namely macronutrients and micronutrients. The following elements are classified as macronutrients and are essential to sustain plant growth: calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), nitrogen (N), and sulphur (S). Micronutrients are also essential to plant growth but in lesser amounts than macronutrients and include the following: copper (Cu), molybdenum (Mo), zinc (Zn) and nickel (Ni) (FSSA, 2007).

Nutrient availability depends on the chemical properties of soils, and certain chemical constraints limit nutrient availability, such as soil organic matter (SOM), salinity, and acidity, among others. These chemical factors influence the transportability, mineralisation, fixation, and adsorption rates of the nutrients that are present in the substrate or growth medium (Baligar *et al*., 2001).

Phosphorus (P) is a vital macronutrient and is responsible for metabolic processes like photosynthesis (Hopkins and Hürner, 2008; Sabrina *et al*., 2013). P is insoluble, and roughly only 2% of phosphorus in the soil is plant available, which is governed by pH (Sabrina *et al*., 2013; Sparks, 2003).

Nitrogen (N), another macronutrient, is used as a constituent for several plant proteins and chlorophyll (FSSA, 2007; Hopkins and Hürner, 2008; Davies, 1995).

Plants acquire soil nitrogen in the nitrate (NO_3^-) or ammonium (NH_4^+) chemical form; however, nitrogen is limited to plants due to the competition with soil microorganisms (Hopkins and Hürner, 2008). Factors that also effect nitrogen availability in soil include water availability and pH, which in turn influence microbial activity that results in nitrification, nitrogen fixation and ammonification (Brady and Weil, 2008; Hopkins and Hürner, 2008).

Potassium (K), an essential macronutrient, is a copious cellular cation and acts as an activator for enzymes involved in photosynthesis and is also vital for protein synthesis (Hopkins and Hürner, 2008; Sparks, 2003). Potassium controls the osmotic potential in plant cells and controls the opening and closure of the stomata (Hopkins and Hürner, 2008).

Awad *et al*. (2014) concluded that high potassium-rich fertiliser application rates resulted in increased biomass production. Other macronutrients that are important in plant growth include carbon (C), hydrogen (H), oxygen (O), calcium (Ca), magnesium (Mg) and sulphur (S).

Plants take up magnesium as a divalent cation (Mg^{2+}) and link Adenosine triphosphate (ATP) molecules to the enzyme in ATP reactions (Hopkins and Hürner, 2008). Sulphur is an important component in the proteins and plant structure and is absorbed by plants as a divalent sulphate anion (SO_4^{2-}), whereas calcium (Ca) is taken up as a divalent cation (Ca^{2+}) and is vital for cell division (Hopkins and Hürner, 2008; Sparks, 2003).

Plants are autotrophic, and sufficient concentrations of macro and micronutrients need to be available in the soil or growth medium to sustain plant growth.

Sulphur is a predominant element in coal mines but is still essential for plants. Soil organisms mineralise organic sulphur forms before they become plant soluble. The mineralisation rate is subordinate to soil-pH, -moisture, -temperature and -aeration, among others. Sulphate esters are more effortless to decompose by microorganisms to release sulphate ions directly. The sulphur in a reduced state bonds to the carbon atoms contained in amino acid compounds and proteins within the soil (Brady and Weil, 2008). Sulphur is essential for growth and physiological processes and must be reduced before metabolising it (De Kok *et al*., 2005).

1.2.5 Micronutrients and trace elements

Some trace elements are required by plants in small concentrations to complete healthy life cycles and are also known as micronutrients (Hopkins and Hürner, 2008).

The primary sources of trace elements in the soil are derived from the weathering of rocks, minerals, and atmospheric deposition (Hopkins and Hürner, 2008; Qin *et al*., 2014; Sreekanth *et al*., 2013). Anthropogenic sources of trace elements include mining, burning fossil fuels, and applying chemical fertilisers and pesticides, to name a few (Qin *et al.,* 2014).

Soil pH is a predominant characteristic that influences the availability and mobility of trace elements. The oxidation of pyrite results in the generation of AMD and acid sulphate soils.

The acidity associated with mine waste or contaminated soil results in the mobilisation of trace elements, leading to phytotoxicity.

Trace elements like copper (Cu), nickel (Ni), zinc (Zn), Iron (Fe) and arsenic (As) are chalcophiles and have a strong geochemical association with sulphide-bearing minerals (Aucamp and Van Schalkwyk, 2003; Akinyeye *et al*., 2016; Finkelman, 1993). Copper (Cu) is plant-available as a divalent cupric ion (Cu^{2+}) and is primarily immobile because it is strongly adsorbed to the colloidal surface (Hopkins and Hürner, 2008; Lasat, 2000).

Copper is a plant cofactor for numerous oxidative enzymes and is responsible for physiological functions such as carbohydrate metabolisms, photosynthesis and respiration (Hopkins and Hürner, 2008; Sreekanth *et al*., 2013). Copper deficiency presents as stunted growth in most plants.

Zinc (Zn) contributes to processes similar to copper and is absorbed by plants as a divalent cation (Zn^{2+}) (Hopkins and Hürner, 2008). Zinc deficiency in plants results in smaller leaves and shortened internodes. Like copper (Cu), zinc is retained by clay and organic matter, resulting in the accumulating in the upper horizons of the soil (Hopkins and Hürner, 2008; Herselman, 2007). Symptoms of zinc toxicity include discolouration in new leaves as well as stunted growth (Kabata-Pendias, 2011).

Iron (Fe) is an essential micronutrient and is taken up as the ferric (Fe^{3+}) or ferrous (Fe^{2+}) ion (Hopkins and Hürner, 2008). Iron is critical in energy transformations needed to synthesise cells and produce chlorophyll in plants (Hopkins and Hürner, 2008; Lasat, 2000). Iron deficiency results in stunted plant growth and reduced yield, and iron toxicity is characterised by necrosis of leaves (Kabata-Pendias, 2011).

Nickel (Ni) is present in the soil as compounds like nickel carbonate, nickel acetate, and nickel oxide, which are soluble at low pH levels (Kabata-Pendias, 2011; Keeling and Werren, 2005). Nickel is an essential component in metabolic activities and plant enzymes (Sreekanth *et al*., 2013). Toxicity results in stunted growth, chlorosis, and impaired nutrient balance (Sreekanth *et al*., 2013; Kabata-Pendias, 2011).

Manganese (Mn) is an essential micronutrient in the electron transport system and is encompassed in the oxygen-evolving complex associated with photosystem II (Hopkins and Hürner, 2008; Kabata-Pendias, 2011). Manganese deficiencies are associated with calcareous-rich soils with higher pH values (Hopkins and Hürner, 2008). Deficiencies in Mn include chlorosis in younger leaves, reddish spots on leaves, browning of roots, and stunted plant growth (Kabata-Pendias, 2011). Like all the other micronutrients, manganese (Mn) mobility depends on soil pH and is mobile at low ranges, becoming less mobile as the pH is raised (Kabata-Pendias, 2011).

1.2.6 Formation factors of acid mine drainage

Acid rock drainage, or AMD generation, from mine waste dumps and structures such as mine opencast pits and underground workings is principally a function of the mineralogy of the parent material, the presence of bacteria (*Thiobacillus ferrooxidans*) and the availability of oxygen and water (Ferguson and Erickson, 1988). Acid mine drainage generation is a complicated and expensive dilemma that coal mining operations must face during mining and rehabilitation. Apart from the contamination of surface and groundwater resources, AMD can also mobilise metals like arsenic (As), cadmium (Cd), copper (Cu), and zinc (Zn), which pose health risks.

Because mineralogy and other AMD generation factors are highly variable and site-specific, AMD generation predictions are complex, costly, and debatable in terms of reliability. Predictive tests are progressively more relied on to assess the probability of AMD generation in the long run.

The issue of AMD generation at historic and active mines focused on improving AMD prediction methods to assess the potential during the exploration phase for sufficient long-term planning regarding water quality and pollution prevention.

The physical and chemical characteristics of coal refuse from a single source can also differ tremendously and are usually attributed to the different processing and deposition methods (McCarthy, 2011; Rinaldo *et al*., 2017). Table 1-2 below tabulates the differences between waste rock dumps, which consist of unevenly crushed material and tailing materials, which consist of fine material.

Table 1-2: Comparison of acid-generating factors in waste rock dumps and tailing storage facilities (*adapted from* USEPA, 1994; Durkin and Hermann, 1994; Rinaldo *et al*., 2017)

Acid generation factors	**Waste rock dumps**	**Tailings material**
pH	The pH is highly variable over short distances.	The pH tends to be uniform in horizontal zones.
Sulphide	Variable concentrations of sulphide, depending on the geological seam and parent material.	The sulphide concentration is often uniform with high sulphide contents due to the increased contact surface.

Acid generation factors	Waste rock dumps	Tailings material
Particle size	The average discard rock is 20 cm in diameter but varies in size, allowing air circulation between the rocks.	The material is roughly 0.2 mm in size and exposes more area to oxidation.
Initiation of oxidation	Oxidation tends to initiate directly after placement.	Oxidation initiates after tailing placement ceases and the tailings dry out, as tailings are usually placed in a saturated state.
Oxygen and water entry	There is seasonal variation in the flow paths of oxygen and water entry, resulting in peaks in concentrations.	Seepage is typically slow, and uniform and concentration peaks are not a normal occurrence.
AMD releases	Large infiltration rates due to particle size results in the rapid release of AMD into the receiving water resources.	The releases tend to be early surface AMD runoff with lower infiltration rates and gradual transition in seeps from process water to low pH AMD.

Acid mine drainage results from the oxidation of sulphide-bearing minerals and, without anthropogenic contributions, occurs naturally at a vastly slower rate and does not threaten the receiving environment. The most predominant sulphide-bearing minerals associated with coal mines in South Africa are Pyrite (FeS2) and Arsenopyrite (FeAsS); however, other minerals include Chaclopyrite ($CuFeS_2$), Galena (PbS), Marcasite (FES_2) and Cinnabar (HgS) amongst others (Johnson *et al.,* 2006).

The following chemical reactions explain the process from pyrite oxidation to AMD (Ferguson and Erickson, 1988; Durkin and Hermann, 1994). The formulae representations are:

- FeS_2 - pyrite;
- $Fe_2(SO_4)_3$ - ferric sulphate.
- $FeSO_4$ - ferrous sulphate;
- S - sulphur;
- H_2SO_4 - sulphuric acid;

$$2FeS_2 + 7O_2 + 2H_2O \text{ ----> } 2\,FeSO_4 + 2H_2SO_4 \qquad \text{(Eq. 1-1)}$$

Sulphide $(S_2^{2})^-$ oxidises to form hydrogen ions and sulphate and the dissociation products of sulphuric acid, which is in solution. Soluble Fe^{2+} can also react further. Oxidation of the ferrous ion to ferric ions reduces as the pH lowers (Kuyucak, 1999; Ferguson and Erickson,1988):

$$4FeSO_4 + 2H_2SO_4 + O_2 \text{ ---------> } 2Fe_2(SO_4)_3 + 2H_2O \qquad \text{(Eq. 1-2)}$$

At pH values between 3.5 and 4.5, iron oxidation is catalysed by a variety of fibrous bacteria, and at pH levels below 3.5, the iron bacterium (*Thiobacillus ferrooxidans*) catalyses the reaction. If the ferric ion is formed in contact with pyrite, the following reaction can occur, dissolving the pyrite (Durkin and Hermann, 1994; Ferguson and Erickson,1988):

$$2FeS_{2\,(s)} + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2\,SO_4^{2-} + 16H^+ \qquad \text{(Eq. 1-3)}$$

The reaction above generates more acid, and the ferric iron (Fe^{3+}) precipitates as hydrated iron oxide (Ferguson and Erickson,1988):

$$Fe^{3+} + 3H_2O \leftarrow\text{--}\rightarrow Fe(OH)_3 + 3H^+ \qquad \text{(Eq. 1-4)}$$

Fe(OH) precipitates and is yellow, orange or red. The end products are sulphuric acid and ferric sulphate. The pH quickly reduces from the inception of pyrite oxidation, after which it stabilises (pH of 2.5 to 3.0) and is determined by the optimal habitat of the specific bacteria strain.

Many coal mines in the Mpumalanga region are abandoned, and most mines decant acidic water into the receiving environment. The acidic water enters the local river systems like the Olifants River, which is diluted and neutralised, to a certain degree, by several biological and chemical reactions (McCarthy, 2011; Durkin and Hermann, 1994). However, the water remains highly saline with elevated sulphate concentrations, and the remanence of this can be seen in the salinity and sulphate levels in the Witbank dam (Mentis, 1999).

The dilemma South Africa now faces is the increasing deterioration of water quality in the rivers and streams in the Highveld due to AMD emanating from the coal mines. Reverse Osmosis (RO) water treatment plants have been commissioned; however, the operational costs are extremely high and cannot be used to improve the quality of rivers. The long-term impacts of coal mines in South Africa are suspected to be worse than in other countries due to the country's unique combination of climate, geography, and the magnitude of deposits (McCarthy, 2011).

Ideally, the solution is to target the problem at the source utilising successful vegetation establishment and/ or phytoremediation to prevent runoff and seepage emanating from the coal refuse, to name a few.

1.2.7 Predicting AMD generation using different laboratory tests

Predictive testing determines whether the material can generate acid and predict the drainage quality based on the acid formation rate. The factors affecting the capability of a material to generate acid are as follows (USEPA, 1994; Akabzaa *et al*., 2007):

- Quantity of acid-generating minerals;
- Quantity of acid-neutralising minerals; and
- Type and quantity of constituents of potential concern (CoPC) present.

The rate and degree of AMD generation are affected by the following components (Modabberi *et al*., 2013; USEPA, 1994):

- Type of sulphide-bearing mineral;
- Type of carbonate mineral present (neutralising minerals);
- The magnitude of mineral surface area (particle size);
- Available water and oxygen; and
- Available bacteria.

Acid mine drainage generating potential of a material can be determined by means of two different tests, namely, static and kinetic tests. The two tests are briefly described below.

1.2.7.1 *Static tests*

Static tests compare the maximum acid neutralising potential (NP) and its maximum acid production potential (AP) to determine the drainage quality. The AP is determined by multiplying the total Sulphur or Sulphide percentage with a conversion factor (AP = %S x 31.25). Neutralising potential is determined by adding acid to the sample and back-titrating it (pH of 3.5) to establish the acid consumption amount (USEPA, 1994; Brady *et al*., 1994).

The net neutralisation potential (NNP) is calculated by subtracting the AP from the NP amounts to the net neutralisation potential (NNP) (NNP = NP - AP). If the difference between NP and AP is positive, there is a low risk of acid formation, and if the difference is negative, the sample has the potential to form acid (USEPA, 1994).

1.2.7.2 Kinetic tests

Kinetic tests differ from static tests in that the test attempts to simulate the natural oxidation reactions, which requires more time. This test can assess the impacts of different variables contributing to acid generation, like introducing certain bacteria and regulating temperature (USEPA, 1994). The most popular kinetic tests include the humidity cells and the column tests.

Besides the chemical constraints, coal refuse material also affects plant growth and vegetation establishment, hindering successful rehabilitation. Plant stress can be engendered by biotic and abiotic factors, also known as stressors.

1.2.8 Abiotic and biotic stressors on plants

Plants encounter two types of stresses and are classified as biotic and abiotic. Abiotic stress results in plant losses due to non-living factors like salinity, drought, floods, extreme temperatures, and heavy metals, to name a few. Biotic stresses are caused by living organisms like nematodes, bacteria, herbivores, and fungi. Plants have developed countless mechanisms to overcome biotic and abiotic stresses. Some of the abiotic stressors associated with coal mines are described below.

- Soil acidity – Soil pH is the most profound aspect because microbial activity, nutrient solubility and availability are all pH-dependent (Gentili *et al*., 2018; Bray *et al*., 2000).
- Soil salinity – Vegetation growth is reduced by salt stress, driven by two primary effects: osmotic stress and ion toxicity.
  The osmotic stress pressure in solution, in saline soil, exceeds the osmotic pressure in the plant and inhibits the plant’s ability to take up minerals and water. Soil salinity leads to secondary effects like decreased cytosolic metabolism, reduced cell expansion, and membrane functions (Gull *et al*., 2019; Lichtenthaler, 1998).
- Drought – Rainfall distribution is uneven due to changes in climate and contributes to drought stress in plants. During dry conditions, plant growth reduces metabolic

demands, and protective compounds are synthesized by mobilizing metabolites required for osmotic adjustment (Bray *et al*., 2000; Gull *et al*., 2019).

- Toxicity – Increased levels of trace elements in soils as a result of anthropogenic activities cause harmful effects on plants and within the soil-plant environmental system. Effects on plants can range from stunted growth to chlorosis and, in severe cases, necrosis and mortality (Gull *et al*., 2019).

Plants experience plant stress due to stressors, resulting in stressor-specific and non-specific general responses. Plant stress is an environmental factor that results in unfavourable living conditions for plants (Lichtenthaler, 1998). Larcher (1987) defines plant stress as a state of increasing demand on the plant that results in the initial destabilisation of normal metabolic functions. Larcher (1987) also mentioned that should the limits of tolerance and the adaptive capability of the plant be exceeded, it could result in permanent damage and even death.

Before stress exposure, plants are in a form of homeostasis, and stressors will then lead to stress responses. Stress responses can be separated into four phases, namely (adapted from Lichtenthaler, 1998):

1. Alarm phase: This is also known as the response phase, where the plant undergoes a decline of vitality, and the physiological functions deviate from the norm. During this phase, the catabolic processes exceed anabolism.
2. Resistance phase: During this phase, the plant continues to experience stress, resulting in the initiation of adaptation and repairing processes, followed by hardening or reactivation.
3. Exhaustion phase: This phase results from long-term stress; if the stress intensity exceeds the plant's adaptation capacity, then it results in permanent damage or death.
4. Regeneration phase: During this phase, the plant undergoes partial or complete reinvigoration of physiological functions when the stressors are removed before the damage becomes lethal.

The phases are represented in Figure 1-5. At the onset of stress, the plant responds with a decline in one or numerous physiological functions, eventually deviating from normal physiological activities. Acute damage is rapid in plants with low or zero stress tolerance. The plants activate various defence mechanisms and responses as the stress continues.

After removing the stressors, new physiological standards can be reached, depending on the interval and intensity of the stress.

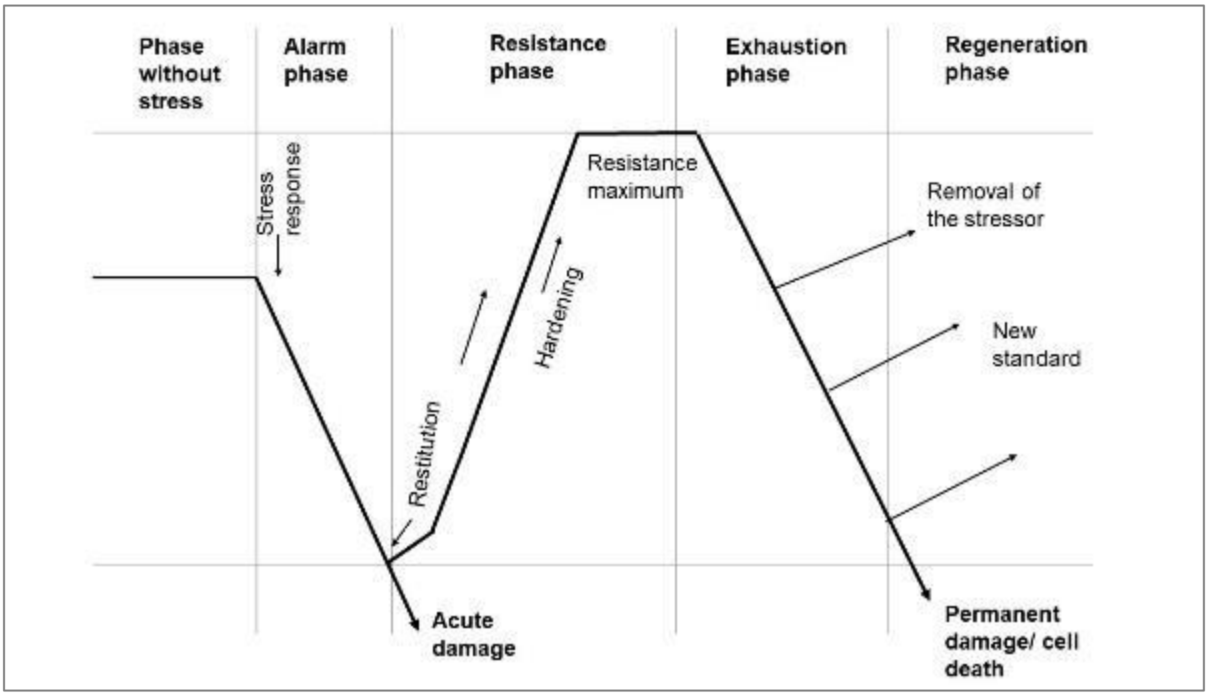


Figure 1-5: Stress response phases of plants (adapted from Lichtenthaler, 1998)

The most critical stressors of concern for this specific project are low pH, high salinity and trace element contamination, which especially becomes problematic with phytoremediation.

Plants have numerous mechanisms to restrict entry to the roots and the movement to the xylem, and these include, but are not limited to (White and Pongrac, 2017; Fageria *et al*., 2011):

- Avoid areas in the soil that contain high metal concentrations.
- Render the metals less plant available in the soil employing root exudates.
- Producing mucilage that can precipitate trace elements in the rhizosphere, rendering them insoluble.
- Foster a wide array of mycorrhizae and other soil microbe species that can sequestrate the trace elements, making them immobile.
- Develop physical barriers to confine the apoplastic movement of trace elements from the rhizosphere to the cortex and then the xylem.
- Pump the trace elements that enter the root cells back into the apoplast or rhizosphere.

- Restrict metals by producing chelates in the cytoplasm of the root cells and sequester trace elements and associated chelates in the root cell vacuoles.
- Reduce the abundance of transport proteins, catalysing the mobility and uptake of trace elements or CoPC.

1.2.9 Constituents of potential concern (CoPC)

Based on X-ray Fluorescence (XRF) results from a recent study, high concentrations of arsenic (As), barium (Ba) and strontium (Sr) were observed in the coal (Akinyeye *et al*., 2016). High concentrations of silicon dioxide and aluminium oxide were also recorded in previous studies on coal (Akinyeye *et al*., 2016; Finkelman, 1993).

Based on the literature, the following section elaborates on the CoPc that are expected to be associated with coal deposits in South Africa.

1.2.9.1 *Aluminium (Al)*

Aluminium is a CoPC that should be considered as it has been identified as having very high concentrations in coal in numerous publications (Akinyeye *et al*., 2016; Finkelman, 1993; Neenu and Karthika, 2019). The following section describes Al's behaviour in both the plant and the soil.

At low soil pH (<5.5), aluminium (Al) goes into soil solution and becomes plant available and high concentrations can become toxic to plants (Sparks, 2003; Rout *et al*., 2000). Aluminium toxicity largely depends on the Al species within the soil, and Al also plays a central role in soil acidity (Brady and Weil, 2008; Akinyeye *et al*., 2016). Many soil minerals are aluminium oxides and aluminosilicates, and H^+ ions adsorbed on clay surfaces, replacing the Al^{3+} ions in the mineral structure, resulting in the release of Al^{3+} ions that are in equilibrium with dissolved Al^{3+} in soil solution (Brady and Weil, 2008). Aluminium (Al^{3+}) ions have a strong tendency to hydrolyse water molecules and split them into H^+ and OH^- ions, and the Al^{3+} ions bond with the OH^- ions, leaving H^+ in solution, which further reduces the soil's pH (Rout *et al*., 2000). The following reversible reaction explains the substitution (Brady and Weil, 2008):

$\mathbf{Al^{3+}}$	$\rightleftharpoons$	$\mathbf{AlOH^{2+}}$	$\rightleftharpoons$	$\mathbf{Al(OH)_2^{+}}$	$\rightleftharpoons$	$\mathbf{Al(OH)_3^{0}}$
	H_2O		H_2O		H_2O	Gibbsite or
	H^+		H^+		H^+	amorphous

The hydroxyl aluminium ions precipitate (Al(OH)30) and become insoluble as the pH increases. Elevated levels of soluble Al in soil solution result in stunted root and shoot growth in susceptible plants (Spark, 2003). The roots absorb aluminium, and only small quantities are transported to the leaves. The primary target area in the plant is the plasma membranes, which are mediated by Al's ability to bind to carboxyl and phosphate groups of cell walls and membranes, respectively (Rout *et al*., 2000). Signs of Al toxicity in plants include dark green leaves, yellowing and dead leaf tips, purpling of the stem and overall stunted growth. Al forms complexes with phosphorous, making it less plant-available, and it also inhibits cell division in the roots and uptake and transport of other essential nutrients (Rout *et al*., 2000).

1.2.9.2 *Arsenic (As)*

Naturally occurring arsenic occurs in the earth's crust, minerals, rocks, and soils; however, in South Africa, organic arsenic is not abundant. Traces of arsenic have been recorded in minerals in sedimentary environments (Akinyeye *et al*., 2016). Arsenic is usually the product of the oxidation of sulphide deposits where As is combined with some metal (e.g., Fe, Pb, and Cu). Sulphide–arsenopyrite (FeAsS) is the post-prevalent As mineral and As-rich pyrite associated with sedimentary formations like shale, coal, and peat deposits. Other prevalent minerals are orpiment (As_2S_3), arenolite (As_2O_3), realgar (AsS), nickiline (NiAs); and sperrylite ($PtAs_2$) (Chibuike and Obiora, 2014; García-Salgado, 2012).

The total As concentration does not always reflect the plant-available As concentrations, and the chemical composition of the soil or growth medium plays a significant role in the availability of As. In soils containing Al, Ca and Fee, the As availability is usually low due to the formation of As carbonates and oxides or hydroxides (Kabata-Pendias, 2011; García-Salgado, 2012). Arsenic is stable under varying redox environments, and trivalent arsenate (As(III)) is prevalent in reducing conditions. Under oxidizing conditions, pentavalent arsenate (As(V)) is widespread; however, As is more soluble under reducing conditions (Smedley and Kinningburgh, 2002). The availability of As for plant uptake depends on the speciation, and the solubility of the speciation are as follows, in order of magnitude: $As^{org} > As^{5+} > As^{3+}$ (Kabata-Pendias, 2011; Chibuike and Obiora, 2014; García-Salgado, 2012).

Toxicity signs in plants include reduced seed germination, decreased seedling height, and reduced biomass production (Chibuike and Obiora, 2014). With increasing As concentrations, elevated As concentrations were recorded in the older leaves and roots, but higher As accumulation was noted in leaves at lower concentrations. Symptoms of As toxicity includes leaf wilting, violet colouration (increased anthocyanin), root discolouration, and cell plasmolysis (Kabata-Pendias, 2011).

1.2.9.3 *Barium (Ba)*

Barium has an affinity for silica-rich minerals and is concentrated in acid igneous and sedimentary rocks. Its ionic radius is similar to the ionic radius of K. Barium also undergoes similar geochemical processes as K. Barium is mainly mined from intrusions in granites and shale, and the most common Ba minerals in nature are barite ($BaSO_4$), hollandite ($Ba_2Mn_8O_{16}$), and carbonate-Ba compound—witherite ($BaCO_3$) also occurs relatively often (Kabata-Pendias, 2011). Barium has been recorded as being present in certain silicate minerals as impurities.

Barium is not mobile during weathering because it precipitates as sulphates and carbonates and is strongly adsorbed by the colloidal fractions. Barium is relatively mobile in acidic soils and has a great affinity for the concentration in Mn soil. It easily displaces other alkaline earth metals from some oxides (MnO_2, TiO_2) and is displaced from Al_2O_3 by alkaline earth metals. Hollandite ($Ba_2Mn_8O_{16}$) is responsible for the migration of Ba in aridic soils, where it is known to accumulate in the surface layers. Ba forms complexes with Fe and Mn hydrous oxides and becomes immobile. Plants can extract Ba quite easily from acid soils; however, there are only a few reports on Ba toxicity in plants, and symptoms include inhibited photosynthesis and K transport (Kabata-Pendias, 2011; Chibuike and Obiora, 2014).

1.2.9.4 *Copper (Cu)*

Copper is essential for plant growth and plays vital roles in photosynthesis, in respiratory electron transport chains, and cell wall metabolism, amongst others. Copper fixes organic matter and minerals in the soil, making it immobile (Mengel and Kirkby, 2001). Copper concentration in soil is linked to acidity and the availability of organic matter. High levels of Cu alter soil microbial activities and impede the decomposition of organic matter (Hopkins and Hürner, 2008'; Vardaki and Kelepertsis, 1999).

Copper is associated with sulphide, which can potentially generate acid mine drainage. Excess Cu in the soil inhibits plant development and impairs essential cellular processes. Plants have evolved to correctly regulate the homeostasis of the copper concentrations in the plant since Cu is both an essential and toxic element transport (Kabata-Pendias, 2011).

Young leaves turn yellow, and the plant has rolled or dead leaf tips with stunted leaf development if the plant has a Cu deficiency (Hopkins and Hürner, 2008). Copper toxicity results in stunted plants with pale leaves with red colouration on the leaf margins. Roots are usually short with barbed-wire patterns (Chibuike and Obiora, 2014).

1.2.9.5 Iron (Fe)

Due to the presence of pyrite (FeS_2) in the Mpumalanga coalfields, iron is a CoPC to consider, and its behaviour in soil and plants is briefly described below. Iron is an insoluble hydroxide, particularly in calcareous soils, limiting plant availability (Rodriguez, 2018; Vardaki and Kelepertsis, 1999). The iron cation in soils is soluble, and as the pH increases, it becomes less soluble as per the following example (Brady and Weil, 2008):

$$\mathbf{Fe^{3+}} \xrightarrow{OH^-} \mathbf{Fe(OH)^{2+}} \xrightarrow{OH^-} \mathbf{Fe(OH)_2^{+}} \xrightarrow{OH^-} \mathbf{Fe(OH)_3}$$

Cation (soluble)	*Hydroxy metal cations (soluble)*	*Hydroxide (insoluble)*

Leaf chlorosis is a sign of iron deficiency, indicating a reduction in photosynthesis that negatively affects crop yields (Rodriguez, 2018). The leaves of plants become brown due to excessive iron in the soil (Mengel and Kirkby, 2001; Vardaki and Kelepertsis, 1999). The solubility of iron is pH-dependent, and as acidity increases, the solubility also increases. Iron is usually associated with sulphide. Young leaves show interveinal chlorosis, and the entire leaf turns white or yellow if the deficiency worsens. Roots grow profuse root hairs. Symptoms of toxicity include bronze or black discolouration of leaf margins and dark, slim roots (Chibuike and Obiora, 2014).

1.2.9.6 Manganese (Mn)

Manganese, part of the iron family, is closely associated with Fe in geochemical processes, and Mn cycles follow Fe cycles. The predominant Mn minerals, mainly in association with metals like Fe include pyrolusite (β-MnO_2), manganite (γ-MnOOH), hausmannite (Mn_3O_4) and rodochrozite ($MnCO_3$).

Manganese also has a large adsorption capacity to some metals (Cu, Co, Cd, Pb, Zn), and during weathering, Mn in minerals is oxidised and released, while Mn-oxides are re-precipitated and readily concentrated in secondary Mn minerals (Kabata-Pendias, 2011; Chibuike and Obiora, 2014).

Manganese is a plant micronutrient and assists in the water-splitting system of photosystem II (PS II), providing the necessary electrons for photosynthesis. In elevated concentrations, Mn becomes toxic. Toxic levels of Mn in plants result in swelling of cell walls and brown spots on the leaves (Mengel and Kirkby, 2001). Excessive Mn also leads to iron deficiencies. If Mn is deficient, young leaves turn yellow with greener veins, and the tissue near the veins remains green. The leaves get a mottled appearance, which later turns into dead tissue. Deficiency also results in the reduction of lateral roots (Mengel and Kirkby, 2001).

The leaves turn dark green with red flecks during the initial phases of toxicity, after which the interveinal tissue turns yellow. Patchy green colours on the leaves are also common signs of manganese toxicity and can induce Iron deficiency (Chibuike and Obiora, 2014).

1.2.9.7 *Nickel (Ni)*

Nickel has both a chalcophilic and siderophilic affinity and readily combines with iron, forming Ni–Fe compounds. The affinity of Ni for S accounts for the high amounts of Ni-S minerals in nature, and abundant forms of Ni include millerite (NiS), niccolite (NiAs), and antimonides (NiSbS), amongst others. After weathering, Ni co-precipitates with Fe and Mn oxides and is included in the mineral lattices of goethite, limonite, serpentinite, and other Fe minerals. Ni is also associated with carbonates, phosphates, and silicates (Kabata-Pendias, 2011; Chibuike and Obiora, 2014). Nickel becomes concentrated in the soil because it attaches to the soil particle surface and renders it immobile. Nickel toxicity results in interveinal chlorosis, browning and stunted root development (Vardaki and Kelepertsis, 1999). Micro-organism growth declines due to high nickel concentrations (Vardaki and Kelepertsis, 1999).

During weathering, nickel is easily mobilised, and unlike Mn^{2+} and Fe^{2+}, Ni^{2+}, is stable in aqueous solutions and can easily migrate within soil profiles. Elevated Ni concentrations in soil decrease enzyme activities like dehydrogenase, urease, and acid and alkaline phosphatases. Nickel toxicities are linked with serpentine or other Ni-rich soils, and Ni concentrations of 18,000 $mg{\cdot}kg^{-1}$ in plants growing on Ni-enriched ultramafic soils have been recorded in South Africa (Kabata-Pendias, 2011; Chibuike and Obiora, 2014).

Deficiencies result in newer plant leaves turning chlorotic and later resulting in the death of meristems (Vardaki and Kelepertsis, 1999). White interveinal banding on young leaflets is a common symptom of Nickel toxicity as well as dark green veins. Brown patches and stunted roots are other common symptoms in plants (Chibuike and Obiora, 2014).

1.2.9.8 Strontium (Sr)

Strontium is concentrated in mafic igneous rocks, calcareous sediments and clay due to clay's capacity to absorb Sr. Strontium's geochemical and biochemical characteristics are like Ca, hence Strontium's lithophilic affinity for Ca, and to a lesser extent for Mg. In specific environments, the Ca to Sr ratio can, to a certain degree, reflect the abundance of Sr and Its associated biogeochemical properties (Kabata-Pendias, 2011; Chibuike and Obiora, 2014).

Strontium tends to precipitate as biogenic carbonates and is associated with calcareous rocks and sulphur deposits. During weathering, Sr is mobilised as strontianite ($SrCO_3$), which is soluble, and later deposited as celestite ($SrSO_4$), which are both commercially essential minerals. Sr is easily mobilised in oxidising and acidic soils. Strontium's distribution in soil profiles varies, and in acid soils, Sr is readily leached in the soil profile, while in calcareous soils and organic soil, Sr is concentrated in the upper horizons. Strontium is not readily translocated from the plant roots to shoots, but most often, Sr concentrations in the plant tops are relatively high compared to the roots (Kabata-Pendias, 2011).

Phytoremediation can reduce the concentrations of the CoPCs and decontaminate contaminated growth mediums.

1.2.10 Phytoremediation

Phytoremediation involves using plants and accompanying microbes to reduce the concentration of contaminants from a growth medium that requires plant biological processes and physical characteristics (Barrutia *et al.*, 2008; Renault *et al.*, 2000; Greipsson, 2011; Pivetz, 2001; Keeling and Werren, 2005).

Plants can decontaminate substrate by one or more of the following mechanisms:

1. The plant can take up contaminants from the soil solution or the soil particles into the plant roots;
2. Plants can physically or chemically bind the contaminants into the plant roots; and

3. The plant can transport the contaminants from the plant's roots into the growing shoots, preventing leaching and further mobilising the pollutants.

According to Cowan *et al.* (2016), phytoremediation is a plant-based remediation method driven by bio-stimulants in the rhizosphere through the mineralisation of exudates ideal for the degradation of carbonaceous wastes as its carbon source.

Phytoremediation includes water and chemical uptake of CoPCs, metabolic activities in the plant, exudate from the growth medium leading to contaminant loss and the biochemical and physical impacts on the plant root (Pivetz, 2001).

Implementing phytoremediation technologies in polluted mine soils is onerous due to the high levels of contaminants in certain soils leading to unsuccessful vegetation establishment, as well as external environmental factors like the absence of topsoil, compaction, drought, temperature fluctuation and shortage of essential nutrients, amongst other (Barrutia *et al.*, 2008; Tordoff *et al*., 2000).

Coal discard, spoils and coal-contaminated soil contain variable amounts of sulphide minerals, mainly pyrite, which is also of concern in the coalfields of Mpumalanga. Oxidation of pyrite decreases pH, which increases the solubility of metals and trace elements, rendering them plant-available. Pyrite oxidation is controlled by establishing vegetation on discard dumps to serve as a cover in the mining industry. The cover stabilises the material in terms of erosion, prevents new acid-generating materials from being exposed and reduces the amount of water percolating through the medium employing transpirational water movement (Renault *et al.*, 2000; Fourie, 2007).

Phytoremediation is limited to the plant's root zone and could be a limiting factor in the effective amelioration of contaminated mediums, especially regarding the depth of contaminants (Greipsson, 2011).

Different forms of phytoremediation are described below in terms of the processes that occur within the plant and the specific types of contaminants that apply to each.

1.2.10.1 *Phytoextraction*

Phytoextraction involves the uptake of contaminants by roots and, subsequently, the accumulation in the plant's shoots, followed by the harvesting and disposal of the biomass (McGrath and Zhao, 2003; Pivetz, 2001; Hunt *et al.*, 2014). Phytoextraction applies to metals, non-metals, metalloids and radionuclides, which are not degraded or altered within the plant. Organic and nutrient contaminants are altered, volatilised and metabolised by plants, which is not associated with phytoextraction (McGrath and Zhao, 2003; USEPA, 2000). Certain studies demonstrated the successful accumulation of unaltered organic pollutants in the plant shoots by means of phytoaccumulation, phytoabsorption and phytosequestration (Pivetz, 2001; USEPA, 2000).

Hyperaccumulator plants can tolerate phytotoxic metals and are widely used in phytoextraction. The roots of the plants usually contain higher concentrations compared to the shoots, regardless of the translocation mechanisms (Pivetz, 2001; Barrutia *et al.*, 2008). The metal concentration distribution is usually uneven throughout the plant. Phytoextraction is localised in the plant's root zone, which is generally relatively shallow. The effectiveness of phytoextraction is still somewhat uncertain since only low concentrations of metals are removed from the substrate. Solutions to increase efficiency include the genetic transfer of abilities to hyperaccumulate with higher biomass production potential. Other solutions include screening hyperaccumulation candidates using faster-growing plants. Factors that influence phytoextraction consist of the sorption of metals on the colloidal particle and the solubility of the metal (McGrath and Zhao, 2003). Chelating agents are usually used to assist in the solubilisation of metals to allow uptake by the plant. Some studies proved that high metal uptakes are achieved using hydroponic systems (McGrath and Zhao, 2003). Comparing the concentration removal results from a hydroponic solution with soils is misleading, even if the same plant species are used. The bioavailability is much more significant in the solution than in the soil. Based on previous studies, the phytoextraction coefficient was less under field conditions than under laboratory conditions (Pivetz, 2001).

1.2.10.2 Phytostabilisation

Phytostabilisation refers to the use of vegetation to retain contaminants *in-situ* or the modifications of the conditions of the substrate (USEPA, 2000; Jadia and Fulekar, 2009). This process is also known as phyto-immobilisation, as it immobilises the contaminants or metals. Metals can be modified from a soluble state to an insoluble oxidised state in the root zone (Pivetz, 2001). Phytostabilisation involves the immobilisation of contaminants in the soil through absorption by the roots and shoots, adsorption onto roots, and precipitation within the root zone (USEPA, 2000). Plants reduce leaching through hydraulic control, enabling the precipitations of elements to less soluble forms and absorbing metals into root tissues, consequently decreasing the bioavailability and potential toxicity of the elements or metals (Mendez and Maier, 2008; Hazrat *et al*., 2013).

Stabilisation also includes non-biological processes of sorption due to adsorption, ion exchange and chelation. Soil pH is altered by carbon dioxide (CO_2) production resulting from the microbes that degrade the substances secreted from the rhizosphere by the roots, or better known as root exudates, which alters the solubility and mobility of metals in the soil (USEPA, 2000). A good understanding of root zone chemistry, fertiliser types, contaminants and root exudates is necessary for effective stabilisation to prevent unintended contaminant solubility and mobilisation increases. Phytostabilisation is cost-effective and is considered to be a green remediation method and has numerous advantages, which include: (1) it improves ecosystem restoration; (2) remediation can be conducted *in-situ* (3) it can be used for a wide array of pollutants; (4) it reduces visual impacts; (5) and plants enhance carbon sequestration and microbial life (USEPA, 2000; Renault *et al*., 2000; Mendez and Maier, 2008;). A disadvantage of phytostabilisation is long-term maintenance and potential re-seeding to sustain the vegetation cover. Plant uptake of metals and the translocation in shoots is undesirable, resulting in phytotoxification. To stabilise contaminants, a plant must be able to grow in contaminated soils and with a deep enough root system to reach the zone of contamination. Another prerequisite for successful stabilisation is the capability of plants to alter the chemical, physical and biological soil properties (Pivetz, 2001). Phytostabilisation reduces erosion by means of water or wind and promotes the restoration of local ecosystems.

Plants considered for phytostabilisation at coal mines should be tolerant to plant stressors like substrate salinity, acidity, elemental toxicity, nutrient deficiencies, and drought. Using pasture species for phytostabilisation is common in South Africa, as certain species are known to tolerate stress associated with coal mine waste mediums. The rapid growth of grass leads to immediate stabilisation of the underlying material.

1.2.10.3 *Rhizofiltration*

Rhizofiltration is similar to phytoextraction and also known as phytofiltration, which involves the removal of contaminants by the roots of the plant through adsorption or precipitation (Pivetz, 2001; USEPA, 2000; Dushenkov *et al*., 1995).

Root exudates produce biogeochemical conditions that enable the precipitation of contaminants onto the roots. The contaminants either remain on the roots or are absorbed and translocated to other sections of the plant depending on contaminant concentration, plant type, and the type of constituent (Pivetz, 2001; Dushenkov *et al*., 1995).

Rhizofiltration encompasses the removal of contaminants by harvesting the roots and, if necessary, the above-ground portion of the plant. In previous studies, this method was primarily used on soils to remove radionuclides and metals. This method is somewhat versatile and can be conducted *in-situ* and *ex-situ*; however, some modifications in soil acidity might be required for effective Rhizofiltration (Pivetz, 2001; USEPA, 2000).

1.2.10.4 Rhizodegradation

Rhizodegradation involves biodegradation that is influenced by plant roots, resulting in the detoxification or deconstruction of organic contaminants (USEPA, 2000; Singh and Jain, 2004). The organic pollutants are broken down into decay products or mineralised. The rhizosphere, the area around the roots, has an increased variety of microbial populations due to the growth stimulation by plant exudates compared to the soil further from the roots.

Exudate includes amino acids, sugar, fatty acids, and flavonoids. The increase in diversity and density of microbial activity accounts for increased biodegradation (Pivetz, 2001; USEPA, 2000).

The plant stimulates soil microbial growth and can result in alterations in geochemical soil conditions like soil pH, affecting contaminants' mobility and bioavailability. The advantage of *in-situ* rhizodegradation is dismantling contaminants and mineralising organic contaminants by forming organic complexes, making translocation less likely. A benefit of this method is that no harvesting is necessary because no pollutants accumulate in the plant but are degraded in the soil (Singh and Jain, 2004; Pivetz, 2001).

A drawback of this method is that only a small amount of soil makes direct contact with the plant roots at a given time, making this an interminable process.

Due to high concentrations of contaminants, inhospitable soil conditions limit root and plant growth, resulting in the accumulation of pollutants at deeper soil depths. It should be noted that plant exudates do not constantly stimulate degradation organisms and that non-degradation organism populations can also increase at the expense of the degraders. Inter-competition between organisms and plants can also impact the extent of biodegradation (Pivetz, 2001; USEPA, 2000). Organic matter from the plant can also be used as a carbon source, reducing the degradation of the contaminants.

1.2.10.5 Phytodegradation

Phytodegradation involves the degradation of contaminants in a growth medium by the enzymes that are produced and released by the plants, or it can affect the uptake, metabolisation and degradation of contaminants within the plant (USEPA, 2000; Longley, 2022). This degradation method does not depend on the microorganisms within the rhizosphere (Pivetz, 2001).

Contaminants include organic compounds like insecticides, herbicides and inorganic nutrients. Phytodegradation is better known as phytotransformation and is a process in which contaminants are destructed. For phytodegradation to occur, the plant must be able to accumulate the bioavailable compounds. Plant enzymes that metabolise the contaminant are released into the rhizosphere and can remain active during the transformation of pollutants (Pivetz, 2001; Greipsson, 2011; USEPA, 2000).

1.2.10.6 Phytovolatilisation

Phytovolatilisation is the uptake of contaminants by the plant and the release of volatile contaminants or a volatile form of an initial non-volatile compound (USEPA, 2000; Baeder-Bederski-Antenda, 2003). The produced volatile must be less toxic than the original contaminants for effective phytoremediation. This process involves the removal of contaminants from the growth medium and transferring the contaminant into a volatile form that is released into the surrounding atmosphere.

An example is the reduction of toxic mercury species to elemental mercury (Hg) or selenium (Se) to a less toxic dimethyl selenide gas. This process sometimes acts as a catalyst in processes like phytodegradation. Phytovolatilisation can also be used on organic contaminants, especially with other phytoremediation methods (Pivetz, 2001; Baeder-Bederski-Antenda, 2003).

Plants ideal for phytoremediation should fulfil the following requirements (Kadukova and Kavuličova, 2011; Schnoor, 1996):

1. The plants should grow fast and have high root turn-over rates and biomass production capabilities;
2. The plants should have deep roots to access trace elements deeper in the soil profile;
3. The plants should be easily harvestable to simplify long-term maintenance; and
4. The plants should accumulate large quantities of metals in their aerial organs.

Currently, the most effective remediation method is the phytoextraction of trace elements using hyperaccumulating plants. The trace element–plant interactions need to be understood to improve the technique. As with any rehabilitation technique, phytoremediation has advantages and constraints, as tabulated below in Table 1-3.

Table 1-3: Advantages and limitations of phytoremediation types (adapted from Jadia and Fulekar, 2009; USEPA, 2000; Pivetz, 2001 and Gratão *et al*., 2005)

Advantages	Limitations
Phytoremediation	
It is a cost-saving remediation technology compared to traditional technologies.	Root contact or depth limitation is the most severe constraint. Remediation is only possible when the contaminant is in contact with the root zone. A way to overcome this is to enlarge the root zone or select a plant with a big root zone.
This method is environmentally friendly and low-tech, and thereby publicly acceptable.	Phytoremediation is a very lengthy process because it depends on plant growth rates and environmental conditions. One way to overcome this constraint is to select fast-growing plants with high biomass or harvest plants more frequently.
Compared to traditional technologies, phytoremediation does not severely impact soils' physical and chemical characteristics, like texture, structure, and fertility.	The effectiveness of phytoremediation reduces during winter months, extreme weather conditions or pest or disease breakouts.
	A high concentration of contaminants in the substrate can be phytotoxic and hinder vegetation growth or even seedling germination.
	Transfer of contaminants from one medium to another or the environment or food chains is undesirable and should be prevented.
	Phytoremediation is not a prefabricated

Advantages	Limitations
	technology and should be tailored as plant species are dependent on site-specific conditions of the growth medium and climate.
	Phytoremediation requires more extensive areas than removal remedial methods and requires constant maintenance throughout.

Phytostabilisation	
It reduces the transportation of contaminants through wind, water and air.	The contaminants remain *in-situ*, and the vegetation requires long-term maintenance to prevent the mobilisation of pollutants.
Contaminated soil does not have to be excavated, resulting in a low remediation cost.	The uptake of contaminants by the plants could lead to transferring the toxicity to livestock.
The method is less disruptive than the other, more vigorous technologies.	The vegetation requires excessive fertiliser, and soil modifications might be necessary.
Rhizodegradation	
The translocation of the contaminant to the atmosphere is reduced.	Root depth is limited, and remediation is achieved only after extended periods.
The installation and maintenance costs are low compared to other treatment technologies.	The exudates secreted in the rhizosphere stimulate non-degrading microorganisms at the expense of degraders.
Phytovolatilisation	
Contaminants are transformed into chemical forms of reduced toxicity.	Hazardous substances can be released into the atmosphere.
The released volatiles might be subject to rapid and effective natural degradation through photodegradation.	Contaminants can accumulate in vegetation and be transferred to succeeding products.
Phytoextraction	
The plant material can be used as a resource; for example, selenium (Se) contained in the biomass can be used in selenium-deficient areas in animal feed.	Hyperaccumulator plants are predominantly slow-growing plant species.
	Biomass needs to be harvested, removed and in most cases, disposed of, which has

Advantages	Limitations
	additional financial and environmental implications.

In order for plants to extract COPCs and remediate contaminated substrates, the CoPCs need to be plant-available.

1.2.11 Bioavailability of trace elements

Nutrient uptake pathways can absorb Constituents of Potential Concern (CoPCs) similar to essential plant nutrients in terms of chemical behaviour and –form (Pivetz, 2001). Cadmium (Cd) is subject to plant uptake due to its likeness to the nutrients calcium (Ca) and zinc (Zn). Arsenic is also plant-absorbent due to its similarities to the plant nutrient phosphate. On the other hand, Selenium can replace plant nutrient sulphur; however, it does not serve the same physiological functions (Pivetz, 2001; Fairweather *et al*., 1996).

Soil and water near mining sites usually contain above-average abundances of chromium, copper, nickel, manganese, lead, cadmium and other metalloids like arsenic, selenium and antimony (John and Leventhal, 1995; Fairweather *et al*., 1996). The formation of numerous bioavailable forms of these elements is inevitable at these sites. To estimate the potential effects of the increased concentrations of these elements, the total bioavailable abundance of the elements needs to be determined. Bioavailability is the proportion of the element available for bioaccumulation that plants can absorb (John and Leventhal, 1995).

Elements are occasionally incorporated in mineral lattices or adsorbed to soil or clay particles, rendering them not readily available for incorporation in biota. Sulphide minerals associated with coal mines are predominantly present in the ore deposit but can also form by bacterial reduction of sulphate in the oxidizing substrate. In their undisturbed natural state, sulphide minerals have little environmental impact and are immobile in chemical-reducing environments (John and Leventhal, 1995’ Keeling and Werren, 2005).

The bioavailability of elements is influenced by numerous seasonal and temporal variables like total concentration, speciation (physical-chemical forms), pH, redox potential, mineralogy, total organic content, temperature, and water's presence and volume availability (John and Leventhal, 1995; Keeling and Werren, 2005). In solid phases, metals and soil are partitioned into different fractions: carbonate, crystalline, dissolved, exchangeable, iron-manganese oxide and organic (Figure 1-6).

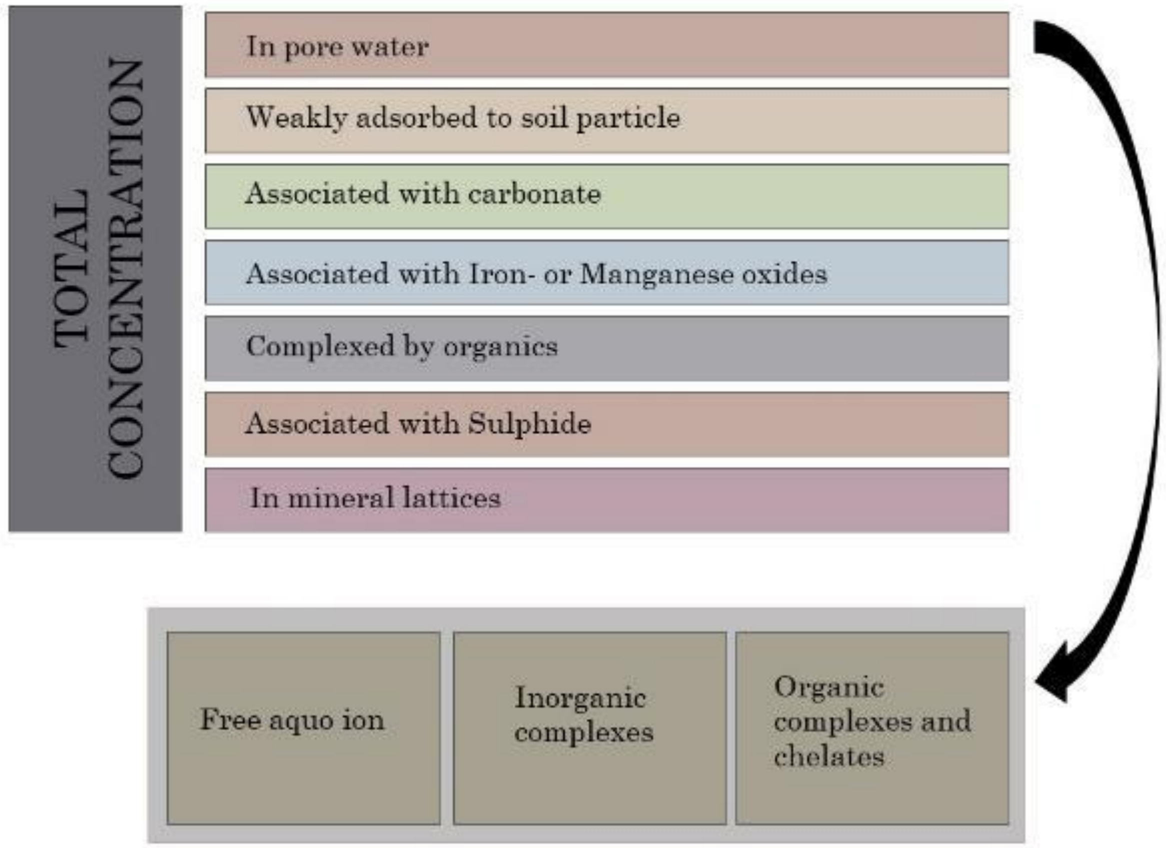


Figure 1-6: Chemical forms of metals in solid phases (adapted from John and Leventhal, 1995)

Several factors influence metal bioavailability in soil, and the uptake of elements by plants depends on the following (John and Leventhal, 1995; Audet and Charest, 2008):

- The elemental movement from the substrate to the root;
- Elements crossing the epidermal cell membrane of the roots;
- Element transportation from epidermal cells to the xylem are transported from roots to shoots; and
- Mobilisation from leaves to tissues in the phloem transport system.

The relative mobility of trace elements in terms of speciation and association are tabulated below in Table 1-4.

Table 1-4: Trace element mobility (adapted from John and Leventhal, 1995; Keeling and Werren, 2005; Audet and Charest, 2008)

Metal speciation and association	Metal mobility
Exchangeable cations	Charges in cationic composition bring on high cation exchanges.
Trace elements associated with Fe-Mn oxides	Medium modifications in redox conditions cause the release of some metals and precipitation if sulphide minerals are insoluble.
Trace elements associated with organic matter	The decomposition and soil organic matter results in metal mobility from medium to high.
Trace elements associated with sulphide minerals	Low to high and is dependent on environmental conditions. The oxidation of sulphide minerals releases metals.
Trace elements fixed in a crystalline phase	Low metals are only available after physical and chemical weathering.

Metals associated with carbonate minerals in sedimentary rocks and soil. The iron-manganese oxides consist of metals attached to iron-manganese oxide particles, while organic fractions consist of metals bound to differing forms of organic matter. The crystalline structure consists of metals in minerals' crystal lattices and is usually unavailable to biota. Substrate or soil solution pH is the most critical factor controlling metal speciation, the solubility, mobility, and plant availability of metals in solutions (Audet and Charest, 2008).

The bioavailability of nutrients at varying pH values is illustrated in Figure 1-7. Soil pH also strongly affects nutrient availability for plant uptake and predicts deficiencies and toxicities. If soil pH is >7.5, nutrients (Cu, Mn, Fe) form insoluble complexes, and bioavailability reduces, and deficiencies of these elements become more likely. The availability of Ca, P and Mg also becomes compromised at pH <6 (Muhammad *et al*., 2017)

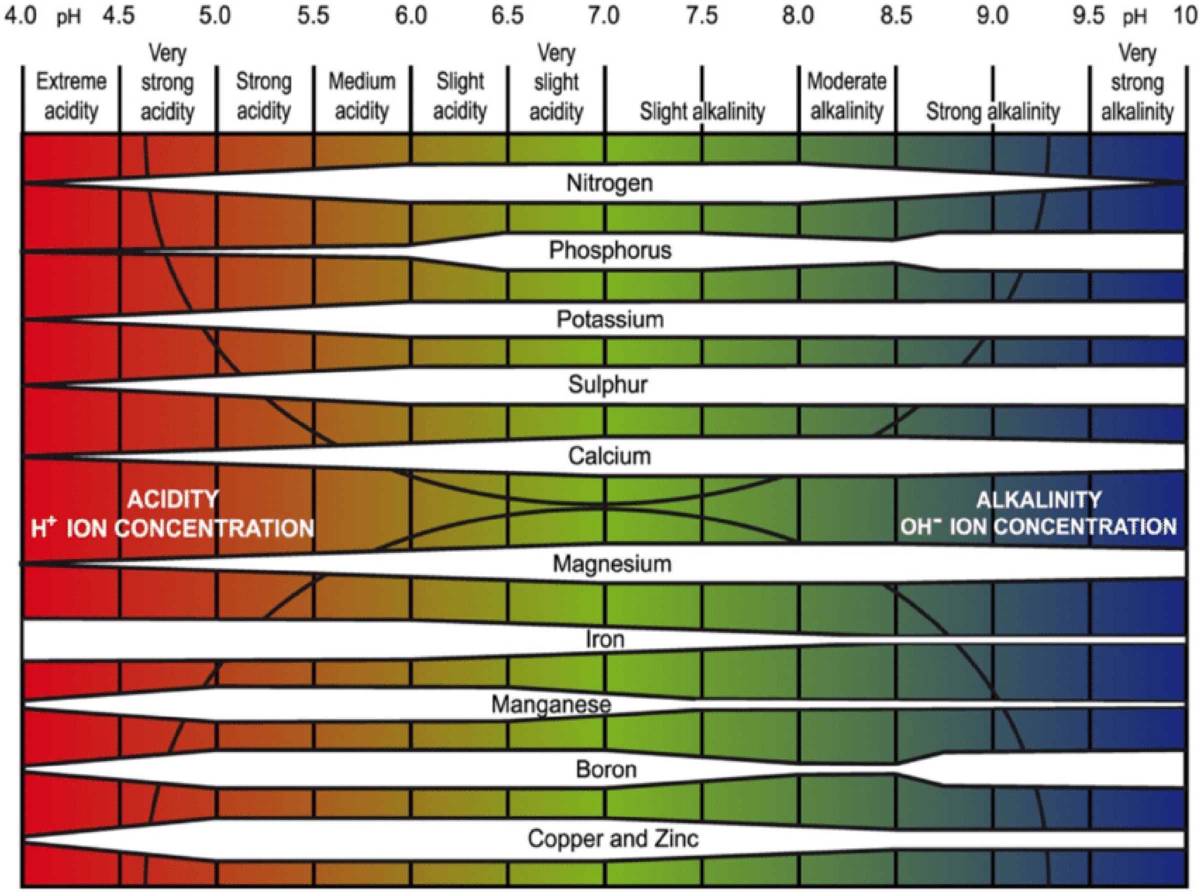


Figure 1-7: Element availability with varying pH (Muhammad *et al*., 2017)

Likewise, Kebata-Pendias (2011) also suggested that other potentially toxic elements (PTE) are more mobile in acidic soil conditions, as depicted in Figure 1-8. It can be anticipated that the PTE or trace elements or elements are more plant-available with decreasing soil pH (Kebata-Pendias, 2011).

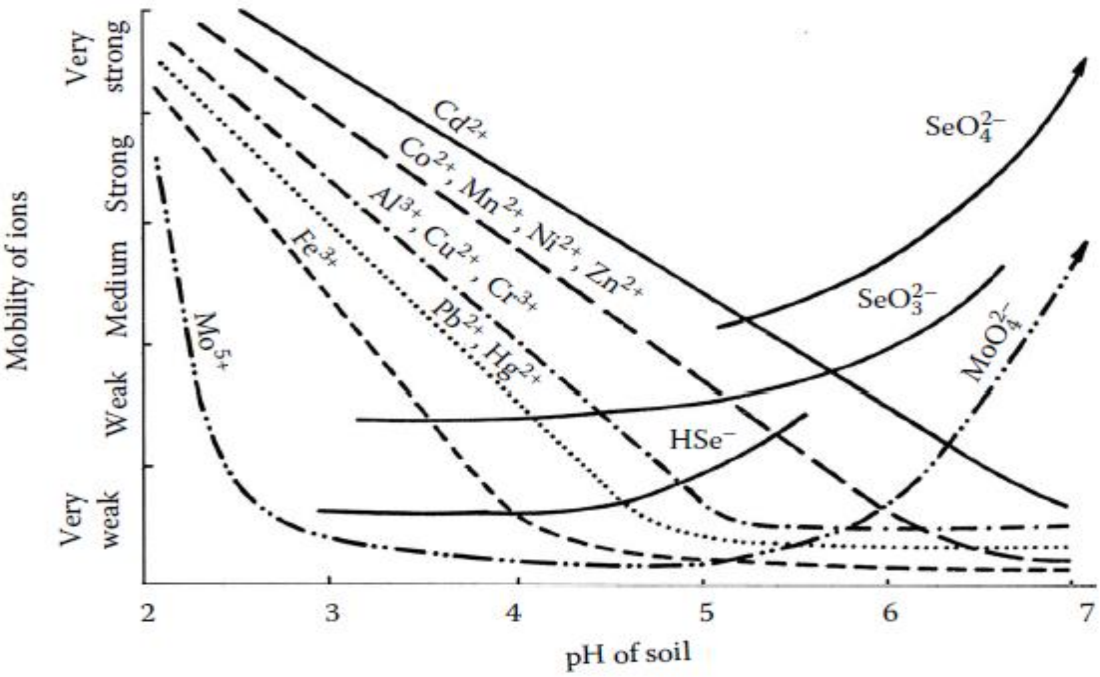


Figure 1-8: Trace element mobility with varying pH (Kebata-Pendias, 2011)

1.2.12 Plant response to metal stress

Element toxicity transpires in the following symptoms in plants: reduced biomass and photosynthesis resulting in discolouration or biochemical disorders like oxidative stress, amongst others (García-Salgado, 2012).

The reaction of plants to trace metal stress includes an assortment of mechanisms, ranging from deviations in gene expression to metabolic or biochemical adjustments, with the final objective of scavenging potentially toxic ions and ameliorating stress effects and associated damages (Raskin *et al*., 1994; Kadukova and Kavuličova, 2011; García-Salgado, 2012).

Plants have one of three responses when introduced to soil or growth medium with elevated concentrations of metals. Based on the different responses, plants are metal excluders, metal indicators or hyperaccumulators (Figure 1-9).

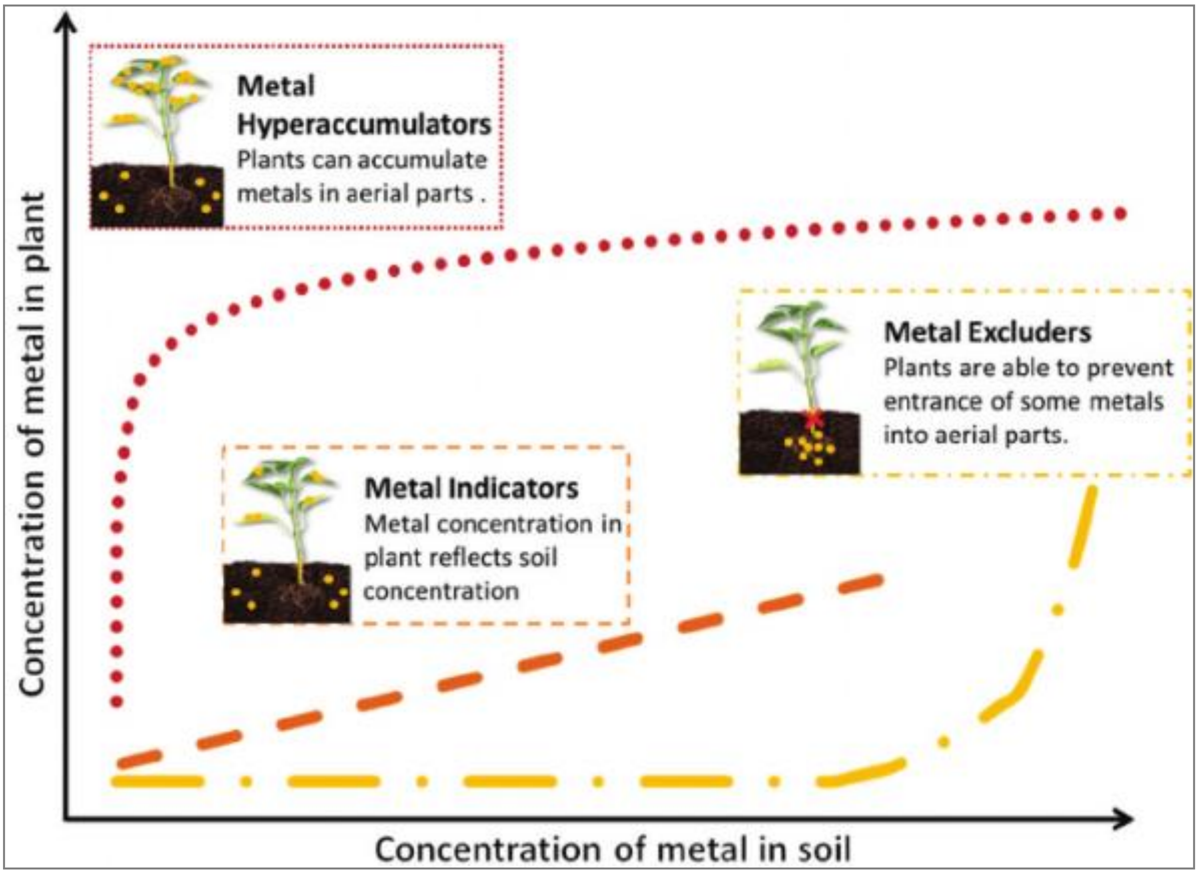


Figure 1-9: Three response strategies (Hunt *et al.*, 2014)

Metal excluders do not import metals into the aerial organs (shoot/ leaves); however, elevated concentrations of metals can be observed in the plant's root system. Metal excluders can be defined as a plant with elevated concentrations of metals in the roots but have a shoot/root ratio of less than one.

Metal indicators can accumulate metals in aerial organs at roughly the same concentration as the surrounding soil (linear relationship in Figure 1-9). Metal hyperaccumulators can accumulate elevated concentrations of metals in relation to the surrounding soil (McGrath and Zhao, 2003). Hyperaccumulators are termed as plants containing more than 0.1% of Co, Ni, Cr and Pb or 1% of Zn in the aerial organs of the plant on the dry weight basis, regardless of the elemental concentration in the growth medium (Raskin *et al*., 1994).

Metal toxicity can sometimes be observed in the field, and the two most profound symptoms of toxicity in plants are growth reduction and pigment alterations in the plant. Metal accumulation and toxicity in plants usually result in plant growth reduction and are expressed as reduced biomass production and reduced growth rates (Kadukova and Kavuličova, 2011; García-Salgado, 2012).

The decline in growth can be attributed to specific toxicity and opposition with other nutrients. An equation to assess the phytotoxicity was suggested by Leita *et al*. (1993) and is expressed as (Kadukova and Kavuličova, 2011):

$$\boldsymbol{Grade\ of\ Growth\ Inhibition}\ (\mathbf{GGI}) = \frac{(\mathbf{C-T})}{\mathbf{C}} \qquad \text{(Eq. 1-5)}$$

Where C represents the dry weight of tissues for the control, and T represents the dry weight of the metal-treated plants. The GGI should equate to zero if the plant growth was not inhibited and the plants did not experience any stress.

A proposed outcome for vegetation establishment for rehabilitation is its resilience to natural disturbances, including defoliation like grazing and harvesting (Ruiz-Jean and Aide, 2005). Vegetation cover attenuates and reduces surface runoff, erosion and improves soil stability by mechanically strengthening the soil structure through root systems (Morgenthal, 2004; Fourie, 2007). The above-ground regrowth of plants after defoliation was assessed to establish the ability of grasses to regrow biomass.

Chlorophylls, primary photosynthetic pigments, are essential for radiance fixation and photosynthesis. The quantity and composition of photosynthetic pigments are imperative indicators of photosynthesis status and depend on the growth conditions, plant species, and mineral nutrition. A typical response to plant stress is the reduction in chlorophyll content and, subsequently, reduced photosynthesis and biomass production. The decreased chlorophyll quantity in the presence of metals may be due to an obstruction of chlorophyll biosynthesis. Numerous studies recorded a reduction in chlorophyll *a* and *b* upon exposure to trace elements like copper, zinc, cadmium, and lead (Prasad *et al*., 2001; Cenkci *et al*., 2010; Ghnaya *et al*., 2009; Mishra *et al*., 2006).

High levels of iron influence chlorophyll content and might result from reduced chloroplast density, phosphorus deficiency and decreased manganese transport (Kadukova and Kavuličova, 2011). Leaf chlorophyll concentration is a critical component frequently measured as an indicator of photosynthetic capacity, chloroplast development, leaf nitrogen content and general plant health. The chlorophyll content is usually determined photometrically by extracting the pigments utilising an organic solvent, such as *dimethylformamide* or *acetone* (Shah *et al*., 2017). The method is well-proven and accurate; however, it is destructive, time-consuming and necessitates toxic chemicals.

A Soil Plant Analysis Development (SPAD) chlorophyll meter is a diagnostic tool used to measure crop nitrogen status and plant stress. Abiotic stress like drought, salinity or nutrient deficiency alters essential physiological functions (Shah *et al*., 2017). There is a strong correlation between plant pigments and the photosynthetic process and physiological conditions, which can be used to determine plant stress response (Shah *et al*., 2017; Uddling *et al*., 2007). Estimating leaf photosynthetic pigments is essential in plant stress and management monitoring. The chlorophyll content is indicative of photosynthetic capacity and, in conjunction with leaf area index, is a proxy for productivity and plant stress (Shah *et al*., 2017). Salinity stress alters biochemical and physiological processes that disturb osmotic potential in the soil-plant system, resulting in reduced water uptake by the plant roots, like drought stress (Shah *et al*., 2017). SPAD meters are rapid and non-destructive apparatuses that measure comparative chlorophyll content, whereas traditional methods entail complicated solvent extraction procedures and vitro-spectrophotometric determination (Shah *et al*., 2017).

The correlation between SPAD values (unitless) and leaf pigment content is not universal due to variations in measurement procedures, leaf direction or exposure, sensor type and plant species (Shah *et al*., 2017; Uddling *et al*., 2007). A previous study by Shah *et al*. in 2017 found a relationship between total chlorophyll and carotenoids, which are carotenes and xanthophylls that represent a photosynthetic pigment group, which is also illustrated in the graph below. The study also found a direct correlation between fertiliser and pigment content per unit leaf area and an inverted correlation between soil salinity and photosynthetic pigments (Figure 1-10).

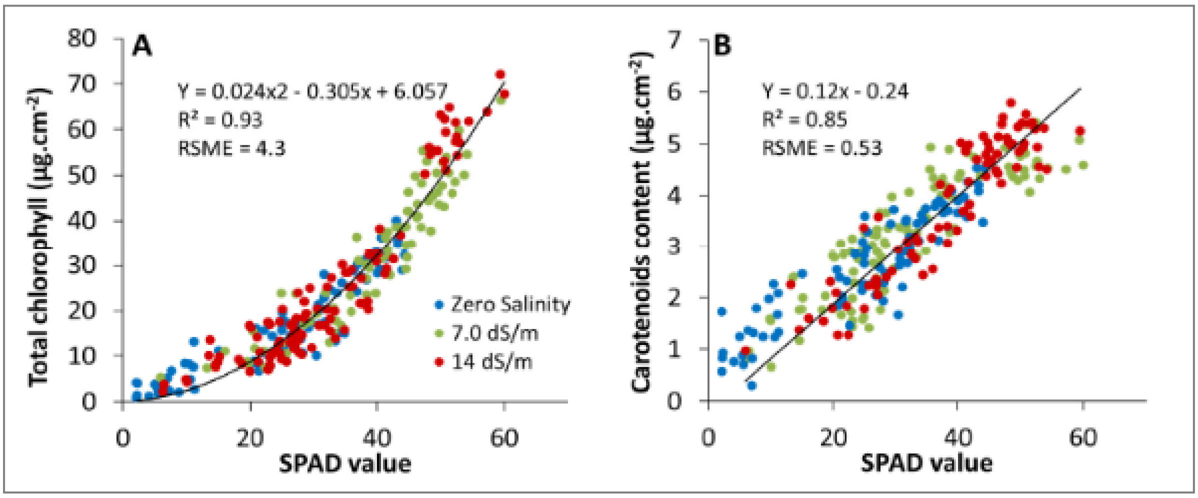


Figure 1-10: Relationship between SPAD readings and a) total chlorophyll and b) carotenoids content (Shah *et al*., 2017)

The SPAD meter has two light-emitting diodes (LED) and a photodiode receptor.

It measures leaf transmittance in the electromagnetic spectrum's red and infrared regions, 650 nm and 940 nm, respectively (Shah *et al*., 2017; Uddling *et al*., 2007). The transmittance value is used to determine the SPAD meter value proportionate to the chlorophyll constant (Shah *et al*., 2017).

The efficiency of metal removal by plant species can be determined by numerous factors and indices that have been developed over the past two decades. Some of the equations used presently are discussed below.

1.2.13 Trace element removal and accumulation factors and indices

Trace element removal efficiency and Translocation Factor (TF) are calculated for the CoPC using the following calculations (Gayatri *et al.*, 2019):

Efficiency of removal = [Concentration (shoot) + Concentration (roots)/ Concentration (soil)] * 100 (Eq. 1-6)

Translocation Factor = Concentration (root) / Concentration (shoot) (Eq. 1-7)

The translocation factor is the ability of the trace element to migrate from the roots in the plant to the shoots and the ability to accumulate trace elements in the upper physiological parts of the plant. A plant is classified as a hyperaccumulator if it has a translocation factor close to one.

The Accumulation Factor (AF) is an indication of the trace element concentrations absorbed from the soil and is calculated using the following equation (Selvaraj *et al*., 2015; Chuan *et al*., 2016):

Accumulation Factor = Concentration in tissue of whole plant/ Initial concentration in substrate (Eq. 1-8)

Mobility Index (MI) determines the bio-mobility of trace elements in different parts of the plant, with Level 1 being the soil – roots, Level 2 (roots – stems), and Level 3 being the stems - leaves. The MI can be calculated as follows (Selvaraj *et al*., 2015):

$$\text{Mobility Index} = \text{Concentration in the receiving level/ concentration in the source level} \quad \text{(Eq. 1-9)}$$

Tolerance index (TI) is another index that is indicative of metal tolerance and biomass production and is calculated as follows (Chuan *et al*., 2016):

$$\text{Tolerance Index} = \text{Dry matter yield in metal soils/ dry matter yield in control soil} \quad \text{(Eq. 1-10)}$$

1.2.13.1 *Hyperaccumulation*

Some plants can hyperaccumulate trace elements through phytomining or potentially ameliorate substrates using phytoremediation (Brooks *et al*., 1999). A hyperaccumulator can also be defined by a threshold value of concentrations roughly 10 to 100 times higher in the plant's aboveground parts than the concentrations in non-hyperaccumulator plants growing in unpolluted soils (Lasat, 2000; Bert *et al*., 2002).

In numerous plant species, the accumulated concentrations of trace elements or metalloids (As, Cd, Co, Cu, Mn, Ni, Sb, Se and Zn) in the aboveground biomass are more than one and, in some instances, in comparison with adjacent plants, roughly four orders of magnitude higher (Krämer 2010; Reeves and Baker, 2000; Baker and Brooks, 1989). In unique cases of approximately 500 plant taxa, it has been reported that the accumulation of contaminants can exceed the macronutrient cations (Ca and K) concentrations (Bert *et al*., 2002).

Metal hyperaccumulation is coupled with a vigorous improved capability to detoxify the accumulated metals in the plant's aboveground section, rendering the plant metal hyper-tolerant. Metal hyper-tolerance is an abiotic stress-resistant attribute, and the selective advantage, beyond the physiological need, remains elusive (Boyd and Martens, 1992).

Previous studies hypothesised that metal hyperaccumulation acts as a defence against pathogen attacks and herbivory (Pollard and Baker, 1997; Freeman *et al*., 2006;).

A benefit of investigating hyperaccumulators is that future research can expedience the accessibility of links connecting biotic stresses and metal homeostasis in hyperaccumulator plants. The amount of trace elements a plant can take up is dependent on the concentrations in the substrate, and it has been noted that trace elements are predominantly accumulated in the plant roots.

Plants can be divided into three categories based on the degree of trace element tolerance, namely (Ernst *et al*., 2008):

1. Hypo-tolerance
2. Basal tolerance
3. Hyper-tolerance

Some plants are apparent hyperaccumulators for Cd, Cu or Zn; however, no single species can store excess concentrations of all trace elements. The periphery between basal tolerance and hyper-tolerance is indistinct. Kabata-Pendias (2011) stated that the risks associated with potentially toxic elements (PTE) can be calculated by quantifying the bioaccumulation capabilities and environmental toxicity. The laboratory results of the substrate samples were used to quantify indices like availability percentage (A%), bioaccumulation index (BAI), threshold exceedance ratio (TER), contamination load index (CLI), and hyperaccumulation threshold exceedance ratio (HTER). The indices and the associated calculations are explained hereafter.

1.2.13.2 Availability %

The contamination status of CoPCs in substrates can be determined by calculating the extraction or mobility percentage, also known as the availability percentage (Rösner *et al.,* 2001). The availability percentages (A%) were determined based on the following equation:

$$A\% = (CoPC_A / CoPC_T)^* \ 100 \qquad \text{(Eq. 1-11)}$$

Where:

A% = availability percentage

A = available concentration

T = total concentration

1.2.13.3 Bioaccumulation Index

The accumulation efficiency of plants, to define the bioaccumulator status, is determined by the bioaccumulation index (BAI) (García-Salgado, 2012; Kabata-Pendias, 2011). Bioaccumulation indices can be classified according to four broad categories, as per the table below (Table 1-5), based on the degree of accumulation (Różanowski *et al*., 2012; Kabata-Pendias, 2011).

Table 1-5: Bioaccumulation index categories

Degree of accumulation	Bioaccumulation index (BAI)
Lack	0.001 – 0.01
Slight	0.01 – 0.1
Medium	0.1 – 1
Intensive	1 - 10

The total and available bioaccumulation indices can be calculated using the following equations (Różanowski *et al*., 2012; Kabata-Pendias, 2011):

$$BAI_T = \text{Concentration in plant/ Total Concentration} \qquad \text{(Eq. 1-12)}$$

$$BAI_A = \text{Concentration in plant/ Available Concentration} \qquad \text{(Eq. 1-13)}$$

Where:

A = available concentration

BAI = bioaccumulation index

T = total concentration

1.2.13.4 Threshold exceedance ratio (TER)

The status of contamination compared to specified thresholds can be determined by employing the following equations (Dung *et al.,* 2013):

$$TER_T = \frac{\text{Concentration in plant}}{\text{Total Concentration}} \qquad \text{(Eq. 1-14)}$$

$$TER_A = \frac{\text{Concentration in plant}}{\text{Available Concentration}} \qquad \text{(Eq. 1-15)}$$

Dung *et al.* (2013) stated that threshold exceedances are the simplest method to determine the degree of contamination in soils and sediments.

1.2.13.5 Contamination Load Index (CLI)

The degree of contamination in crops or feed can be determined using the CLI equation below (Moradi *et al*., 2016):

$$CLI = \text{Concentration in plant/ MPC} \qquad \text{(Eq. 1-16)}$$

Where:

CLI = Contamination Load Index

MPC = maximum permitted concentrations

1.2.13.6 Hyperaccumulation threshold exceedance ratio (HTER)

The accumulation efficiency of plant species or the hyperaccumulator status can be determined by calculating the HTER. If an HTER value of ≥ 1 is obtained, the plant can be classified as a hyperaccumulator (García-Salgado, 2012). HTER can be calculated as follows:

$$\text{HTER} = \frac{\text{Concentration in plant}}{\text{Hyperaccumulation threshold}} \qquad \text{(Eq. 1-17)}$$

1.2.14 Pasture species as phytoremediators

Grasses, belonging to the Gramineae family, are currently the most popular seeded plants used in mine rehabilitation. They produce large amounts of biomass with rapid re-growth potential, and the roots of the grass are fibrous and prevent erosion by holding the soil in place and reducing the effects of raindrop impacts. Forbs, herbaceous flowering plants that include Legumes, are sometimes used for rehabilitation in conjunction with grasses. Legumes can utilise nitrogen from the atmosphere to supplement nutrient requirements and transfer the fixed nitrogen to other components within the soil and plant. Perennial grasses have been reported to be more suitable for phytoremediation because of the capacity to stabilise surfaces and the ability to build up soil organic matter (SOM) in short periods due to the quick turnover time (Pandey and Maiti, 2020). Factors to further support the use of perennial grasses for phytoremediation can be summarised as follows (adapted from Pandey and Maiti, 2020):

- Tolerant to harsh conditions

- Provide rapid ground cover
- High biomass production abilities
- Low greenhouse emissions
- Ability to contribute to carbon sequestration
- Propagates vigorously and requires low amounts of fertilizer
- Have phytostabilisation and phytoremediation capabilities
- Have high aesthetic value;
- Can be used as a source of income; and
- It can be used for grazing, which is the most popular land capability for rehabilitated coal mines.

Based on current rehabilitation practices and numerous studies, the following grass species were initially identified as potential hyperaccumulators (Scotney and McPhee, 1992; Heuzé *et al.*, 2016; Cowan *et al.*, 2016; Pivetz, 2001):

- *Eragrostis curvula* (Weeping love grass)
- *Digitaria eriantha* (Smuts finger grass)
- *Cenchrus ciliaris* (Blue buffalo grass)
- *Cynodon dactylon* (Couch grass)
- *Panicum maximum* (White buffalo grass)
- *Chloris gayana* (Rhodes grass)
- *Hyparrhenia tamba (*Blue thatch grass*)*
- *Hyparrhenia hirta (*Common thatch grass*)*
- *Chrysopogon zizanioides (*Vetiver*)*
- *Paspalum dilatatum (*Common Paspalum*)*
- *Pennisetum clandestinum (*Kikuyu*)*
- *Pennisetum purpureum (*Elephant grass*)*
- *Eragrostis tef (*Teff*)*

The most popular grass species used in mine rehabilitation were assessed according to survival factors, including tolerance to extreme soil conditions and past success in similar projects and other tertiary uses, as potential post-closure land uses. The results of the assessment are summarised in Table 1-6 below.

Table 1-6: Preliminary grass species (adopted from Scotney and McPhee, 1992; van Oudtshoorn, 2014; Heuzé *et al*., 2014; Heuzé *et al*., 2015; Heuzé *et al*., 2016; Magonoro *et al*., 2011; Okereafor *et al*., 2020)

Grass	Perenniality	Grazing potential	Plant succession	Grazing status	Flower dates	Resistance				Suitability		Tertiary use
						Acid	Drought	Low pH	Salt	Disturbed	Undisturbed	
***Eragrostis curvula* (Weeping love grass)**	Perennial tufted grass	Average grazing value	Subclimax/ Climax grass	Increaser II grass	Aug - June	x		x		x		Grazing/ good quality hay – poor palatability
***Digitaria eriantha* (Smuts finger grass)**	Perennial tufted grass	High grazing value	Climax grass	Decreaser grass	Jan - Apr	x	x	x		x	x	Grazing sometimes silage
***Cenchrus ciliaris* (Blue buffalo grass)**	Perennial tufted grass	High grazing value	Subclimax/ Climax grass	Decreaser grass	Aug - Apr		x			x	x	Grazing sometimes hay
***Cynodon dactylon* (Couch grass)**	Perennial (creeping grass)	High grazing value	Pioneer grass	Increaser II grass	Sep - May	x	x		x	x		Grazing sometimes hay
***Panicum maximum* (White buffalo grass)**	Perennial tufted grass	High grazing value	Subclimax/ Climax grass	Decreaser grass	Sep - Mar	x				x	x	Grazing, hay and silage
***Chloris gayana* (Rhodes grass)**	Perennial tufted grass	High grazing value	Subclimax grass	Decreaser grass	Nov - May	x				x	x	Grazing sometimes hay
Hyparrhenia tamba (Blue thatch grass)	Perennial tufted grass	Low grazing value	Climax grass	Increaser I grass	Jan - Jun	x		x	x	x	x	Thatching, very hard and

Grass	Perenniality	Grazing potential	Plant succession	Grazing status	Flower dates	Resistance				Suitability		Tertiary use
						Acid	Drought	Low pH	Salt	Disturbed	Undisturbed	
												unpalatable
Hyparrhenia hirta (Common thatch grass)	Perennial tufted grass	Average grazing value	Subclimax/ Climax grass	Increaser I grass	Nov - May	x	x	x		x	x	Thatching and weaving (mature) and grazing (early stages)
Chrysopogon zizanioides (Vetiver)	Perennial tufted grass	High grazing value	Exotic grass - Climax grass	Increaser II grass		x		x	x	x		Forage grass that is drought resistant (hay and silage), essential oils
***Paspalum dilatatum* (Common Paspalum)**	Weak perennial tufted grass	High grazing value	Exotic grass		Nov - Feb	x		x		x		Difficult to eradicate once maturity is reached
***Pennisetum clandestinum* (Kikuyu)**	Perennial (creeping grass)	High grazing value	Exotic grass - Climax grass	Decreaser grass	Aug - Apr	x		x		x		Productive under heavy grazing
***Pennisetum purpereum* (Elephant grass)**	Perennial tufted grass	High grazing value	Climax grass	Increaser II grass		x		x				Cereal and forage
Pennisetum	Annual	High			Sep -	x	x	x		x		Grazing,

Grass	**Perenniality**	**Grazing potential**	**Plant succession**	**Grazing status**	**Flower dates**	**Resistance**				**Suitability**		**Tertiary use**
						Acid	**Drought**	**Low pH**	**Salt**	**Disturbed**	**Undisturbed**	
glaucum (Babala grass/ Pearl Millet)	tufted grass	grazing value			Oct							silage Flour, bread, porridge and couscous
***Eragrostis tef* (Teff)**	Annual tufted grass	High grazing value	Pioneer grass	Decreaser grass	Nov - May			x		x	x	A popular source of fodder for horses, bread (Injera)

The grass species were shortlisted based on the type of elements they are known to accumulate and the structural locality of the concentrations. Based on previous studies, Table 1-7 lists the grass species and the Constituent of Potential Concern (CoPC) they are known to accumulate and the accumulation area. No information could be sourced for the grass species that were omitted from the table prior to the finalisation of this Chapter.

Table 1-7: Grass species accumulation potential of different CoPCs

Grass species	CoPC	Accumulation area
Cynodon dactylon	As (Pivetz, 2001) Mn, Zn, Cu, Cd, Pb (Mulugisi *et al*., 2009)	No literature was found.
Hyparrhenia hirta	Cu, Zn, Pb, Cr, Co, Ni (Okereafor. *et al*., 2020) (Mulugisi *et al*., 2009) As, Mn, Cd (Magonoro *et al*., 2011)	Roots > shoots (Magonoro *et al*., 2011)
Chrysopogon zizanioides	As, Cd, Cu, Cr, Pb, Ni, Zn (Truong, 1999; Paz-Alberto and Sigua, 2013)	Roots > shoots (Truong, 1999)
Eragrostis curvula	Lead (Dushenkov *et al*., 1995)	No literature found.
Paspalum dilatatum	Mn, Zn, Cu, Cd, Pb, Co (Mulugisi *et al*., 2009)	No literature was found.
Hyparrhenia tamba	Mn, Zn, Cu, Lead, Cd, Co (Mulugisi *et al*., 2009)	No literature was found.
Digitaria eriantha	Zn, Mn, Fe, Cr (Okedeyi *et al*., 2013)	No literature was found.
Pennisetum glaucum	Cd, Pb, Cr (Wuana *et al.,* 2013)	No literature was found.
Chloris gayana	Ag, As, Zn, Pb, Cd, (Keeling and Werren, 2005)	Roots > shoots (Keeling and Werren, 2005)

The final pasture species were selected based on their hyperaccumulating capabilities, high forage potential for livestock, and ability to tolerate drought, acidity, or low soil fertility. The following species were selected as part of the summer growth term, and each will be discussed in detail hereafter regarding characteristics and success in rehabilitation practices:

I. *Cynodon dactylon* (Couch grass)

II. *Chrysopogon zizanioides* (Vertiver grass)

III. *Chloris gayana* (Rhodes grass)

IV. *Pennisetum glaucum* (Pearl Millet grass)

V. *Digitaria eriantha (*Smuts finger grass*)*

VI. *Medicago sativa (*Lucerne/ Legume)

VII. *Eragrostis tef* (Teff grass)

1.2.14.1 *Chrysopogon zizanioides*

Vetiver grass (*Chrysopogon zizanioides*) is a perennial grass and can grow up to two meters high with a root system reaching up to five meters (van Oudtshoorn, 2014). On average, yield levels of the leaves are 15 to 30 tons·ha^{-1} while the roots can produce a dry matter yield of up to 2142.9 kg·ha^{-1} (Mekonen, 2000). *C. zizanioides* is famous for its effectiveness in sediment and erosion control and tolerance in extreme soil conditions (Truong, 1999). *C. zizanioides* belongs to the same grass family as lemongrass, maise, sorghum and sugarcane and has numerous unique characteristics. Previous studies suggested that if *C. zizanioides* has an adequate supply of phosphorus and nitrogen, the grass can grow in highly acidic soils.

Chrysopogon zizanioides has been able to extract 890 mg·kg^{-1} of manganese (Mn) in the plant tops from a substrate containing an Mn concentration of 578 mg·kg^{-1} (Truong, 1999).

Chrysopogon zizanioides is salt-tolerant, and vigorous growth is observed with sufficient water. *C. zizanioides* is also tolerant to elevated concentrations of trace elements. Based on results, it translocates small concentrations of trace elements to the plant's shoots, making it safe for animal grazing.

Chrysopogon zizanioides is highly suitable for phytoremediation because it grows in hydrophilic and xerophytic conditions and can tolerate extreme climatic conditions like prolonged drought, fire, submergence, flood, and temperatures ranging from -20ºC to 55ºC. Previous studies determined that *Chrysopogon zizanioides* thrives in precipitation ranging from 300 mm to 6000 mm per annum and can tolerate a broad spectrum of physiochemical properties like varying pH levels (3 to 10.5), sodicity and salinity (Truong, 1994; Truong and Baker, 1996; Truong. 1999). It was also recorded that *C. zizanioides* can grow in soils with elevated Al, As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Se and Zn in soils (Truong and Baker, 1998).

1.2.14.2 *Cynodon dactylon*

Cynodon dactylon is native to Africa and is commonly found in sub-tropical or warm temperate areas frequently disturbed by grazing, floods, or fires (Paul *et al*., 2012; Dickinson *et al*., 2010). It is known to remedy soil erosion by virtue of its creeping-, and drought-resistant nature as well as its soil-holding capabilities (Van Oudtshoorn, 2014). It is an excellent grass for annual production and grows best at pH (KCl) levels ranging from 5 to 6.5.

Cynodon dactylon is exceptional because it grows in all soil types and even grows well in disturbed areas. *Cynodon dactylon* is a valuable grass and performs well in pastures because it is tolerant to heavy grazing to a certain extent. The root system is ideal for stabilising surfaces during rehabilitation.

Cynodon dactylon proved effective in acid mine rehabilitation, is aluminium tolerant, and can grow without any visible restriction in soil with an aluminium saturation percentage of 68 and a pH of 3.8 (Truong, 1999; Truong and Baker, 1998). A study on coal substrates was conducted from 2003 to 2007 by Platt (2010) and it concluded that *C. dactylon* had 100% survivability by the end of a three-year trial.

1.2.14.3 *Chloris gayana*

Chloris gayana (Rhodes) is abundant in the bushveld and grassland and grows in wet areas like wetlands in dry regions or disturbed areas with higher rainfall (Van Oudtshoorn, 2014). It is palatable, tolerant to overgrazing and established with ease. Rhodes is also said to behave like a weed, appearing when soil fertility is deficient (Mentis, 1999)

Chloris gayana is a perennial, stoloniferous tufted grass and shoots at the nodes, resulting in quick ground coverage (Dickinson *et al*., 2010). Lifespan ranges from three to five years. The grass has smooth leave blades 1450 mm long, which gradually tapers to a fine point. The flower stalks range from 1000 mm and end in 10 to 20 racemes. The spikes consist of pointed flowers, which have a fertile and infertile inflorescence with a beard each (Dickinson *et al*., 2010).

Chloris gayana is cultivated in nearly all tropical countries and, to a lesser extent, certain sub-tropical countries. It has good seed production capabilities and is established with ease due to its creeping growth habit (Van Oudtshoorn, 2014).

It is not a high-quality grass; however, it is used where ease of establishment prioritises high-quality production. It is indigenous to eastern provinces like Natal in Southern Africa and can be found in areas with relatively low rainfall (Van Oudtshoorn, 2014). *Chloris gayana* can grow in numerous soil types, varying in texture, from structureless soils like sand to highly structured soils. *Chloris gayana* grows well in soils with pH(KCl) ranging from 5.5 to 7 (Dickinson *et al*., 2010). It is not as drought-resistant as *Cenchrus ciliaris* or *Panicum maximum*. *Chloris gayana* is more readily utilised for grazing than hay production (Dickinson *et al*., 2010). The grass can be established during the rainy season; however, October to November and February to March is the best time.

Chloris gayana is hypertolerant to high concentration of trace elements and poor nutrient status. *Chloris gayana* can grow in high salinity mediums with low pH, making them adequate to establish (Truong, 1999).

1.2.14.4 *Pennisetum glaucum*

Pennisetum glaucum (Pearl millet) has a relatively fast-growing deep root system that enables the scavenging of residual nutrients (Dickinson *et al*., 2010; Anash *et al*., 2010). *Pennisetum glaucum* is an upright annual tufted grass and usually has only one stem which can grow to a height of more than 3 000 mm (Dickinson *et al*., 2010). *Pennisetum glaucum* has a firm stem from which the leaves grow that can be up to 50 mm broad and 1 000 mm long. The inflorescence comprises a dense spike (Dickinson *et al*., 2010; Heuzé *et al*., 2014). *Pennisetum glaucum* can grow in a wide variety of soil and can survive in acidic soils with low fertility and has been proven to be drought resistant (Sheahan, 2014; Dickinson *et al*., 2010). Even though *P. glaucum* can grow in various soil, and it is said that the grass performs better in soils with less structure and flourish in high temperatures (Dickinson *et al*., 2010). Sheahan (2014) stated that *P. glaucum* could accumulate toxic nitrate levels in the lower part of their shoots (150 mm). Its high vegetative vigour, root dry matter, and root density make it well-suited for loosening compacted soil, which is a fundamental constraint in rehabilitation practices (Sheahan, 2014; Anash *et al*., 2010).

1.2.14.5 *Digitaria eriantha*

Digitaria eriantha is a perennial, tufted grass that grows in undisturbed fields, with gravelly and sandy soils with high rainfall (Van Oudtshoorn, 2014).

The branched stalks can grow to a height of 1 200 mm with inflorescence, including 6 to 10 finger-shaped clusters that can be 130 mm long (Dickinson *et al*., 2010). Only the base of the leaves are hairy, and can grow up to 13 mm wide and 600 mm long (Dickinson *et al*., 2010).

Digitaria eriantha has both rhizomes and stolons and grows in the undisturbed veld. When *D. eriantha* dominates in an area, it indicates good veld condition. It responds well to fertiliser and can endure heavy grazing for prolonged periods (Dickinson *et al*., 2010; Mentis, 2005; Mentis, 2019).

Digitaria eriantha has an extensive root system and can grow up to 2 meters in depth, depending on the soil conditions, making it extremely drought-resistant (Mosebi *et al*., 2018). It is a palatable grass and is considered one of the best cultivated and natural pastures. *Digitaria eriantha* can grow in various soil conditions, including medium and low potential soils. The grass thrives in regions with an annual rainfall of 500 mm and higher. The grass can grow in shallow stony, and clayey soils. It is a pasture grass utilised from mid-summer and keeps its palatability well into winter, making it excellent as fodder. The seeds can be sown throughout the summer months; however, November, January and February are the best (Dickinson *et al*., 2010).

1.2.14.6 *Eragrostis tef*

Eragrostis tef is a tufted annual grass that grows in different soil types and disturbed areas *and* is especially suitable for hay and is used as fodder for horses. It is widely used in mine rehabilitation practices along with *Eragrostis curvula* and *Cynodon dactylon*, because it establishes rapidly (Van Oudtshoorn, 2014; Mentis, 2020).).

Eragrostis tef has delicate leaves and slender stems and can grow up to 900 mm long. It is a summer-growing annual grass, and the inflorescence has compacted open panicles (Dickinson *et al*., 2010). *E. tef* can be grown in a wide variety of soil, from sand to turf; however, not in waterlogged soil conditions. *Eragrostis tef* can grow in regions with rainfall as low as 400 mm per annum and is usually sown after November (Dickinson *et al*., 2010). It is exotic and often occurs in disturbed areas and has become famous for the temporary re-vegetation of exposed growth mediums (Van Oudtshoorn, 2014).

1.2.14.7 *Medicago sativa*

Medicago sativa is a perennial flowering plant that is mainly utilised for its characteristics as a nitrogen-fixing legume (Hopkins and Hürner, 2008; Dickinson *et al*., 2010). It resembles clover, has purple flowers and grows in deep, well-drained soil due to its deep penetrating roots.

It can fix nitrogen in the soil by employing symbiosis with bacteria, like *rhizobia*, rendering the nitrogen plant soluble. It is usually planted along with grasses on rehabilitated areas for this reason and because it enriches the soil in terms of nutrient status (Dickinson *et al*., 2010; Donaldson, 2001; Mentis, 2005; Mentis, 2019; Mentis, 2020). *M. sativa* yields high-quality and large volumes of protein-rich hay, making it commercially viable. It is difficult to achieve a 50:50 grass-lucerne mix, and it can cause bloat in animals (Donaldson, 2001).

Legumes, part of the botanical family Leguminosae, are an essential plant group used in agriculture (Dickinson *et al*., 2010). Legumes can draw nitrogen from the air and make it plant available through symbiotic relationships with nodular bacteria.

1.2.15 Pasture Quality

Near-Infrared Reflectance Spectroscopy (NIRS) has recently been employed to determine pasture quality. The NIRS can predict protein content, neutral detergent fibre (NDF), and acid detergent fibre (ADF), amongst other. The NDF predicts animal intake, and the higher the NDF level, the lower the animal intake. The ADF is a factor of digestibility and lower ADF levels indicate higher digestibility. Differences between different forage species are presented in Figure 1-11 (Hoffman *et al*., 2008). Legumes tend to have lower NDF % and lower NDF digestibility compared to grasses due to greater lignification associated with legumes (Hoffman *et al*., 2008). Pasture species has a wide range of NDF digestibility because of their diversity and because they are utilised at different maturity stages.

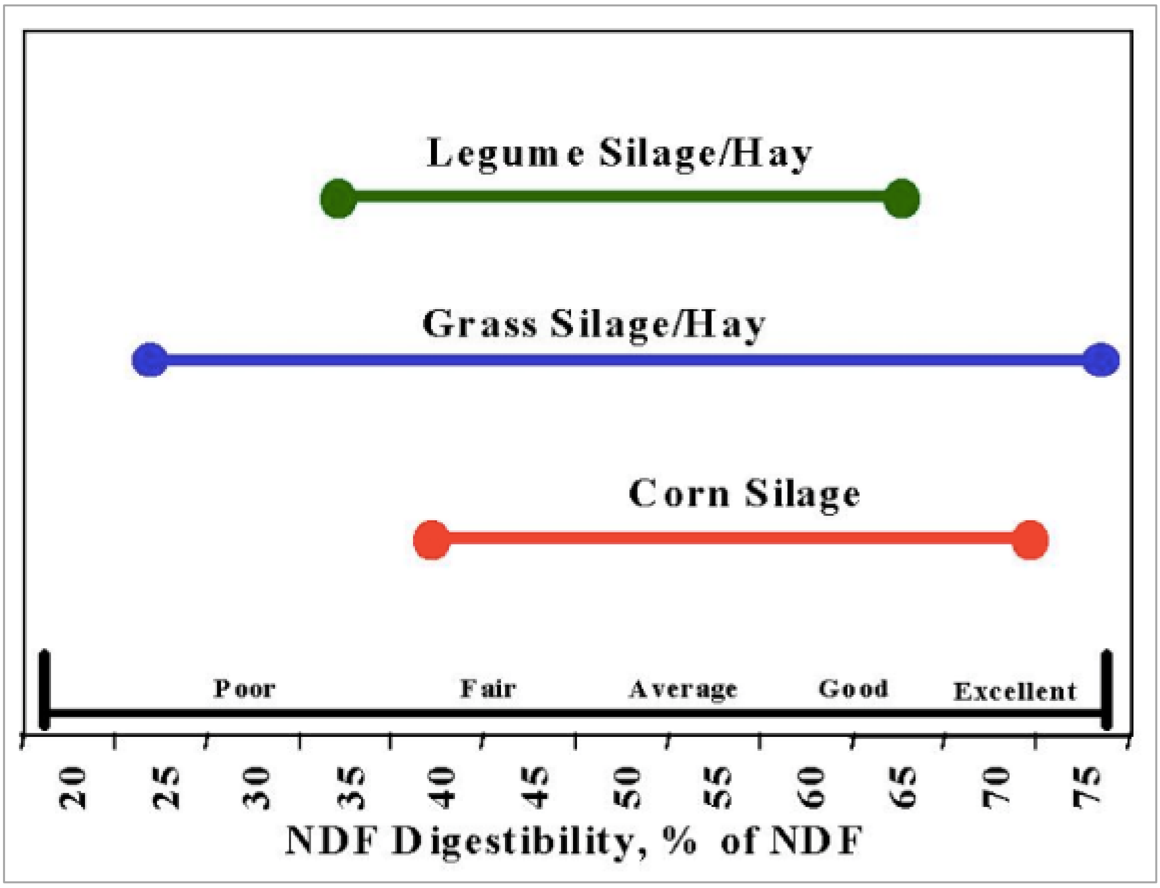


Figure 1-11: NDF digestibility of forage (Hoffman *et al*., 2008)

Acid detergent fibre predicts the energy content of the pasture species. Acid detergent fibre is a good indicator of feed quality; higher values suggest lower-quality feed (Van Saun, 2013; Hoffman *et al*., 2008). A goal would be to have ADF below 35% in pasture species (Van Saun, 2013).

Traditional methods for forage quality analysis are costly, time-consuming and technically demanding. Total nitrogen and ADF contents vary within a growing season and require constant evaluation. All the parameters that are measured for forage quality are essential for the controlled feeding of animals. Numerous methods have been developed to estimate the digestible nutrient content of the forage. Near-Infrared Reflectance Spectroscopy has been investigated and identified as a method for evaluating the chemical composition of forages by several authors (Arzani *et al*., 2015; Aiken *et al*., 2005; Zhang *et al*., 2018).

Contrasting to most conventional analytical methods, NIRS is quick and non-destructive. No chemicals are used, and no waste is generated as part of the analysing process (Zhang *et al*., 2018; Aiken *et al*., 2005).

A recent study focused on the accuracy of NIRS results compared to the conventional chemical analysis and found that the average N compositions of roughly 171 samples were 1.54 and 1.50% by NIRS determination and chemical determination, respectively (Arzani *et al*., 2015). The study also noted that the average ADF composition varied from 42.14% with the NIRS to 44.72% with the chemical determination (Arzani *et al*., 2015). The following table (Table 1-8) summarises the comparison results between NIRS and chemical methods.

Table 1-8: Overall mean and range of nitrogen and ADF concentrations analysed by NIRS and conventional chemical methods for forage grasses (Arzani *et al*., 2015).

Variable (concentration as % dry weight)	Mean		Range	
	NIRS	Chemical	NIRS	Chemical
N	1.54	1.50	0.31 - 4.19	0.33 - 4.17
ADF	42.14	44.72	26.25 - 50.9	24.77 - 61.84

1.3 Concluding summary

It is evident that topsoil for rehabilitation practices is a scarce resource and that alternative materials should be investigated like phytoremediation to render the substrate a viable growth medium. Coal refuse material, including discard and spoils, poses significant threats to the receiving environment, and effective stabilisation of these materials with vegetation is warranted. To successfully rehabilitate a medium, the chemical and certain physical properties need to be quantified, after which the appropriate plant species should be selected. The selection process should consider the chemical and physical results as well as past success in terms of vegetation establishment in similar growth mediums. Pasture species will be adequate for phytoremediation of contaminated growth mediums, because of numerous characteristics like, high biomass production, tolerance to drought, economic uses and documented hyperaccumulation potential. After a growth season, the concentrations of trace elements in the plants will give an indication of the plant’s ability to accumulate and ultimately phytoremediate the substrate. The chemical properties after a growth season should also be evaluated to determine whether the selected plants improved the growth medium and could potentially render it non-polluting and harmless in the future. The pasture quality of the plant species will also have to be evaluated in conjunction with the metal concentrations, with a focus on financially viable post-closure land-uses. The following study will investigate the unknowns above and identify shortcomings that should be considered for future research.

1.4 Problem Statement

Regarding rehabilitation, there is a knowledge gap in a consolidated conceptualisation of the chemical and physical constraints associated with coal spoils and coal contaminated soils. Previous studies on coal mines only focused on isolated constituents, and there exists a need to link all the constraints to derive a holistic solution.

Environmental contamination with trace elements has become a global problem affecting soil biomass, fertility, crop yields and has contributed to the biomagnifications or bioaccumulation in the food chain (Gratão *et al*., 2005).

In-situ and *ex-situ* soil decontamination techniques have been investigated and utilised in the industry for the past few years. Due to the array of different contaminants, the decontamination and rehabilitation processes are arduous and costly. A need exists for effective soil remediation for beneficial alternative land uses of mined areas or areas affected by coal mines.

1.5 Aims and Objectives

The aim is to quantify the magnitude of phytoremediation and hyperaccumulation of CoPCs, within selected pasture species. The quantification will enable effective remediation of coal spoils and soils contaminated by acid mine drainage (AMD), to sustain long-term plant growth, effective and sustainable rehabilitation, and potential post-closure land uses.

The objectives relevant to this study are to:

- identify the substrate disturbances associated with coal mines in South Africa;
- quantify the chemical and physical properties of coal spoils, and AMD-contaminated soils and assess the results against other growth mediums;
- determine the initial concentrations of the CoPC in the growth mediums;
- select the appropriate plant species to use that can also be used for alternative land uses, post-closure like forage, essential oil production etc.;
- quantify the CoPC concentrations in the plant species and substrates after a growing season;

- compare concentrations in the substrate before and after growing season along with concentrations in plants to calculate a mass balance; and
- determine which plants can be seen as outliers in terms of hyperaccumulation by comparing absorbed concentrations within each plant type.

1.6 Research Questions

The following research questions were identified:

1. Which plant species are the best hyperaccumulators of the CoPCs associated with coal mining?
2. Can plant species that have hyperaccumulation properties effectively ameliorate coal-contaminated soils and coal discard to potentially sustain long-term plant growth?
3. How do the chemical and physical properties of the growth mediums affect plant growth?
4. How do vegetation performance and plant growth differ between the growth mediums?
5. Which plant species can ameliorate the substrate and have a viable potential post-closure land use/ tertiary use?
6. Can a mass balance be calculated from the results obtained from the concentrations in the grass or selected plant species and the substrate?

1.7 Hypothesis

It can be hypothesised that if a growth medium or coal mine substrate, which serves as a growth medium, has a high concentration of a certain CoPC, the plant species will mimic the high CoPC concentration to prove their phytoremediation potential. It is also envisioned that the calculated mass balance of the concentrations of the CoPCs within the substrate before planting and that within the plant and substrate after harvesting will be balanced.

1.8 Dissertation structure and content

This dissertation provides a good understanding of constraints associated with the rehabilitation and restoration of coal mines and linked environmental impacts, especially on soil, water, and plants. It also describes the chemical constituents associated with coal ore and how certain plant species can remediate a coal-contaminated growth medium through numerous remediation methods.

Chapter 1: This chapter provides a literature review of all the different facets of the project. This chapter elaborates on all the impacts and constraints associated with the rehabilitation of coal mines, the constituents (CoPC) associated with coal deposits, and general rehabilitation practices. Phytoremediation types are explained in detail as well as the bioavailability of trace elements and plant responses to stressors. The phytoremediation capabilities of the different grass species are discussed in-depth, and outliers in terms of hyperaccumulators are identified.

Chapter 2: This chapter gives an overview of the different macro- and micronutrients also known as trace elements. This chapter also investigates the physical and chemical properties of the four growth mediums that were assessed in detail. The results and discussions elaborate on the different CoPC identified in the growth mediums and compare them to plant available concentrations.

Chapter 3: Chapter 3 explains the impact of the potential use of grass planted in contaminated growth mediums as a source of forage. The effect of the elevated levels of trace elements, associated with coal mines in the Highveld on forage quality is also discussed. An in-depth overview of the selected pasture species and the associated selection method is contained in this chapter, as well as species performance in terms of the specified variables that were measured during the project.

Chapter 4: Chapter 4 discusses the different types of phytoremediation and the benefits and constraints associated with each. The chapter also elaborates on the phytoremediation and phytomining capabilities of the plant species.

Chapter 5: This chapter includes concepts that were considered and the knowledge gaps encountered during the setup and implementation of the experimental component of this study. This chapter is the concluding chapter encompassing the concluding remarks based on the experiment and recommendations. A philosophical component as to where and how phytoremediation can be applied to ameliorate third-world problems is also included in this chapter.

1.9 References

Aiken, G.E., Pote, D.H., Tabler, S.F., and Tabler, T.C. 2005. Application of near-infrared reflectance spectroscopy to estimate chemical constituents in broiler litter. Communications in Soil Science and Plant Analysis, 36(17-18): 2529-2539. DOI: https://doi.org/10.1080/00103620500257028.

Akabzaa, T.M., Armah, T.E.K., and Baneong-Yakubo, B.K. 2007. Prediction of acid mine drainage generation potential in selected mines in the Ashanti metallogenic belt using static geochemical methods. Environmental Geology, 52(5): 957-964. DOI: https://doi.org/10.1007/s00254-006-0537-1.

Akinyeye, R.O., and Omoniyi, P., 2016. Comparative chemical and trace element composition of coal samples from Nigeria and South Africa. American Journal of Innovative Research and Applied Sciences. *https://american-jiras. com/Akinyeye% 20ManuscritRef*. (accessed 10 October 2022).

Anash, T., Osafo, E.L.K. and Hansen, H.,H. 2010. Herbage yield and chemical composition of four varieties of Napier grass (Pennisetum purpureum) grass harvested at three different days after planting. Agricultural and Biological Journal of NOrth America, 1(5): 923–929. DOI: http://scihub. org/ABJNA/PDF/2010/5/ABJNA-1-5-923-929.

Arzani ,H., Sanaei, A., Barker, A.V., Ghafario, S. and Motamedir, J. 2015. Estimating nitrogen and acid detergent fiber contents of grass species using near infrared reflectance spectroscopy (NIRS). Journal for Rangeland Science, 5(4): 260-268.

Aucamp, P. and van Schalkwyk, A. 2003. Trace element pollution of soils by abandoned gold mine tailings, near Potchefstroom, South Africa.Bulletin of Engineering Geology and the Environment, 62(2): 123-134. DOI: https://doi.org/10.1007/s10064-002-0179-9.

Audet, P. and Charest, C. 2008. Allocation plasticity and plant metal partitioning: meta-analytical perspectives in phytoremediation. Environmental Pollution, 156(2): 290 – 296. DOI: https://doi.org/10.1016/j.envpol.2008.02.010.

Australian Government, Department of Industry Innovation and Science, Department of Foreign Affairs and Trade. 2016. Mine rehabilitation: Leading practice sustainable development program for the mining industry. Leading practice sustainability program for the mining industry: Canberra, Australia.

Awad, Y.M., Kim, S.C. El-Azeem, A., Samy, A.M., Kim, K.H., Kim, K.R., Kim, K., Jeon, C., Lee, S.S. and Ok, Y.S. 2014. Veterinary antibiotics contamination in water, sediment, and soil near a swine manure composting facility. Environmental Earth Sciences, 71(3): 1433-1440. DOI: https://doi.org/10.1007/s12665-013-2548-z.

Baeder-Bederski-Anteda, O. 2003. Phytovolatilisation of organic chemicals. Journal of Soils and Sediments, 3(2): 65-71. DOI: https://doi.org/10.1007/BF02991068.

Baker, A.J.M. and Brooks, R.R. 1989. Terrestrial higher plants which hyperaccumulate metallic elements. a review of their distribution, ecology and phytochemistry. Biorecovery, 1: 81-126. DOI: http://dx.doi.org/10.1080/01904168109362867.

Baligar, V.C., Fageria, N.K. and He, Z.L. 2001. Nutrient use efficiency in plants. Communications in Soil Science and Plant Analysis, 32(7-8): 921-950. DOI: https://doi.org/10.1081/CSS-100104098.

Balková, M., Kubalíková, L., Prokopová, M., Sedlák, P. and Bajer, A. 2021. Ecosystem services of vegetation features as the multifunction anti-erosion measures in the Czech Republic in 2019 and Its 30-Year Prediction. Agriculture 2021, 11(2): 105. DOI: https://doi.org/10.3390/agriculture11020105.

Barrutia, O., Epelde, L., Garcia-Plazaola, J.I., Garbisu, C. and Becerril, J.M. 2008. Phytoextraction potential of two *Rumex acestro L*. accession collected from metalliferous and non-metalliferous sites: Effect of fertilisation. Chemosphere, 74(2): 259-264. DOI: https://doi.org/10.1016/j.chemosphere.2008.09.036.

Bert, V., Bonnin, I., Saumitou-Laprade, P., De Laguérie, P. and Petit, D. 2002. Do Arabidopsis halleri from non-metallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? The New Phytologist, 146(2): 225-233. DOI: https://doi.org/10.1046/j.1469-8137.2000.00634.

Boyd, R.S. and Martens, S.N. 1992. The raison d'etre for metal hyperaccumulation in plants. In: Baker, A.J.M., Proctor, J., and Reeves, R.D., eds. The vegetation of ultramafic (Serpentine) soils, Andover, Hampshire: Intercept Ltd. p. 279–289.

Bradshaw, A.D. 1998. Restoration of mined lands – using natural processes. Ecological Engineering, 8(4): 255- 269. DOI: https://doi.org/10.1016/S0925-8574(97)00022-0.

Brady, K., Perry, E., Beam, R., Bisko, D., Gardner, M.D. and Tarantino, J.M. 1994. Evaluation of acid-base accounting to predict the quality of drainage at surface coal mines in Pennsylvania, USA. Journal American Society of Mining and Reclamation, 1: 138-147. DOI: 10.21000/JASMR94010138.

Brady, N.C. and Weil, R.R. 2008. The nature and properties of soil. 14th ed Upper Saddle River, NJ: Prentice Hall. ISBN: -013227938.

Bray, E.A., Bailey-Serres, J. and Weretilnyk, E. 2000. Responses to abiotic stresses. In: Buchanan, B.B., Gruissem, W. and Jones, R.L., eds. Biochemistry and molecular biology of plants, vol. 40. Rockville, MD: American Society of Plant Physiologists. p 1160.

British Petroleum Company [Internet]. BP statistical review of world energy. London: British Petroleum Co. [cited 2021 July 25]. Available from https://www.bp.com/en/global/corporate/energy-economics/statistical-review-of-world-energy.html.

Brooks, R., Anderson, C., Stewart, R. and Robinson, B. 1999. Phytomining: growing a crop of a metal. Biologis*t*, 46(5): 201- 205.

Cenkci, S., Cigerci, I.H., Yildiz, M., Özay, C., Bozdag, A. and Terzi, H. 2010. Lead contamination reduces chlorophyll biosynthesis and genomic template stability in Brassica rapa L. Environmental and Experimental Botany. 67(3): 467-473.

Chamber of Mines of South Africa. 1979. Handbook of Guidelines for Environmental Protection, Volume 2/1979: The Vegetation of Residue Deposits against Water and Wind Erosion. July 1979.

Chibuike, G.U. and Obiora, S.C. 2014. Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods. Applied and Environmental Soil Science. 2014: 1-12.

Chuan, N.C., Rahman, M., Boyce, A.N. and Bin Abas, M.R. 2016. Heavy metals phyto-assessment in commonly grown vegetables: water spinach (I. aquatica) and okra (A.

esculentus). SpringerPlus. 5(1): 1-9. DOI: https://doi.org/10.1186/s40064-016-2125-5.

Cowan, A.K., Lodewijks, H.M., Sekhohola, L.M., Edeki, O.G. 2016. In situ bioremediation of South African coal discard dumps. Paper presented at the Mine Closure 11th International Conference on Mine Closure; 2016, March15-17; Perth: Australia.

Davies, R., Hodgkinson, R., and Chapman, R. 1995. Nitrogen loss from a soil restored after surface mining. Journal of Environmental Quality. 24(6): 1215-1222.

De Kok, L,J., Castro, A., Durenkamp, M., Koralewska, A., Posthumus, F,S., Stuiver, C.E., Yang, L. and Stulen, I. 2005. Pathways of plant sulphur uptake and metabolism - an overview. Landbauforschung Völkenrode, Special Issue, 283: 5-13.

Dickinson, E.B., Hyam, G.F.S., Breytenbach, W.A.S., Metcalf, W.D., Basson, W.D., Williams, F.R., Scheepers, L.J., Plint, A.P., Smith, H.R.H., Smith, P.J., van Vuuren, P.J., Viljoen, J.H., Archibald, K.P., and Els, J.N. 2010. Kynoch pasture handbook. Maanhaarrand: Kejafa Knowledge Works.

Donaldson, C.H. 2001. A Practical Guide to Planted Pastures. Kalbas Publishers.

Dung, T.T.T., Cappuyns, V., Swennen, R. and Phung, N.K. 2013. From geochemical background determination to pollution assessment of heavy metals in sediments and soils. Reviews in Environmental Science and Bio/Technology. 12(4): 335-353.

Durkin, T.V., and Herrmann, J.G. 1994. Focusing on the Problem of Mining Wastes: An Introduction to Acid Mine Drainage. In: Managing Environmental Problems at Inactive and Abandoned Mine Sites. USEPA Seminar Publication. p. 1-3.

Dushenkov, V., Kumar, P., Motto, H. and Raskin, I. 1995. Rhizofiltration: The Use of Plants to Remove Heavy Metals from Aqueous Streams. Environmental Science and Technology. 29(5): 1239-1245. DOI: 1239-45. 10.1021/es00005a015.

Egan, D. Hjerpe, E.E. and Abrams, J. 2011. Human Dimensions of Ecological Restoration: Integrating Science, Nature and Culture. Society for Ecological Restoration. Washington: Island Press.

Ernst, W.H.O., Krauss, G.J., Verkleij, J.A.C., and Wesenberg, D. 2008. Interaction of heavy metals with deregulation, and higher gene copy number for genes in metal homeostasis of the hyperaccumulator populations of the metallophyte Thlaspi caerulescens J. and C. Presl (Brassicaceae). New Phytologist. 127:61–68.

Fageria, N.K., Moraes, O.P., and Vasconcelos, M.J. 2011. Yield and yield components of upland rice as influenced by nitrogen sources. Journal of Plant Nutrition. 34(3): 361-370.

Fairweather-Tait, S., Hurrell, R., Dael, P., Dokkum, W., Ducros, V., Cashman, K., Flynn, T.A., Alegría, A., Barbéra, R., Farré, R., Lagarda, M., Macleod, F., McGaw, B.A. and Sandström, B. 1996. Bioavailability of Minerals and Trace Elements: Members of EC flair concerted action no. 10: Measurements of micronutrient absorption and status. Nutrition Research Reviews. 9(1): 295-32. DOI: 10.1079/NRR19960016.

Fanourakis, G.C. 1991. *Engineering soil properties from pedological data*. L Tech Thesis: Technicon Witwatersrand.

Ferguson, K.D. and Erickson P.M. 1988. Pre-Mine Prediction of Acid Mine Drainage. In: Salomons, W. and Forstner. U., eds. Dredged Material and Mine Tailings. Berlin Heidelberg: Springer-Verlag.

Finkelman, R.B. 1993. Trace and Minor Elements in Coal. In: Engel, M.H. and Macko, S.A., eds. Organic Geochemistry. Topics in Geobiology, vol 11. Boston, MA.: Springer. DOI: https://doi.org/10.1007/978-1-4615-2890-6_28.

Fourie, A.B. 2007. The engineering contribution of vegetation to the stability of cover systems. In Mine Closure 2007. Santiago, Chile: Salviat Impresores. 1st Ed. p. 483-494.

Freeman, J.L, Quinn, C.F., Marcus, M.A., Fakra, S. and Pilon-Smits, E.A.H. 2006. Selenium-tolerant diamondback moth disarms hyperaccumulator plant defence. Current Biology. 16(22): 2181-2192.

FSSA (Fertilizer Society of South Africa). 2007. Fertilizer Handbook. Lynwood Ridge, Pretoria, South Africa: The Fertilizer Society of South Africa.

García-Salgado, S., García-Casillas, D., Quijano-Nieto, A. and Bonilla-Simón, M. 2012. Arsenic and heavy metal uptake and accumulation in native plant species from soils polluted by mining activities. Water, Air and Soil Pollution. 223: 559-572.

Gayatri, N., Sailesh, A.R. and Srinivas, N. 2019. Phytoremediation potential of Brassica juncea for removal of selected heavy metals in urban soil amended with cow dung. Journal of Materials and Environmental Sciences. 10(5): 463-469.

Gentili, R., Ambrosini, R., Montagnani, C., Caronni, S. and Citterio, S. 2018. Effect of Soil pH on the Growth, Reproductive Investment and Pollen Allergenicity of Ambrosia artemisiifolia L. Frontiers in Plant Science, 9: 1335.

Ghnaya, A.B., Charles, G., Hourmant, A., Hamita, J.B. and Branchard, M. 2009. Physiological behaviour of four rapeseed cultivar (Brassica napus L.) submitted to metal stress. Comptes Rendus Biologies, 332(4): 363-370.

Gratão, P.L., Prasad, M.N.V., Cardoso, P.F., Lea, P.J. and Azevedo, R.A. 2005. Phytoremediation: Green technology for the cleanup of toxic metals in the environment. Plant Physiology.17(1): 53- 64.

Greipsson, S. 2011. Phytoremediation. Nature Education Knowledge. 3(10): 7.

Grimshaw, S. 2007. Planning for closure as part of project approval. In: Mine Closure 2007. 1st Ed, Santiago, Chile: Salviat Impresores. p. 289-300.

Gull, A., Lone, A.A. and Wani, N.U.I. 2019. Biotic and Abiotic Stresses in Plants. In: De Oliveira, A., ed. Abiotic and biotic stress in plants. London: Intech Open. DOI: 10.5772/intechopen.85832.

Haagner, A.S.H. The role of vegetation in characterising landscape function on rehabilitating gold tailings [dissertation]. Potchefstroom: North-West University; 2008.

Hancox, J.P. and Gotz, A.E. 2014. South Africa's coalfields - a 2014 perspective. International Journal of Coal Geology. 132(1): 170-254.

Harmse, H.J von M and Hattingh, J.M. 1985. Pedological profile classes. In: Brink, A.B.A., ed. Engineering geology of Southern Africa, Volume 4, Pretoria, South Africa: Building Publications.

Hattingh, J.M. and Van Deventer, P.W. 2004. The effect of the chemical properties of tailings and water application on the establishment of a vegetative cover on gold tailings dams. Water Research Commission Report No. 899/1/04. Pretoria.

Hattingh, R., Tanner, P. and Aken, M. 2019. Land Rehabilitation Guidelines for Surface Coal Mines. Land Rehabilitation Society of Southern Africa (LaRSSA), Coaltech Research Association and the Minerals Council of South Africa.

Hazrat, A., Ezzat, K and Muhammad, A.S. 2013. Phytoremediation of heavy metals-concepts and applications. Chemosphere. 91(7): 869-881. DOI: 10.1016/j.chemosphere.2013.01.075.

Herselman, J.E. The concentration of selected trace elements in South African soils [thesis]. Stellenbosch: University of Stellenbosch; 2007.

Heuzé V., Tran G., Boval M [Internet]. Kikuyu (Pennisetum clandestinum). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO; [updated 2015 July 30; cited 2022 June 15]. Available from: https://www.feedipedia.org/node/398.

Heuzé V., Tran G., Giger-Reverdin S., Lebas F [Internet]. Elephant grass (Pennisetum purpureum). Feedipedia, a programme by INRA, CIRAD, AFZ and FAO. [updated 2016 July 23; cited 2022 June 15]. Available from: https://www.feedipedia.org/node/395.

Heuzé, V. and Tran, G [Internet]. Pearl millet (Pennisetum glaucum), grain. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO; [updated 2015 Sept 30; cited 2022 June 15]. Available from: https://feedipedia.org/node/724.

Hickey, M. and King, C. 2000. The Cambridge Illustrated Glossary of Botanical Terms. 1st Ed. Cambridge, United Kingdom: Cambridge University Press. 208 pages. ISBN: 9780521794015.

Hoffman, P.C., Shaver, R.D., Combs, D.K., Undersander, D.J., Bauman, L.M. and Seeger, T.K. 2008. Understanding NDF Digestibility of Forages. Focus on Forage. 3(10): 1-3.

Hopkins, W.G. and Hürner, N.P.A. 2008. Introduction to plant physiology. 4th ed. New York, USA: Wiley Publishers. p. 503.

Hunt, A.J., Christopher, W.N., Bruce, N., Muñoz, A., Graedel, T.E., Hodson, M., Meech, J., Nassar, N., Parker, H.M., Rylott, E.L., Sotiriou, K., Zhang, Q. and Clark, J. 2014. Phytoextraction as a tool for green chemistry. Green Processing and Synthesis. 3(1): 3-22. DOI: 10.1515/gps-2013-0103.

Jadia, C.D. and Fulekar, M.H. 2009. Phytoremediation of heavy metals: recent techniques. African Journal of Biotechnology. 8(6): 921-928.

John, D.A. and Leventhal, J.S. 1995. Bioavailability of Metals. Preliminary compilation of descriptive geoenvironmental mineral deposit models. In: du Bray, E.A., ed. Preliminary compilation of descriptive geoenvironmental mineral deposit models. U.S. Geological Survey: U.S. Department of Interior. p.10-18.

Johnson, M.R., Van Vuuren, C.J., Visser, J.N.J., Cole, D.I., Wickens, H. de V., Christie, A.D.M., Roberts, D.L. and Brandl, G. 2006. Sedimentary rocks of the Karoo Supergroup. In: Johnson, M.R., Anhaeusser, C.R. and Thomas, R.J., eds. *The* Geology of South Africa. Pretoria: Geological Society of South Africa, Johannesburg/ Council for Geoscience, p. 461-499.

Kabata-Pendias, A. 2011. Trace elements in soils and plants. 4th ed. Boca Raton: Taylor and Francis. p. 432.

Kadukova, J. and Kavuličova, J. 2011. Phytoremediation of Heavy Metal Contaminated Soils - Plant Stress Assessment. Handbook of Phytoremediation. ISBN: 978-1-61728-753-4.

Keeling, S.M. and Werren, G. 2005. Phytoremediation: The Uptake of Metals and Metalloids by Rhodes Grass Grown on Metal-Contaminated Soil. Remediation Journal: The Journal of Environmental Cleanup Costs, Technologies and Techniques. 15(2): 53-61.

Kellner, K. Rangeland Degradation and Restoration: A Land Care Approach. Part 2: The Concept of Rangeland Restoration. Paper presented at the Landcare Conference; 2015 Sept 5-8, Potchefstroom.

Krämer, U. 2010. Metal hyperaccumulation in plants. Annual Review of Plant Biology. 61(1): 517-534. DOI: 10.1146/annurev-arplant-042809-112156.

Kuyucak, N. 1999. Acid Mine Drainage Prevention and Control Options. International Mine Water Association 2012. CIM bulletin, p.96-102.

Lange, C.A., Kotte, K., Smit, M., van Deventer, P.W. and van Rensburg, L. 2012. Effects of different soil ameliorants on karee trees (Searsia lancea) growing on mine tailings dump soil - part 1: pot trails. International Journal of Phytoremediation. 14(9): 908-924.

Larcher, W. 1987. Streß bei Pflanzen. Naturwissenschaften. 74(4): 158-167.

Lasat, M.M. 2000. Phytoextraction of Metals from Contaminated Soil: A Review of Plant/Soil/Metal Interaction and Assessment of Pertinent Agronomic Issues. Journal of Hazardous Substance Research, 2(5):1-25.

Leita, L., Nobili, M., Mühlbachová, G., Mondini, C., Marchiol, L. and Zerbi, G. 1995. Bioavailability and Effects of Heavy Metals on Soil Microbial Biomass Survival during Laboratory Incubation. Biology and Fertility of Soils. 19(2): 103-108. DOI: 10.1007/BF00336144.

Lichtenthaler, H.K. 1998. The stress concept in plants: an introduction. Annals of the New York Academy of Sciences. 851: 187-198

Limpitlaw, D. and Briel, A. 2015. Post-mining land use opportunities in developing countries- a review. The Journal of The Southern African Institute of Mining and Metallurgy. 114: 1-5.

Longley, K. The Feasibility of Poplars for Phytoremediation of TCE Contaminated Groundwater: A Cost-Effective And Natural Alternative Means Of Groundwater Treatment [dissertation]. Olympia, Washington, USA: Evergreen State College; 2022.

Magonoro, F.A., Gumbo, J.R., Chigayo, K., Dacosta, F.A. and Mojapelo, P. 2011. Bioaccumulation of Toxic Metals by Hyparrhenia Grass Species: A Case Study of New Union Gold Mine Tailings and Makhado Town, Limpopo, South Africa. International Mine Water Conference 2011. Department of Chemistry; University of Venda.

McCarthy T.S. 2011. The impact of acid mine drainage in South Africa. South African Journal of Science. 107(5): 1-7. DOI: 10.4102/sajs.v107i5/6.712.

McGrath, S.P. and Zhao, F.J. 2003. Phytoextraction of metals and metalloids from contaminated soils. Current Opinion in Biotechnology. 14(3): 277-282.

Mekonen, A. 2000. Erosion control in agricultural areas An Ethiopian perspective, GTZ International Food security Program: Ethiopia.

Mendez, M.O. and Maier, R.M. 2008. Phytoremediation of mine tailings in temperate and arid environments. Review of Environmental Sciences of Biotechnologies. 7: 47-59.

Mengel, K. and Kirkby, E.A. 2001. Principles of Plant Nutrition. 5th Ed. Dordrecht: Kluwer Academic Publishers. 849 pp.

Mentis, M. 2019. Environmental Rehabilitation Guide for South Africa. Cape Town: Quickfox Publishing. 205 pp.

Mentis, M. 2020. Environmental rehabilitation of damaged land. Forest Ecosystems. 7(1): 1-16. DOI: https://doi.org/10.1186/s40663-020-00233-4.

Mentis, M.T. 1999. Diagnosis of the Rehabilitation of Opencast Coal Mines on the Highveld of South Africa. South African Journal of Science. 95(5): 210-217.

Mentis, M.T. 2005. Restoring native grassland on land disturbed by coal mining on the Eastern Highveld of South Africa. South African Journal of Science. 102(5): 193-197.

Meyer, T., Kellner, K. and Viljoen, C. 2003. Land transformation and soil quality. In Magold, S., Kalule-Sabiti, M., eds. North West province state of the environment report: overview 2002. Mafikeng: Department of Agriculture, Conservation and Environment, Northwest Province.

Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S. and Gupta, D.K. 2006. Lead detoxification by coontail (Ceratophyllum demersum L.) involves induction of phytochelatins and antioxidant system in response to its accumulation. Chemosphere. 65(6): 1027-1039.

Modabberi, S., Alizadegan, A., Mirnejad, H. and Esmaeilzadeh, E. 2013. Prediction of AMD generation potential in mining waste piles, in the Sarcheshmeh porphyry copper deposit, Iran. Environmental Monitoring and Assessment. 185(11): 9077-9087.

Moradi, A., Honarjoo, N., Najafi, P. and Fallahzade, J. 2016. A human health risk assessment of soil and crops contaminated by heavy metals in industrial regions, central Iran. Human and Ecological Risk Assessment. 22(1): 153-167.

Morgenthal, T., Maboeta, M. and van Rensburg, L. 2004. Revegetation of heavy metal contaminated mine dumps using locally serpentine-adapted grassland species. South African Journal of Botany.70(5): 784-789.

Mosebi, P.E., Truter, W.F. and Madakadze, I.C. 2018. Smuts Finger Grass (Digitaria Eriantha Cv Irene) Root Growth Assessment and Some Physicochemical Characteristics on Coal Mined Land Compacted Soil. Journal of Applied Science Environmental Management. 22(8): 1293-1296.

Muhammad, S., Sebastian, S., Sat Darshan, K., Weinbaum, S. W. and Patrick, B. 2017. Chapter 14 -1 - 14 Almond Tree Nutrition CABI Press. 10.1079/9781780643540.0000.

Mulugisi, G., Gumbo, J.R., Dacosta, F.A. and Muzerengi, C. The Use of Indigenous Grass Species as Part of Rehabilitation of Mine Tailings: A Case Study of New Union Gold Mine. Paper presented at the International Mine Water; 2009; Pretoria, South Africa.

Munnik, V. 2010. The Social and Environmental Consequences of Coal Mining in South Africa. Cape Town: Environmental Monitoring Group.

Myburgh, D. Development of guidelines for the management of groundwater in and around discard facilities [dissertation]. Bloemfontein: University of the Free State; 2017.

Neenu, S. and Karthika, K.S. 2019. Aluminium toxicity in soil and plants. Soil Health/Fertility Management: Knowledge - HARIT DHARA. 2(1): 15-19.

Nortcliff, S. 2001. Reclaimed Land-Erosion Control, Soils and Ecology. European Journal of Soil Science 52: 525-526. DOI: 10.1046/j.1365-2389.2001.00418-7.

Okedeyi, O., Dube, S., Awofolu, O. and Nindi, M. 2013. Assessing the enrichment of heavy metals in surface soil and plant (Digitaria eriantha) around coal-fired power plants in South Africa. Environmental Science and Pollution Research. 21(6): 4686-4696. DOI: 10.1007/s11356-013-2432-0.

Okereafor, G.U., Makhatha, M. E., Mekuto, L. and Mavumengwana, V. Assessing the effectiveness of Hyparrhenia hirta in the rehabilitation of the ecosystem of a gold mine dump. Paper presented at the 7th International Conference on Environment Pollution and Prevention (ICEPP 2019) 158; E3S Web of Conferences 158, 04004; 2020. DOI: https://doi.org/10.1051/e3sconf/202015804004.

Palmer, M.A., Allan, J.D., Meyer, J.L. and Bernhardt, E.S. 2007. River restoration in the twenty-first century: data and experiential future efforts. Restoration Ecology. 15: 472-481.

Pandey, V.C. and Maiti, D. 2020. Perennial grasses in phytoremediation-challenges and opportunities. Phytoremediation Potential of Perennial Grasses. p.1.

Paul, R., Mandal, A. and Datta, A. 2012. An updated overview on Cynodon dactylon (L.) Pers. International Journal of Research in Ayurveda and Pharmacy. 3(1): 11-14.

Paz-Alberto, A.M. and Sigua, G.C. 2013. Phytoremediation: A green Technology to Remove Environmental Pollutants. American Journal of Climate Change, 2(1): 71-86. DOI: 10.4236/ajcc.2013.21008.

Pivetz, B.E. 2001. Phytoremediation of Contaminated Soil and Groundwater at Hazardous Waste sites. EPA/540/S-01/500. Washington, USA: United States Environmental Protection Agency.

Pollard, J.A. and Baker, A.J. 1997. Deterrence of Herbivory by Zinc Hyperaccumulation in Thlaspicaerulescens. New Phytologist, 134(4): 655-658. DOI:10.1046/j.1469-8137.1997.00689.

Pone, J.D.N., Hein, K.A., Stracher, G.B., Annegarn, H.J., Finkleman, R.B., Blake, D.R., McCormack, J.K. and Schroeder, P., 2007. The spontaneous combustion of coal and its by-products in the Witbank and Sasolburg coalfields of South Africa. International Journal of Coal Geology. 72(2): 124-140. Prasad, M.N.V., Malec, P., Waloszek, A., Bojko, M. and Strzalka, K. 2001. Physiological responses of Lemna trisulca L. (duckweed) to cadmium and copper bioaccumulation. Plant Science, 161.

Qin, F., Ji, H., Li, Q., Guo, X., Tang, L. and Feng, J., 2014. Evaluation of trace elements and identification of pollution sources in particle size fractions of soil from iron ore areas along the Chao River. Journal of Geochemical Exploration. 138: 33-49.

Raskin, I., Nanda Kumar, P.B.A, Dushenkov, S. and Salt, D.E. 1994. Bioconcentration of Heavy Metals by Plants. Current Opinion in Biotechnology, 5(3): 285-290.

Reeves, R.D. and Baker, A.J.M. 2000. Metal-accumulating plants. In: Raskin, I. and Ensley, B.D. Phytoremediation of Toxic Metals-Using Plants to Clean Up the Environment, New York: Wiley, p. 193-229.

Renault, S., Sailerova, E. and Fedikow, M.A.F. 2000. Phytoremediation and phytomining in Manitoba: Preliminary observations from an orientation survey at the Central Manitoba (Au) minesite (NTS 52L/13). Report of Activities 2000, Manitoba Industry, Trade and Mines, Manitoba Geological Survey, p.179-188.

Rinaldo, J., Varsha, S., Meenu, G., Pardeep, K. and Ankit, S. 2017. Acid Mine Drainage: Sources, Impacts and Prevention [internet]. Environmental Biotechnology. [cited 2022 Aug 7]. Available from Biotech Articles: https://www.biotecharticles.com/Environmental-Biotechnology-Article/Acid-Mine-Drainage-Sources-Impacts-and-Prevention-3801.html.

Rodriguez, D.C. Grass-specific Mechanisms of Iron Uptake: Investigation of Phytosiderophore Transporters and Discovery of Novel Iron Deficiency Loci. [dissertation] Amherst, Massachusetts, USA: University of Massachusetts Amherst; 2018.

Rösner, T., Boer, R., Reyneke, R., Aucamp, P. and Vermaak, J. 2001. A preliminary assessment of pollution contained in the unsaturated and saturated zone beneath reclaimed gold mine residue deposits. WRC Report No. 797/1/01, Pretoria.

Rout, G., Samantaray, S. and Das, P. 2001. Aluminum toxicity in plants: a review. Agronomie. EDP Sciences. 21(1): 3-21. DOI: ff10.1051/agro:2001105ff. ffhal-00886101f.

Różanowski, B., Michałowski, M., Tora, B., Cablik, V. and Cernotova, L. 2012. Effectiveness of the use of willow tree (Salix viminalis) for wastewater treatment. AGH Journal of Mining and Geoengineering, 36(4):159-165.

RSA (The Republic of South Africa). 1983. Conservation of Agricultural Resources Act 43 of 1983 [Internet]. Pretoria: Government Printer. [cited 2022 Jan 23]. Available from South African Government: https://www.gov.za/documents/conservation-agricultural-resources-act-1-apr-2015-0926.

RSA (The Republic of South Africa). 2002. Mineral and Petroleum Resources Development Act, 28 of 2002 [Internet]. Pretoria: Government Printer. [cited 2022 Jan 23]. Available from South African Government: https://www.gov.za/documents/mineral-and-petroleum-resources-development-act.

RSA (The Republic of South Africa).1998. National Environmental Act 107 of 1998 [Internet]. Pretoria: Government Printer. [cited 2022 Jan 23]. Available from South African Government: https://www.gov.za/documents/national-environmental-management-act.

Ruiz-Jaen, M. and Aide, T.M. 2005. Restoration Success: How Is It Being Measured?. Restoration Ecology. 13(3): 569-577. DOI: 10.1111/j.1526-100X.2005.00072.x.

Sabrina, D.T., Hanafi, M.M., Gandahi, A.W., Mohamed, M.T.M. and Aziz, N.A.A. 2013. Effect of mixed organic-inorganic fertilizer on growth and phosphorus uptake of setaria grass (*Setaria splendida*). Australian Journal of Crop Science. 7 (1): 75-83.

Scotney, D.M. and McPhee, P.J. 1992. Soil Erosion and Conservation. In: van Oudtshoom, F.P., ed. Guide to Grasses of South Africa. South Africa: Briza Publikasies.

Selvaraj, K., Sevugaperumal, R. and Ramasubramanian, V. 2015. Phytoextraction: Using Brassica as a Hyper Accumulator. Biochemistry and Physiology. 4(3): 174.

SER (Society for Ecological Restoration). 2004. The SER International Primer on Ecological Restoration [internet]. International Science and Policy Working Group. [cited 2022 Jan 23] SER: Tucson: Arizona. Available from Society for Ecological Restoration: http//www.ser.org.

Shah, S.S., Houborg, R. and McCabe, M.F. 2017. Response of Chlorophyll, Caronoid and SPAD-502 Measurement to Salinity and Nutrient Stress in Wheat (*Triticum aestivum* L.). Agronomy. 7(61).

Sheahan, C.M. 2014. Plant guide for pearl millet (*Pennisetum glaucum)*. USDA-Natural Resources Conservation Service, Cape May Plant Materials Center, Cape Mya, NJ.

Singh, O. and Jain, R. 2004. Phytoremediation of toxic aromatic pollutants from soil. Applied Microbiology and Biotechnology. 63: 128-35. DOI: 10.1007/s00253-003-1425-1.

Smedley, P.L. and Kinningburgh, D.G. 2002. Source and Behaviour of Arsenic in Natural Waters. British Geological Survey, Wallingford, OX10 8BB, UK.

Sparks, D. L. 2003. Environmental Soil Chemistry. London: Academic Press.

Sreekanth, T.V.M., Nagajyothi, P.C., Lee, K.D. and Prasad, T.N.V.K.V. 2013. Occurrence, physiological responses and toxicity of nickel in plants. International Journal of Environmental Sciences and Technology. 10: 1129-1140.

Tanner, P. 2007. Guidelines for the Rehabilitation of Mined Land. Johannesburg, South Africa: Coaltech/Chamber of Mines of South Africa.

Tordoff, G.M., Baker, A.J.M. and Willis, A.J. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere. 41: 219-228.

Truong, P.N. 1994. Vetiver grass, its potential in the stabilisation and rehabilitation of degraded and saline lands. In: Squire, V.R. and Ayoub A.T., eds. Halophytes a resource for livestock and for rehabilitation of degraded land, Netherlands: Kluwer Academics Publisher.p. 293-296.

Truong, P.N. 1999. Vetiver Grass Technology for Mine Rehabilitation, Tech. Bull. No. 1999/2, PRVN / RDPB, Bangkok, Thailand.

Truong, P.N. and Baker, D. 1996. Vetiver grass for the stabilisation and rehabilitation of acid Sulphate soils. Proc. Second National Conference on Acid Sulphate Soils, Coffs Harbour, Australia.

Uddling, J., Gelang-Alfredsson, J., Piikki, K. and Pleijel, H. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynthesis research. 91: 37-46. DOI: 10.1007/s11120-006-9077-5.

USEPA (U.S. Environmental Protection Agency). 1994. Technical Document - Acid Mine Drainage Prediction. EPA 530-R-94-036 [internet] Washington, DC 20460; [cited 22 March 2022]. Available from: https://www.epa.gov/sites/default/files/2015-09/documents/amd.pdf.

USEPA (United States Environmental Protection Agency). 2000. Introduction to phytoremediation. Office of Research and Development. Report No. EPA/600/R-99/107 [internet]. Washington DC. [cited 15 Sept 2022]. Available from: https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NRMRL&dirEntryId=63433.

Van Deventer, P. W., Hattingh, J.M. and Harris, P.M. 2021. Agreenco Glossary: Environmental Rehabilitation of Mines. 1st Edt. Agreenco: Potchefstroom, South Africa.

Van Deventer, P.W., Hattingh, J. and Bloem, D. 2008. Some rehabilitation issues on Witwatersrand gold tailings dam. Civil Engineering. August: 25-29.

Van Oudtshoorn, F. 2014. Guide to grasses of Southern Africa. Pretoria: Briza Publications. 3rd Edition. ISBN: 978-1-920217-35-8.

Van Saun, R.J. 2013. Determining Forage Quality: Understanding Feed Analysis [internet]. [cited 3 Jan 2020]. Avalable from: https://extension.psu.edu/determining-forage-quality-understanding-feed-analysis.

Vardaki, C. and Kelepertsis, A. 1999. Environmental impact of heavy metals (Fe, Ni, Cr, Co) in soils waters and plants of Triada in Euboea from ultrabasic rocks and nickeliferous mineralisation. Environmental Geochemistry and Health, 21(3): 211-226.

Weaver. T.R., Fridell. P.P., Ospina, M.B., Brooker, R., Schenkel. M.K. and Scrase, A.M. 2019. Contamination assessment of mine infrastructure areas for closure and relinquishment: Hazelwood Coal Mine, Victoria, Australia. Australian Centre for Geomechanics: Perth Australia. ISBN 978-0-9876389-3-9.

Weiersbye, I.M. 2007. Global review and cost comparison of conventional and phyto-technologies for mine closure. In Mine Closure 2007. Santiago, Chile: Salviat Impresores. 1st Ed. p. 13-30.

Welsh, D., Bianco, A. and Roe, P. 2007. A risk assessment approach for comparing mine rehabilitation and closure options at coal mines in central Queensland, Australia. In: Mine Closure 2007. Santiago, Chile: Salviat Impresores. 1st Ed. p. 167-178.

Wescott, P. 2004. The Inaugural UNSW/ Mitsubishi Lecture: Dragline or Truck and Shovel? - Some technical and business considerations. Presented to the School of Mining Engineering: University of New South Wales.

White, P.J. and Pongrac, P. 2017. Heavy metal toxicity in plants. In:Shabala, S., ed. Plant Stress Physiology, 2nd Edn. Wallingford: CABI. p. 301-332.

Wu, Q., Wang, S., Thangavel, P., Li, Q., Zheng, H., Bai, J. and Qiu, R. 2011. Phytostabilization potential of Jatropha curcas L. in polymetallic acid mine tailings. International Journal of Phytoremediation. 13: 788-804.

Wuana, R.A., Adie, P., Abah, J. and Ejeh, M.A. 2013. Screening of Pearl Millet for Phytoextraction Potential in Soil Contaminated with Cadmium and Lead. International Journal of Science and Technology. 2: 1-4.

Zhang, J., Li, S., Lin, M., Yang, E. and Chen, X. 2018. A near-infrared reflectance spectroscopic method for the direct analysis of several fodder-related chemical components in drumstick (Moringaoleifera Lam.) leaves, Bioscience, Biotechnology, and Biochemistry. 82: 1-5. DOI: 10.1080/09168451.2018.1445519.

Chapter 2 Characteristics of spoils and contaminated soils associated with the Highveld coalfields

Schmidhuber[1], B.E and Truter, W.F[2]

[1]Centre for Environmental Studies, University of Pretoria

[2]Department of Plant and Soil Sciences, University of Pretoria

Abstract

The Witbank coalfields are one of the most critical coal-producing regions in South Africa and are also one of the most significant contributors to deteriorated water quality in the Mpumalanga province. The pollution of the environment by coal mines, especially when mining activities cease, is attributed to ineffective rehabilitation and unsuccessful vegetation establishment.

Chemical and physical properties of coal spoils (ESP) and soil contaminated with Acid Mine Drainage (AMD) (EAMD) obtained from a mine in the Witbank coalfields, along with two other growth mediums, were assessed. The other two growth mediums were agricultural soil sampled in the proximity of the mine (EAGRI) as a reference soil, and a silica or quartz sand (ESI) as a sterile reference. The focus was on the chemical properties as they are the most limiting factor in terms of vegetation establishment and can be ameliorated more easily than physical characteristics. Basic soil chemical analyses were conducted that included pH(KCl), pH(H_2O), electrical conductivity (EC) and nutrient status to inform the type and quantity of fertiliser and/ or soil conditioners that will be required prior to vegetation establishment. The growth medium samples were also analysed for particle size distribution (PSD), and total concentrations of a wide array of elements including Barium (Ba), Copper (Cu), Zinc (Zn) and Lead (Pb), to name a few, using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The identification of trace elements and corresponding concentrations aided in the selection of appropriate vegetation selection for phytoremediation or phytostabilisation.

The results of the ICP-MS analyses were compared to the national norms and standards or soil screening values (SSVs) for the remediation of contaminated land and soil quality published in the Government Gazette on 2 May 2014, by the South African Department of Environmental Affairs (DEA).

Based on the results, the growth mediums had deficient macronutrient concentrations and none of the growth mediums, including coal spoils and AMD contaminated soil were classified as contaminated soil according to the SSV values.

Keywords: *Trace elements, ICP-MS,* Particle Size Distribution*, Constituents of Potential Concern*

2.1 Introduction

Countless studies have been conducted on the AMD and acid-generating potential of coal refuse material and coal-contaminated soils (Modabberi *et al*., 2013; Rinaldo *et al*, 2017; USEPA, 1994; Akabzaa *et al*., 2007); however, based on a literature review, there is a knowledge gap in the South African coalfields and the associated trace elements and concentrations. Soil as a growing medium for plants is an important natural resource for sustainable development, and the geochemical and physical characteristics of soils have undergone significant changes due to anthropogenic activities. The geochemical properties of four different growth mediums were assessed, including soil nutrient status, basic soil analyses, total- and plant-available elements. One of the factors affecting vegetation growth is the soil textural class, and it was considered worthwhile to investigate the physical property, of the different growth mediums, to determine the effect on vegetation establishment. The four growth mediums included acid mine drainage (AMD) contaminated soils (EAMD), coal spoils (ESP), agricultural soil (EAGRI), and quartz (silica) sand (ESI).

Most studies pertaining to the rehabilitation of coal mines in South Africa focused on pH, AMD quality and AMD generation potential (Welsh *et al*., 2007; Hattingh *et al*., 2019; Mentis, 1999). Very few studies focused on the physical attributes, or the trace elements associated with coal. Different soils and growth mediums have varying characteristics, and it is essential to determine the characteristics to establish potential implications associated with each medium for sustainable vegetation establishment.

Due to the diverse nature of soils and underlying geology, the physical and chemical properties of growth mediums can differ over short distances. The properties of growth mediums or substrates need to be quantified to successfully apply the correct type and quantity of soil ameliorants or soil conditioners to sustain plant growth. The concentrations of constituents of potential concern (CoPC) or trace elements should also be known to identify the appropriate adapted vegetation species that can be considered in the rehabilitation process.

Apart from the trace elemental and micronutrient concentrations, it is also essential to consider the macronutrient concentrations. If macronutrient concentrations are insufficient in the growth medium, soil ameliorants like fertiliser will have to be applied.

Due to the high sulphate content associated with coal deposits, it can be hypothesised that the other elements that will have elevated concentrations are chalcophile elements. According to the Goldschmidt (1923) classification, chalcophile elements are concentrated in sulphide minerals and include Ag. As, Bi, Cu, Se, Pb, Zn, amongst others.

It is also anticipated that the coal spoil material and, to a lesser degree, the AMD contaminated soil, will have elevated levels of barium (Ba), strontium (Sr), silicon dioxide (SiO_2) and aluminium oxide (Al_2O_3), based on previous studies (Akinyeye *et al*., 2016; Finkelman, 1993; Cowan *et al*., 2016; Hancox and Gotz, 2014).

The aim is to define the substrates' chemical and applicable physical attributes that could potentially affect vegetation establishment. The total and plant-available concentrations of the trace metals, also referred to as constituents of potential concern (CoPC), will be quantified in all the growth mediums. Another aim is to compare the total concentrations to published screening values to assess the degree of contamination as well as to use the concentration results in concentration indices (refer to Chapter 1).

2.2 Materials and Methods

The following section elaborates on the description of the site from where the growth mediums were derived, the sampling and analytical procedures and the analysis conducted on the results.

2.2.1 Site description

Composite soil samples were taken from four different growth mediums, namely, soil contaminated by AMD (EAMD), coal spoil material (ESP), medium silica sand (ESI), as a sterile control, and ordinary agricultural soil (EAGRI).

Two of the growth mediums, namely the AMD-contaminated soil (EAMD) and the coal spoil material (ESP), were sampled from a coal operation roughly 25 km south of eMalahleni in the Mpumalanga province.

The agricultural soil (EAGRI) is a blended product to represent the optimal growth medium, and laboratory quartz sand (ESI) represents a sterile growth medium, and both were used as controls. The following section gives an overview of the geological and pedological setting of the area where the two samples were collected.

Figure 2-1: Hutton soil type at the mining colliery

The dominant soils encountered in the area are Avalon, Hutton (Figure 2-1), Glencoe, Mispah, Clovelly, and Wasbank.

According to Martin Fey's classification (2010), the AMD polluted soil, which is a Hutton, is an oxidic soil. These soils are red due to iron oxidation and good drainage, and where there is periodic soil saturation, soils tend to have a yellow hue due to the reducing state. This is an example of typical unpolluted soils that can be found in the Highveld.

The oxidic horizons are more or less uniform in colour but can vary in structure (Fey, 2010).

The dominant minerals in these soil types are hematite (Fe_2O_3) and goethite (Fe(OH)O) (Fey, 2010).

2.2.2 Sampling and analytical procedures

Soil samples were dried in an oven, after which it was sieved <2 mm and analysed. In accordance with The Non-Affiliated Soil Analysis Work Committee (1990) and Soil and Plant Analysis Council (1999), the methods for the analyses are as follows:

- Exchangeable cations were determined using the leaching method using ammonium acetate (1 mol/dm^3 and pH buffered at 7.0), with the use of an Atomic Absorption Spectrophotometer (AAS).
- Both exchangeable acidity pH(KCl) and actual acidity pH(H_2O) were determined using an electrometric method using a pH and EC meter. The pH was determined

using the 1:2.5 substrate samples to de-ionised water as well as KCl suspension on a mass basis ratio.

- Electrical conductivity (EC) was determined by means of saturated substrate paste with a calibrated EC meter.
- Plant available concentrations were analysed employing the DIN 19730 [NH_4NO_3] method (Prüeβ, 1997).
- Total trace element concentrations were determined using the HNO_3/H_2O_2 method (EPA3051A method) and measured by means of inductively coupled plasma mass spectrometry (ICP-MS).
- The particle size distribution (PSD) was determined by drying, sieving (< 2mm) and analysing the samples according to standard methods (The Non-Affiliated Soil Analysis Work Committee,1990).

Optimal and critical threshold limits were used as references to fully comprehend the chemical characteristics of the different substrates or growth mediums. As discussed below, the laboratory results were compared to different threshold and guideline values.

2.2.2.1 *Soil Screening Values*

The South African Department of Environmental Affairs (DEA) published national norms and standards or soil screening values (SSVs) for the remediation of contaminated land and soil quality in the Government Gazette on 2 May 2014 (RSA, 2014). SSVs were used to assess whether the concentrations of the CoPC present in the substrate could pose a potential risk to the receiving environment. The SSVs are conventional concentrations of three potential source-pathway-receptor model calculations:

- The direct pathways for the protection of sensitive receptors to potentially high exposures anticipated in informal residential settlements;
- Indirect pathway for the protection of water resources based on human consumption. The soil-water transfer model is based on simplified partitioning with allowance for finite, limited dilution and dispersion, with water mediums, assuming a porous sand aquifer and a shallow water table; and
- Indirect pathway for the protection of aquatic ecosystems with the same assumptions defined above to determine aquatic eco-toxicology.

The lowest concentration provided by the three pathway-receptor models listed above is the SSV 1. It is a conservative multi-functional soil quality criterion under many potential exposure scenarios (DEA, 2010).

The SSV 1 values are appropriate for assessing potential soil contamination when (DEA, 2010):

- There is a potential quality risk to the groundwater;
- There are groundwater users within a 1 km radius from the site; and
- There are surface water resources that could be impacted by means of off-site migration of the potential contaminants.

During the determination of SSV 1 a Dilution Factor (DF), that accounts for the dilution caused by groundwater recharge and amalgamation, is used (DEA, 2010).

The SSV 1 are calculated as follows (DEA, 2010):

$$\boldsymbol{Y = C_W \; X \; K_d \; X \; DF} \qquad \textbf{(Eq. 2-1)}$$

Where:

Y = total contaminant concentration in soil at equilibrium with pore water at defined water quality standard

C_w = water quality standard (aquatic ecosystem/domestic drinking water use guideline)

K_d = partition coefficient

DF = dilution attenuation factor

The SSV 1 represents soil values required to achieve Department of Water and Sanitation (DWS) *Water Quality Guideline levels for aquatic ecosystem protection and domestic water use* (RSA, 2014).

Water-soluble soil screening is conducted to determine the risk CoPC poses to the environment and refine the evaluation of risk conducted using the total analysis. Water-soluble soil screening values (Soluble SSV) are determined using the same methodology as presented in the Framework, without using the partitioning coefficient. Water quality objectives from the DWS Water Quality Guidelines for aquatic ecosystem protection and domestic water use were used to calculate SSVs.

Total concentrations screening

The screening results of the total metals in the substrate were compared to the GN R. 331 soil screening values for the protection of water resources (SSV 1) as tabulated below.

Table 2-1: Soil Screening Values (RSA, 2014)

Variable	Units	SSV 1	SSV2 Informal Residential	SSV2 Standard Residential	SSV2 Commercial Industrial
Arsenic	$mg \cdot kg^{-1}$	5.8	23	48	150
Cadmium		7.5	15	32	260
Chromium		46000	46000	96000	790000
Cobalt		300	300	630	5000
Copper		16	1100	2300	19000
Lead		20	1100	2300	19000
Manganese		740	740	1500	12000
Mercury		0.9	0.93	1	6.5
Nickel		91	620	1200	10000
Vanadium		150	150	320	2600
Zinc		240	9200	19000	150000

2.2.2.2 Concentration Indices

Kabata-Pendias (2011) stated that the risks associated with CoPCs can be calculated by quantifying the bioaccumulation capabilities and environmental toxicity. The laboratory results of the substrate samples were used to quantify the availability percentage (A%).

Availability %

The contamination status of CoPCs in substrates can be determined by calculating the extraction or mobility percentage, also known as the availability percentage (Rösner *et al.,* 2001). The availability percentages (A%) were determined based on the following equation:

$$A\% = (CoPC_A / CoPC_T) * 100 \qquad \text{(Eq. 2-2)}$$

Where:

A% = availability percentage

A = available concentration

T = total concentration

2.2.2.3 Particle size

Particle size is an informative factor because it reflects the soil's drainage properties, which influence the permeability (Burland *et al*., 2012). With increased clay content, the permeability of soil decreases due to the plate-like particles and the compressibility increases as a result of the high porosity associated with clayey soils (Winegardner, 2000). Clay and silt particles in the growth medium influence hydromechanical properties since particle-fluid interaction (PFI) becomes pertinent in materials with high specific surfaces and is only relevant in the case of fine soils (Montoro and Francisca, 2010).

The coefficient of uniformity is used to determine the range of different particle sizes that are contained in the soil. By making use of a particle size distribution (PSD) curve, the coefficient of uniformity (C_u) can be calculated by utilizing certain grain diameters (D), namely D10 and D60 (Holtz *et al*., 1981). To determine Cu, D10 and D60 are used, representing grain sizes that correlate to 10 and 60% of the sample passing in weight through the sieves, respectively (Holtz *et al*., 1981; Winegardner, 2000). The following PSD curve aids in the determination of gradation.

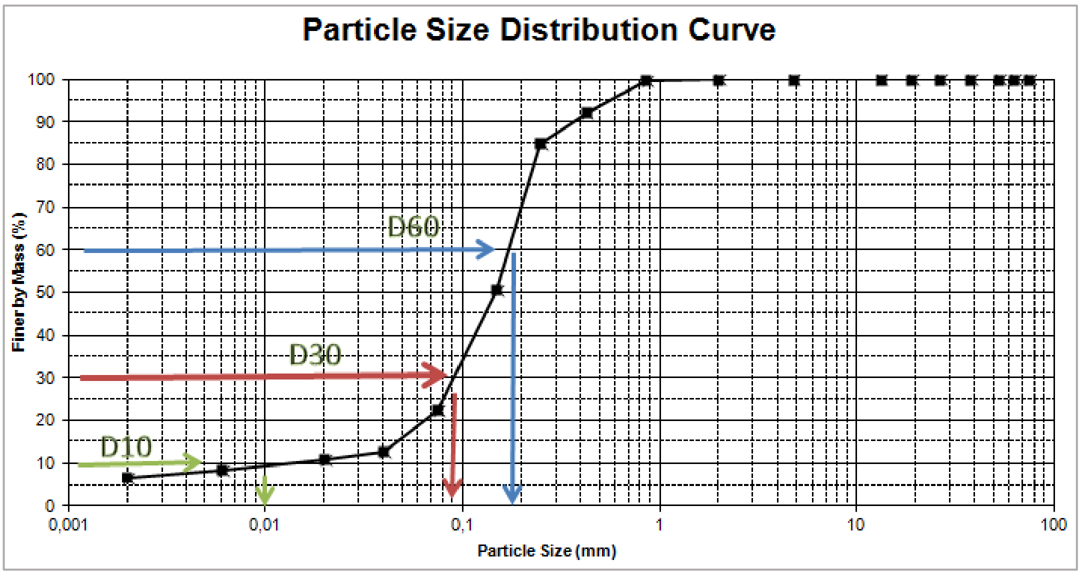


Figure 2-2: Particle size distribution curve

The following equation is used to calculate the coefficient of uniformity (Holtz *et al*., 1981):

$$\mathrm{Cu} = \frac{D60}{D10} \qquad \text{(Eq. 2-3)}$$

The greater the difference between D60 and D10, the more graded the soil and the smaller the coefficient of uniformity, the more uniform the soil. Another equation that is used to express the particle size distribution of soil is the coefficient of curvature (Cc) (Das and Sobhan, 2012).

$$\mathrm{Cc} = \frac{(D30)^2}{D60 \times D10} \qquad \text{(Eq. 2-4)}$$

Where D30 represents the grain size that correlates with 30% of the sample passing through the sieves. If the Cc value is between 1 and 3 it is termed a well-graded soil or poorly sorted soil. The criteria for defining well and poorly graded growth mediums are tabulated below in Table 2-2.

Table 2-2: Grading of growth mediums based on C_u and Cc parameters (FHWA, 2006)

Degree of gradation	Growth mediums
Well-graded	$C_u \geq 4$ and $1 < Cc < 3$
Poorly graded	$C_u < 4$ and $1 < Cc < 3$
Gap graded* Cc not between 1 and 3. *Gap-graded soils may be well-graded or poorly graded.	

As the figure below represents, poorly sorted (well graded) soils undergo compression more readily than well sorted (poorly graded) soils. Alternatively, well sorted soils are more permeable than poorly sorted soils due to the decreased void ratio in well graded soils.

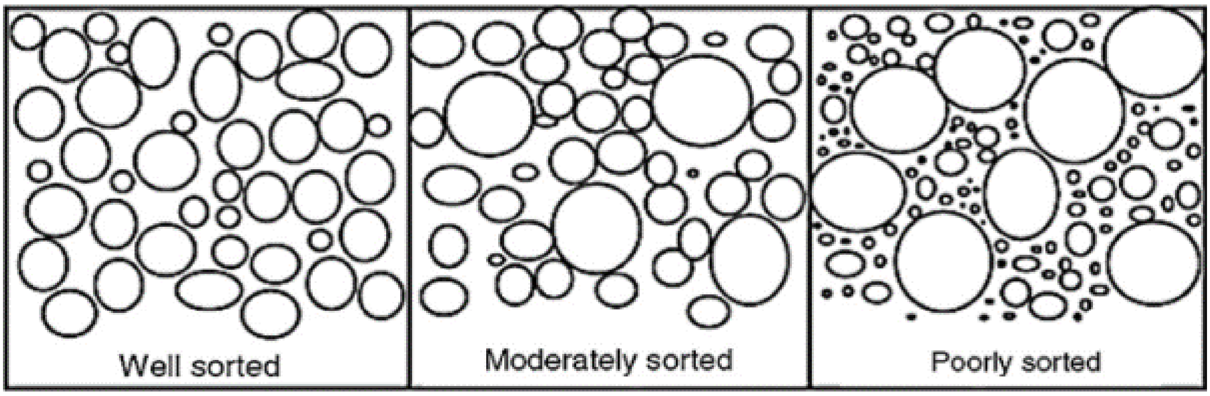


Figure 2-3: Schematic representing soils of different degrees of sorting (Bradaway and Ganat, 2022)

2.2.3 Statistical analysis

The results are displayed with a standard error of 5 %, and a PROC GLM (SAS) procedure was used. A principal component analysis (PCA) and a resemblance cluster diagram (Bray Curtis) were generated using Primer-E Version 7.

2.3 Results and discussions

The chemical and physical results of the AMD-contaminated soil, coal spoils, agricultural soils and silica or quartz sand, with the latter two being controls, are discussed below.

2.3.1 Chemical results

The samples were analysed for macro and micronutrients and other chemical constituents like electrical conductivity (EC) and pH, among others. The results are tabulated below and discussed thereafter.

Table 2-3: Chemical results of substrates

Variables	Unit	EAGRI	ESI	EAMD	ESP
Ca	$mg{\cdot}kg^{-1}$	90.74	4.23	31.76	131.57
Mg		19.63	1.39	10.56	16.51
K		19.04	3.84	1.08	0.66
Na		27.41	1.15	1.59	0.47
PO_4		1.89	0.72	0.72	0.72
SO_4		175.45	15.95	306.67	393.66
NO_3		194.75	3.28	6.55	2.93
NH_4		0.26	0.66	3.97	1.28
Cl		20.60	3.21	1.07	0.35
HCO_3		23.02	2.30	0.00	0.00
Fe		0.12	0.04	0.10	0.11
Mn		0.00	0.30	6.28	3.95
Cu		0.05	0.02	0.22	0.27
Zn		0.02	0.06	0.20	1.06
P-BRAY 1		69.75	1.50	1.50	2.25
B		0.10	0.11	0.08	0.08
pH (H_2O)	-	7.68	5.37	3.23	3.55
pH (KCl)		6.10	5.90	2.5	3.00
EC	(mS/cm)	1.04	0.07	0.87	1.09

- *This is a representative sample pooled from 10 samples, no statistical analysis was conducted, and values are used as a baseline reference value.*

2.3.1.1 pH

The pH is indicative of the hydrogen ion (H^+) and hydroxyl ions (OH^-) present in the soil solution. Soil or substrate pH (H_2O) is indicative of the acidity of the soil solution, while pH (KCl) refers to the soil solution acidity and the reserve acidity in the colloidal particles. Soil or substrate pH influences chemical processes, toxicities, deficiencies, and the soil's ability to support a healthy environment. The chemical activity is the lowest at neutral pH (7). Figure 2-4 illustrates the pH (H_2O) and pH (KCl) of the four different substrates. The optimal pH range for vegetation growth is between the maximum (red line) and the minimum (blue line) limits (Läuchli and Grattan, 2012). A composite sample, comprising ten samples of each growth medium, was analysed and the 5% error margin was added to the graph below.

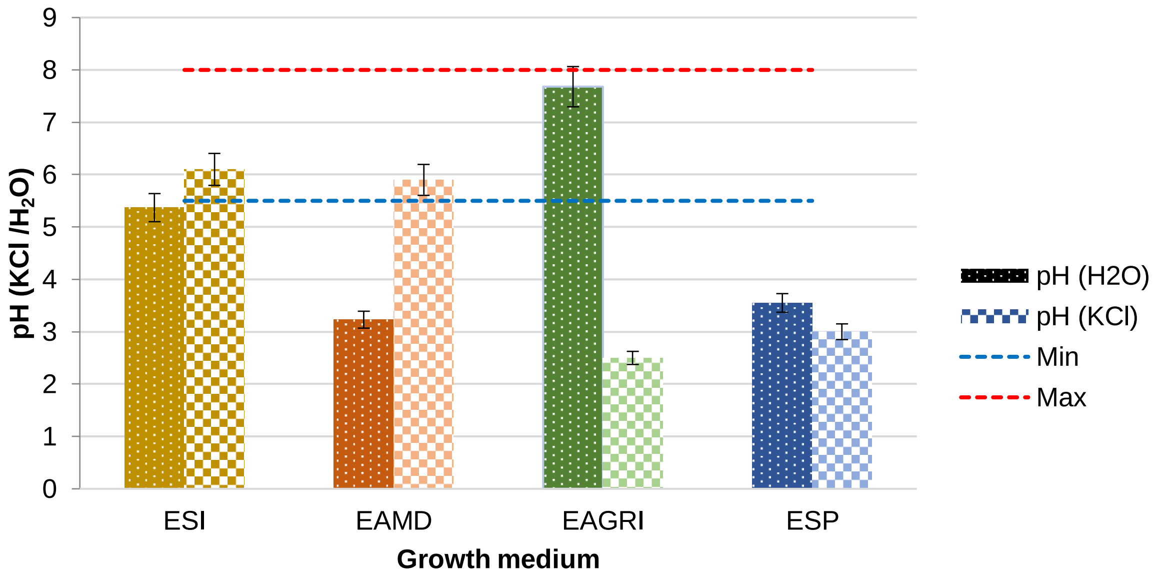


Figure 2-4: pH (H_2O and KCl) of growth mediums *(Standard error calculated using PROC GLM)*

The range between the minimum and maximum threshold is the optimal pH range, and ESI and EAMD had pH (KCl) values within the optimum threshold. The pH (H_2O) of EAGRI was also within the optimal range. To sufficiently sustain plant growth, EAMD and ESP will require lime to raise the pH (H_2O). Trace element mobility might also be associated with EAMD and ESP.

2.3.1.2 Electrical Conductivity (EC)

Electrical conductivity is a measure of the salinity or salts of the soil solution. The higher the EC value in the soil, the greater the concentration of dissolved salts due to the increased conductivity associated with dissolved salts. Soils with poor drainage often tend to have higher EC values, while well-draining soils do not contain a build-up of salts due to leaching. Electrical conductivity has an inverse relationship with nutrient uptake in most plant species, and only salt-tolerant plants, termed halophytes, can grow in soils with high EC levels. Plant vitality and performance decrease with increasing EC, and only a few plants can survive EC levels around 400 mS/m (Williamson *et al*., 1982; Cummings and Elliott, 1991). The results of the EC of the soils are presented in Figure 2-5.

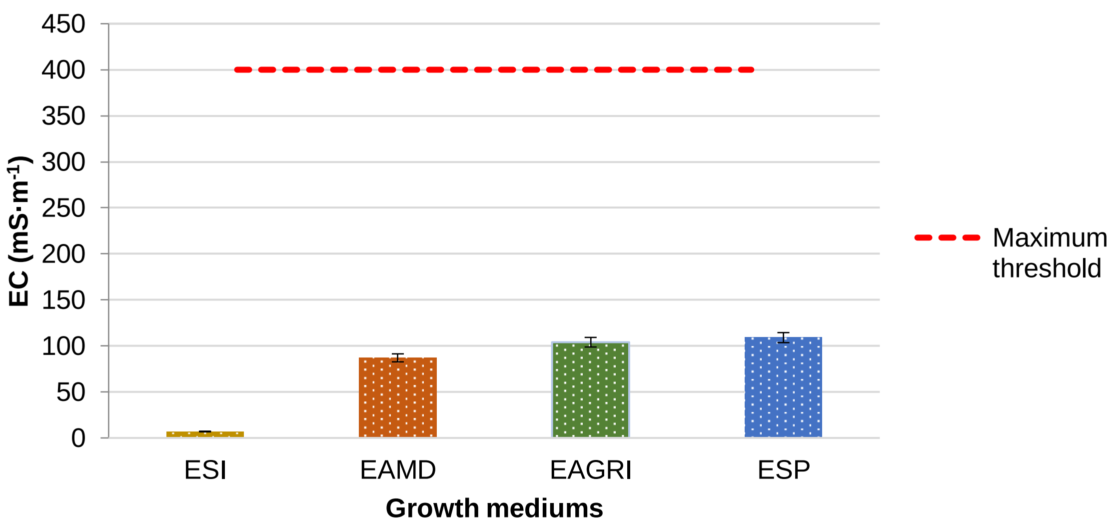


Figure 2-5: Electrical conductivity of growth mediums *(Standard error calculated using PROC GLM)*

The EC of all the growth mediums was well below the maximum threshold, and the dissolved salts in the soil solution will not impact on the nutrient uptake and ultimately, plant performance.

2.3.1.3 Calcium (Ca)

Calcium is present at significant concentrations and is a macro-nutrient. Calcium is essential for root development and becomes less crucial as the plant matures. In substrates or soils with pH above 5, Ca concentration is generally very high compared to other cations (Marschner, 1974).

The quantity of dissolved Ca^{+2} is dependent on the presence of Ca-containing minerals, the soil pH and the soil cation exchange capacity (CEC). Soils with high pH (>7.5) habitually contain higher concentrations of Ca due to precipitated Ca salts (lime and gypsum). Calcium concentrations of less than 200 mg·kg^{-1} are usually moderately low (Marschner, 1974). The Ca concentrations in the growth mediums are presented in Figure 2-6.

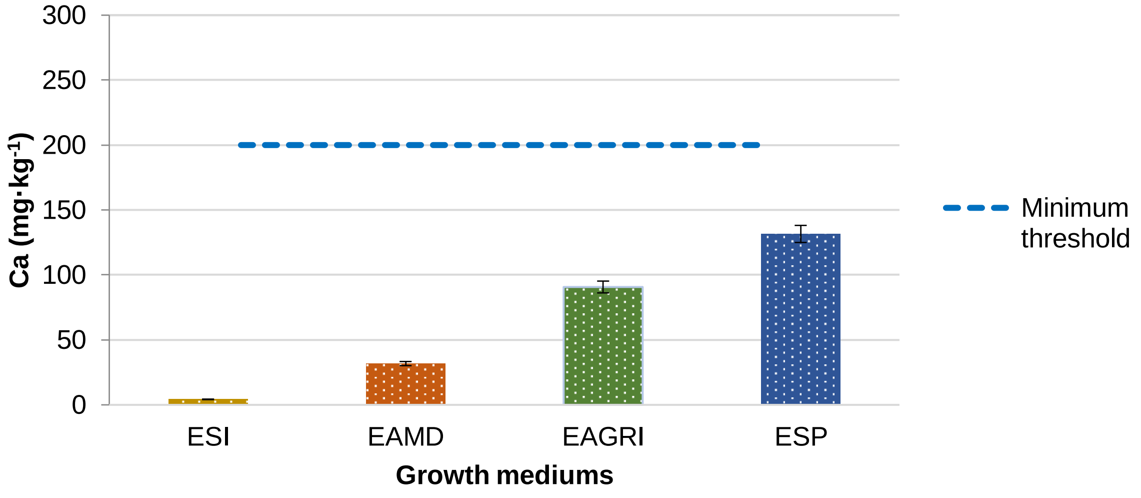


Figure 2-6: Calcium concentration (mg.kg^{-1}) in growth mediums *(Standard error calculated using PROC GLM)*

The calcium content in all the growth mediums was below the minimum threshold; however, this can be corrected by lime application.

2.3.1.4 Phosphorus (P)

Phosphorus is primarily insoluble and strongly bonds to particle surfaces, resulting in small available P concentrations in the soil solution. Due to low concentrations in the soil solution, small quantities are lost to leaching and do not pose a significant threat to water contamination. Phosphorus concentrations less than 10 mg·kg^{-1} are considered moderately low by Epstein (1965), and the P concentrations (P Bray) are presented below (Figure 2-7).

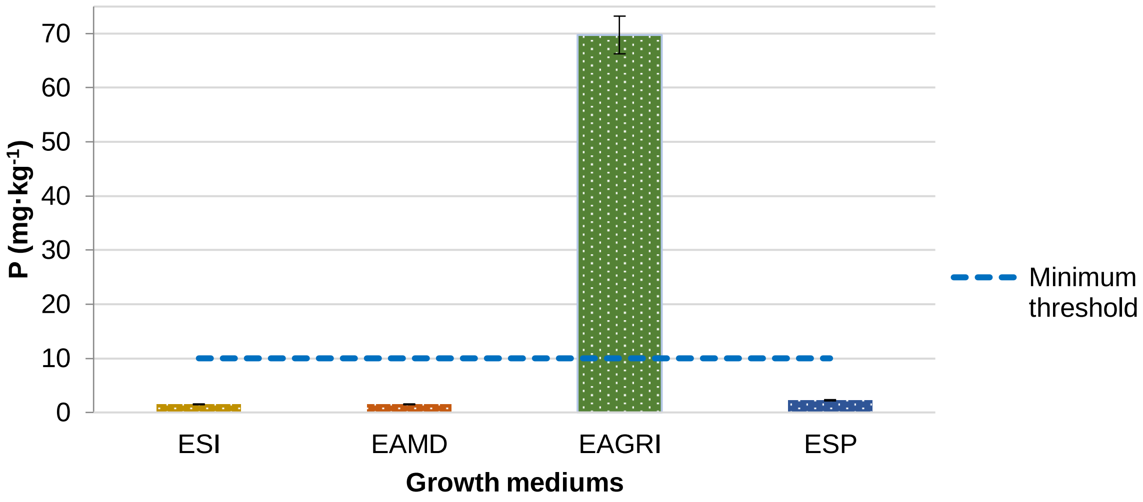


Figure 2-7: Phosphorous concentration (mg.kg^{-1}) in growth mediums *(Standard error calculated using PROC GLM)*

2.3.1.5 Potassium (K)

Potassium (K) is a macronutrient crucial in sustainable plant growth and is not readily available as a free element in nature due to its reactivity with water. According to Mengel and Steffens (1985), potassium in the soil solution is extremely mobile and can leach beyond the root depth by means of precipitation. The soil moisture deficiency also reduces K uptake and can lead to K deficiency. Potassium is a very mobile element in the soil solution, and concentrations lower than 75 mg·kg^{-1} are considered moderately low (PDA, 2011). The results on the K content of the growth mediums are illustrated in Figure 2-8.

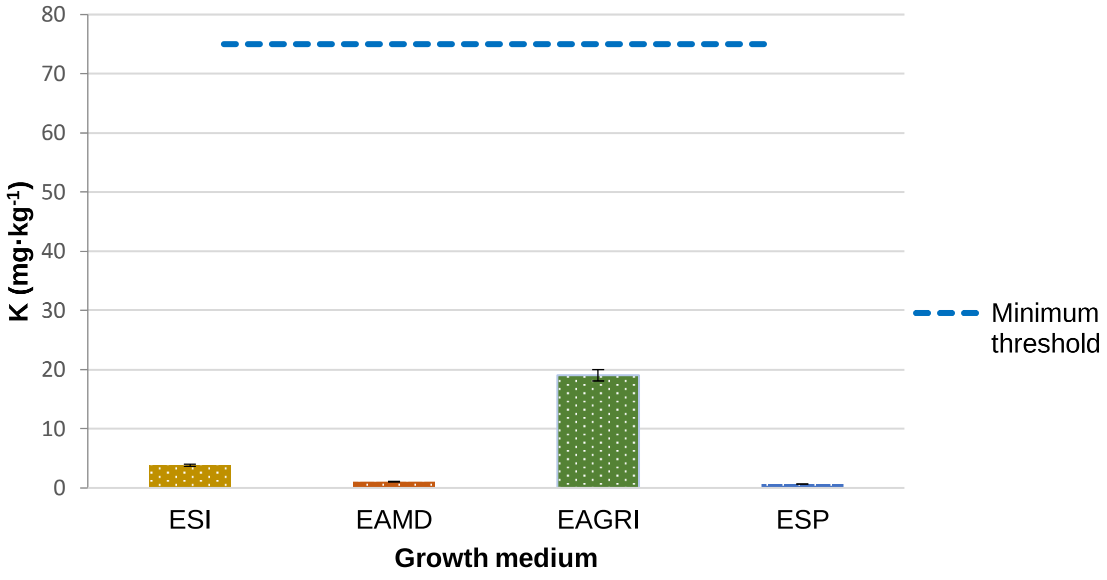


Figure 2-8: Potassium concentration (mg.kg^{-1}) in growth mediums *(Standard error calculated using PROC GLM)*

The K concentration in all the growth mediums was low, and below the minimum threshold, and K fertiliser will be required for successful vegetation establishment.

2.3.1.6 Magnesium (Mg)

Magnesium is an essential element that is present in the soil solution and on reactive colloidal surfaces as soluble and exchangeable Mg^{+2}. Soils with a slightly acidic to neutral pH have higher levels of available Mg since Mg is soluble at low pH levels. In soils with higher pH levels, Ca tends to dominate in the exchangeable cation adsorption complex, possibly causing Mg deficiency. Magnesium concentrations of less than 60 mg·kg^{-1} are considered moderately low (PDA, 2011), and the Mg concentrations in the substrate are presented in Figure 2-9.

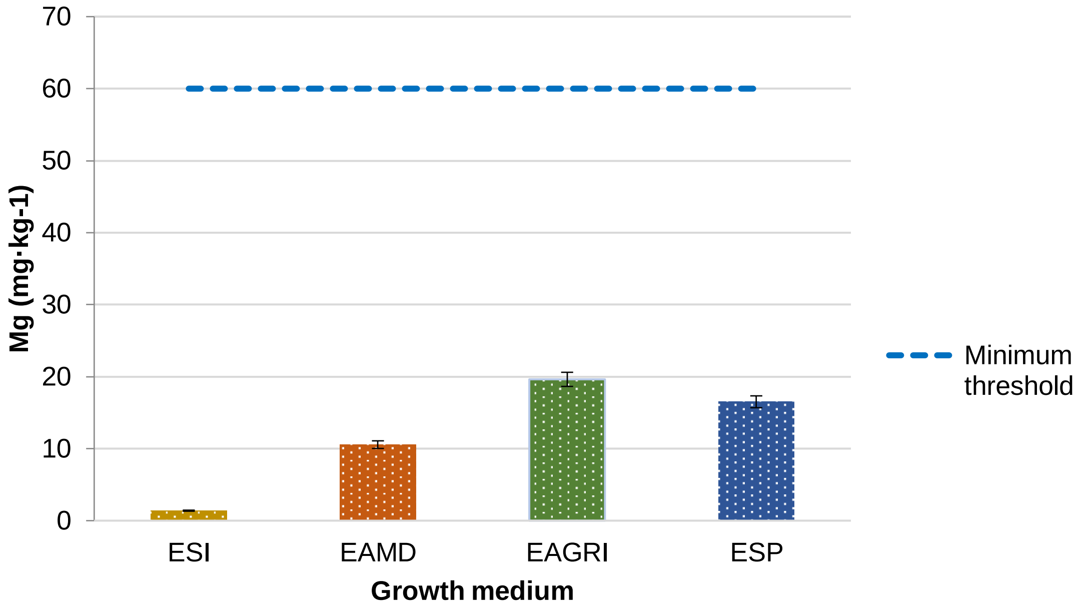


Figure 2-9: Magnesium concentration (mg.kg^{-1}) in growth mediums *(Standard error calculated using PROC GLM)*

2.3.1.7 Sodium (Na)

Sodium is an vital mineral element for plant growth, although in minimal concentrations, as most plants do not have responses to limit Na uptake. Na concentrations should generally be 40 mg·kg^{-1} or less. Exchangeable sodium percentage is an alternative expression for Na. The results on the Na content of the soils are presented in Figure 2-10.

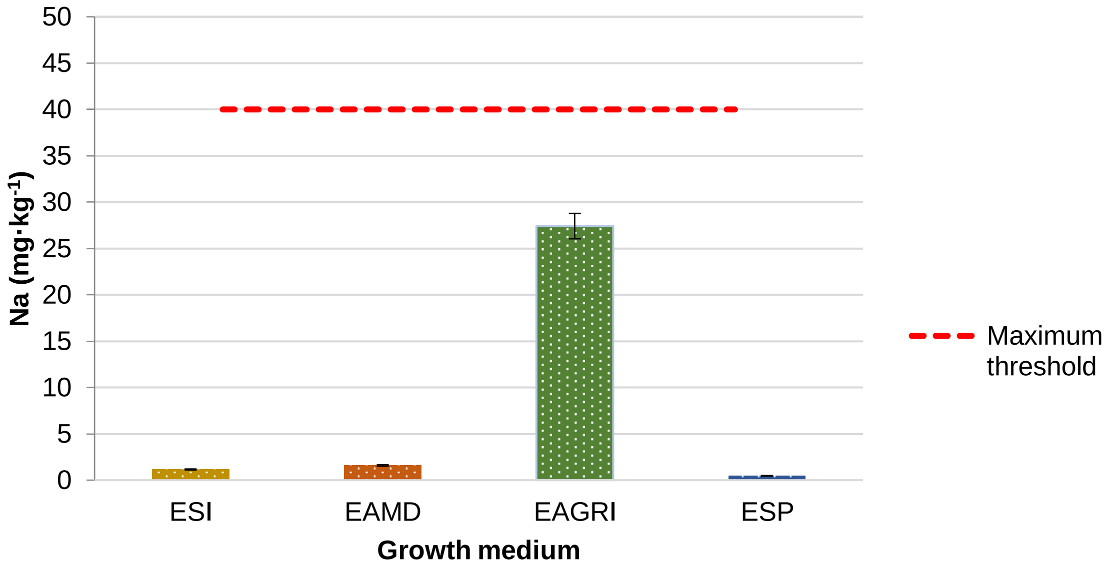


Figure 2-10: Sodium concentration (mg.kg^{-1}) in growth mediums *(Standard error calculated using PROC GLM)*

2.3.2 Total and plant-available trace elemental concentrations

The total and plant available (PA) metal concentrations in the growth mediums were analysed using ICP-MS, and the results are presented in the table below (Table 2-4).

Table 2-4: Total and plant available concentrations

Sample	**EAGRI**		**ESI**		**EAMD**		**ESP**	
	Total	***PA***	***Total***	***PA***	***Total***	***PA***	***Total***	***PA***
Unit	*mg·kg^{-1}*							
Be	0.22	ND	0.01	ND	0.54	0.04	0.91	0.02
B	1.75	0.40	ND	0.32	0.56	0.29	7.94	0.28
Na	134.40	60.06	25.70	1.95	79.78	10.19	77.49	3.34
Mg	1501.00	173.30	14.25	2.32	404.20	29.43	304.30	44.53
Al	8716.00	2.58	277.20	21.93	14990.00	297.60	5133.00	92.21
P	758.30	13.13	16.43	0.45	109.10	0.40	328.60	0.37
K	1163.00	117.50	54.01	3.74	1125.00	15.27	1284.00	8.11
Ca	10020.00	1337.00	31.61	10.62	163.70	66.17	3010.00	459.80
Ti	240.40	0.06	4.56	0.01	348.30	0.03	234.50	0.01
V	25.83	0.01	1.10	ND	71.59	0.00	39.42	ND
Cr	48.24	0.02	3.99	0.01	64.82	0.04	27.98	0.02
Mn	197.30	0.25	2.60	1.03	152.60	18.31	38.65	10.80

Sample	EAGRI		ESI		EAMD		ESP	
	Total	*PA*	*Total*	*PA*	*Total*	*PA*	*Total*	*PA*
Unit	*mg·kg^{-1}*							
Fe	9656.00	1.32	556.50	0.16	42990.00	3.32	8904.00	8.47
Co	6.39	0.01	0.13	0.01	4.93	0.66	4.67	0.55
Ni	33.23	0.03	5.13	0.03	15.84	0.67	12.63	0.84
Cu	29.37	0.13	6.35	0.05	22.44	0.33	20.02	0.33
Zn	79.50	0.10	36.60	0.20	46.88	0.51	52.03	2.60
As	2.05	0.01	0.12	0.01	6.65	0.04	4.92	0.03
Se	8.12	0.03	0.95	0.12	4.72	0.56	13.36	0.65
Rb	14.87	0.09	0.40	0.02	27.11	0.62	9.70	0.18
Sr	16.64	3.56	0.20	0.03	7.39	0.55	168.30	1.56
Mo	4.65	0.01	1.30	ND	1.65	ND	1.96	ND
Pd	0.13	0.00	0.01	0.00	0.06	0.01	0.36	0.01
Ag	0.14	0.00	0.01	0.00	0.12	0.00	0.41	0.00
Cd	0.03	0.02	ND	0.02	0.02	0.02	0.08	0.02
Sn	67.57	0.27	38.12	0.19	74.71	0.33	73.28	0.26
Sb	0.23	0.00	0.09	ND	0.16	ND	0.13	0.00
Ba	41.41	2.94	2.53	0.72	295.40	2.12	238.70	0.82
Pt	ND	ND	ND	ND	ND	ND	ND	ND
Au	0.00	ND	0.00	ND	ND	ND	0.01	ND
Hg	0.04	0.00	0.03	ND	0.04	ND	0.29	ND
Tl	0.12	0.00	ND	ND	1.01	0.06	0.28	0.02
Pb	8.38	0.01	0.48	0.02	29.53	0.73	28.74	0.02
Bi	0.10	ND	0.01	ND	0.11	ND	0.45	ND
Th	7.89	0.00	0.68	0.00	8.09	0.00	10.26	ND
U	1.00	0.00	0.06	ND	1.01	0.07	1.91	0.01

**ND – Not detectable*

- This is a representative sample pooled from 10 samples, no statistical analysis was conducted, and values are used as a baseline reference value.

The total concentrations were compared to the SSVs as measurable thresholds to determine the degree of potential contamination and the potential for phytotoxicity. Only the metals with specified SSV limits are discussed below and presented on logarithmic scales. These include arsenic, cadmium, chromium, cobalt, copper, manganese, mercury, nickel, vanadium and zinc.

2.3.2.1 Arsenic (As)

The total arsenic concentrations for all the growth mediums were between 0.1 mg·kg^{-1} and 6.6 mg·kg^{-1}, and only EAMD exceeded the SSV 1 guideline value of 5.8 mg·kg^{-1}. The plant-available arsenic concentrations are negligibly low and will not affect the development and growth of plants.

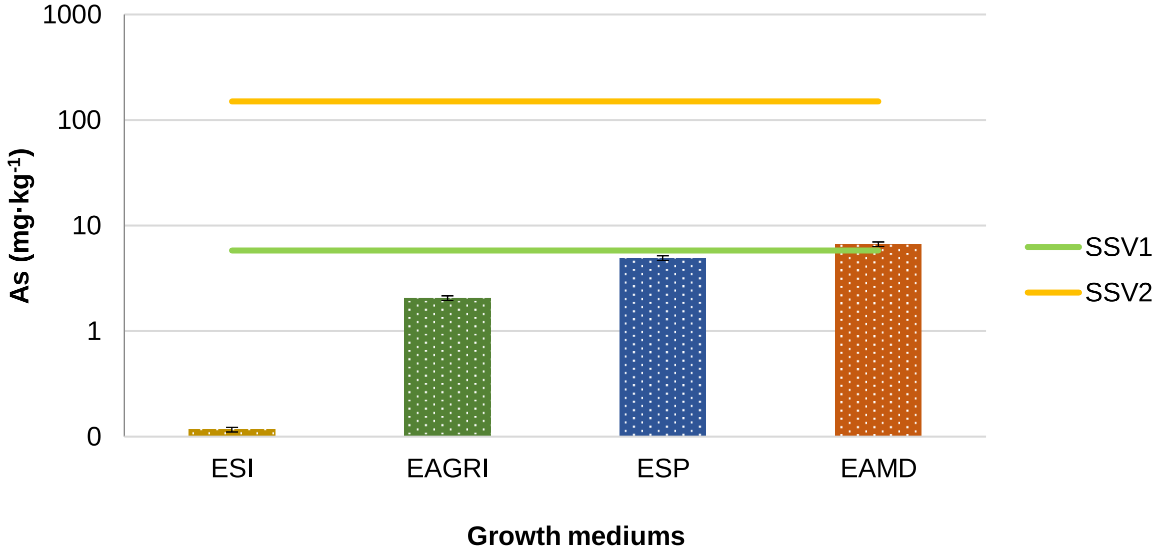


Figure 2-11: Total Arsenic concentration (mg.kg^{-1}) in growth mediums

Some chemical speciations of arsenic that are toxic include, arsenine (AsH_3), arsenite (AsO_3^{3-}) and arsenate (AsO_4^{3-}) (Garcia-Salgado *et al*., 2012). Arsenic is a naturally occurring element; however, concentrations can be elevated due to anthropogenic activities. Arsenic has a strong affinity for nickel, iron, and cobalt.

2.3.2.2 Cadmium (Cd)

The total cadmium concentration for ESI, EAMD, EAGRI and ESP was 0 mg·kg^{-1}, 0.01 mg·kg^{-1}, 0.03 mg·kg^{-1} and 0.07 mg·kg^{-1}, respectively and well below the SSV 1 threshold of 7.5 mg·kg^{-1}. The plant-available cadmium concentrations were negligibly low.

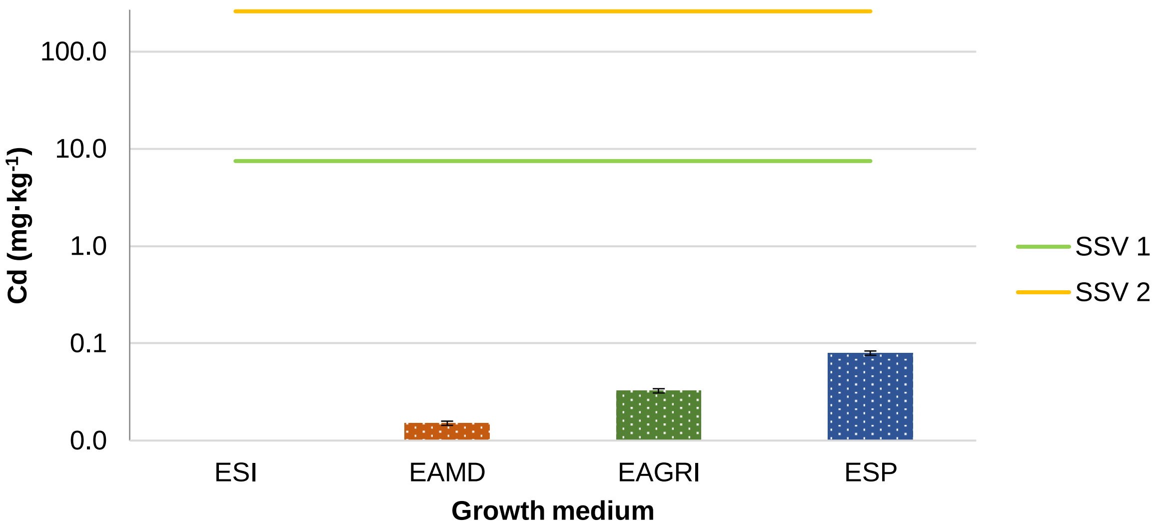


Figure 2-12: Total Cadmium concentration (mg.kg^{-1}) in growth mediums

Cadmium concentrations in soils are dependent on the underlying geology and Cd is more mobile in acidic soils. Cadmium has the same valence and has a similar ionic radius as Ca; however, there is no substitution between Cd and Ca in soil. Cadmium chemical forms, such as $Cd(OH)_2$, $CdCl_2$, CdO, and CdF_2, are soluble in soil (Prasad *et al*., 2001).

2.3.2.3 Chromium (Cr)

The chromium concentration in the growth mediums ranged from 3.9 mg·kg^{-1} to 64.8 mg·kg^{-1}, which is well below the SSV 1 limit of 46 000 mg·kg^{-1}. The concentration of plant-available was negligibly low and ranged from 0.01 mg·kg^{-1} and 0.4 mg·kg^{-1}.

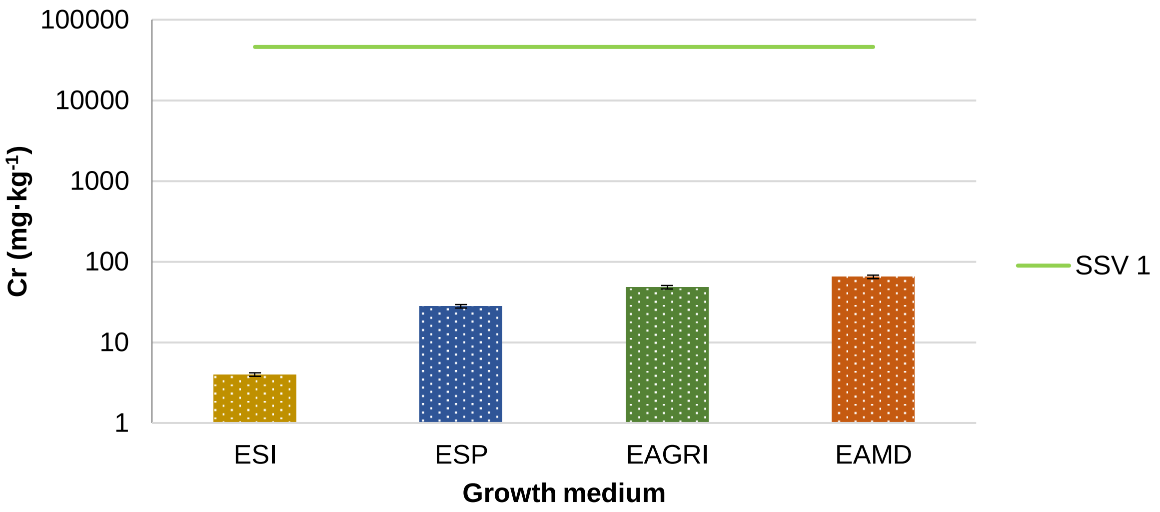


Figure 2-13: Total Chromium concentration (mg.kg^{-1}) in growth mediums

Chromium is known to be slightly mobile in very acidic soils and starts precipitating and becoming less plant-available in soils with a pH of 5.5 and above; however, Cr can also become mobile in highly alkaline soils (Vardaki and Kelepertsis, 1999).

2.3.2.4 Cobalt (Co)

The highest total cobalt concentration was recorded in ESP with a concentration of 84.1 mg·kg$^{-1,}$ which was well below the SSV 1 limit of 300 mg·kg^{-1}. The plant-available concentrations were all negligibly low.

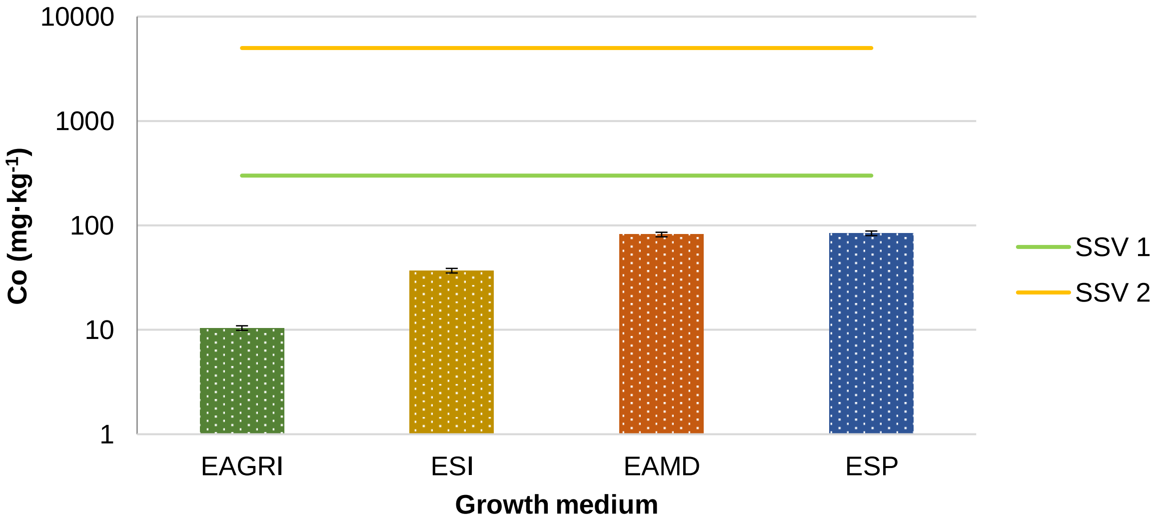


Figure 2-14: Total Cobalt concentration (mg.kg^{-1}) in growth mediums

Cobalt oxidises from Co^{2+} to Co^{3+} to the complex anion $Co(OH)_3^{-3}$ in erosive environments (Vardaki and Kelepertsis, 1999). Cobalt is readily adsorbed by organic substances, creating organic chelates, which increase the solubility and makes it more plant-available.

2.3.2.5 Copper (Cu)

The total copper concentrations in the growth mediums ranged from 1 mg·kg^{-1} to 6.3 mg·kg^{-1} and were well below the 16 mg·kg^{-1} threshold (SSV 1). The highest plant-available concentration was 0.3 mg·kg^{-1} and is negligibly low and pose no risk for vegetation.

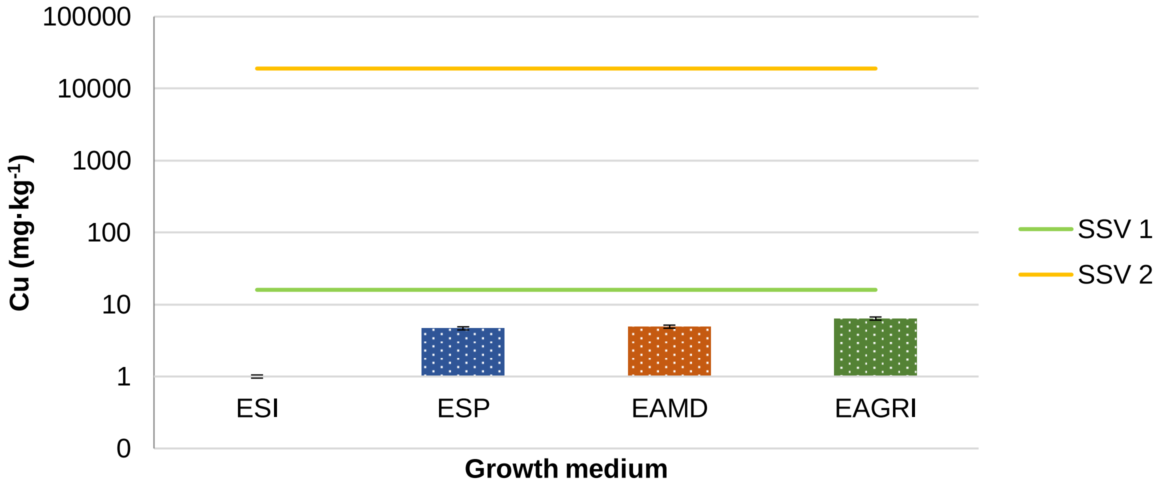


Figure 2-15: Total Copper concentration (mg.kg^{-1}) in growth mediums

Copper is retained in the soil through substitution and adsorption mechanisms, and the precipitates are usually unstable in aqueous solutions. Copper is predominantly adsorbed by soils and soil constituents and, to a lesser extent, by other metals, with the exemption of Pb. Copper has a high affinity for soluble organic ligands, which increases the mobility of copper (Prasad *et al*., 2001).

2.3.2.6 Manganese (Mn)

The growth mediums' manganese concentrations were well below the SSV 1 threshold of 740 mg·kg^{-1} and ranged from 2.5 mg·kg^{-1} to 197.3 mg·kg^{-1}. The plant available manganese concentration for EAMD and ESP was slightly elevated compared to the other two growth mediums and were 18.3 mg·kg^{-1} and 10.8 mg·kg^{-1}, respectively. Since Mn is a micronutrient in plants, these concentrations will not harm vegetation establishment and growth.

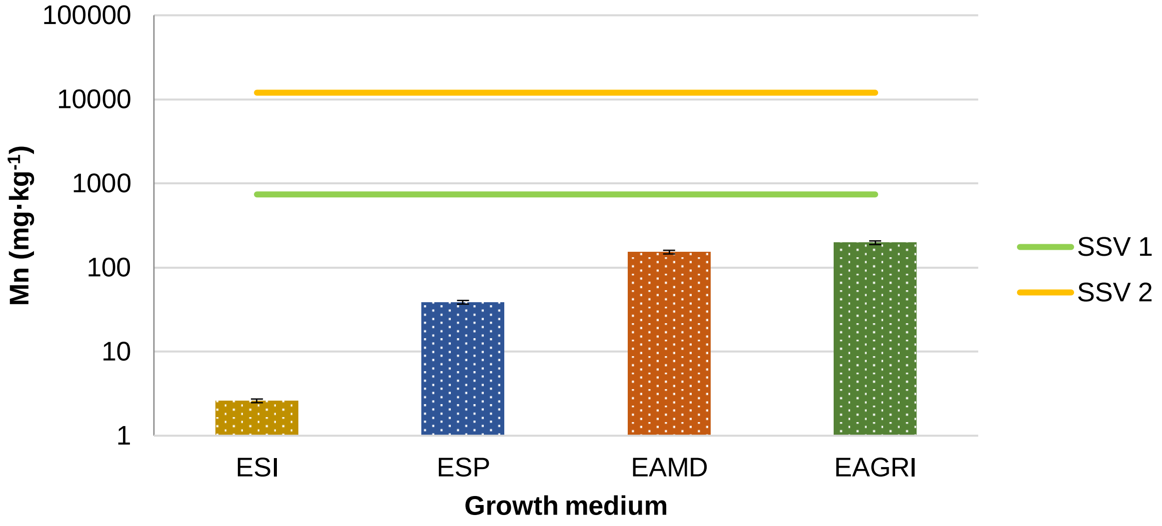


Figure 2-16: Total Manganese concentration (mg.kg^{-1}) in growth mediums

2.3.2.7 Mercury (Hg)

The total mercury concentration in the substrates did not exceed 0.02 mg·kg^{-1} and was well below the SSV 1 guideline value of 0.93 mg·kg^{-1}. The plant-available mercury concentrations in the growth mediums were not detectable and would not influence physiological processes.

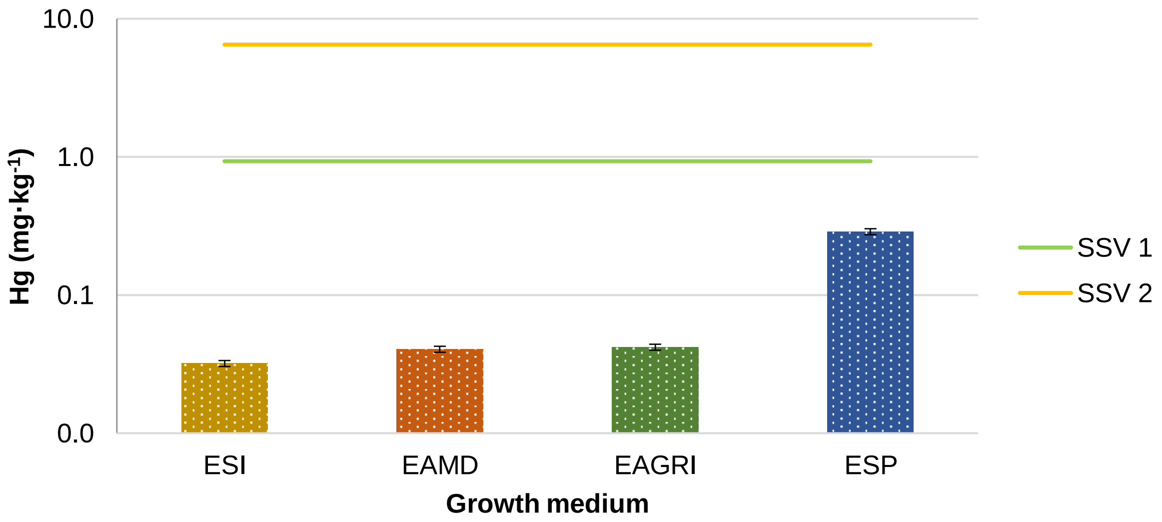


Figure 2-17: Total Mercury concentration (mg.kg^{-1}) in growth mediums

Mercury is a chalcophile metal with a strong affinity for forming sulphur bonds. It is also known to form organic complexes, which are relatively stable in aqueous mediums even though they are volatile.

2.3.2.8 Nickel (Ni)

The total Ni concentration in the different growth mediums ranged from 5.1 mg·kg^{-1} and 33.2 mg·kg^{-1}. None of the Ni concentrations exceeded the SSV 1 guideline value of 91 mg·kg^{-1}. The highest plant-available nickel concentration was 0.08 mg·kg^{-1} in ESP and will not affect plant growth.

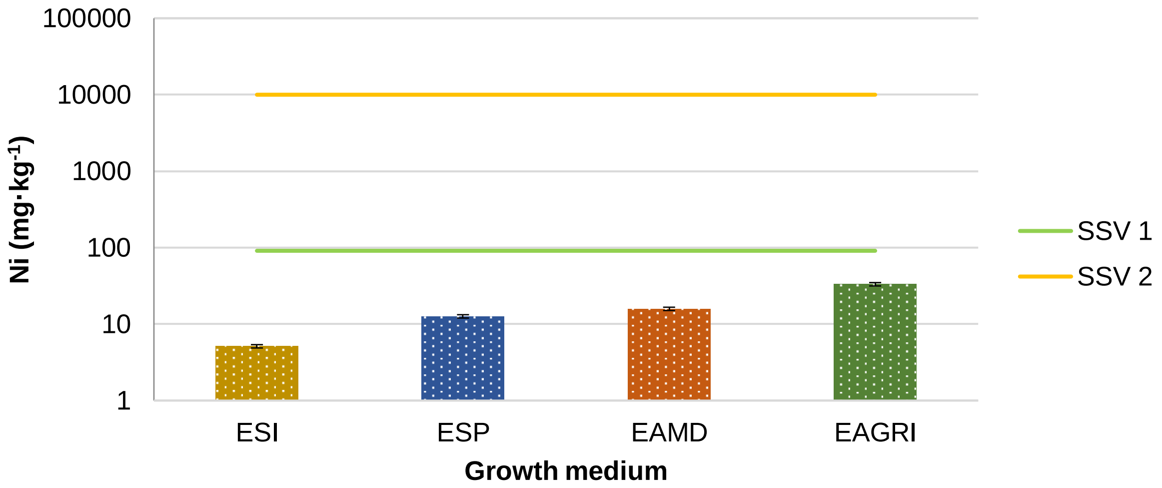


Figure 2-18: Total Nickel concentration (mg.kg^{-1}) in growth mediums

Nickel is both a siderophile and chalcophile and has a strong affinity for sulphur and iron. During weathering, the majority of Ni is coprecipitated with iron- and manganese oxides; however, it is also associated with phosphates, carbonates, and silicates (Vardaki and Kelepertsis, 1999).

2.3.2.9 Vanadium (V)

The highest total V concentration was noted in EAMD, and the vanadium content was 71.5 mg·kg^{-1}, however, it was well below the SSV 1 guideline value of 150 mg·kg^{-1}. The plant-available vanadium concentration is negligibly low.

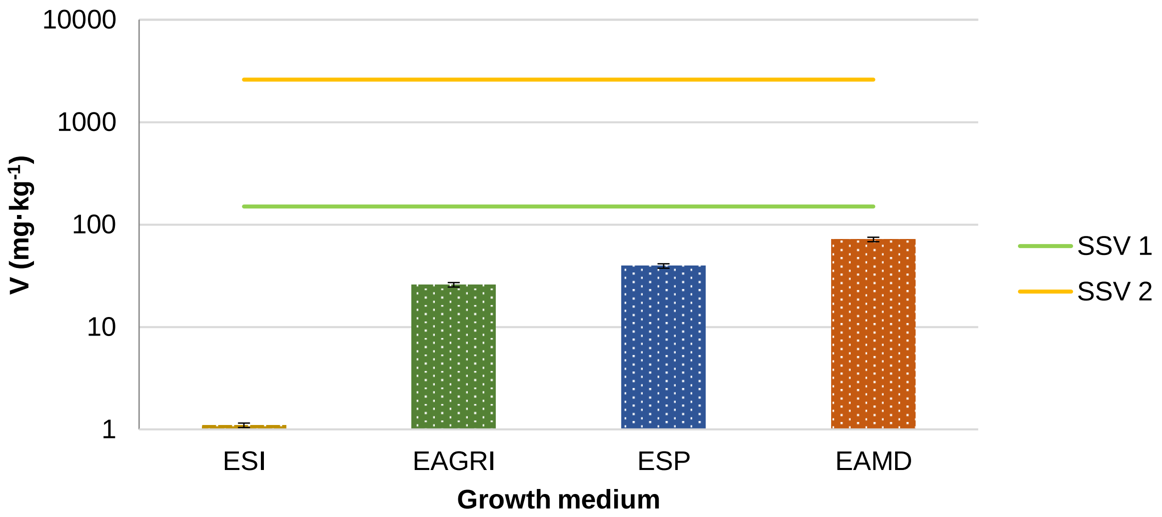


Figure 2-19: Total Vanadium concentration (mg.kg^{-1}) in growth mediums

Geochemically, the solubility of vanadium is dependent on the oxidation state and the acidity of the growth medium. In the oxidising state, vanadium depicts an isomorphic relation to other cations like MO^{5+} Fe^{2+}, amongst others.

2.3.2.10 *Zinc (Zn)*

The total Zn concentrations in the growth mediums ranged from 36.6 mg·kg^{-1} to 79.5 mg·kg^{-1} and were well below the SSV 1 guideline value of 240 mg·kg^{-1}. The plant-available Zn concentrations were negligible.

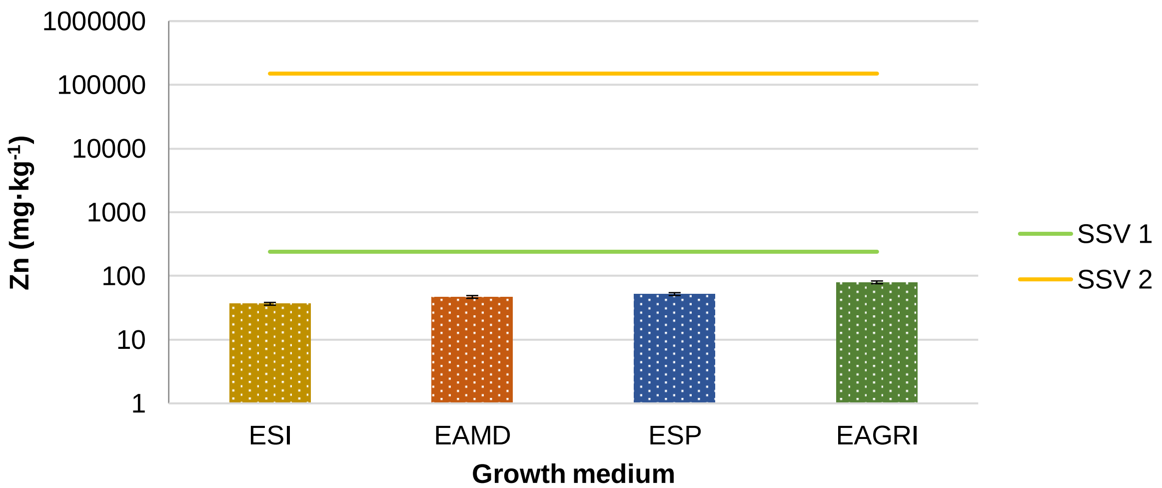


Figure 2-20: Total Zinc concentration (mg.kg^{-1}) in growth mediums

Zinc is very mobile during the weathering process and readily precipitated by reactions with carbonates or by absorption of minerals or organic compounds, especially in the presence of sulphur anions.

Based on the Principal Component Analysis (PCA) graph below, which considered all the CoPCs (Figure 2-21), both the soils contaminated with AMD and the coal spoils were chemically similar and associated with elements like Cu, Co, Ni, U, Zn, Ti, B and Be.

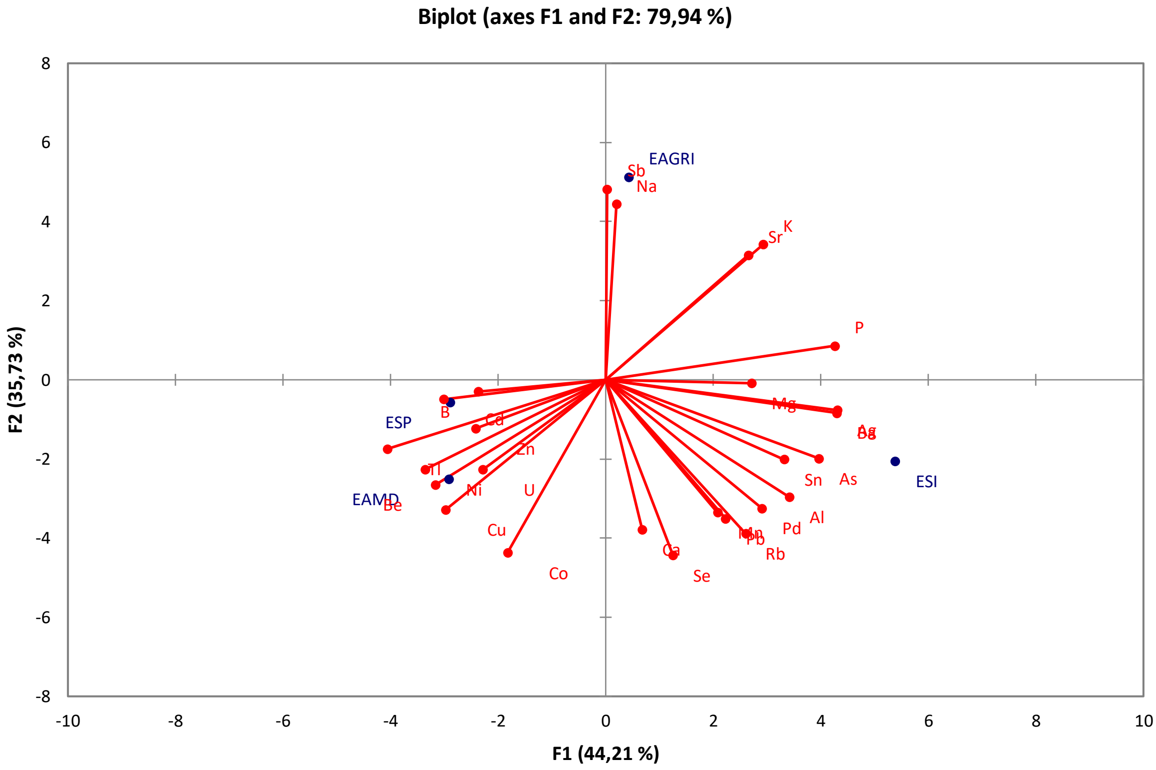


Figure 2-21: PCA graph of associated elements in the substrates

	F1	F2	F3
Eigenvalue	11.496	9.289	5.215
Variability (%)	44.215	35.727	20.058
Cumulative %	44.215	79.942	100.000

The PCA was merely used for indicative purposes to see what CoPCs are of importance for each of the growth mediums.

2.3.3 Particle size distribution of the growth mediums

The most important physical attribute of growth mediums is particle size distribution (PSD), and the results are discussed below.

Particle size distribution is a crucial component to quantify and will give a good indication of the substrate's water holding capacity and, in turn, the leaching potential.

The PSD diagrams for all four substrates are displayed and briefly discussed below. The coefficient of uniformity (C_u) and coefficient of curvature (Cc) were also used to describe the degree of grading and sorting.

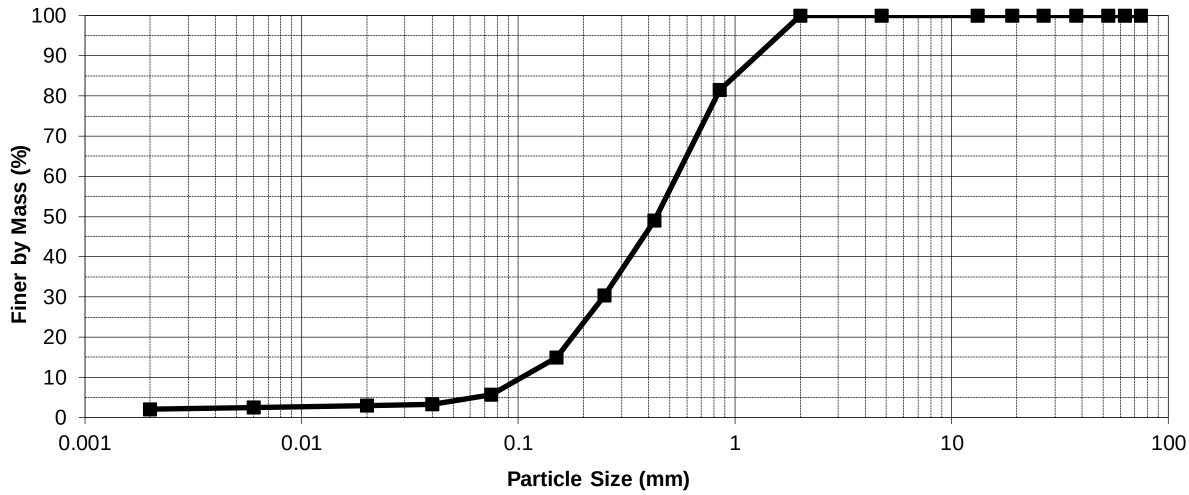


Figure 2-22: Particle size distribution diagram for EAGRI

In terms of particle size distribution (PSD), more than 40% of the particles in EAGRI are smaller than 0.3 mm in diameter. The Cc value is 1.25 and a Cu of 5, indicating the soil is well graded.

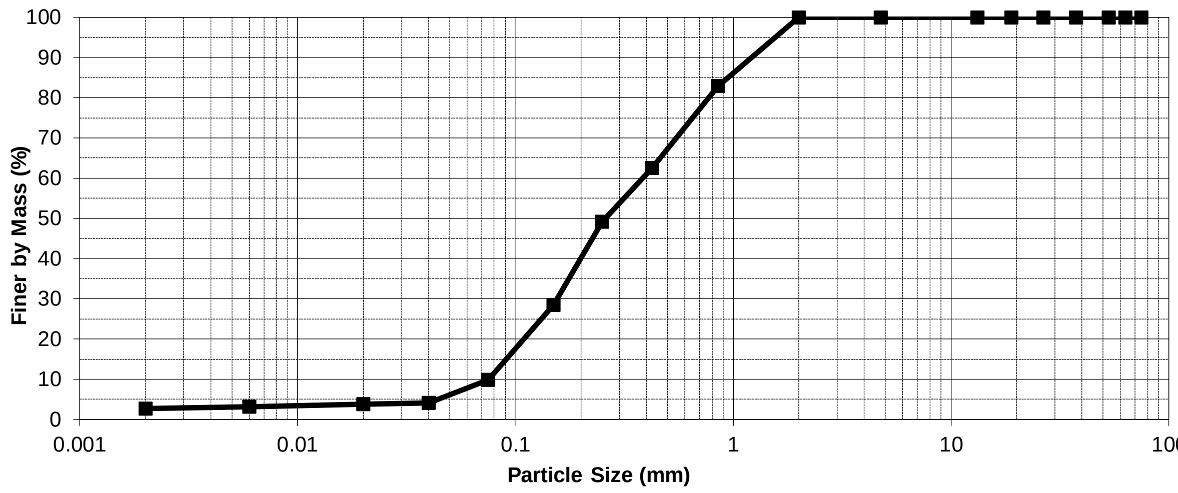


Figure 2-23: Particle size distribution diagram for ESI

The silica soil (ESI) had a Cu of 5.7 and a Cc of 0.8, indicating the growth medium is well sorted and poorly graded. Since all the particles are more or less uniform, it can be predicted that the soil water retention will be low.

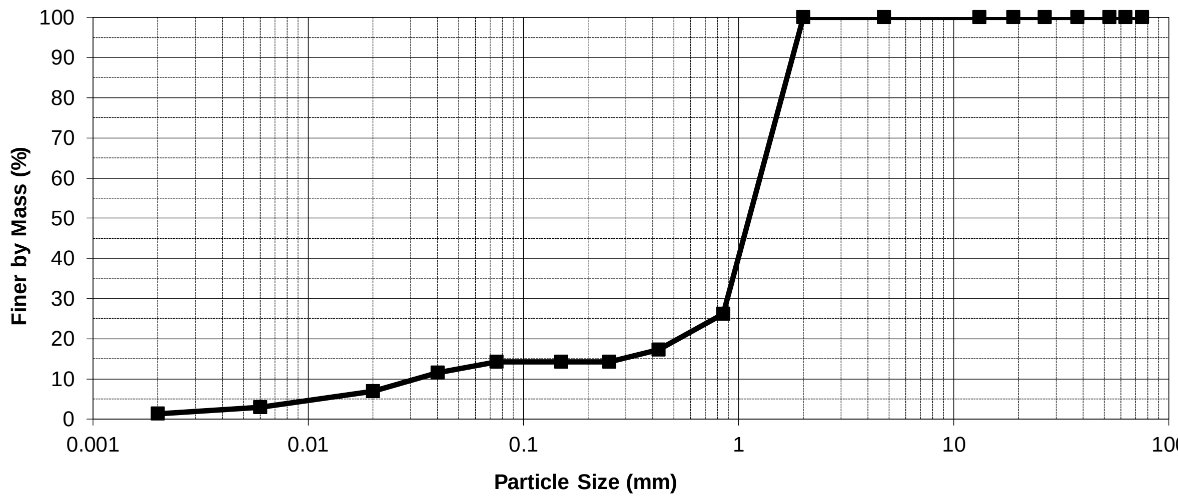


Figure 2-24: Particle size distribution diagram for EAMD

The particle size distribution curve indicates the soil is gap-graded. The AMD-contaminated soil has a large Cu (43.3), indicating a large range of particles. The soil has a Cc of 20.7, indicating the soil is moderate to poorly graded.

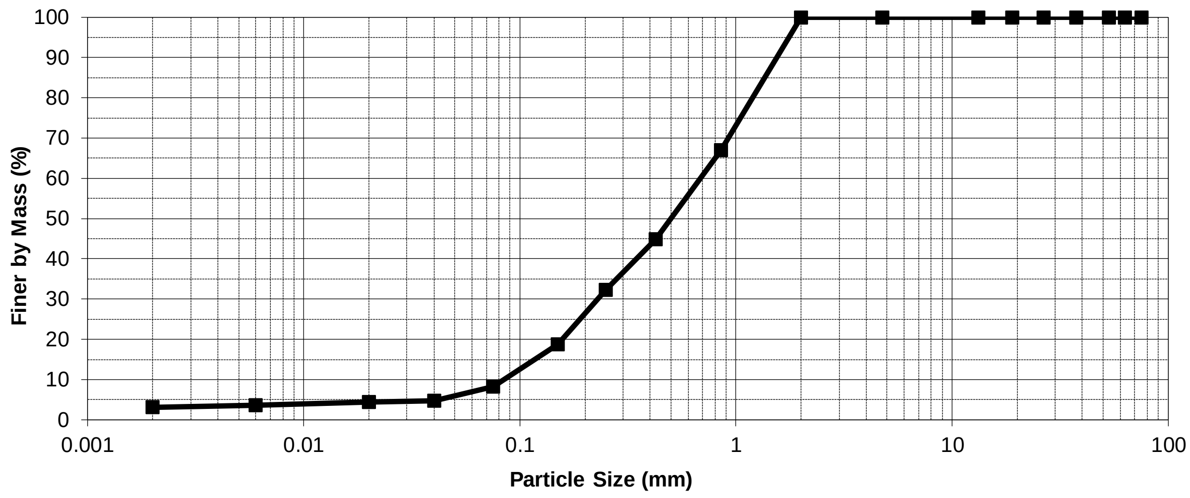


Figure 2-25: Particle size distribution diagram for ESP

The spoils are well graded with a CC of 1.1 and a Cu of 8.7, indicating there is a broad range of particles. The particle size distribution curve indicates the soil is well graded and forms a uniform line.

2.3.4 Concentration indices

The availability percentages (A%) were calculated for all the elements in the growth medium, and the results are tabulated below in a colour gradient (Table 2-5), where low is depicted in green, moderate in yellow, moderate to high in orange and high in red. It should be noted that the colour scale was merely used for comparative purposes.

Table 2-5: Availability percentages of elements in growth mediums

Sample:	**EAGRI**	**ESI**	**EAMD**	**ESP**
	Availability %			
Be	NC	NC	6,61%	2,53%
B	22,58%	NC	51,58%	3,48%
Na	44,69%	7,58%	12,77%	4,31%
Mg	11,55%	16,26%	7,28%	14,63%
Al	0,03%	7,91%	1,99%	1,80%
P	1,73%	2,72%	0,37%	0,11%
K	10,10%	6,93%	1,36%	0,63%
Ca	13,34%	33,60%	40,42%	15,28%
Ti	0,03%	0,19%	0,01%	0,00%

Sample:	EAGRI	ESI	EAMD	ESP
	Availability %			
V	0,05%	NC	0,00%	NC
Cr	0,05%	0,32%	0,07%	0,08%
Mn	0,13%	39,52%	12,00%	27,94%
Fe	0,01%	0,03%	0,01%	0,10%
Co	0,09%	8,16%	13,30%	11,89%
Ni	0,10%	0,66%	4,26%	6,64%
Cu	0,46%	0,82%	1,48%	1,64%
Zn	0,13%	0,55%	1,08%	4,99%
As	0,45%	5,34%	0,58%	0,51%
Se	0,33%	12,71%	11,85%	4,89%
Rb	0,61%	4,05%	2,29%	1,88%
Sr	21,41%	14,57%	7,42%	0,93%
Mo	0,22%	NC	NC	NC
Pd	3,60%	21,10%	13,25%	3,43%
Ag	0,38%	1,14%	0,15%	0,07%
Cd	53,18%	NC	124,65%	30,95%
Sn	0,40%	0,50%	0,44%	0,35%
Sb	0,60%	NC	NC	0,08%
Ba	7,09%	28,32%	0,72%	0,34%
Pt	NC	NC	NC	NC
Au	NC	NC	NC	NC
Hg	0,58%	NC	NC	NC
Tl	1,42%	NC	6,34%	5,41%
Pb	0,10%	3,13%	2,46%	0,06%
Bi	NC	NC	NC	NC
Th	0,01%	0,01%	0,00%	NC
U	0,15%	NC	7,29%	0,29%

**NC – Not calculated due to total concentration being not detectable*

Key : Very low, low, medium, medium to high, high and very high.

The Cd availability percentage in EAMD was exceptionally high and relatively high in both the ESP and EAGRI. Cadmium alters plants' functional and structural properties, inhibits the uptake and utilisation of nutrients in the soil, and could result in plant tissue death in severe cases. Following Cd, Mn and Pb also had high availability percentages, indicating these elements are plant-available and could affect vegetation growth. A cluster diagram was generated (Figure 2-26), based on the availability percentages.

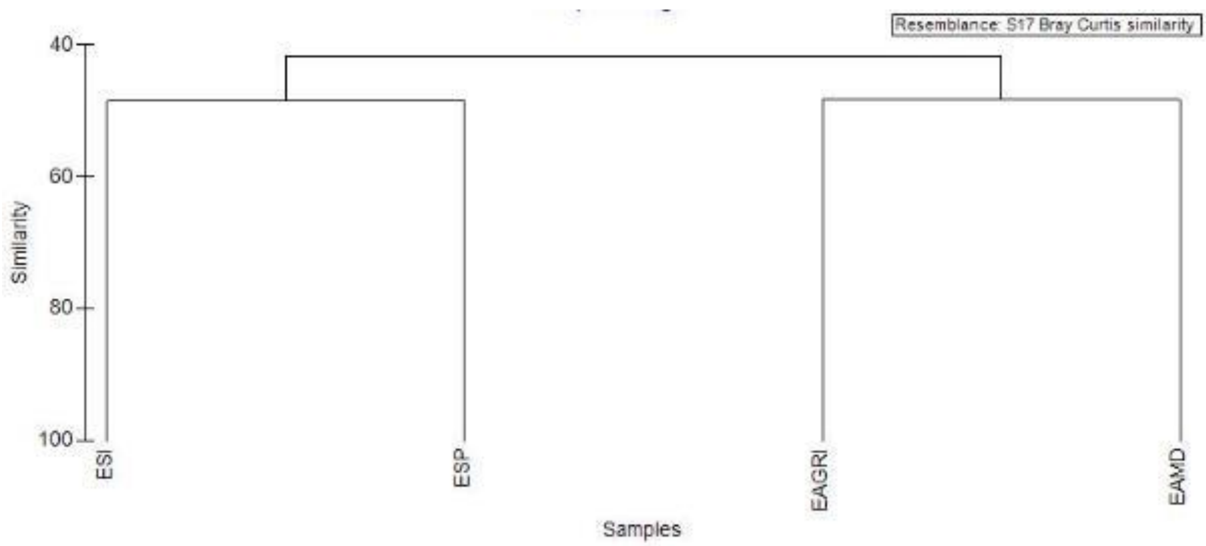


Figure 2-26: Cluster diagram of Availability of CoPCs

It is evident from the cluster diagram that no definitive similarities were observed between the growth mediums in terms of availability percentages, and the growth mediums had a similarity of roughly 40%.

2.4 Conclusion

The particle size distribution of the growth mediums will not be a significant limiting factor for plant growth. The successful establishment of vegetation should improve the soil structure due to root penetration and carbon sequestration. Other soil physical properties like bulk density, aggregate stability, and porosity, amongst others, should be investigated to define the physical constraints in more depth.

Based on the basic chemical analyses, the AMD-polluted soil (EAMD) and coal spoil materials (ESP) were acidic, and lime will have to be applied to increase the soil pH. The chemical results indicated that soil ameliorants in the form of fertiliser would also be required to sustain plant growth. Iron, followed closely by aluminium, had the highest concentrations in the AMD-contaminated soil and coal spoil material. The excess iron might cause bronzing of the younger leaves of the plants, and at toxicity stages, necrosis may occur, and eventually, the leaves could die.

None of the growth mediums, including the AMD-polluted soil and coal spoils, is classified as contaminated, based on the comparison with the SSV thresholds. This might be an indication that the threshold values are not stringent enough. Certain elements did not have SSV threshold values. As part of a future study, it is recommended that thresholds be allocated for all the CoPC associated with coal or that a tailored screening tool be developed to assess contamination in coal and coal-contaminated soils. The specific focus should be on iron, aluminium, titanium and barium.

Cadmium had the highest availability percentage, indicating that in relation to the total concentration, the majority, if not all, of the cadmium in the growth mediums were plant-available.

Based on the results of the growth mediums, it can be concluded that hyperaccumulator plant species that are salt-tolerant and can grow in acidic soils will need to be selected for sustainable long-term rehabilitation success. The plant species should be chosen based on their ability to thrive in mediums with similar concentrations and should be steered by previous studies.

2.5 References

Akabzaa, T.M., Armah, T.E.K., and Baneong-Yakubo, B.K. 2007. Prediction of acid mine drainage generation potential in selected mines in the Ashanti metallogenic belt using static geochemical methods. Environmental Geology, 52(5): 957-964. DOI: https://doi.org/10.1007/s00254-006-0537-1.

Akinyeye, R.O., and Omoniyi, P., 2016. Comparative chemical and trace element composition of coal samples from Nigeria and South Africa. American Journal of Innovative Research and Applied Sciences. *https://american-jiras. com/Akinyeye% 20ManuscritRef*. (accessed 10 October 2022).

Bradawy, A.M., Ganat, T.A.A.O. (2022). Porosity. In: Rock Properties and Reservoir Engineering: A Practical View. Petroleum Engineering. Springer, Cham. https://doi.org/10.1007/978-3-030-87462-9_3.

Burland, J., Chapman, T., Skinner, H. and Brown, M. 2012. ICE manual of geotechnical engineering. Volume 1: Geotechnical Engineering Principles, Problematic Soils and Site Investigation: ICE Publishing. ISBN: 9780727757074.

Cowan, A.K., Lodewijks, H.M., Sekhohola, L.M., Edeki, O.G. 2016. In situ bioremediation of South African coal discard dumps. Paper presented at the Mine Closure 11th International Conference on Mine Closure; 2016, March15-17; Perth: Australia.

Cummings, R.W. and Elliott, G.L. 1991. Soil chemical properties. In: Charman, P.E.V. and Murphy. B.W., eds. Soils: their properties and management. A soil conservation handbook for New South Wales. Sydney, Australia: Sydney University Press. p. 193-205.

Das, B.M. and Sobhan, K. 2012. Principles of Geotechnical Engineering. 8th ed. Cengage Learning. USA.

DEA (Department of Environmental Affairs). 2010. Framework for the Management of Contaminated Land. [cited 2022 Sept 4] Available from: http://sawic.environment.gov.za/documents/562.pdf.

Epstein, E. 1965. Mineral metabolism. In: Bonner, J., and Varner, J.E., eds. Plant Biochemistry London: Academic Press. p 438-466.

Fay, M. 2010. Soils of South Africa. Distribution, properties, classification, genesis, use and environmental significance. Cape Town: Cambridge University Press. ISBN: 9781107000506.

FHWA, (Federal Highway Administration) [internet]. 2006. Soils and Foundation: Reference Manual – Volume 1. National Highway Institute. U.S. Department of Transportation. [cited 2021 Oct 4]. Available from: https://www.fhwa.dot.gov/engineering/geotech/pubs/nhi06088.pdf.

Finkelman, R.B. 1993. Trace and Minor Elements in Coal. In: Engel, M.H. and Macko, S.A., eds. Organic Geochemistry. Topics in Geobiology, vol 11. Boston, MA.: Springer. DOI: https://doi.org/10.1007/978-1-4615-2890-6_28.

Goldschmidt, V., M. 1923. Geochemische Verteilungsgesetze der ElementeSkrifter utg. av det Norske Visenskaps-Akademii i Oslo I. Mat-Naturv Klasse 2:1–17.

Hancox, J.P. and Gotz, A.E. 2014. South Africa's coalfields - a 2014 perspective. International Journal of Coal Geology. 132(1): 170-254.

Hattingh, R., Tanner, P. and Aken, M. 2019. Land Rehabilitation Guidelines for Surface Coal Mines. Land Rehabilitation Society of Southern Africa (LaRSSA), Coaltech Research Association and the Minerals Council of South Africa.

Holtz, R.D. Kovacs, W.D. and Sheahan, T.C. 1981. An Introduction to Geotechnical Engineering. Vol. 733. Englewood Cliffs: Prentice-Hall. p. 773.

Kabata-Pendias, A. 2011. Trace elements in soils and plants. 4th ed. Boca Raton: Taylor and Francis. p. 432.

Läuchli, A. and Grattan, S. 2012. Soil pH extremes. In: Shabala, S., ed. Plant stress physiology. Boston, MA: CABI.

Marschner, H. 1974. Calcium nutrients of higher plants Netherlands Journal of Agricultural Science. 22(4): 275-282.

Mengel, K. and Steffens, D. 1985. Potassium uptake of rye-grass (Lolium perenne) and red clover (Trifolium pratense) as related to root parameters. Biology and Fertility of Soils. 1(1): 53-58.

Mentis, M.T. 1999. Diagnosis of the Rehabilitation of Opencast Coal Mines on the Highveld of South Africa. South African Journal of Science. 95(5): 210-217.

Modabberi, S., Alizadegan, A., Mirnejad, H. and Esmaeilzadeh, E. 2013. Prediction of AMD generation potential in mining waste piles, in the Sarcheshmeh porphyry copper deposit, Iran. Environmental Monitoring and Assessment. 185(11): 9077-9087.

Montoro, M.A. and Francisca, F.M. 2010. Soil Permeability Controlled by Particle-Fluid Interaction. Geotechnical and Geological Engineering. 28: 851-864. DOI: 10.1007/s10706-010-9348-y.

PDA (Potash Development Association) [Internet]. Soil Analysis: Key to Nutrient Management Planning. Leaflet No. 24;[updated 2011 Feb 17; cited 2022 April 16]. Available from:https://www.pda.org.uk.

Prasad, M.N.V., Malec, P., Waloszek, A., Bojko, M. and Strzalka, K. 2001. Physiological responses of Lemna trisulca L. (duckweed) to cadmium and copper bioaccumulation. Plant Science, 161.

Prüeβ, A. 1997. Action values for mobile (NH4NO3-extractable) trace elements in soils based on the German national standard DIN 19730. In Prost, R., ed. Contaminated soils. 3rd International conference on the biogeochemistry of trace elements. Institut National de la Recherche Agronomique, Paris. p. 415-423.

Rinaldo, J., Varsha, S., Meenu, G., Pardeep, K. and Ankit, S. 2017. Acid Mine Drainage: Sources, Impacts and Prevention [internet]. Environmental Biotechnology. [cited 2022 Aug 7]. Available from Biotech Articles: https://www.biotecharticles.com/Environmental-Biotechnology-Article/Acid-Mine-Drainage-Sources-Impacts-and-Prevention-3801.html.

Rösner, T., Boer, R., Reyneke, R., Aucamp, P. and Vermaak, J. 2001. A preliminary assessment of pollution contained in the unsaturated and saturated zone beneath reclaimed gold mine residue deposits. WRC Report No. 797/1/01, Pretoria.

RSA (The Republic of South Africa). 2014. NEMWA (National Environmental Management: Waste Act [59/2008]: National norms and standards for the remediation of contaminated land and soil quality, 2014. (Notice 331). Government gazette, 37603:3 [Internet]. Pretoria: Government Printer. [cited 2022 Jan 23]. Available from South African Government: https://www.gov.za/documents/national-environmental-management-waste-act-national-norms-and-standards-remediation-1.

The Non-Affiliated Soil Analysis Work Committee. 1990. Handbook of standard soil testing methods for advisory purposes. Soil Science Society of South Africa. Sunnyside, Pretoria.

USEPA (U.S. Environmental Protection Agency). 1994. Technical Document - Acid Mine Drainage Prediction. EPA 530-R-94-036 [internet] Washington, DC 20460; [cited 22 March 2022]. Available from: https://www.epa.gov/sites/default/files/2015-09/documents/amd.pdf.

Vardaki, C. and Kelepertsis, A. 1999. Environmental impact of heavy metals (Fe, Ni, Cr, Co) in soils waters and plants of Triada in Euboea from ultrabasic rocks and nickeliferous mineralisation. Environmental Geochemistry and Health, 21(3): 211-226.

Welsh, D., Bianco, A. and Roe, P. 2007. A risk assessment approach for comparing mine rehabilitation and closure options at coal mines in central Queensland, Australia. In: Mine Closure 2007. Santiago, Chile: Salviat Impresores. 1st Ed. p. 167-178.

Williamson, N.A., Johnson, M.S. and Bradshaw, A.D. 1982. Mine Waste Reclamation. London, UK: Mineral Industry Research Organisation.

Winegardner, D.L. 2000. An Introduction to Soils for Environmental Professionals. New York: Routledge. 288 pp. ISBN: 0873719395.

Chapter 3 Plant growth response factors of pasture species grown in coal mine substrates

Schmidhuber[1], B.E and Truter, W.F[2]

[1]Centre for Environmental Studies, University of Pretoria

[2]Department of Plant and Soil Sciences, University of Pretoria

Abstract

The rehabilitation of coal mines is pertinent to prevent pollution and the potential migration of pollutants that impact the receiving environment. Vegetation covers, especially grass covers, are the most popular covers concerning coal mine rehabilitation in South Africa due to the covers' vast array of functions. Grasses growing in coal-contaminated soil experience a selection of plant stresses that contribute to the difficulty to successfully establish vegetation on mined areas. The most common factors that contribute to plant stress on coal mines are extreme weather conditions like drought, unfavourable soil conditions like low pH, high salinity, and high concentrations of trace elements, to name a few. In turn, high trace element concentrations bring about symptoms in plants like reduced biomass and/or reduced photosynthesis, resulting in discolouration and biochemical disorders.

Stress in different plant species in several growth mediums was assessed. Two contaminated growth mediums, namely coal spoil material (ESP) and soil contaminated with acid mine drainage (EAMD), were sourced from a mine situated in the eastern Highveld grasslands in Mpumalanga. Several soil ameliorants were added to the two contaminated mediums and two control growth mediums, one of optimal quality and one of substandard, namely EAGRI (agricultural soil) and ESI (quartz sand), respectively. The four growth mediums were used in a pot trial, consisting of six different plant species and/or mixtures of species and four replications of each. During the study, biomass production, plant height, chlorophyll employing a Soil Plant Analysis Development (SPAD) chlorophyll meter and forage quality were assessed utilising Near-Infrared Reflectance Spectroscopy (NIRS). The growth mediums' chemical composition did not have definite effects on the forage quality, based on the results and no signs of plant stress were observed in the pasture species grown in the contaminated growth mediums.

Keywords: Plant stress, coal mines, pasture species, SPAD, NIRS,

3.1 Introduction

The contamination of the environment by coal mines, especially in the Highveld, poses serious health risks to humans and animals alike and has become a critical subject. The establishment of vegetation on mined areas is a common practice in the coal rehabilitation industry of South Africa. The aim of rehabilitation is to re-establish functional ecosystems by utilising plants to stabilise and increase biodiversity for sustainability. Another benefit of using plants is that the ecosystem gets restored naturally, which is also more economical, than the excavation and disposal of contaminated substrates.

Ideally, mine rehabilitation entails the transformation of an active mine into a safe and stable landform that is non-polluting and provides animal habitats and ecosystem services, as well as support economic activities associated with the new land use in accordance with regulatory requirements (Tanner, 2007; van Deventer *et al*., 2008; Australian Government *et al*., 2016; Hattingh *et al*., 2019). These activities and habitats that are created may differ from those historically present on the site before mining commenced.

The newly created rehabilitated environment should allow for alternative end land use opportunities, not lead to additional environmental deterioration and degradation, and should be aligned with the surrounding aesthetic values of the area. The focus will be coal mine rehabilitation in the Mpumalanga Highveld region, and it is only fitting that grasses be the focal point in plant species selection, as the area falls in the grassland biome. The use of grasses also has alternative end land-use opportunities like grazing, forage production, and, for certain species, the production of essential oils, to name a few.

To determine the prospects of using grasses as the basis for an alternative land use like grazing, the stress responses in the grass growing in polluted growth mediums need to be assessed over time. Plant stress responses usually include reduced biomass production, discolouration, or chlorosis of the leaves. Suppose forage production and grazing are considered viable land uses, the changes in forage quality and quantity of the grasses growing in coal contaminated growth mediums should be determined.

A specific objective is to identify suitable pasture species that will be able to grow sustainably in the contaminated soil while also providing adequate surface coverage in a short period. Another objective is to compare biomass production in the two contaminated growth mediums with the two control growth mediums to assess the overall performance of the plants and to evaluate the above ground regrowth after cutting, by measuring the plant height.

The forage quality of grasses grown in contaminated growth mediums was compared to the forage quality of grasses grown in the control growth mediums. The last objective was to determine if the growth mediums' chemical characteristics influence leaf chlorophyll concentrations as an indicator of plant stress.

It can be hypothesised that the pasture species growing in the coal-contaminated substrates namely, the coal spoils (ESP) and acid mine drainage (AMD) contaminated soil, will experience more plant stress than the plants growing in both the optimal and substandard controls, namely EAGRI and ESI. Due to the predicted low plant stress in the control mediums, the grass in the control mediums is anticipated to have higher forage quality and chlorophyll concentrations than the grasses in the contaminated growth mediums.

3.2 Materials and methods

This study included pot trials, chemical analyses and data processing, and the summative experimental element will be explained below.

The growth mediums were sourced from a coal mine situated in the eastern Highveld grasslands (Gm12), encompassing Mpumalanga and Gauteng. The topography has slight to moderate undulating plains and includes low hills and pan depressions (Mucina and Rutherford, 2011). The dense grasslands are usually dominated by species like *Eragrostis, Digitaria, Themeda* and *Aristida* (Mucina and Rutherford, 2011).

It is a summer rainfall area with mean annual precipitation (MAP) from 600 mm to 900 mm and has dry winters (Mucina and Rutherford, 2011).

3.2.1 Experimental Layout

Critical components considered during the plant selection phase were the composition of trace elements in the substrate, the characteristics of the substrate, and the plant's ability to accumulate trace elements in shoots and roots. Other aspects considered included fast-growing nature, high biomass production, the plant's tolerance to elevated elemental concentrations and the capacity to remove trace elements from the substrate.

The pot trial consisted of four (4) substrates: silica or quartz sand, agricultural soil, coal mine spoil material, and AMD-contaminated soil (refer to Table 3-1 and Figure 3-2). Six different plant species and/or mixtures of species were used during the experiment.

The species used included (Figure 3-1), starting from the left, *Cynodon dactylon* (Couch grass), *Chrysopogon zizanioides* (Vetiver), *Chloris gayana* (Rhodes), *Pennisetum glaucum* (Babala), *Medicago sativa* (Lucerne) and a mixture which included *C. dactylon, C. gayana, Digitaria eriantha* (Smuts), *M. sativa* and *Eragrostis tef* (Teff). Details on the pasture species can be found in Chapter 1.

Figure 3-1: Plant species selected for the pot trial

The trial consisted of four replicates of each species for each growth medium or substrate, e.g., EAMD-B1, EAMD-B2, EAMD-3 and EAMD-B4. Five seedling plugs were planted in the 96 separate pots, amounting to 480 individual plants.

Table 3-1: Treatment descriptions and abbreviations

Abbreviation	Description
	Substrate
EAMD	AMD contaminated soil
EAGRI	Agricultural soil (Optimal control)
ESP	Coal Spoils
ESI	Silica sand (Substandard/ sterile control)
	Species
B	*Pennisetum glaucum*
C	*Cynodon dactylon*
M	*Cynodon dactylon, Chloris gayana, Digitaria eriantha, Medicago sativa , Eragrostis tef*
L	*Medicago sativa*
R	*Chloris gayana*
V	*Chrysopogon zizanioides*

Figure 3-2 illustrates the different species and treatment combinations, and immediately after planting, the pots were placed in a randomised design in a greenhouse on the University of Pretoria Experimental Farm, Phytotron D at Innovation Africa.

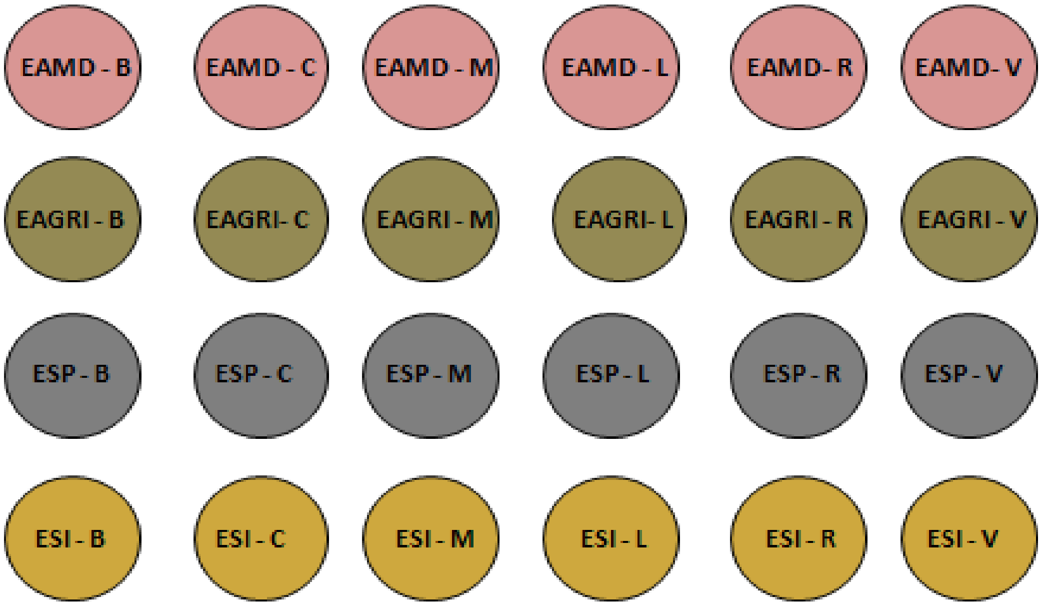


Figure 3-2: Species and treatment combinations

Before planting, lime was applied and mixed into two growth mediums (ESP and EAMD). The lime quantity for ESP equated to 40 t/ha and EAMD to 20 t/ha. The lime and growth medium mixtures were left for approximately one month to react, after which the seedling plugs were planted in all four growth mediums, and then fertilised.

The pots were fertilised three times during the growing season, directly after each harvest. The plugs were planted in the pots in December 2020 and harvested in February, April, and June of the following year.

Often, mine rehabilitation practices ignore the analyses of topsoil to ultimately apply fertiliser according to the initial nutrient concentrations. In terms of the fertiliser quantities, the same approach was taken that has been implemented on most mines. The fertiliser that was applied were as follows; 200 kg N/ha, 200 kg P/ha and 250 kg K/ha, which accounts for common plant productivity. The plants were kept in a controlled environment and watered daily for the first two weeks, thereafter every second day until the experiment was completed.

Composite samples of the substrate were taken to determine CoPCs concentration in the growth medium before vegetation establishment (refer to Chapter 2).

3.2.2 Plant productivity

Each plot was harvested at two-month intervals in February, April and June. A pair of sheers were used, and each plug was cut roughly 5 cm from above the growth medium, as illustrated in Figure 3-3.

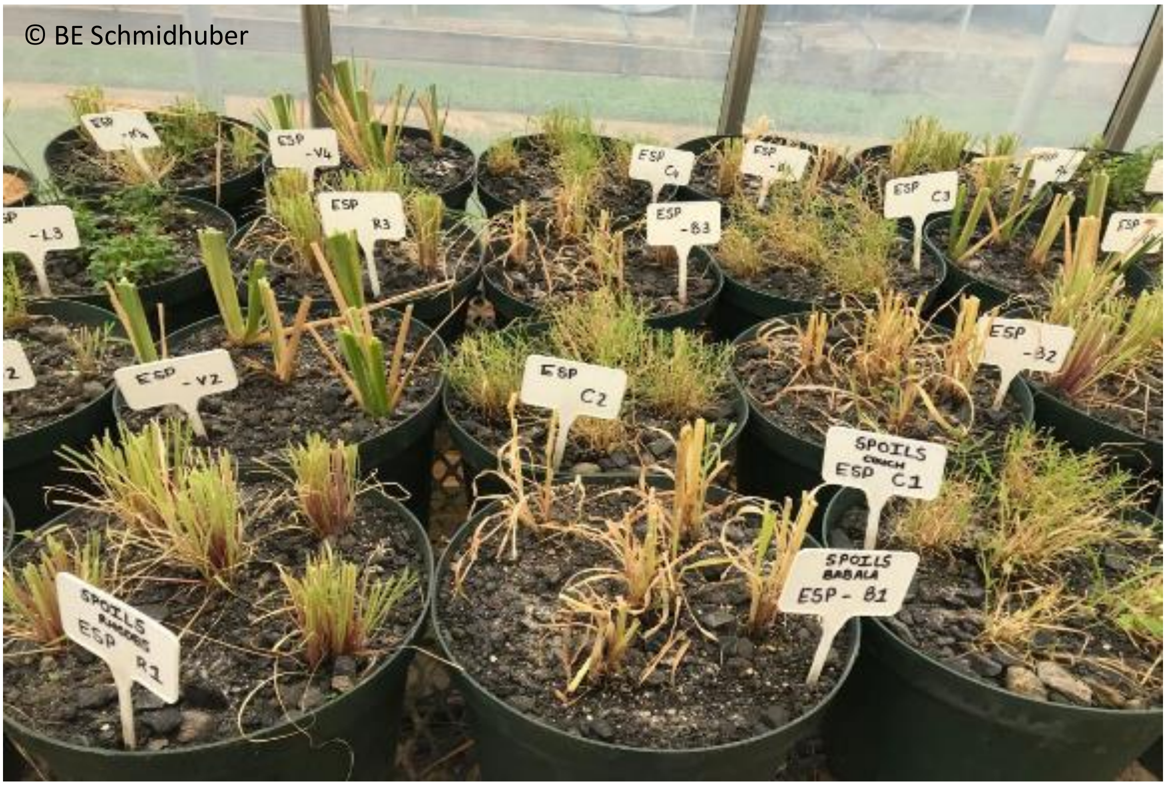


Figure 3-3: Photograph of pots after February harvest

All the plant material collected was placed in brown paper bags, after which it was weighed. The grass samples were then oven-dried at 50°C for approximately 48 hours and weighed to determine total dry biomass.

All the biomass figures in each plot were expressed in $kg{\cdot}ha^{-1}$, $t\ ha^{-1}$ or $g{\cdot}m^{-2}$.

3.2.3 Plant height measurements

The length of the grasses was measured before they were cut roughly 5 cm from the soil surface. The length of the grass is indicative of the regrowth rates and can be used to assess the regrowth of species grown in the different growth mediums.

A tape measure was utilised to measure each plug (96) in all the pots from the substrate surface to the highest or, in the case of *C. dactylon,* the most protracted part of the plant, to determine the plant height. The plant height was measured twice during the growing season, and the results were recorded. The results were processed and expressed as average height in Section 3.3.

3.2.4 Forage quality

Another critical component of this study was to determine the forage quality of the grass to assess the practicality of using rehabilitated areas as a source of income for end land users by grazing or producing animal feed post-closure.

3.2.4.1 *Soil Plant Analysis Development (SPAD) readings*

The Soil Plant Analysis Development (SPAD) chlorophyll meter, in this case, the SPAD-502-meter (Konica-Minolta, Japan), measures relative leaf chlorophyll levels without the mentioned disadvantages, and is inexpensive. It is a hand-held device with two light-emitting diodes and a silicon photodiode receptor, which measures leaf transmittance in infrared (940 nm), red (650 nm), and a reference wavelength. The device uses these transmittance values to derive a relative SPAD meter value (typically between 0 and 50) proportionate to the amount of chlorophyll in the leaf (Uddling *et al*., 2007).

Before the February harvest, the chlorophyll of the plants was analysed by means of a SPAD-502-meter. Before the April and June harvests, the chlorophyll contents were measured with a MC-100 SPAD meter (Figure 3-4).

Figure 3-4: Apogee MC-100 SPAD meter

A single grass leaf was placed between the light-emitting diodes (LEDs) and a photodiode receptor. SPAD meter readings were taken at three different localities on the plant: the bottom section slightly above the ground, the middle section of the plant, and near the tip of the plant. The SPAD reading measurements were done for the three different growth stages of the plants: the vegetative stage, the transition stage, and the reproductive stage. Each pot’s SPAD reading was determined by taking the average of the 15 readings per pot.

3.2.4.2 Near-Infrared Reflectance Spectroscopy (NIRS) scans

To use the grass planted in rehabilitated areas for animal feed, which is the most probable post-closure land use, the nutritional quality of grass grown in polluted growth mediums needs to be understood.

Forage quality analysis is essential and includes parameters like predicted protein, moisture, Neutral Detergent Fibre (NDF) and Acid Detergent Fibre (ADF), among others, to quantify forage quality analytically.

NDF content below approximately 40% would be considered suitable for legume forages, while above 50% would be regarded as poor (Van Saun, 2013). NDF < 50% would be regarded as high quality for grass forages and > 60% as low quality (Van Saun, 2013).

All the sampled pasture species grown in the different growth mediums underwent four scans in the Perten DA 7250 NIRS analyser (PerkiElmer Billerica: Massachusetts USA). Averages of grazing quality constituents like ADF%, protein content, NDF% were calculated from the four scans. Four replicas of each species in the different growth mediums were analysed at the University of Pretoria: African Forage, Fodder, Feed and Food Quality Reference Laboratory.

3.2.5 Statistical analysis

There was a total of 96 pots comprising four different growth mediums, two of which are controls, six plant species and four replications of each. Three data sets exist, one for each of the harvest rounds. The plant-specific analyses were statistically analysed with Gen Stat 64- bit Version 18.2 and the pot results were analysed with SAS Statistical Software.

Because two different SPAD meters were used during the experiment, the results had to be stabilised by means of Logarithmic transformation.

3.3 Results and discussions

The results obtained included the SPAD meter readings, plant heights, forage quality, and dry matter (DM) production.

3.3.1.1 Plant height

Each pot consisted of five plant plugs and the plugs were all measured during April and June. The mean plant height in terms of the growth medium and the species is illustrated in Figure 3-5, along with a 5% error margin. Based on the collective data *C. zizanioides* (V) grew the longest in all four of the growth mediums, followed by *C. gayana* (R).

An overall reduction in plant lengths was observed from April to June and can be attributed to the grass reaching the end of the growing season.

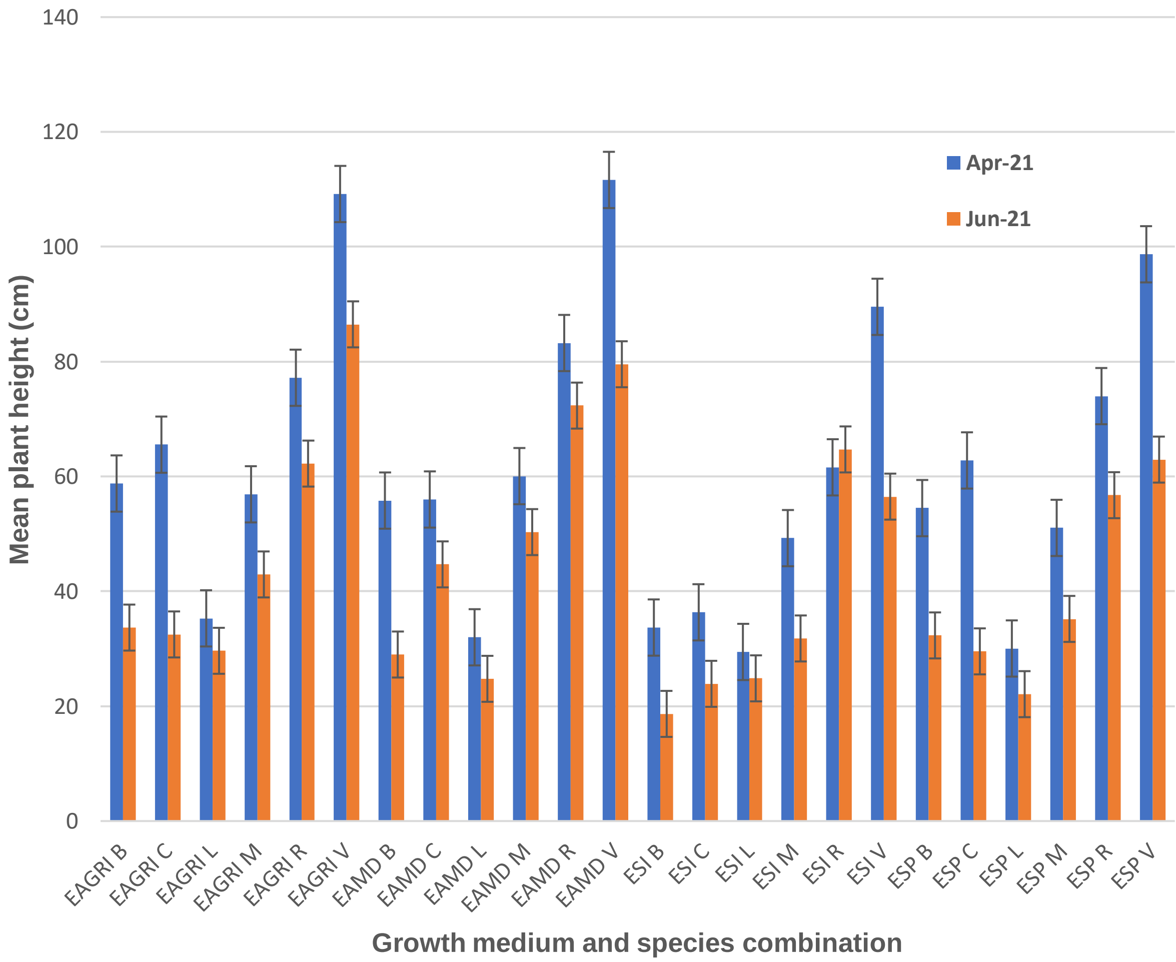


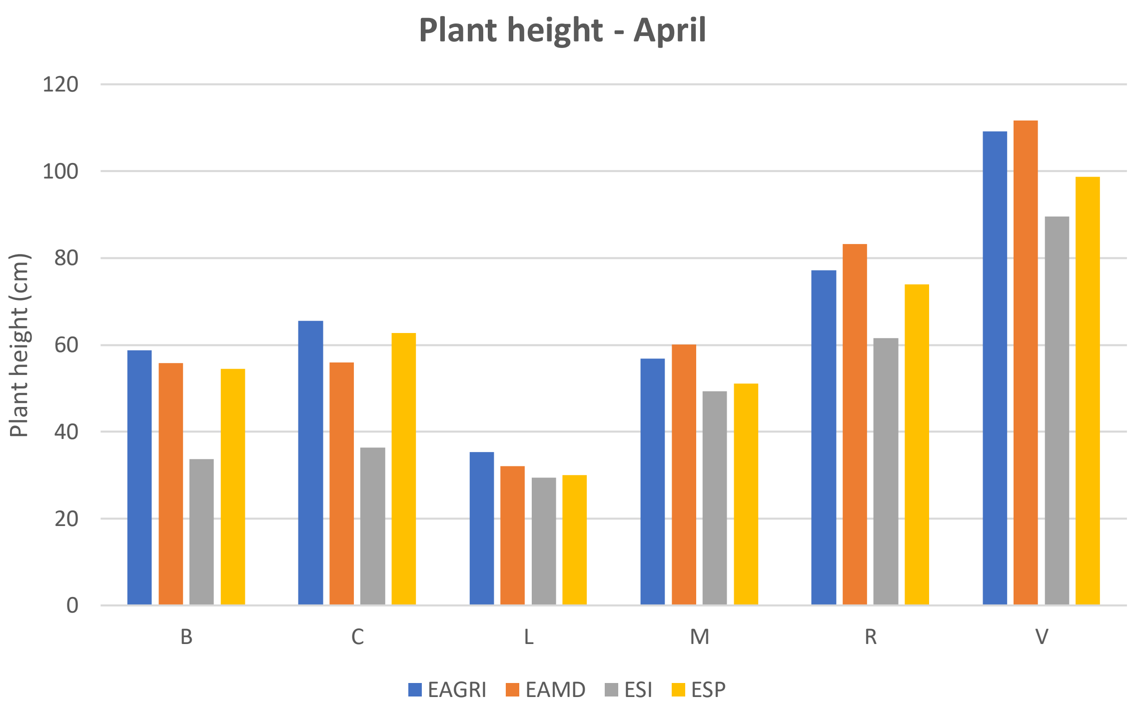
Figure 3-5: Mean plant height (cm) according to species and substrate *(Standard error calculated using PROC GLM)*

It is evident that the quartz sand (ESI) produced the shortest plants compared to the other growth mediums which can be attributed to the sand's low water and nutrient-holding capacity.

The average plant height for the ESI plants was 49 cm and 36 cm for April and June, respectively. The agricultural soils produced the longest grasses with a mean height of 67.1 cm and 47 cm for April and June, respectively, as expected.

The mean plant heights for April and June according to growth medium and species are illustrated in Figure 3-6.

The *M. sativa* (L) had the lowest average plant height compared to the other species, with an average height of 31 cm and 25 cm in April and June, respectively. The *P. glaucum* (B) had an average height of 50 cm and 28 cm in April and June. Even though *C. dactylon* is a creeping grass, it marginally outperformed the mixture of species in terms of average plant height.



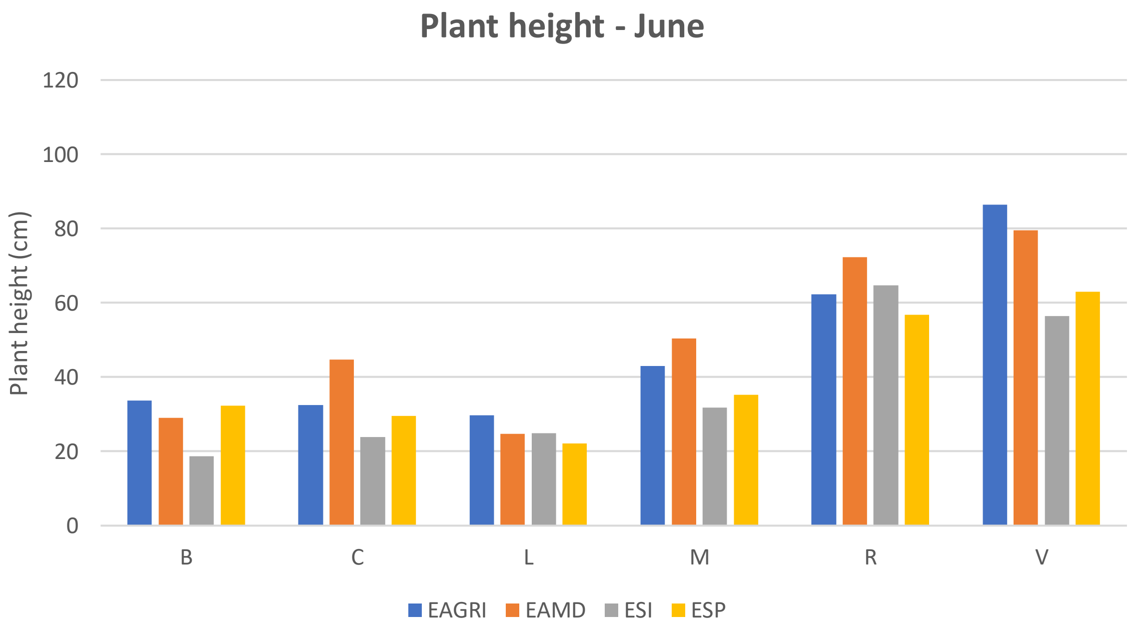


Figure 3-6: Mean plant height (cm) per harvest (Calculated using Genstat)

3.3.1.2 SPAD values

The SPAD chlorophyll meter results before the three harvests are represented according to growth medium and species in Figure 3-7.

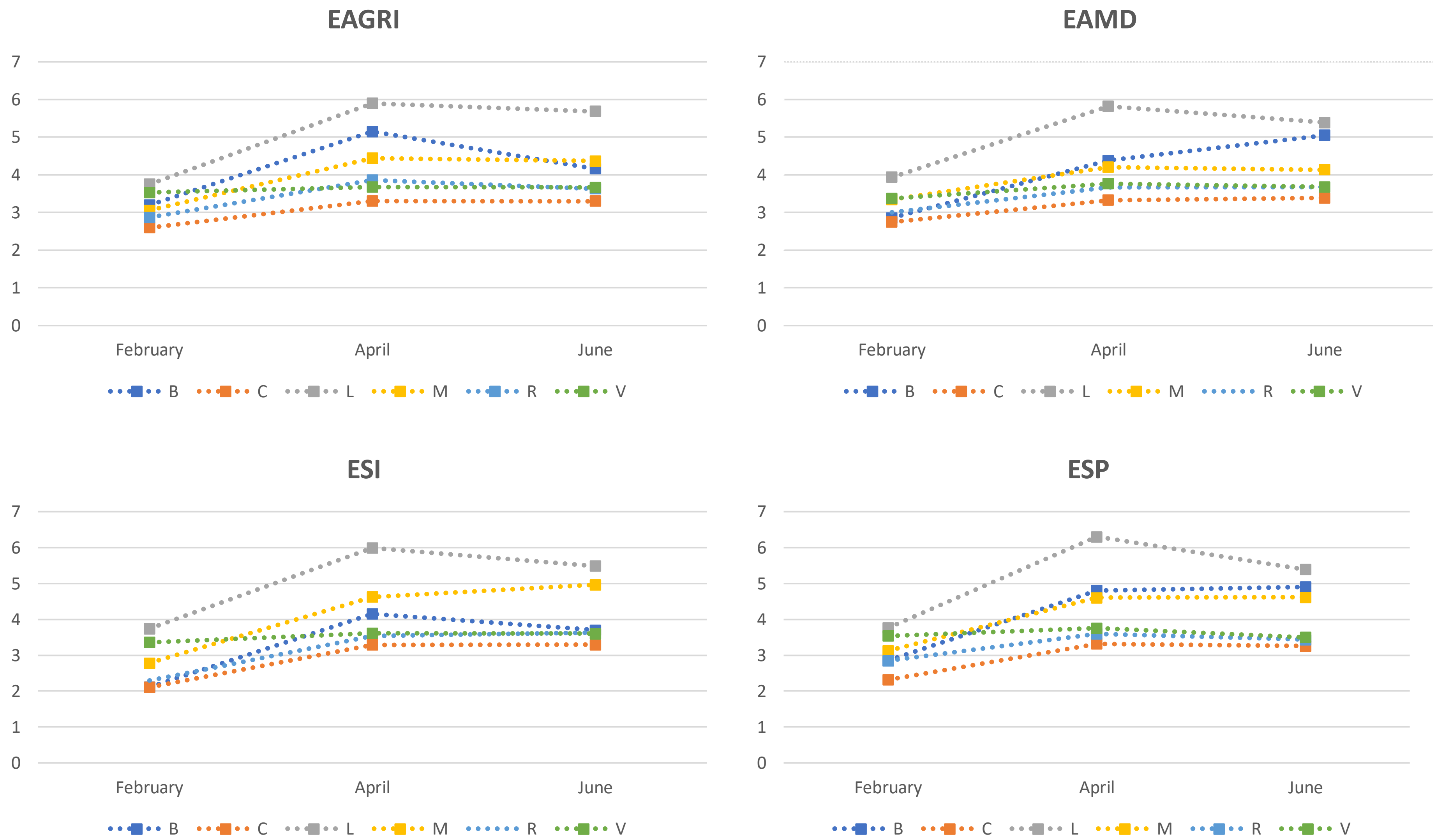


Figure 3-7: Mean SPAD readings according to the growth medium

The SPAD values were the lowest in February, representing the earliest stages, namely the vegetative state. In April, the SPAD values were the highest, indicating a spike in chlorophyll, and it should be noted that the grasses were in the transition phase and approaching winter dormancy. During the reproductive phase in June, the SPAD values reduced slightly and can be attributed to the fact that the species reached maturity.

The plants growing in the sand (ESI) had the lowest SPAD values compared to the other growth mediums, indicating the plants experienced the most stress in the cohesionless and nutrient-deficient sand for the duration of the growing season. As expected, the SPAD meter readings were the highest in the plants growing in the agricultural soils (EAGRI) and experienced the least amount of plant stress compared to the other growth mediums.

Overall, the SPAD meter readings for ESP and EAMD were fairly constant throughout the growing season, with the chlorophyll content of the plants growing in the coal spoils being higher in certain months and the plants growing in the AMD contaminated soil higher in others.

If the different species are compared, it is evident that lucerne produced the highest SPAD values. This can be validated visually, as *M. sativa* is naturally a darker shade of green when compared to grass. Compared to the other species*, C. dactylon* had the lowest SPAD values and could be due to the small leave blades, followed by *C. gayana*.

The SPAD meter readings for all the different species followed a similar trend indicating that the chlorophyll contents varied between the different pasture species and the different maturity stages of the species.

3.3.1.3 Biomass production

The fresh weight, in grams, of the species and growth medium combinations was recorded in February, April and June are tabulated in Table 3-2, along with the calculated standard deviations.

Table 3-2: Fresh weight production of species and growth medium combinations

Medium x Species	February		April		June	
	Mean (g)	Standard deviation	Mean (g)	Standard deviation	Mean (g)	Standard deviation
EAGRIxB	52.18	13.91	27.39	3.17	20.09	12.16
EAGRIxC	49.29	11.60	44.25	5.36	23.65	3.81
EAGRIxL	25.65	3.76	36.80	2.44	51.14	13.76
EAGRIxM	35.49	4.30	63.05	7.07	63.93	14.94

Medium x Species	February		April		June	
	Mean (g)	Standard deviation	Mean (g)	Standard deviation	Mean (g)	Standard deviation
EAGRIxR	76.14	8.65	70.96	9.69	44.89	2.59
EAGRIxV	74.68	21.21	63.25	5.63	41.09	2.92
EAMDxB	57.31	18.90	23.47	7.79	14.83	2.32
EAMDxC	47.70	4.93	39.68	1.43	44.13	3.30
EAMDxL	21.87	8.68	25.43	3.72	26.89	4.00
EAMDxM	44.35	10.45	56.85	11.87	69.28	10.81
EAMDxR	78.64	8.11	72.17	5.20	69.86	7.28
EAMDxV	53.80	12.37	48.64	6.91	40.96	3.07
ESIxB	19.57	1.95	21.73	2.33	13.63	3.10
ESIxC	16.85	0.39	28.07	2.04	23.39	0.77
ESIxL	25.49	2.20	40.95	13.10	43.75	5.14
ESIxM	30.80	3.00	45.12	8.43	45.01	10.71
ESIxR	24.72	3.16	56.93	7.94	41.12	5.53
ESIxV	36.89	3.07	36.28	4.16	22.94	2.86
ESPxB	59.52	19.64	33.94	14.71	ND	ND
ESPxC	30.60	4.82	46.92	4.35	28.82	3.74
ESPxL	28.72	6.90	25.85	3.89	39.78	11.76
ESPxM	42.96	8.25	51.38	7.94	50.77	4.68
ESPxR	44.20	11.13	74.92	10.93	42.33	11.50
ESPxV	51.46	12.02	41.37	4.12	26.24	1.41

The mean dry weight that was weighed during the three harvest rounds and the calculated standard deviations are tabulated in Table 3-3.

Table 3-3: Dry weight production of species and growth medium combinations

Medium x Species	February		April		June	
	Mean (g)	Standard deviation	Mean (g)	Standard deviation	Mean (g)	Standard deviation
EAGRIxB	19.92	2.91	21.10	3.85	12.35	0.32
EAGRIxC	21.57	1.81	24.34	1.76	16.17	1.27
EAGRIxL	15.59	0.86	15.78	1.25	19.78	1.75
EAGRIxM	18.48	0.96	24.31	1.86	23.91	2.57
EAGRIxR	29.98	1.20	30.76	3.04	20.95	1.01
EAGRIxV	29.17	5.26	27.39	0.47	19.60	0.54
EAMDxB	28.04	5.08	14.77	2.56	12.68	0.64
EAMDxC	24.51	2.57	22.10	1.88	22.74	0.74
EAMDxL	14.33	3.36	13.03	0.85	13.94	1.66
EAMDxM	21.88	2.87	24.86	2.81	25.30	1.84
EAMDxR	28.29	5.17	30.45	2.20	27.30	2.40
EAMDxV	26.49	3.14	22.06	2.55	20.41	0.81
ESIxB	14.86	0.53	16.46	1.39	10.69	0.82
ESIxC	14.45	0.21	20.49	1.11	15.48	0.63

ESIxL	17.12	0.43	19.02	2.62	16.98	0.85
ESIxM	18.51	0.69	22.34	1.89	18.28	1.91
ESIxR	16.89	1.08	27.77	2.24	17.96	1.69
ESIxV	21.10	1.17	19.78	2.33	13.11	0.51
ESPxB	24.67	4.52	17.60	4.13	-	-
ESPxC	16.26	2.78	25.74	1.43	17.25	1.33
ESPxL	16.08	1.56	14.27	0.78	17.41	2.40
ESPxM	19.19	3.17	24.30	1.46	21.24	1.32
ESPxR	21.66	2.15	29.44	4.68	21.62	2.07
ESPxV	22.78	1.67	20.75	0.99	16.37	0.67

The extrapolated fresh and dry biomass production from Table 3-2 and Table 3-3, are tabulated according to species and growth mediums in Table 3-4 and illustrated in Figure 3-8.

Table 3-4: Biomass production according to pasture species

Species	Date	Fresh biomass (kg·ha^{-1})	Dry biomass (kg·ha^{-1})
B	Feb-21	9605.54	4456.30
	Apr-21	5415.89	3561.80
	Jun-21	3297.83	2417.76
C	Feb-21	7356.75	3911.17
	Apr-21	8094.87	4720.20
	Jun-21	6111.74	3648.77
L	Feb-21	5181.97	3215.04
	Apr-21	6572.43	3163.34
	Jun-21	8229.67	3469.39
M	Feb-21	7824.25	3976.14
	Apr-21	11022.97	4880.68
	Jun-21	11663.74	4519.56
R	Feb-21	10770.08	4853.44
	Apr-21	14088.95	6057.08
	Jun-21	10095.88	4478.51
V	Feb-21	11044.21	5069.89
	Apr-21	9654.96	4583.58
	Jun-21	6684.35	3540.18

The average dry biomass that was harvested is graphically presented in Figure 3-8, according to growth mediums. The calculated standard deviations (SAS Statistical Software) are also illustrated on the graphs.

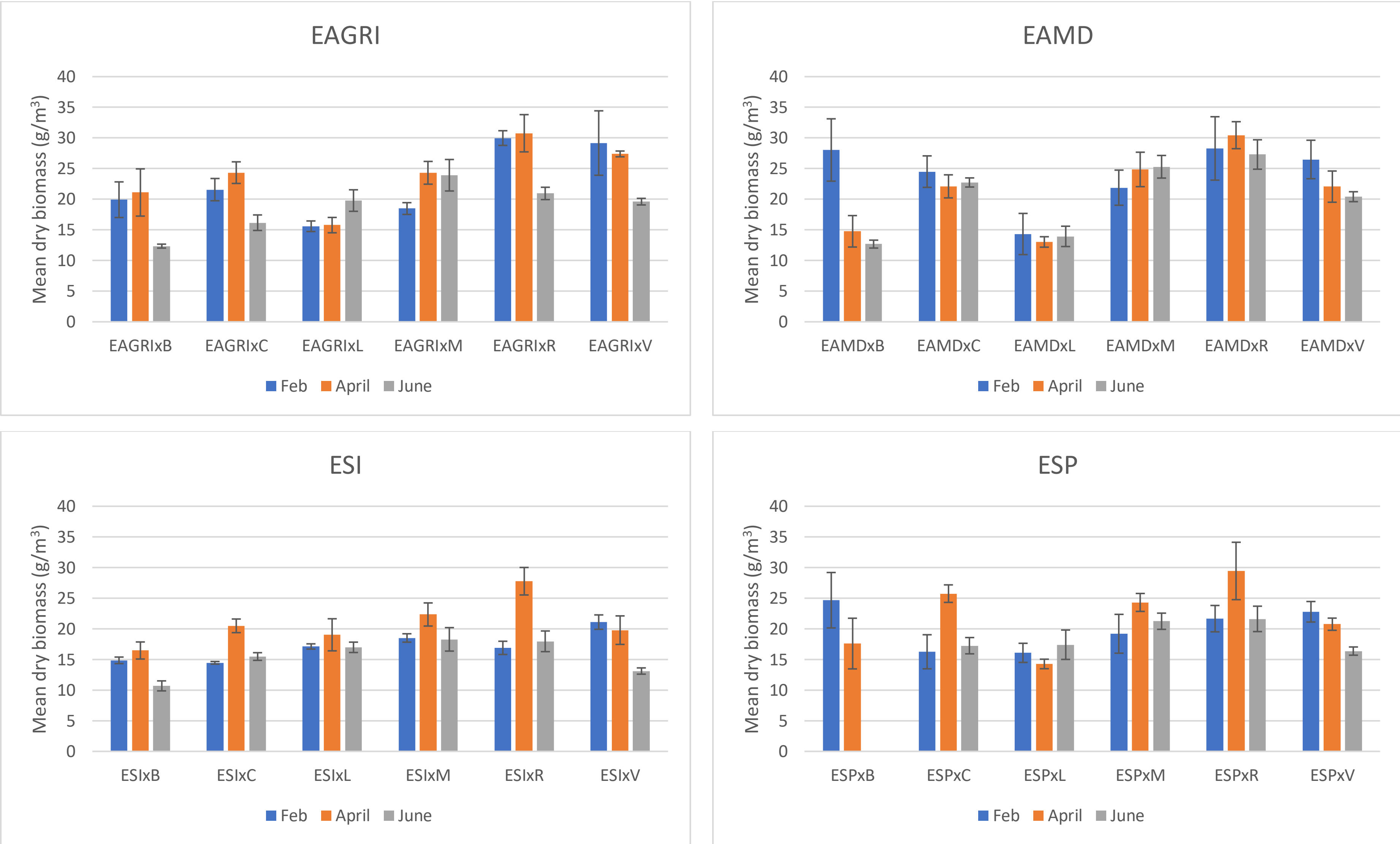


Figure 3-8: Average growth medium dry biomass production

The silica growth medium (ESI) produced the least dry biomass during all three harvests, followed by ESP. In February and in June, EAMD had more biomass than the optimal control (EAGRI); however, the reverse was observed in April. The average fresh biomass that was harvested is graphically presented in Figure 3-9, according to growth mediums.

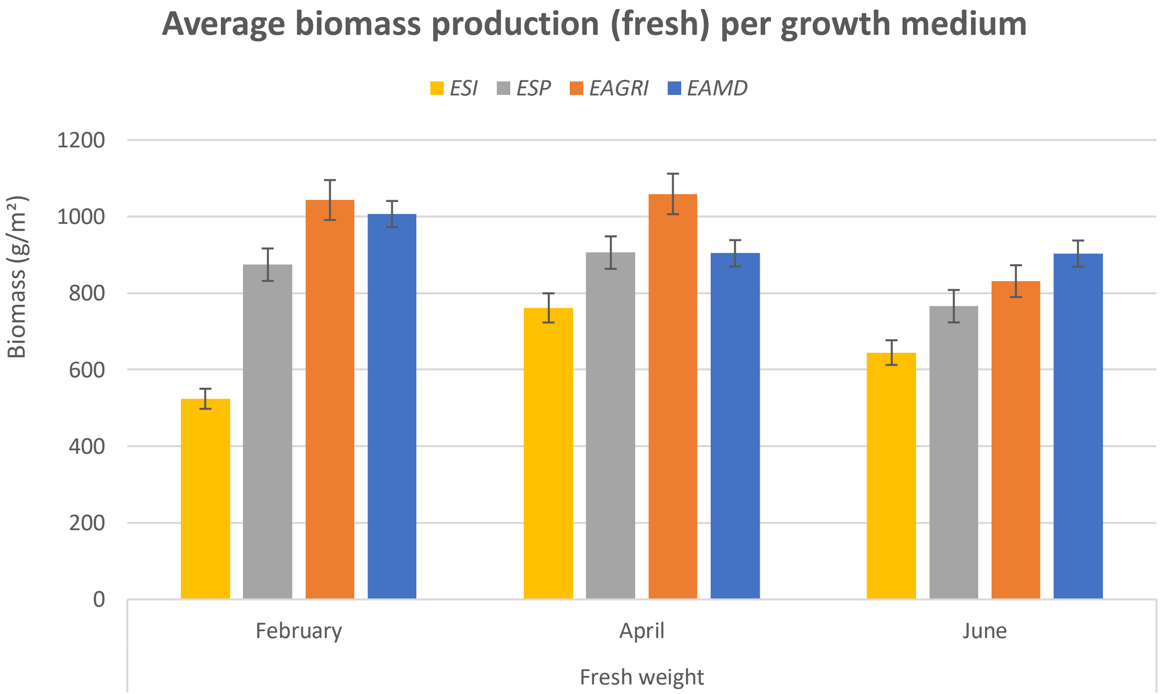


Figure 3-9: Average growth medium fresh biomass production *(Standard error calculated using PROC GLM)*

The agricultural soil (EAGRI) produced the highest quantity of fresh biomass in February and April; however, EAMD outperformed all the growth mediums in June in terms of fresh biomass. The sterile control (ESI) produced the least fresh biomass throughout the entire growing season, followed by ESP.

In February, V produced the most dry biomass, followed by R and B, while L produced the least. In April, R produced the most dry biomass and M the second most. In June, M slightly exceeded R. Overall, a decline in dry biomass reduction was seen for B. This was validated by the fact that the majority of B died progressively after the first harvest.

M and L gradually increased from the beginning of the growing season towards the end, indicating a gradual improvement. This observation strengthens the argument that a nitrogen source is required for increased biomass production as both these had *M. sativa* either solely or in combination with other species.

The cumulative mean dry biomass production for all the different growth mediums and plant species is illustrated in Figure 3-10 in tons(t)·ha^{-1}. Surprisingly, the highest dry biomass was obtained from (EAMDxR) at 17.52 t·ha^{-1} followed by EAGRIxR and EAGRIxV at 16.64 t·ha^{-1} and 15.51 t·ha^{-1}, respectively.

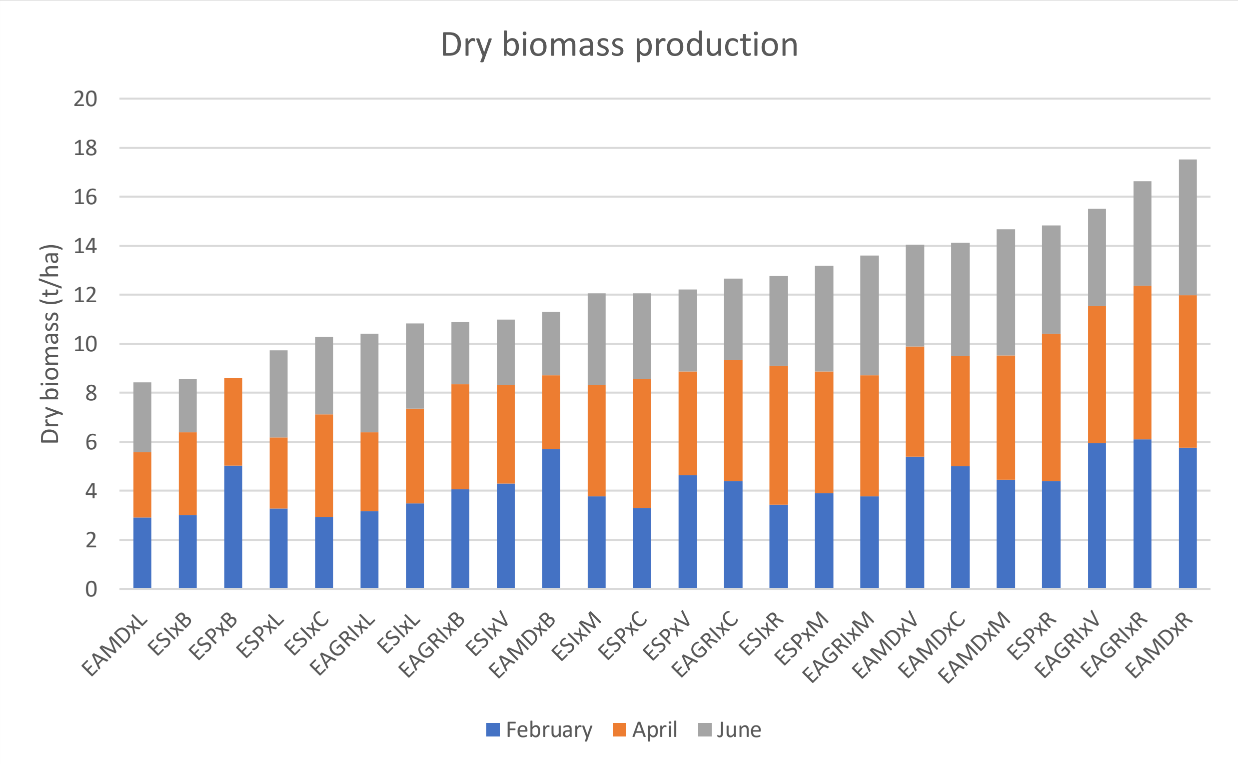


Figure 3-10: Cumulative dry biomass production per species and growth medium combinations

The stacked graph above (Figure 3-10: Cumulative dry biomass production), is purely for visual purposes, and no statistical analyses were conducted on the results.

The plants grown in the agricultural soils had the highest recorded fresh plant mass in February and April; however, in June, the fresh plant weight of the AMD contaminated soils was higher than EAGRI. The moisture content, the difference in weight in the EAGRI plants were also the highest, as expected.

It is evident that the B (*P. glaucum*) production reduced as the project progressed. V (C. zizanioides) production also reduced throughout the growing season. By contrast, the total fresh plant material production increased in the case of *M. sativa* (L) and the mixture (consisting of *C. dactylon, C. gayana, D. eriantha, M. sativa and E. tef).*

The calculated moisture content and weight according to growth medium are tabulated in Table 3-5 below.

Table 3-5: Moisture content and weight of biomass harvested from the different growth mediums

Growth medium	February		April		June	
	Weight of moisture (g)	Moisture content (%)	Weight of moisture (g)	Moisture content (%)	Weight of moisture (g)	Moisture content (%)
ESI	8,56	49,92	16,68	80,67	16,22	105,20
ESP	22,80	113,43	22,44	101,93	18,96	101,81
EAGRI	29,07	131,41	28,02	117,03	21,72	113,88
EAMD	25,47	106,49	23,16	109,19	23,92	117,33

ESI, the sterile cohesionless control, had the lowest moisture content throughout the growing season, followed by ESP. The agricultural soil (EAGRI) had the highest moisture content overall; however, the moisture content of the species grown in EAMD slightly exceeded EAGRI in June.

3.3.1.4 *Grade of growth inhibition*

The agricultural soil (EAGRI) was used as the control to calculate the grade of growth inhibition as depicted in (Eq. 1-5), and the results are illustrated in Figure 3-11. In February and June, the grasses in the AMD-contaminated soil outperformed the control medium in dry weight production. In June, ESP, the coal spoil material, produced roughly the same amount of dry plant material as the agricultural soil. Overall, the coal spoil material performed the worst, and the AMD-contaminated soil performed similarly to the control medium.

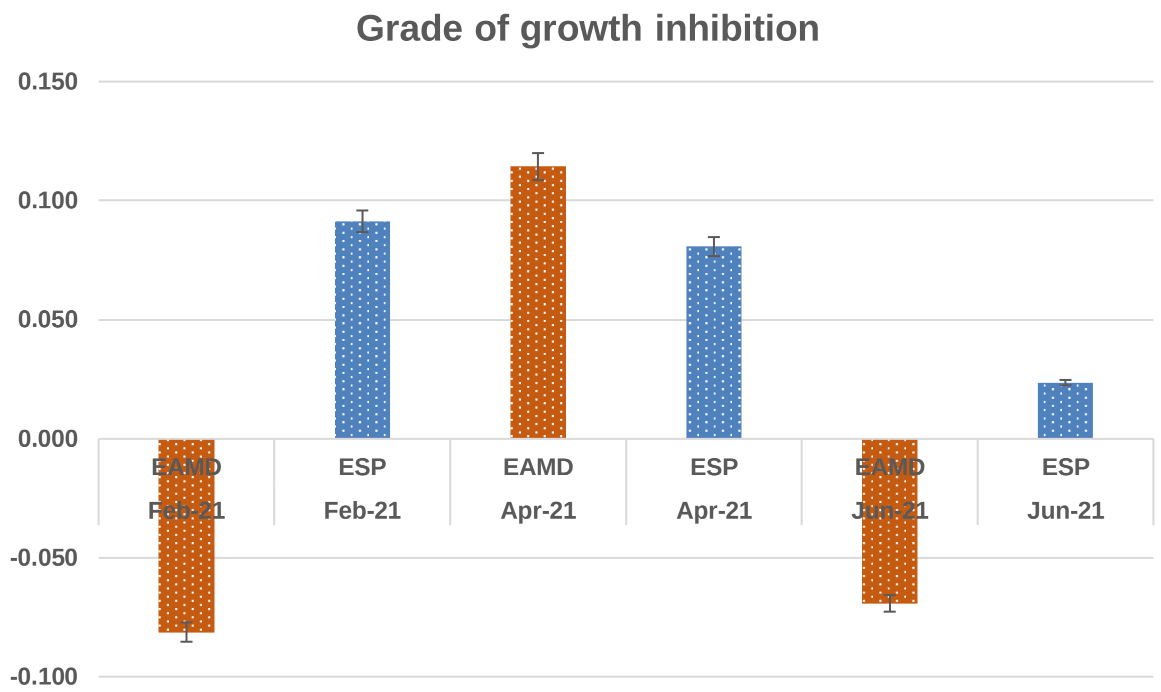


Figure 3-11: Overall grade of growth inhibition according to growth medium during the three harvests (5%)

3.3.1.5 Forage quality

After the three harvests, the dry material was analysed with a NIRS scanner, and the results are presented in column graphs, according to growth medium or treatments (Figure 3-12 to Figure 3-15). The standard deviations were calculated with SAS Statistical Software and are indicated on the graphs. The abbreviations used in the charts are defined below in Table 3-6.

Table 3-6: NIRS graph abbreviations

Abbreviation	Description
Protein	Predicted Protein Dry basis %
Fat	Predicted Fat Dry basis %
Fib	Predicted Fiber Dry basis %
ADF	Predicted ADF Dry basis %
NDF	Predicted NDF Dry basis %
ASH	Predicted Ash Dry basis %

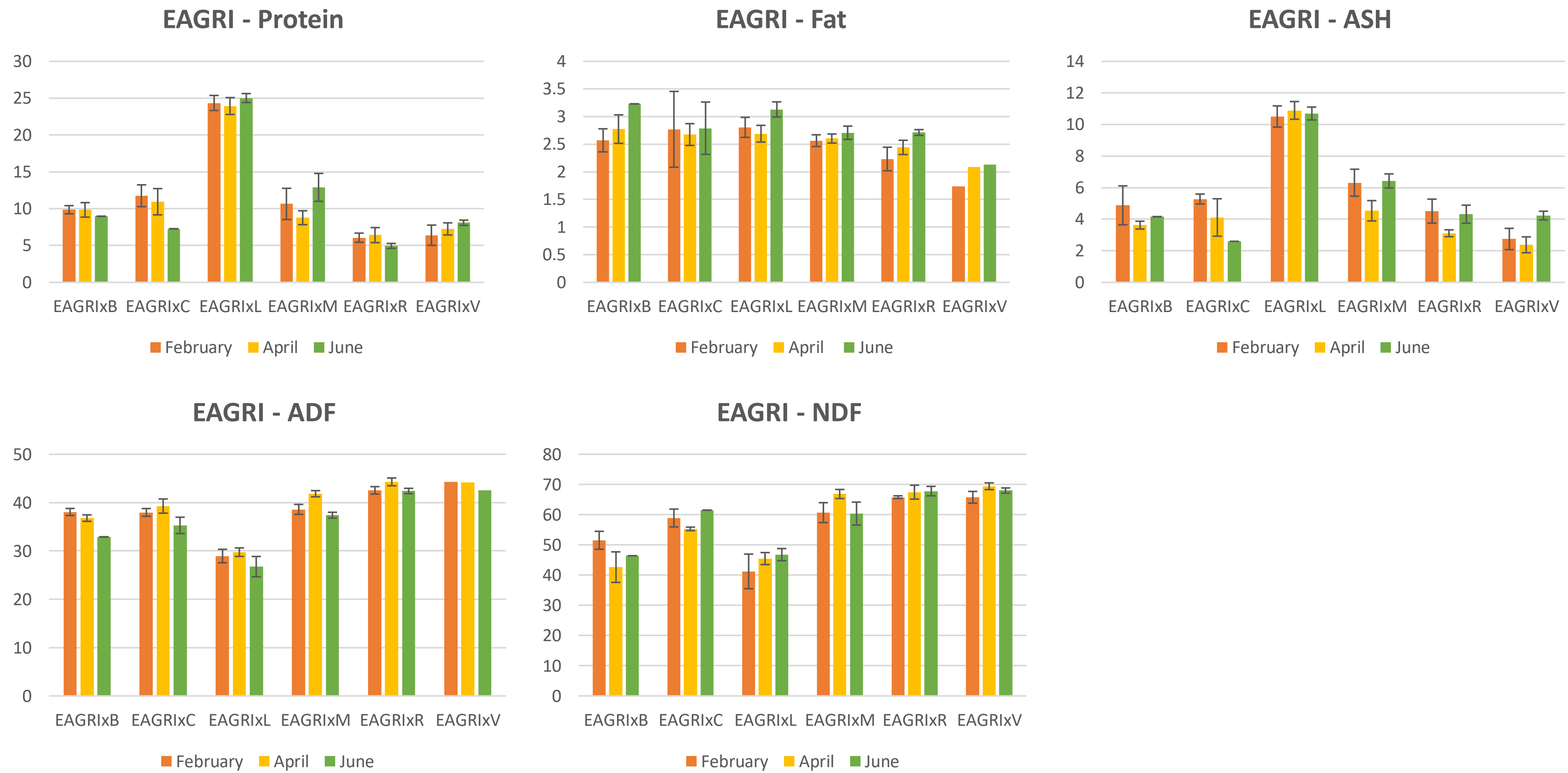


Figure 3-12: NIRS results for EAGRI (in percentage)

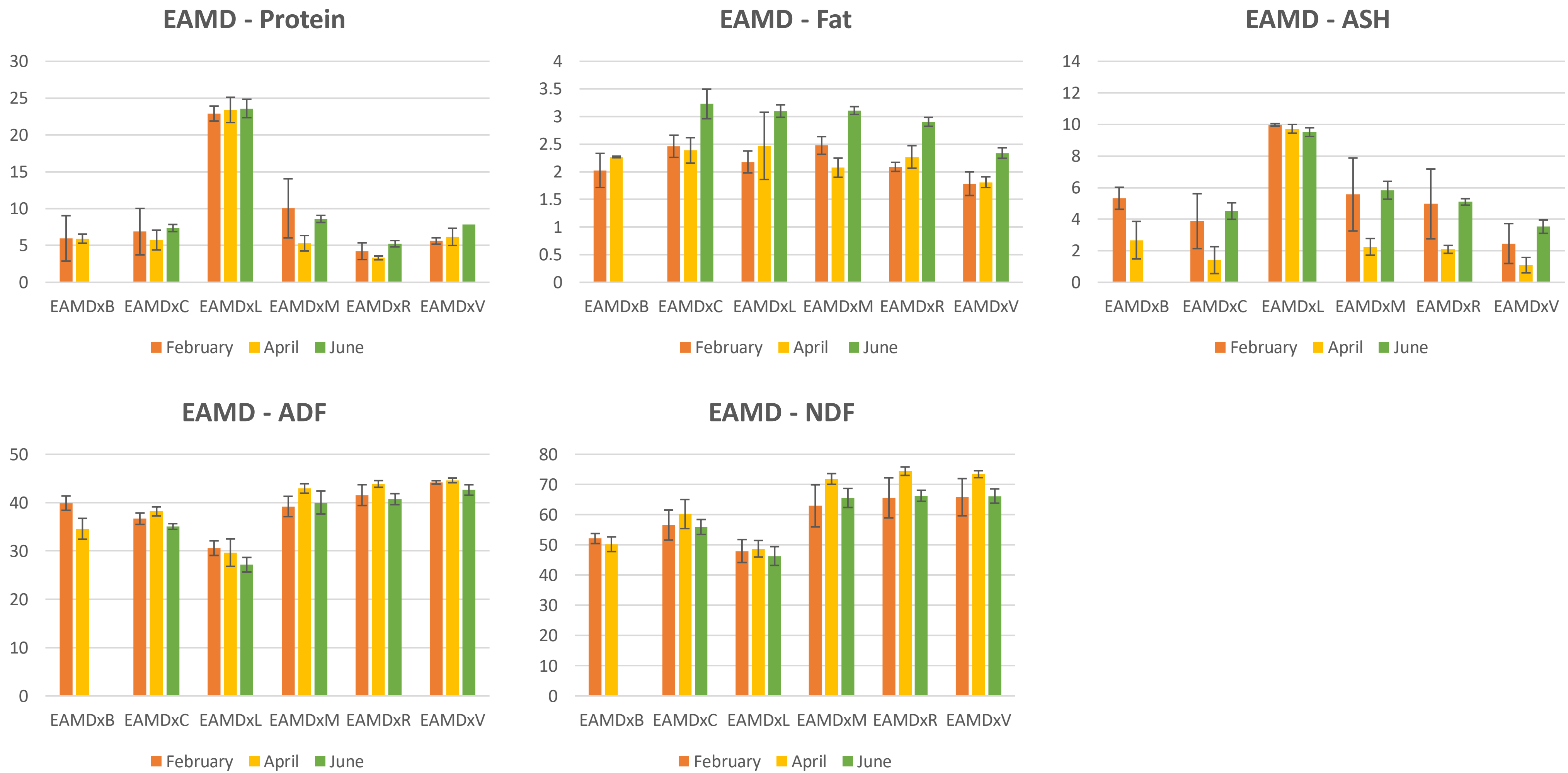


Figure 3-13: NIRS results for EAMD (in percentage)

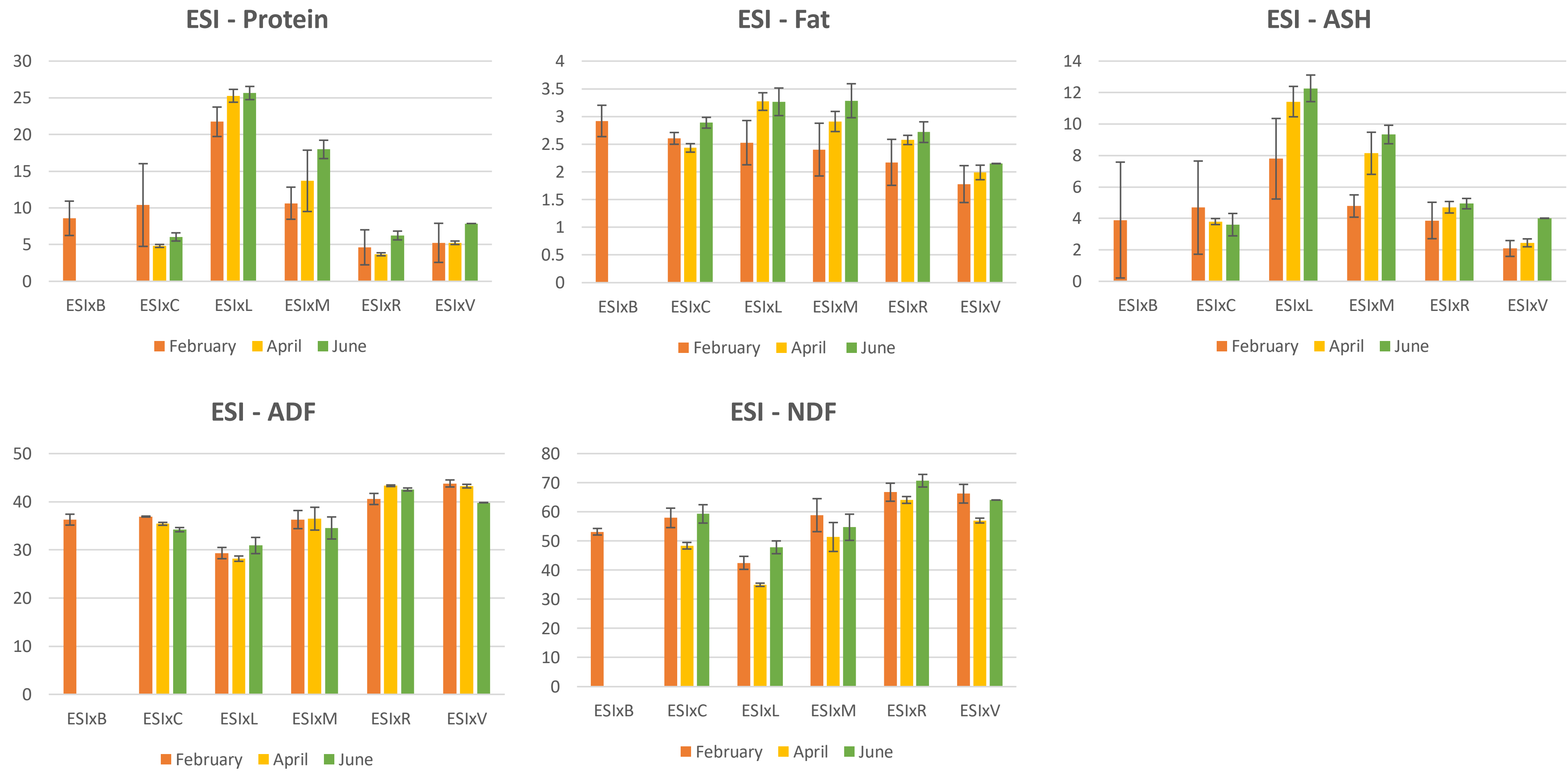


Figure 3-14: NIRS results for ESI (in percentage)

Figure 3-15: NIRS results for ESP (in percentage)

The results from the harvest are presented chronologically and the samples that were too small to assess in the scanner were omitted. It is evident that the B (*P. glaucum*) production reduced, as previously mentioned, and was not scanned in June for EAMD, ESI and ESP. The protein percentage correlates with the nitrogen content.

The predicted NDF was higher in B (*P. glaucum*), M (mixture), R (C. *gayana*) and V (*C. zizaniodes*) compared to the other species; however, it should be noted that the overall NDF percentage increased in April after which it decreased again in June. Overall, the NDF percentages were below 35 %, indicating high-quality forage. The ADF for all the species and mediums varied between 38 % and 41 %, higher than the suggested 35%, meaning the feed is of lower quality.

The graphs show that the L (*M. sativa*) had the highest protein percentage for the duration of the growing season, as expected from the nitrogen-fixing legume. In February, all the species were considered as good quality forage and had protein percentages of >9 % in grasses and > 15% for lucerne, except EAMDxR, ESIxR and ESPxV. In April, the protein percentage declined drastically. The forage quality was low for all the species apart from L (*M. sativa*) and M. In June, and the protein percentages exceeded the thresholds. Overall, the forage quality was good. The predicted calcium percentage was slightly higher in L (*M. sativa*) than in the other species.

In April, the protein percentages decreased in all the growth mediums and increased again in June. Overall, the species grown in EAMD had the lowest protein percentages, and the species in ESI performed slightly better than EAGRI.

Certain plant samples were too small to analyse in the NIRS machine, hence some gaps in the data. As expected, L (*M. sativa*) had the highest predicted protein percentage between 17 % and 22 % in all the growth mediums. R (C. *gayana*) and V (*C. zizaniodes*) both had similar protein percentages, with percentages below ten throughout the year and in all the growth mediums. The predicted protein percentages in the species mixture (M) decreased in April but increased again in June.

The average predicted fat percentage for the different growth mediums is illustrated in Figure 3-16.

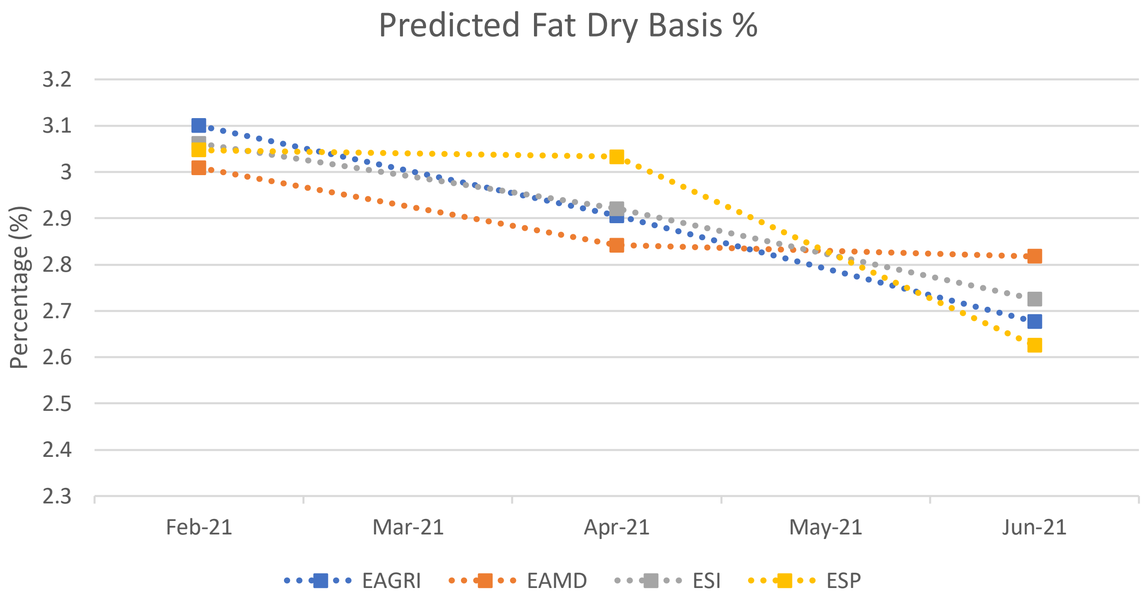


Figure 3-16: Predicted fat dry basis percentage of pasture species grown in different growth mediums

The fat percentage declined in all the growth mediums throughout the growing season from more than 3 % to less than 2.9 %. At the start of the growing season, EAGRI had the highest fat percentage, followed by ESI, while at the end of the season, EAGRI had the second-lowest fat % and ESI the second-highest fat %. ESP had the lowest fat percentage at the end of the growing season, and EAMD had the highest.

The ADF percentages are inversely related to digestibility, and forage with low ADF% are usually higher in energy. The average predicted ADF percentages are depicted in Figure 3-17. ESI and EAGRI had the lowest predicted ADF % at the beginning of the growing season; however, these two growth mediums increased and had the highest ADF% in June. The ESP and EAMD went from having the highest ADF percentages in February to having the lowest ADF % in June. In June, ESP and EAMD yielded better quality forage than the two control growth mediums.

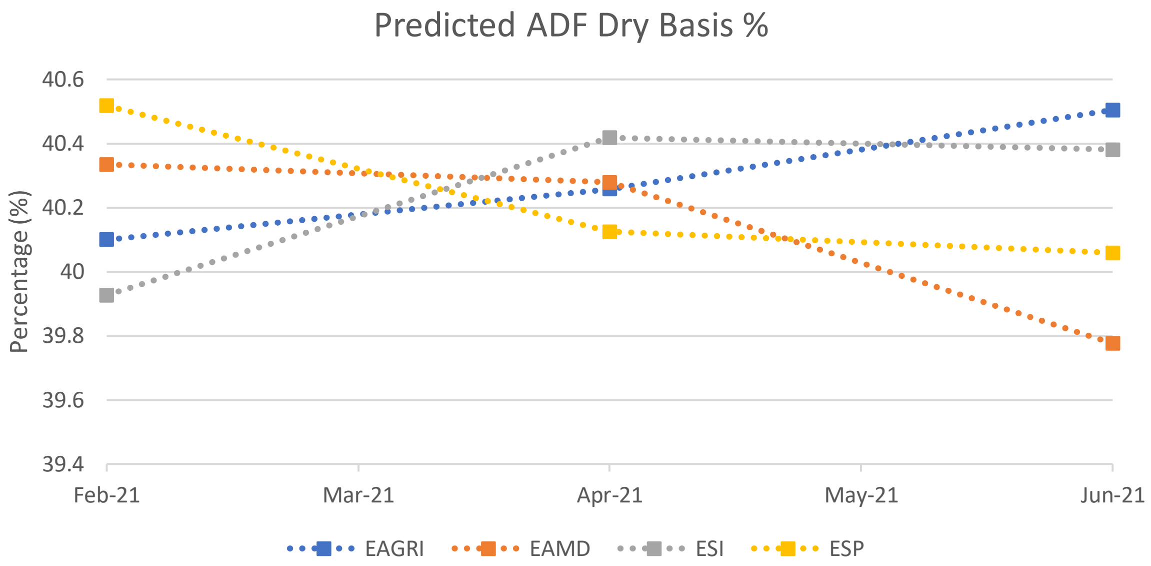


Figure 3-17: Predicted ADF dry basis percentage of pasture species grown in different growth mediums

The average predicted NDF percentages are presented below (Figure 3-18), and it is observed that the NDF in EAGRI and EAMD remained constant throughout the growing season. Low NDF values are desired, and NDF increases as forages mature. The NDF % in ESI and ESP reduced over time, indicating an improvement in forage quality. The average NDF was all below 35 %, indicating high forage quality.

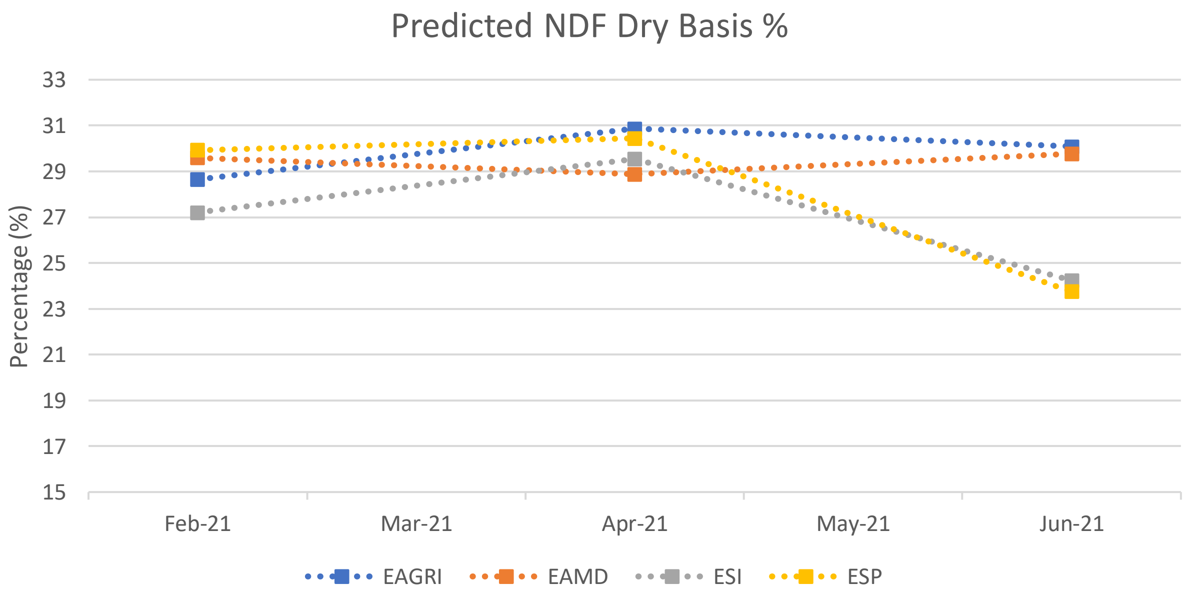


Figure 3-18: Predicted NDF dry basis percentage of pasture species grown in different growth mediums

3.4 Conclusion

Almost no growth inhibition because of metal toxicity was observed in the AMD-contaminated soil, which performed similarly to the control growth medium, namely the agricultural soil, in terms of dry mass production. EAGRI, the optimal growth medium control, produced the longest grass throughout the growing season, followed by EAMD and ESP. In contrast, the substandard control medium performed the worst in terms of plant height. The SPAD meter readings were different for each species, and L (*M. sativa*) had the highest overall SPAD meter reading and can be attributed to the darker hue of the plant compared to the grasses, while C (*C. dactylon)* had the lowest SPAD meter readings. When the SPAD meter readings were compared across the growth mediums, it was observed that the chlorophyll contents in all the growth mediums followed similar trends over time. A similar trend in all the growth mediums indicates the SPAD value and chlorophyll content is a function of growth stage and plant maturity rather than plant stress. In terms of production, EAMD produced more dry material than the optimal control (EAGRI) during two of the three harvests, and it can be concluded that the contaminated growth medium did not experience inhibited growth. The substandard control (ESI) produced the least amount of fresh material, and ESP produced the second least. This suggests that the plants in the ESI experienced more stress than in ESP. The spoils (ESP) had the highest protein percentage at the end of the growing season and EAMD the lowest, indicating no significant trend between the contaminated growth medium and protein content. The protein percentage in all the growth mediums followed a similar trend, suggesting the protein % is dependent on the plant's maturity. The EAMD followed by ESP outperformed the two control growth mediums in ADF %, indicating the forage in the polluted growth medium was of better quality. The grass from all four growth mediums was classified as good quality forage because the NDF % was less than 35%; however, the NDF% for ESP was the lowest, indicating the ESP forage was of better quality. It can be concluded that the coal contaminated growth mediums did not induce significant plant stress to reduce biomass production, induce chlorosis or result in low-grade forage quality. The results of this study suggest that the failure to establish vegetation on coal-contaminated material successfully is not attributed to chemical constraints of the growth medium but could be attributed to insufficient aftercare after implementing rehabilitation measures. It also means that plant performance can increase and be sustained with regular irrigation with effective aftercare until the vegetation has successfully been established, after which native grass species can start being incorporated into the rehabilitated area. To refute this study, a way forward would be to conduct an in-field trial where the grasses received timeous aftercare.

3.5 References

Australian Government, Department of Industry Innovation and Science, Department of Foreign Affairs and Trade. 2016. Mine rehabilitation: Leading practice sustainable development program for the mining industry. Leading practice sustainability program for the mining industry:Canberra, Australia.

Hattingh, R., Tanner, P. and Aken, M. 2019. Land Rehabilitation Guidelines for Surface Coal Mines. Land Rehabilitation Society of Southern Africa (LaRSSA), Coaltech Research Association and the Minerals Council of South Africa.

Mucina, L. and Rutherford, M.C. 2011. The Vegetation of South Africa, Lesotho and Swaziland. Pretoria: South African National Biodiversity Institute, 807 pp. ISBN: 978-1919976-21-1.

Tanner, P. 2007. Guidelines for the Rehabilitation of Mined Land. Johannesburg, South Africa: Coaltech/Chamber of Mines of South Africa.

Uddling, J., Gelang-Alfredsson, J., Piikki, K. and Pleijel, H. 2007. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynthesis research. 91: 37-46. DOI: 10.1007/s11120-006-9077-5.

Van Deventer, P.W., Hattingh, J. and Bloem, D. 2008. Some rehabilitation issues on Witwatersrand gold tailings dam. Civil Engineering. August: 25-29.

Van Saun, R.J. 2013. Determining Forage Quality: Understanding Feed Analysis [internet]. [cited 3 Jan 2020]. Avalable from: https://extension.psu.edu/determining-forage-quality-understanding-feed-analysis.

Chapter 4 Phytoremediation potential of common pasture species

Schmidhuber[1], B.E and Truter, W.F[2]

[1]Centre for Environmental Studies, University of Pretoria

[2]Department of Plant and Soil Sciences, University of Pretoria

Abstract

Phytoremediation has been getting a lot of attention in the past two decades because it is a cost-effective method to immobilise, transform, or extract otherwise harmful trace elements. Certain grass species have been used predominantly for coal mine rehabilitation purposes, and the phytoremediation potential of these grasses in South Africa should be explored.

The primary focus of the study was to investigate the phytoremediation potential and phytotoxicity thresholds of plant species commonly used in the rehabilitation practices of coal mines.

Three different grass species, one legume and one legume and grass mixture, hereafter referred to as pasture species, were planted in contaminated growth mediums. The growth mediums consisted of soil contaminated with acid mine drainage (AMD) and coal spoil material. The initial concentrations of trace elements, total and plant available, were determined by employing Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). All the plant material produced by the replications was harvested three times during the entire growing season and analysed for total concentrations. The growth mediums were sampled after the growing season, and the remaining plant-available concentrations were determined.

Cynodon dactylon (Couch grass), *Chrysopogon zizanioides* (Vetiver), *Chloris gayana* (Rhodes), *Pennisetum glaucum* (Babala), *Medicago sativa* (Lucerne) and a mixture which included *C. dactylon, C. gayana, Digitaria eriantha* (Smuts), *M. sativa* and *Eragrostis tef* (Teff) are all considered hyperaccumulators and can tolerate elevated concentrations of trace elements making them suitable for phytoremediation. The concentration of the elements in the growth medium and irrigation water, if applicable, influence the degree of hyperaccumulation and in turn, influence the phytoremediation rate.

Keywords: Phytoremediation; Pasture species; AMD; Coal mines; Rehabilitation

4.1 Introduction

Phytoremediation, a cost-effective green technology, utilises plants to take up, mobilise, metabolise, accumulate and detoxify pollutants from contaminated mediums (USEPA, 2000; Kabata-Pendias, 2011; Langella *et al*., 2014; Pandey and Maiti, 2020). Bio-stimulants drive the process in the rhizosphere completed by the mineralisation of exudates for the degradation of carbonaceous wastes as a carbon source (Barrutia *et al.*, 2008; Renault *et al*., 2000; Geipsson, 2011; Pivetz, 2001; Cowan *et al.,* 2016).

Four broad different technologies for phytoremediation are identified as follows (Geipsson, 2011; Mahar *et al*., 2016; Keeling and Werren, 2005):

1. Phytostabilisation: This method retains the contaminants in the substrate to prevent the dispersion of the pollutants. The pollutants are stabilised in the rhizosphere or the plant roots.
2. Phytodegradation: This process involves attenuating contaminants into less toxic variations of the contaminant by metabolic activities or the release of enzymes from the roots.
3. Phytovolatilisation: This method involves the uptake of the contaminants by the plant's roots and the conversion of them into a gas that is released into the atmosphere. The evapotranspiration of the plants governs this.
4. Phytoextraction: This involves the ability of the plant to accumulate the contaminants by extracting the pollutants from the growth medium and storing them in the harvestable biomass of the plant.

Phytoremediation is almost never implemented in isolation and is usually applied as part of the rehabilitation and ecological restoration of areas (Geipsson, 2011). Plants that are preferred for phytoremediation should have a high biomass production potential, rapid growth potential, and tolerance to toxic elements and hostile growing conditions (Pandey and Maiti, 2020).

Perennial grasses are important plants to consider for phytoremediation due to their colossal biomass production potential and low fertiliser requirement. Grasses also improve soil structure, reduces erosion, increases biodiversity and the aesthetical value of rehabilitated areas (Pandey and Maiti, 2020). Grasses can also yield economic returns if it is used for grazing and forage purposes, which might be beneficial post-closure land uses.

Perennial grasses have been reported to reduce the toxicity of trace elements in previous studies (Gołda and Korzeniowska, 2016; Cui *et al*., 2018; Ghosh *et al*., 2015; Yasin *et al*., 2015; Ziarati *et al.,* 2015; Suchkova *et al*., 2010 [cited by Pandey and Maiti, 2020]). It was reported that perennial grasses are metal tolerant and can phytostabilise trace elements in their roots.

Other characteristics that make grasses suitable for phytoremediation include the following (Pandey and Maiti, 2020):

- The grass is naturally more drought-resistant compared to other plant species;
- Grass can produce bioproducts and can be used as feedstock; and
- Some grass have phytoliths, siliceous compounds in the epidermal cells, making them resistant to abiotic stresses.

Additionally, grasses have numerous other uses, and the services can be classified according to ecological, economic and societal aspects, as depicted in Figure 4-1 (Pandey and Maiti, 2020). The ecological aspects include the usage of plants for rehabilitation and phytoremediation purposes. The societal factors comprise the use of grass for thatching, forage and crafts, to mention a few. Some pasture species have additional economic value and can be used for essential oil, it can be used for biofuel, and it can also be a source of income, especially considering post-closure land uses.

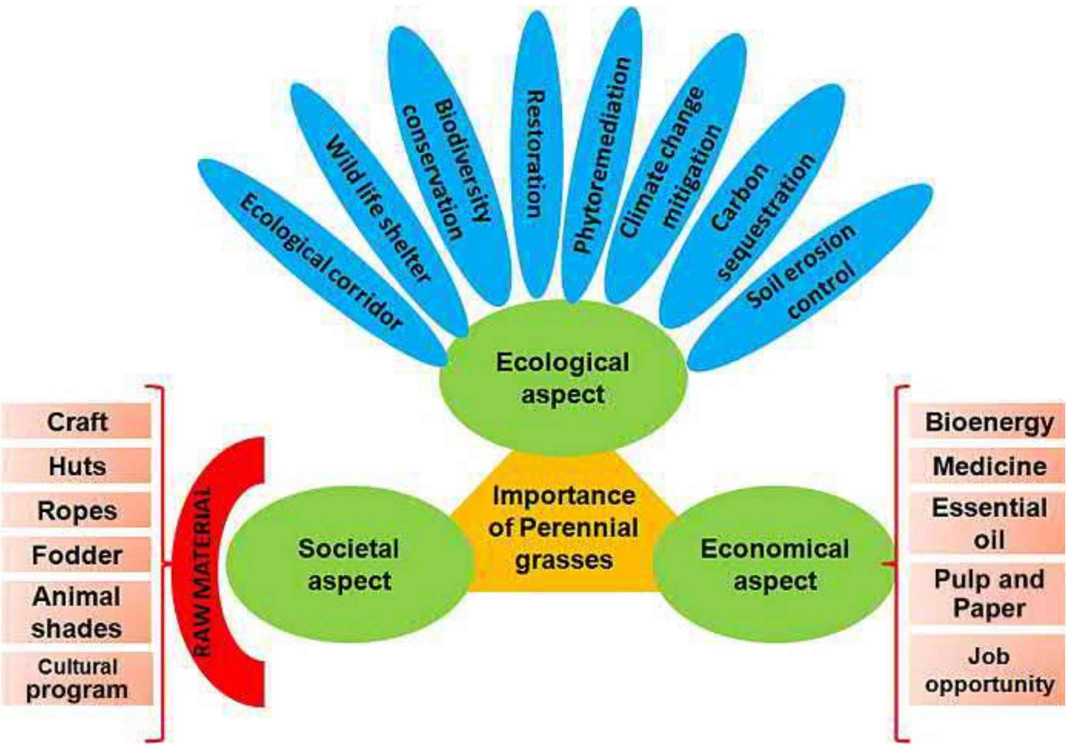


Figure 4-1: Ecosystem services of perennial grasses (Pandey and Maiti, 2020)

The plant's ability to absorb trace elements from a growth medium is determined by evaluating the concentration in the growth medium and comparing that to the concentration in the plant, in order to derive accumulation indices (Hunt *et al*., 2014). The indices that are derived are known as the Biological Absorption Coefficient (BAC), the Index of Bioaccumulation (IBA), or the Transfer Factors (TF) (Kabata-Pendia, 2011; Pandey and Maiti, 2020).

The use of common rehabilitation pasture species as phytoremediators has not been explored in depth in South Africa. For decades, grasses have been used as the primary vegetation type in mine rehabilitation, especially coal mining. Perennial grasses have huge biomass production rates and require low amounts of fertiliser, making them more than suitable for phytoremediation purposes.

The first aim is to quantify the CoPC concentrations, using ICP MS, in the pasture species and substrate after a full growing season following a comparison of the concentrations between growth mediums and plant species, to identify the most appropriate phytoremediation type that is applicable in the usage of pasture species to remediate coal spoil and contaminated soil. Another aim is to determine which pasture species can be seen as outliers in terms of hyperaccumulation, by comparing absorbed concentrations within each plant type and to calculate hyperaccumulation and associated indices.

It is hypothesised that the phytoextraction method will be the most appropriate phytoremediation method as grass has a high biomass production capacity. It is also hypothesised that the species with the largest leave areas and/ or the highest fresh biomass production will have higher trace element concentrations. It is assumed that species grown in EAMD will accumulate more trace elements than ESP, because EAMD had a slightly lower pH, rendering the metals more plant available.

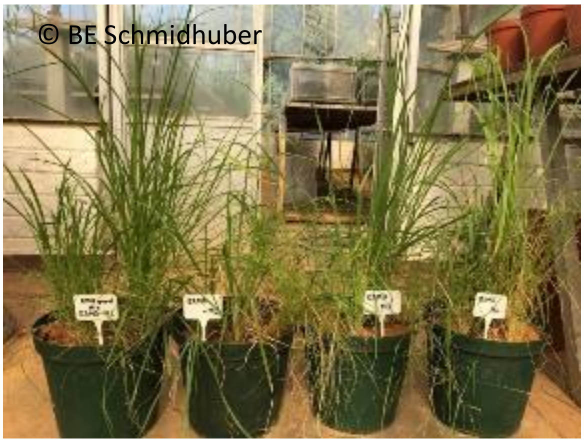
4.2 Experimental design

The pot trial consisted of two (2) polluted growth mediums sourced from a coal mine in the Highveld and one control (EAGRI), which is a natural agricultural soil. The contaminated growth mediums were coal mine spoil material (ESP) and AMD contaminated soil (EAMD). The growth mediums were ameliorated and lime was applied to the growth mediums, ESP at 40 t/ha and EAMD at 20 t/ha (Truter WF – Personal communication). Roughly two months after the lime was applied the growth mediums were fertilised with 200 kg N/ha, 200 kg P/ha and 250 kg K/ha.

Four different plant species and grass-legume mixture were used during the experiment (Figure 4-2). The seeds were germinated, and the two-week-old plugs were planted in the ameliorated growth mediums at the start of December 2020. The species used included, *C. dactylon* (C), *C. zizanioides* (V), *C. gayana* (R), *M. sativa* (L) and a mixture (M) which included *C. dactylon, C. gayana, D. eriantha, M. sativa* and *E. tef*.

EAMD – C

EAMD - L



EAMD - M

ESP R

ESP - V

Figure 4-2: Photographs of pasture species selected for the phytoremediation trial

The randomised pots (Figure 4-3) were kept in the greenhouse at Phytotron D on the University of Pretoria Experimental Farm for a full year and watered every other day. Fertiliser was applied after each of the three harvests.

Figure 4-3: Picture of randomised pots in greenhouse

4.2.1 Sampling procedure

Composite samples were taken from the two different growth mediums namely, EAMD and ESP, and the control growth medium (EAGRI). The contaminated soil (EAMD) is a red structured soil contaminated with acid mine drainage (AMD), and ESP is coal spoils from a coal mine in Mpumalanga.

The plant-available concentrations of elements in the growth mediums were determined with the NH_4NO_3 (DIN 19730 method), and the total concentrations with the HNO_3/H_2O_2 method (EPA3051A method) using an inductively coupled plasma mass spectrometry (ICP-MS).

The concentration of total and plant-available trace elements in the two contaminated growth mediums (ESP and EAMD) and the one control medium (EAGRI) are tabulated below in Table 4-1.

Table 4-1: Total and plant-available concentrations of growth mediums

Sample	EAGRI		EAMD		ESP	
	Total	*Plant-available*	*Total*	*Plant-available*	*Total*	*Plant-available*
	($mg \cdot kg^{-1}$)	*($mg \cdot kg^{-1}$)*	*($mg \cdot kg^{-1}$)*	*($mg \cdot kg^{-1}$)*	*($mg \cdot kg^{-1}$)*	*($mg \cdot kg^{-1}$)*
Be	0,22	ND	0,54	0,04	0,91	0,02
B	1,75	0,40	0,56	0,29	7,94	0,28

Na	134,40	60,06	79,78	10,19	77,49	3,34
Mg	1501,00	173,30	404,20	29,43	304,30	44,53
Al	8716,00	2,58	14990,00	297,60	5133,00	92,21
P	758,30	13,13	109,10	0,40	328,60	0,37
K	1163,00	117,50	1125,00	15,27	1284,00	8,11
Ca	10020,00	1337,00	163,70	66,17	3010,00	459,80
Ti	240,40	0,06	348,30	0,03	234,50	0,01
V	25,83	0,01	71,59	0,00	39,42	ND
Cr	48,24	0,02	64,82	0,04	27,98	0,02
Mn	197,30	0,25	152,60	18,31	38,65	10,80
Fe	9656,00	1,32	42990,00	3,32	8904,00	8,47
Co	6,39	0,01	4,93	0,66	4,67	0,55
Ni	33,23	0,03	15,84	0,67	12,63	0,84
Cu	29,37	0,13	22,44	0,33	20,02	0,33
Zn	79,50	0,10	46,88	0,51	52,03	2,60
As	2,05	0,01	6,65	0,04	4,92	0,03
Se	8,12	0,03	4,72	0,56	13,36	0,65
Rb	14,87	0,09	27,11	0,62	9,70	0,18
Sr	16,64	3,56	7,39	0,55	168,30	1,56
Mo	4,65	0,01	1,65	ND	1,96	ND
Pd	0,13	0,00	0,06	0,01	0,36	0,01
Ag	0,14	0,00	0,12	0,00	0,41	0,00
Cd	0,03	0,02	0,02	0,02	0,08	0,02
Sn	67,57	0,27	74,71	0,33	73,28	0,26
Sb	0,23	0,00	0,16	ND	0,13	0,00
Ba	41,41	2,94	295,40	2,12	238,70	0,82
Pt	ND	ND	ND	ND	ND	ND
Au	0,00	ND	ND	ND	0,01	ND
Hg	0,04	0,00	0,04	ND	0,29	ND
Tl	0,12	0,00	1,01	0,06	0,28	0,02
Pb	8,38	0,01	29,53	0,73	28,74	0,02
Bi	0,10	ND	0,11	ND	0,45	ND
Th	7,89	0,00	8,09	0,00	10,26	ND
U	1,00	0,00	1,01	0,07	1,91	0,01

**ND – Not detectable*

The plant species were harvested three times during the growing season by cutting each plant roughly 5 cm above the surface of the growth medium. Following the three harvests in February, April and June of 2021, the plant material from each pot was oven-dried at 60°C for roughly 48 hours and then milled with an electronic mill. The corresponding sample (C, M, L, R and V) and replications (one to three) over the three harvests were mixed to form a composite sample. The total concentrations in the plant were determined with ICP-MS.

For each species, composite samples of the growth medium were again taken after the last harvest in June, and the plant-available concentrations were again assessed with the NH_4NO_3 method.

4.2.2 Statistical analysis

The results were derived from three replications of each of the plant species- and treatment combinations amounting to 60 samples in total. The statistical analyses were done with SAS Statistical Software and Gen stat 64- bit Version 18.2. The Principal Component Analysis (PCA) was generated using XLStat, an add-on software package for Microsoft Excel.

4.3 Results

The results are contained and explained in the subsections below.

4.3.1 Elemental concentrations at the end of the growing season

After the growing season, which included three harvests roughly two months apart, the various growth mediums were sampled again and subjected to ICP-MS analyses. The results of the residual concentrations are depicted in a PCA graph in Figure 4-4.

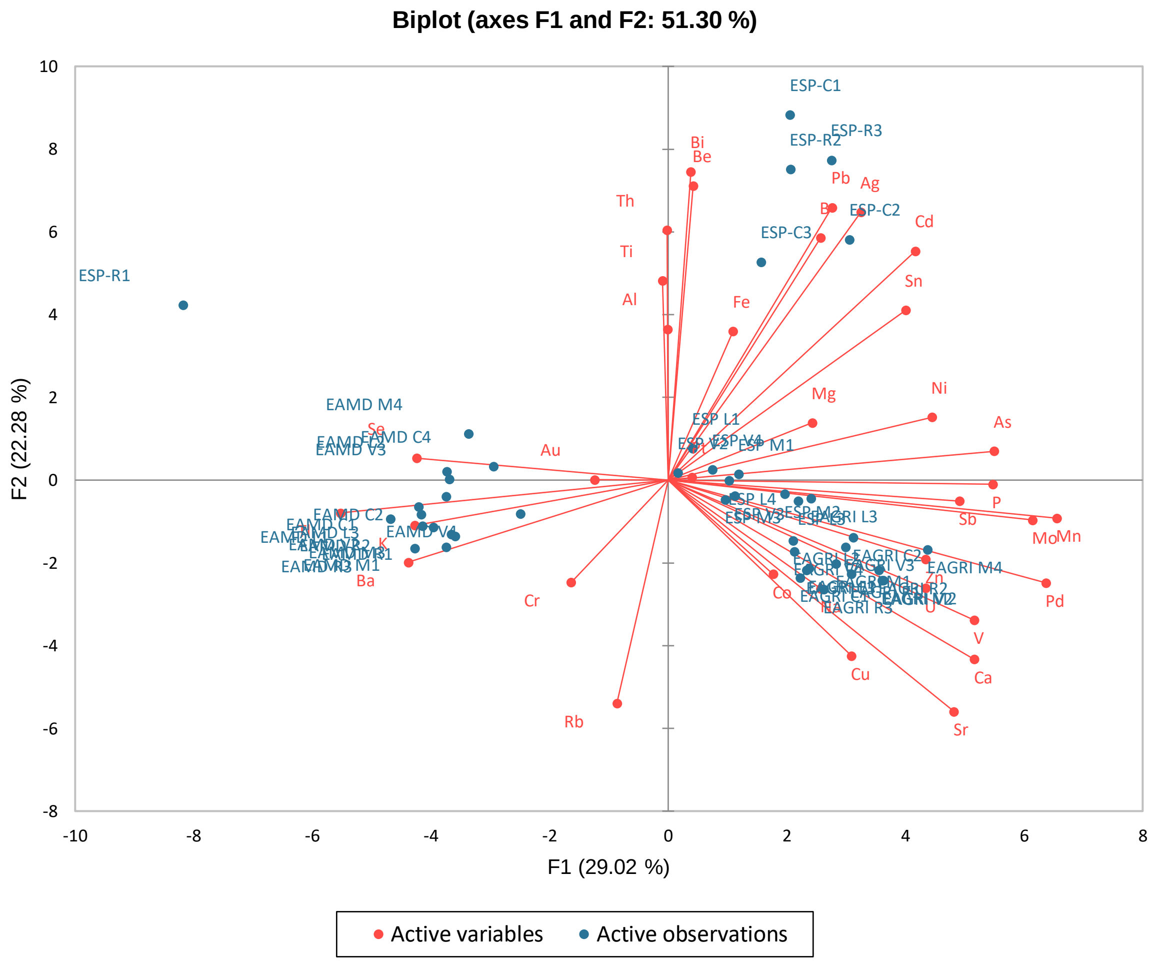


Figure 4-4: Principal component analyses graph of elemental concentrations in growth mediums

The AMD-contaminated soil (EAMD) all grouped close to the Se, Ba and Au axis lines, indicating a strong correlation to those elements. The control medium (EAGRI) is grouped on the opposite side of the graph, which represents a strong dissimilarity between the two growth mediums. The coal spoils (ESP) are mostly grouped closed to the Pb, B and Fe axis, with scattered samples plotting between EAGRI.

After the entire growing season, all the collected plant materials were harvested and milled, and ICP-MS analyses were conducted to determine the elemental concentrations in the different plant species in the various growth mediums. The PCA graph for the concentrations in the plant material is depicted in Figure 4-5.

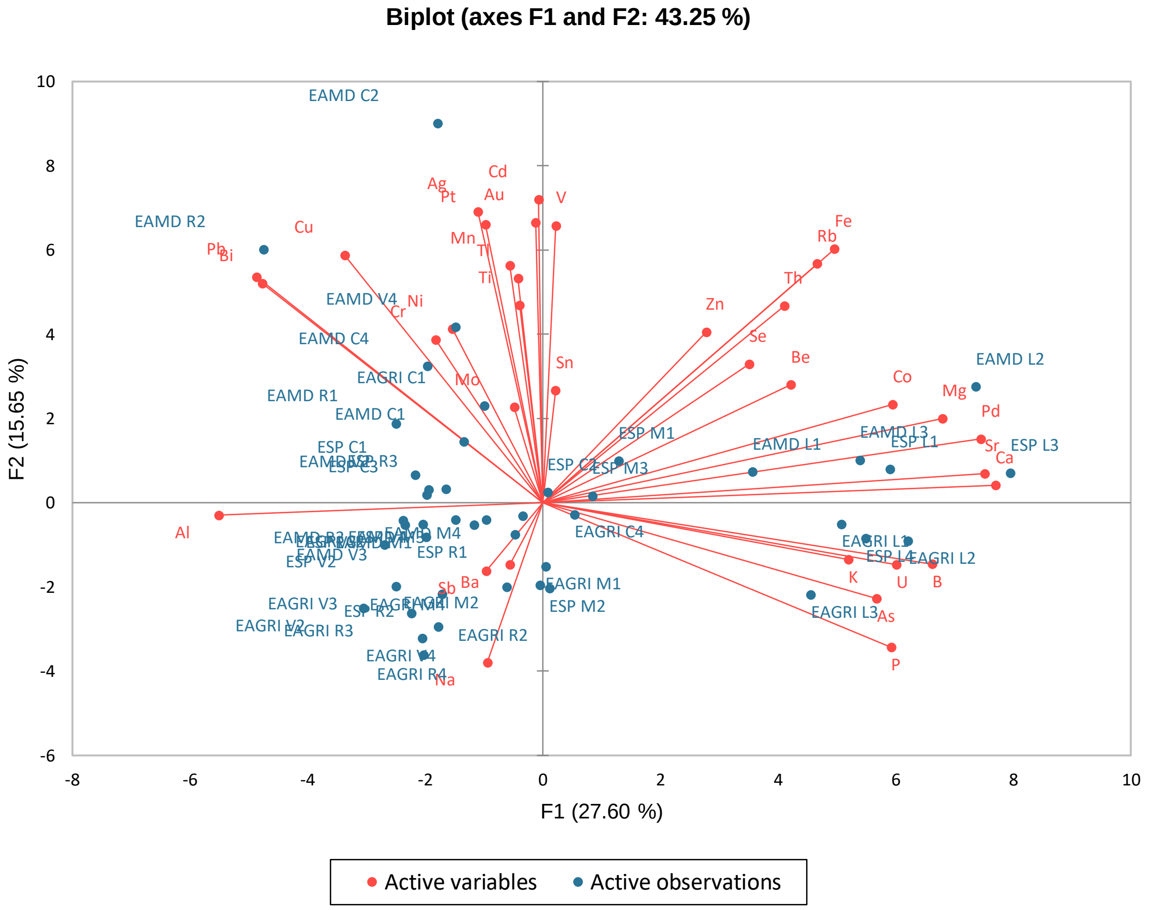


Figure 4-5: Principal component analyses graph of elemental concentrations in plant material

M. sativa (L) is mostly clustered on the right of the graph and are strongly associated with elements like As, U, Co, Mg, Pd and Sr indicating that *M. sativa* can be considered for phytoremediation of the aforementioned elements. Apart from *M. sativa*, no definite patterns in terms of element concentrations were observed.

To conceptualise the initial concentrations in the growth mediums, including the total concentrations and plant-available (PA) concentrations, with the elemental concentrations in the plant and the residual concentration in the medium after the harvest, the results are illustrated on logarithmic radar graphs. The initial concentrations are compared to the concentrations in the plant and the residual growth medium concentrations for each separate growth medium. The control medium is depicted in Figure 4-6, the AMD-contaminated soil depicted in Figure 4-7, and the coal spoil results are depicted in Figure 4-8. The gaps in the logarithmic radar graphs represents the concentrations that were below the detection limits or 0.

It is evident from all the graphs that the elemental concentrations in the growth mediums and the concertation in the plant material all follow independently similar trends. In EAGRI the residual growth medium concentrations of B and Cd were higher than the initial concentrations and could be attributed to the irrigation water quality. Based on the 2021 Rand Water quality report, the average concentrations for B and Cd were 1.48 $mg{\cdot}kg^{-1}$and 1.39 $mg{\cdot}kg^{-1}$, respectively (Rand Water Integrated Annual Report, 2021). The higher concentrations of Na, Rb, Sr and Mo in the plant material as opposed to the initial concentration in the growth medium could also be attributed to the water quality. The elevated P and K concentrations are as a result of the fertilizer that was applied after each of the three harvests throughout the year. It was also observed that higher concentrations of the elements were absorbed by the plants compared to the concentrations that were initially plant available. In the control medium (EAGRI) *C. Gayana* (R) had the overall highest elemental concentrations, followed by *M. sativa* (L). The majority of the element residual concentrations in the growth medium for the species mixture (M) and *C. dactylon* (C) were below the detection levels, indicating these species absorbed the most of the element.

The elemental concentrations in the different plant species in the AMD contaminated soil (EAMD) followed a similar trend and *C. gayana* (R) had slightly higher overall concentrations and *C. zizaniodes* (V) had the lowest concentrations. The plant material had higher concentrations than what was initially plant available in the growth medium. It can be concluded that the pasture species all had phytoremediation and hyperaccumulation abilities based on the fact that the residual concentrations in the growth medium were lower than the initial total concentrations.

Similar to the other growth mediums, the plants absorbed higher concentrations that were plant-available and, in some cases, higher than the total concentrations. *C. dactylon* (C) performed the best in terms of elemental absorption and can be considered a hyperaccumulator, which can be further validated by the fact that the residual concentrations in the growth mediums were mostly undetectable. *C. zizanioides* (V) had the lowest overall concentrations compared to the other plant species and *M. sativa* (L) the overall highest. *C. gayana* (R) underperformed in terms of phytoremediation, compared to the other plant species.

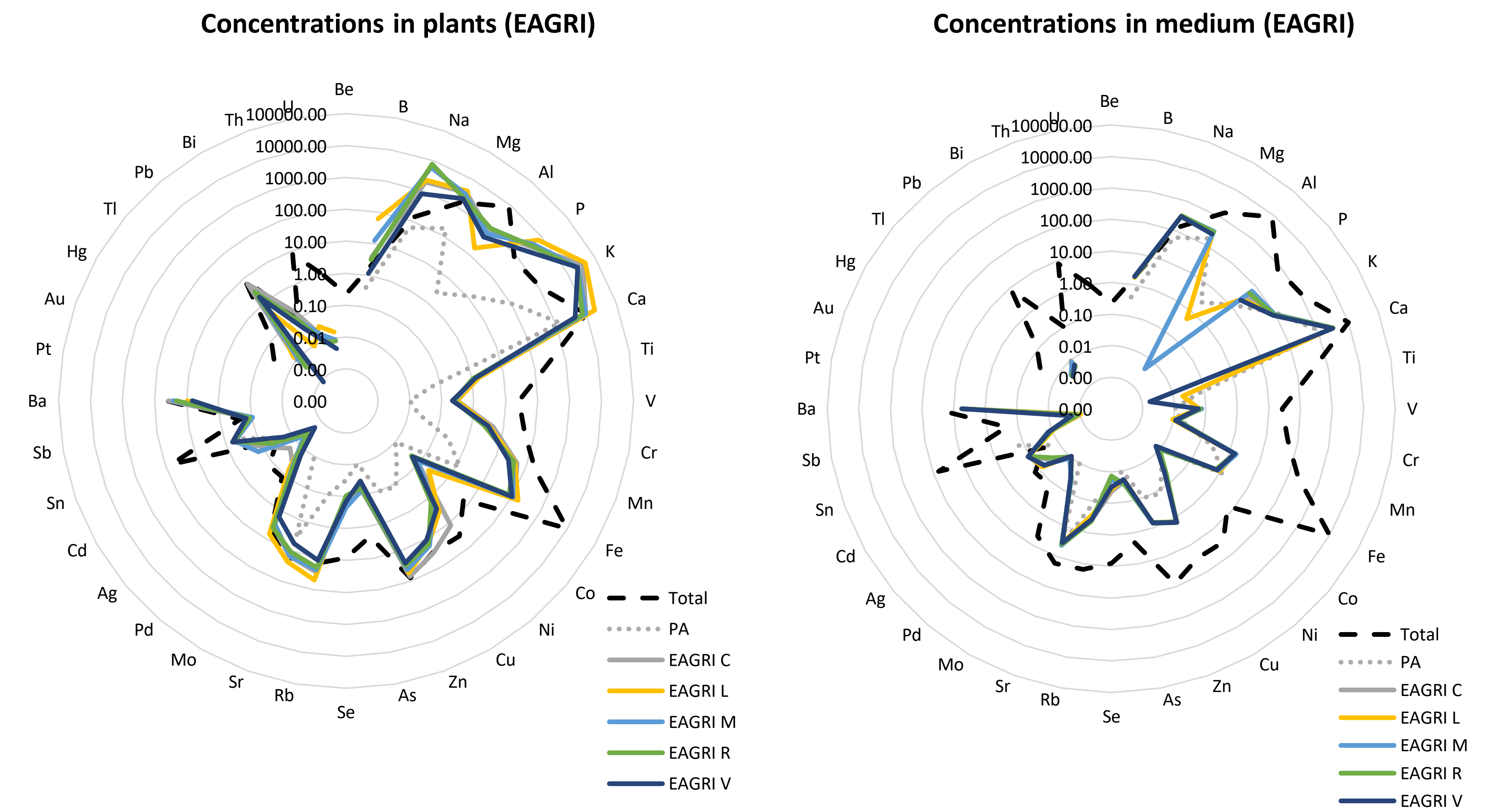


Figure 4-6: Plant and growth medium concentrations (mg·kg^{-1}) in control (EAGRI)

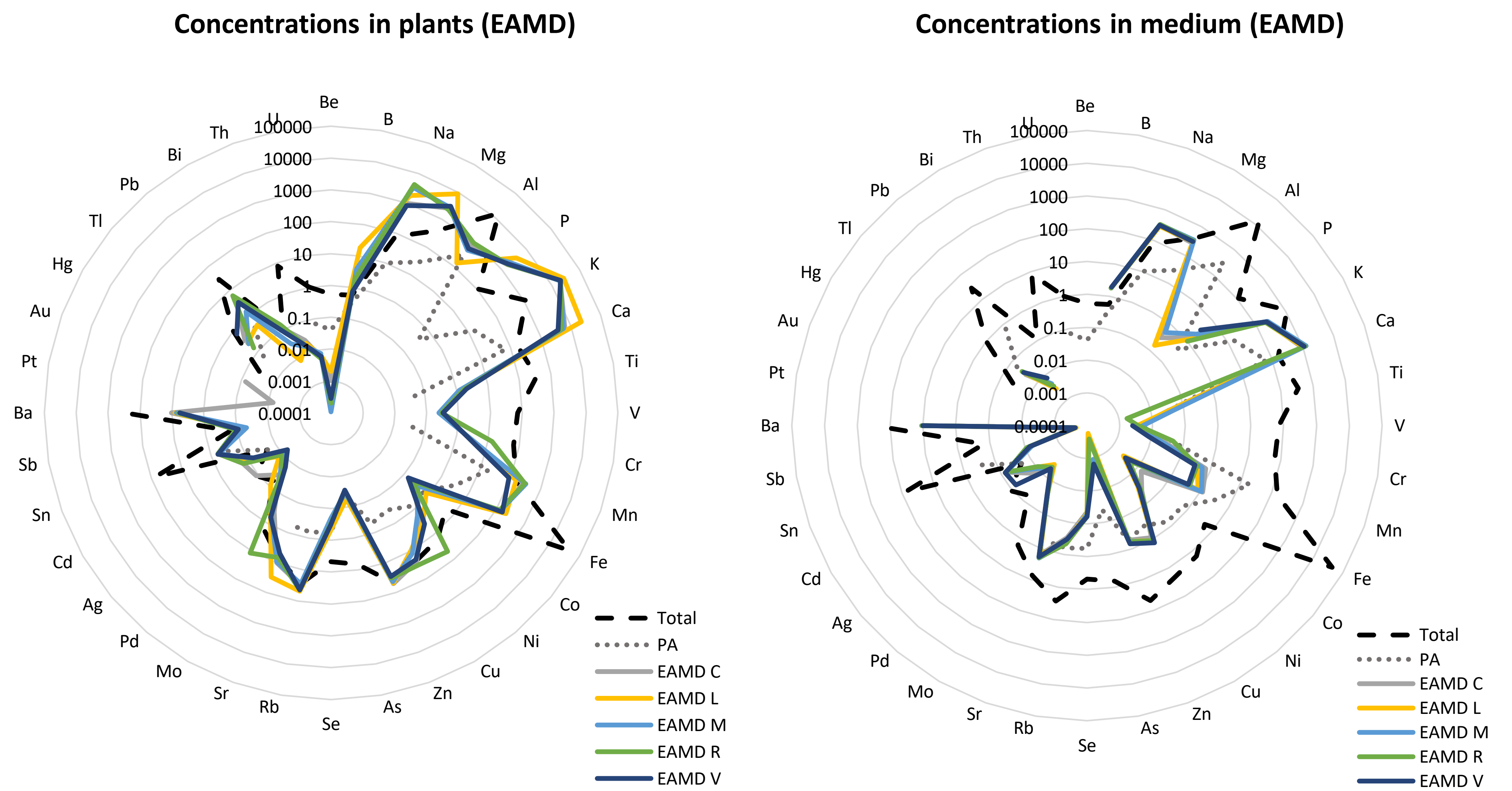


Figure 4-7: Plant and growth medium concentrations (mg·kg⁻¹) in AMD contaminated soil (EAMD)

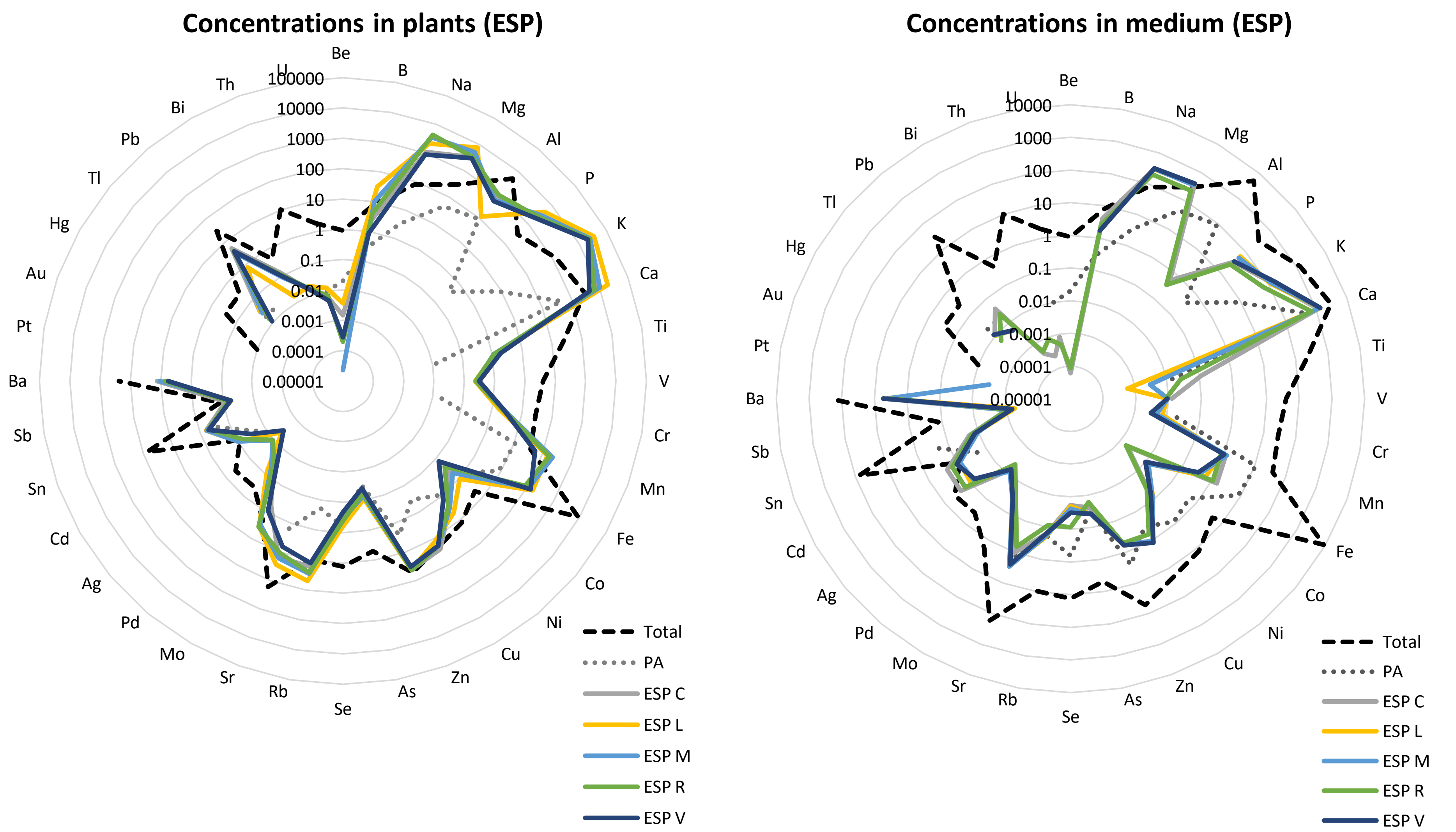


Figure 4-8: Plant and growth medium concentrations (mg·kg^{-1}) in coal spoil (ESP)

4.3.2 Accumulation and tolerance indices

The initial total and plant concentrations, along with the concentration in the plants and residual concentrations in the growth mediums, were used to calculate several indices to quantify the plant species' tolerance to stressors and the hyperaccumulation capabilities. The following section elaborates on the indices and the interpretation thereof.

The tolerance index (TI) reveals the metal tolerance of the plant and is calculated using biomass production. The TI is calculated as follows (Chuan *et al*., 2016):

$$TI = \text{Dry matter yield in metal-rich growth medium/ dry matter yield in control growth medium} \quad \text{(Eq. 4-1)}$$

The average TIs for each growth medium and plant species combination, calculated from four replicas, are depicted in Figure 4-9, with a 5 % error margin.

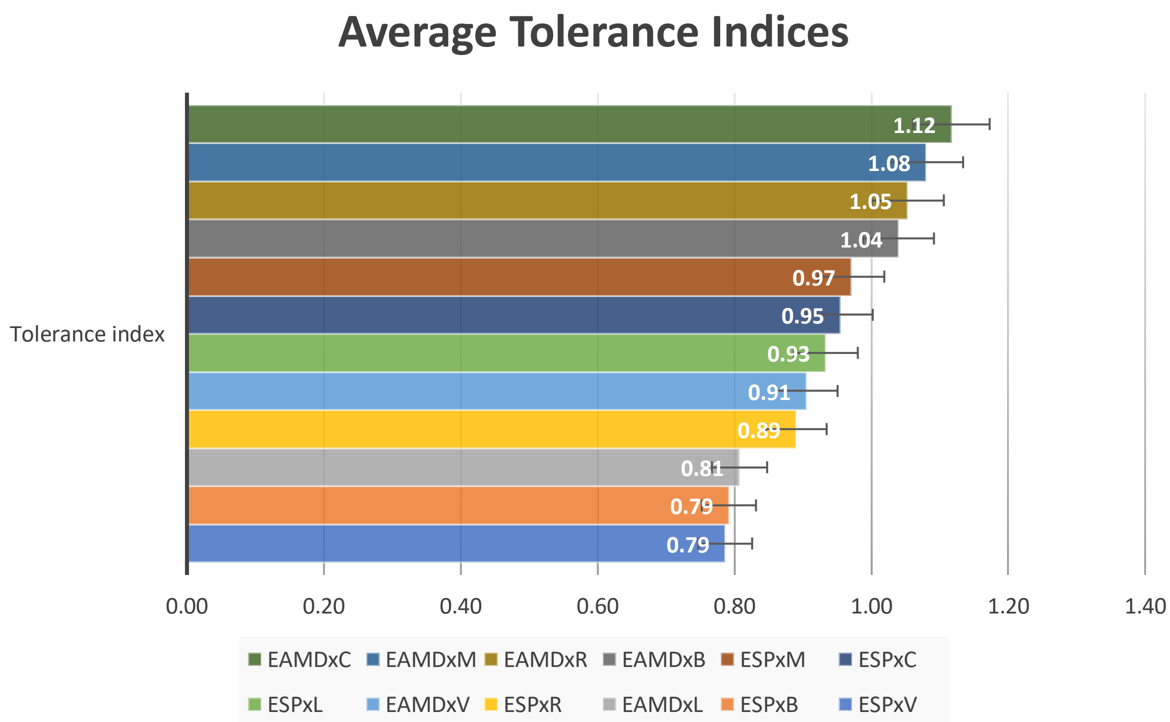


Figure 4-9: Average tolerance index (TI) per growth medium and plant species

The species and growth medium combinations that had TI >1 produced a larger quantity of dry biomass than the control medium (EAGRI), and TI values of <1 indicate the species were less tolerant. It is evident that *C. dactylon* in the AMD-contaminated soil produced 12 %

more dry biomass than the *C. dactylon* in the control. The species mixture (EAMDxM), *C. gayana* (EAMDxR) and *P. glaucum* (EAMDxB) all produced more dry biomass than the controls indicating the plant species tolerated the elevated trace metal concentrations in the growth medium exceptionally well. The fact that the aforementioned species produced more biomass in the AMD polluted soils than in the control growth medium raises a lot of questions and could be attributed to a number of factors, two being: 1) the AMD-polluted soils had better water retention capabilities and remained saturated for longer and; 2) the control medium had a lot of annual weeds in the seedbank that germinated throughout the year, which could have competed with the planted species. Surprisingly, the rest of the species and growth medium combinations produced slightly less dry biomass than the control growth medium with ESPxV and ESPxB producing the least dry biomass, roughly 21% less than in the control.

The total and available bioaccumulation indices were calculated with the following equations (Różanowski *et al*., 2012; Kabata-Pendias, 2011):

$$BAI_T = \text{Concentration in plant/ TC} \qquad \text{(Eq. 4-2)}$$

$$BAI_A = \text{Concentration in plant/ AC} \qquad \text{(Eq. 4-3)}$$

Where:

AC = available concentration

BAI = bioaccumulation index

TC = total concentration

The results for the total and available bioaccumulation indices are tabulated in Table 4-2 and Table 4-3, respectively.

The degree of bioaccumulation is depicted in the following table (Table 4-2), where values shaded in orange indicate intensive degrees of bioaccumulation and highlight elements that hyperaccumulated in the different species.

Table 4-3 tabulates the bioaccumulation index based on the plant-available concentrations, and the elevated BAI_A values suggest that the plant absorbed more of the elements that were initially plant-available and could indicate that the plants produced organic chelates that made the insoluble elements plant available in the root zone.

Table 4-2: Total bioaccumulation index (BAI_T) per element for different pasture species- and growth medium combinations

Sample	EAMD C	EAMD L	EAMD M	EAMD R	EAMD V	ESP C	ESP L	ESP M	ESP R	ESP V	EAGRI C	EAGRI L	EAGRI M	EAGRI R	EAGRI V
*Lack	0.001 – 0.01														
*Slight	0.01 – 0.1														
*Medium	0.1 – 1														
*Intensive	1 - 10														
Be	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B	2.09	33.36	6.82	3.36	1.32	0.31	4.07	1.27	0.51	0.11	2.12	35.69	7.21	1.77	0.65
Na	12.44	23.20	42.43	53.50	10.58	13.90	27.72	46.09	55.53	11.35	14.73	16.94	45.06	58.38	5.99
Mg	6.43	21.59	7.04	5.71	7.67	10.94	25.35	16.46	11.12	9.92	2.29	2.65	2.02	1.50	1.41
Al	0.05	0.01	0.03	0.06	0.04	0.16	0.02	0.12	0.19	0.11	0.10	0.02	0.08	0.13	0.06
P	15.70	34.84	20.00	16.40	16.90	7.68	14.15	9.65	7.95	6.59	4.46	9.29	5.81	4.90	2.82
K	17.86	24.07	17.59	18.83	18.33	16.43	27.14	19.73	18.08	16.71	31.34	37.81	20.57	19.32	20.48
Ca	39.08	138.32	35.43	29.87	23.73	2.43	6.39	3.39	2.20	1.45	0.98	1.91	0.98	0.75	0.43
Ti	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.01
V	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Cr	0.04	0.04	0.04	0.21	0.03	0.09	0.06	0.08	0.08	0.09	0.10	0.07	0.05	0.05	0.07
Mn	1.73	0.98	2.11	1.94	0.57	4.68	3.86	5.79	4.62	1.40	0.26	0.22	0.19	0.20	0.13
Fe	0.00	0.01	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.01	0.01	0.01
Co	0.04	0.16	0.07	0.04	0.03	0.07	0.23	0.11	0.05	0.03	0.01	0.04	0.01	0.01	0.01
Ni	0.14	0.20	0.10	3.07	0.23	0.23	0.37	0.23	0.20	0.11	0.37	0.10	0.05	0.04	0.08
Cu	1.06	0.45	0.55	1.40	0.93	1.28	0.62	0.96	0.95	0.95	1.07	0.49	0.58	0.51	0.37
Zn	1.07	1.04	0.94	0.69	0.63	0.80	0.78	0.76	0.83	0.63	0.90	0.68	0.53	0.39	0.32
As	0.00	0.01	0.01	0.00	0.00	0.01	0.02	0.01	0.01	0.01	0.03	0.03	0.03	0.03	0.02
Se	0.06	0.10	0.04	0.06	0.07	0.02	0.05	0.03	0.03	0.02	0.02	0.01	0.03	0.01	0.02
Rb	1.18	1.79	1.04	1.62	1.63	1.71	5.12	2.91	2.69	1.32	1.84	3.33	1.57	1.35	0.79

Sample	EAMD C	EAMD L	EAMD M	EAMD R	EAMD V	ESP C	ESP L	ESP M	ESP R	ESP V	EAGRI C	EAGRI L	EAGRI M	EAGRI R	EAGRI V
*Lack	0.001 – 0.01														
*Slight	0.01 – 0.1														
*Medium	0.1 – 1														
*Intensive	1 - 10														
Sr	1.35	4.18	1.28	0.93	0.68	0.09	0.16	0.10	0.06	0.04	0.90	1.42	0.87	0.67	0.35
Mo	0.23	0.37	0.33	7.19	0.37	0.28	1.76	1.47	1.77	0.45	0.74	1.38	0.61	0.83	0.34
Pd	0.57	1.48	0.50	0.38	0.28	0.12	0.21	0.13	0.09	0.06	0.31	0.47	0.29	0.23	0.13
Ag	0.96	0.11	0.08	0.09	0.05	0.03	0.01	0.03	0.03	0.01	0.14	0.03	0.04	0.03	0.01
Cd	7.75	5.41	3.56	7.39	3.40	1.13	1.21	1.25	0.84	0.39	2.63	1.23	4.98	1.59	0.60
Sn	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sb	0.40	0.57	0.31	0.68	0.57	0.66	0.42	0.44	0.52	0.44	0.51	0.56	0.40	0.51	0.66
Ba	0.03	0.03	0.02	0.02	0.02	0.06	0.03	0.05	0.03	0.02	0.87	0.23	0.62	0.50	0.16
Pt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Au	-	-	-	-	-	0.00	0.00	0.00	0.00	0.00	-	-	-	-	-
Hg	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Tl	0.34	0.26	0.24	0.15	0.71	0.13	0.14	0.10	0.05	0.04	0.09	0.11	0.06	0.04	0.01
Pb	0.15	0.01	0.05	0.21	0.11	0.18	0.03	0.11	0.14	0.13	0.73	0.04	0.33	0.33	0.21
Bi	1.05	0.07	0.29	1.43	0.73	0.29	0.04	0.18	0.23	0.21	1.59	0.10	0.70	0.70	0.40
Th	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
U	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.00

** Degree of accumulation (Różanowski et al., 2012; Kabata-Pendias, 2011) – refer to Section 1.2.13.3*

- Undetectable limits

Table 4-3: Available bioaccumulation index (BAI_A) per element for different pasture species and growth medium combinations

Sample	EAMD C	EAMD L	EAMD M	EAMD R	EAMD V	ESP C	ESP L	ESP M	ESP R	ESP V	EAGRI C	EAGRI L	EAGRI M	EAGRI R	EAGRI V
Be	0.03	0.05	0.00	0.01	0.01	0.07	0.18	0.00	0.01	0.01	-	-	-	-	-
B	4.04	64.43	13.16	6.49	2.55	8.83	115.35	35.91	14.39	3.15	9.27	156.13	31.54	7.76	2.83
Na	97.40	181.65	332.22	418.84	82.85	322.53	643.11	1069.36	1288.32	263.31	32.96	37.91	100.83	130.63	13.41
Mg	88.30	296.56	96.73	78.41	105.29	74.79	173.20	112.49	75.96	67.77	19.81	22.92	17.50	12.98	12.20
Al	2.27	0.47	1.56	3.07	1.77	8.65	1.30	6.65	10.60	5.91	344.82	70.26	275.35	442.07	197.67
P	4280.8	9502.50	5454.1	4474.1	4609.1	6823.4	12568.47	8566.67	7060.36	5849.55	257.30	536.53	335.80	282.79	162.96
K	1315.6	1773.19	1296.0	1387.2	1350.5	2600.4	4297.16	3123.72	2863.13	2645.29	310.16	374.21	203.60	191.23	202.75
Ca	96.68	342.20	87.66	73.89	58.70	15.91	41.81	22.18	14.41	9.52	7.31	14.33	7.31	5.59	3.25
Ti	50.60	47.43	41.11	57.54	68.47	170.17	120.88	110.10	113.30	189.50	25.84	27.88	20.41	18.44	22.01
V	-	-	-	-	-	-	-	-	-	-	30.45	29.17	24.74	23.30	20.96
Cr	60.89	57.78	61.50	335.10	55.09	120.57	80.12	112.55	106.60	120.70	242.70	175.38	126.33	122.25	166.08
Mn	14.42	8.13	17.60	16.14	4.76	16.73	13.82	20.74	16.53	5.01	201.49	171.00	147.04	157.41	103.79
Fe	44.96	64.95	40.58	39.03	49.14	12.72	19.83	13.61	10.00	15.99	94.87	122.85	81.57	58.34	76.94
Co	0.32	1.19	0.56	0.33	0.22	0.61	1.93	0.97	0.43	0.24	7.41	24.09	7.77	4.60	5.23
Ni	3.21	4.62	2.37	72.48	5.32	3.42	5.56	3.47	3.07	1.61	414.76	114.47	56.51	47.04	85.42
Cu	71.85	30.82	37.42	95.24	63.57	77.86	37.53	58.29	57.76	57.68	241.79	111.82	130.08	114.23	84.22
Zn	98.55	95.97	86.46	63.49	57.59	15.91	15.71	15.21	16.71	12.68	714.07	541.73	424.07	307.53	256.30
As	0.74	1.39	0.86	0.76	0.71	1.33	2.94	2.36	1.89	1.33	5.91	7.05	6.78	5.86	3.46
Se	0.48	0.85	0.36	0.51	0.55	0.50	0.94	0.55	0.62	0.33	5.55	3.31	7.04	3.09	4.59
Rb	51.46	78.11	45.59	70.74	71.17	92.35	276.11	157.00	144.76	70.96	304.56	550.96	260.00	222.74	130.91
Sr	18.20	56.20	17.19	12.46	9.12	9.60	17.77	10.63	6.93	4.19	4.21	6.62	4.06	3.15	1.62
Mo	-	-	-	-	-	-	-	-	-	-	342.25	643.87	284.40	385.37	159.40
Pd	3.42	8.88	3.03	2.31	1.68	4.17	7.71	4.63	3.30	2.09	-	-	-	-	-
Ag	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Sample	EAMD C	EAMD L	EAMD M	EAMD R	EAMD V	ESP C	ESP L	ESP M	ESP R	ESP V	EAGRI C	EAGRI L	EAGRI M	EAGRI R	EAGRI V
Cd	7.75	5.41	3.56	7.39	3.40	4.52	4.83	5.00	3.37	1.55	3.94	1.84	7.47	2.38	0.89
Sn	1.68	1.71	1.67	1.80	1.86	2.39	2.41	2.33	1.93	1.94	2.13	1.86	1.85	2.01	2.25
Sb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ba	4.85	3.77	3.40	2.88	2.65	16.23	8.20	13.16	8.95	6.95	12.25	3.17	8.74	7.10	2.20
Pt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Au	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hg	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tl	5.66	4.46	3.98	2.48	11.92	1.79	1.96	1.46	0.73	0.56	-	-	-	-	-
Pb	6.24	0.54	1.93	8.62	4.63	251.93	39.07	164.40	201.97	188.52	611.97	37.13	279.17	280.17	172.41
Bi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Th	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
U	0.08	0.10	0.11	0.07	0.09	0.62	1.29	0.89	0.72	0.47	-	-	-	-	-

- Undetectable limits

The Accumulation Factor (AF) is the trace element concentrations absorbed by the plant from the growth medium and is calculated using the following equation (Selvaraj *et al*., 2015; Chuan *et al*., 2016):

AF = Concentration in the tissue of plant/ Initial concentration in the growth medium (Eq. 4-4)

The AF for all the plant species grown in the different growth mediums, including the control medium, are tabulated in Table 4-4, per element. An AF value >1 indicates the plant has hyperaccumulation capabilities. The values shaded in green represent the AF values >1. It should be noted that the macronutrients were also included in the list for comparison purposes.

B, Cd, Mn, Mo, Cu, Zn, Rb, Sr, and Bi hyperaccumulated in the various plant species and show promise for phytomining of these elements. There is no specific trend in terms of the specific plant species and their hyperaccumulation capabilities. Based on the results, all the plant species have hyperaccumulation abilities and the degree of hyperaccumulation depended on the elemental concentration in the growth mediums. EAGRI-V outperformed all the other growth medium and plant species combinations in terms of accumulation of elements and had extremely elevated AF values.

Table 4-4: Accumulation factor (AF) per element for different pasture species and growth medium combinations

	EAMD C	EAMD L	EAMD M	EAMD R	EAMD V	ESP C	ESP L	ESP M	ESP R	ESP V	EAGRI C	EAGRI L	EAGRI M	EAGRI R	EAGRI V
Be	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22
B	2.09	33.36	6.82	3.36	1.32	0.31	4.07	1.27	0.51	0.11	2.12	35.69	7.21	1.77	1.75
Na	12.44	23.20	42.43	53.50	10.58	13.90	27.72	46.09	55.53	11.35	14.73	16.94	45.06	58.38	134.40
Mg	6.43	21.59	7.04	5.71	7.67	10.94	25.35	16.46	11.12	9.92	2.29	2.65	2.02	1.50	1501.00
Al	0.05	0.01	0.03	0.06	0.04	0.16	0.02	0.12	0.19	0.11	0.10	0.02	0.08	0.13	8716.00
P	15.70	34.84	20.00	16.40	16.90	7.68	14.15	9.65	7.95	6.59	4.46	9.29	5.81	4.90	758.30
K	17.86	24.07	17.59	18.83	18.33	16.43	27.14	19.73	18.08	16.71	31.34	37.81	20.57	19.32	1163.00
Ca	39.08	138.32	35.43	29.87	23.73	2.43	6.39	3.39	2.20	1.45	0.98	1.91	0.98	0.75	10020.0
Ti	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.00	240.40
V	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	25.83
Cr	0.04	0.04	0.04	0.21	0.03	0.09	0.06	0.08	0.08	0.09	0.10	0.07	0.05	0.05	48.24
Mn	1.73	0.98	2.11	1.94	0.57	4.68	3.86	5.79	4.62	1.40	0.26	0.22	0.19	0.20	197.30
Fe	0.00	0.01	0.00	0.00	0.00	0.01	0.02	0.01	0.01	0.02	0.01	0.02	0.01	0.01	9656.00
Co	0.04	0.16	0.07	0.04	0.03	0.07	0.23	0.11	0.05	0.03	0.01	0.04	0.01	0.01	6.39
Ni	0.14	0.20	0.10	3.07	0.23	0.23	0.37	0.23	0.20	0.11	0.37	0.10	0.05	0.04	33.23
Cu	1.06	0.45	0.55	1.40	0.93	1.28	0.62	0.96	0.95	0.95	1.07	0.49	0.58	0.51	29.37
Zn	1.07	1.04	0.94	0.69	0.63	0.80	0.78	0.76	0.83	0.63	0.90	0.68	0.53	0.39	79.50
As	0.00	0.01	0.01	0.00	0.00	0.01	0.02	0.01	0.01	0.01	0.03	0.03	0.03	0.03	2.05
Se	0.06	0.10	0.04	0.06	0.07	0.02	0.05	0.03	0.03	0.02	0.02	0.01	0.03	0.01	8.12
Rb	1.18	1.79	1.04	1.62	1.63	1.71	5.12	2.91	2.69	1.32	1.84	3.33	1.57	1.35	14.87
Sr	1.35	4.18	1.28	0.93	0.68	0.09	0.16	0.10	0.06	0.04	0.90	1.42	0.87	0.67	16.64
Mo	0.23	0.37	0.33	7.19	0.37	0.28	1.76	1.47	1.77	0.45	0.74	1.38	0.61	0.83	4.65
Pd	0.57	1.48	0.50	0.38	0.28	0.12	0.21	0.13	0.09	0.06	0.31	0.47	0.29	0.23	0.13
Ag	0.96	0.11	0.08	0.09	0.05	0.03	0.01	0.03	0.03	0.01	0.14	0.03	0.04	0.03	0.14

	EAMD C	EAMD L	EAMD M	EAMD R	EAMD V	ESP C	ESP L	ESP M	ESP R	ESP V	EAGRI C	EAGRI L	EAGRI M	EAGRI R	EAGRI V
Cd	7.75	5.41	3.56	7.39	3.40	1.13	1.21	1.25	0.84	0.39	2.63	1.23	4.98	1.59	0.03
Sn	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	67.57
Sb	0.40	0.57	0.31	0.68	0.57	0.66	0.42	0.44	0.52	0.44	0.51	0.56	0.40	0.51	0.23
Ba	0.03	0.03	0.02	0.02	0.02	0.06	0.03	0.05	0.03	0.02	0.87	0.23	0.62	0.50	41.41
Pt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Au	-	-	-	-	-	0.00	0.00	0.00	0.00	0.00	-	-	-	-	0.00
Hg	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
Tl	0.34	0.26	0.24	0.15	0.71	0.13	0.14	0.10	0.05	0.04	0.09	0.11	0.06	0.04	0.12
Pb	0.15	0.01	0.05	0.21	0.11	0.18	0.03	0.11	0.14	0.13	0.73	0.04	0.33	0.33	8.38
Bi	1.05	0.07	0.29	1.43	0.73	0.29	0.04	0.18	0.23	0.21	1.59	0.10	0.70	0.70	0.10
Th	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.89
U	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.02	0.01	0.01	1.00

- Undetectable limits

4.4 Conclusion

Cynodon dactylon (C), *C. zizanioides* (V), *C. gayana* (R), *M. sativa* (L) and a mixture (M) which included *C. dactylon, C. gayana, D. eriantha, M. sativa* and *E. tef* can all be used for phytoremediation and all show potential as hyperaccumulators.

Several factors influenced the accumulation rate of the plant species, including the initial concentrations in the growth medium, and the elemental concentrations in the irrigation water.

The irrigation water quality was not analysed, and the assumption that the water quality resulted in increased concentrations in both the plant material and growth mediums, compared to the initial concentrations, could not be proven.

The complementary development of phytoremediation necessitates an integrated multidisciplinary research effort that syndicates soil microbiology, plant biology, soil biochemistry, agriculture, and environmental engineering. Numerous parameters can be used to assess metal toxicity in plants, like plant growth and photosynthetical health, and the symptoms depend on the specific metal. Numerous studies exist for plant responses for certain metals in isolation however, metal combinations should be assessed further.

4.5 References

Barrutia, O., Epelde, L., Garcia-Plazaola, J.I., Garbisu, C. and Becerril, J.M. 2008. Phytoextraction potential of two *Rumex acestro L*. accession collected from metalliferous and non-metalliferous sites: Effect of fertilisation. Chemosphere, 74(2): 259-264. DOI: https://doi.org/10.1016/j.chemosphere.2008.09.036.

Chuan, N.C., Rahman, M., Boyce, A.N. and Bin Abas, M.R. 2016. Heavy metals phyto-assessment in commonly grown vegetables: water spinach (I. aquatica) and okra (A. esculentus). SpringerPlus. 5(1): 1-9. DOI: https://doi.org/10.1186/s40064-016-2125-5.

Cowan, A.K., Lodewijks, H.M., Sekhohola, L.M., Edeki, O.G. 2016. In situ bioremediation of South African coal discard dumps. Paper presented at the Mine Closure 11th International Conference on Mine Closure; 2016, March15-17; Perth: Australia.

Greipsson, S. 2011. Phytoremediation. Nature Education Knowledge. 3(10): 7.

Hunt, A.J., Christopher, W.N., Bruce, N., Muñoz, A., Graedel, T.E., Hodson, M., Meech, J., Nassar, N., Parker, H.M., Rylott, E.L., Sotiriou, K., Zhang, Q. and Clark, J. 2014. Phytoextraction as a tool for green chemistry. Green Processing and Synthesis. 3(1): 3-22. DOI: 10.1515/gps-2013-0103.

Kabata-Pendias, A. 2011. Trace elements in soils and plants. 4th ed. Boca Raton: Taylor and Francis. p. 432.

Keeling, S.M. and Werren, G. 2005. Phytoremediation: The Uptake of Metals and Metalloids by Rhodes Grass Grown on Metal-Contaminated Soil. Remediation Journal: The Journal of Environmental Cleanup Costs, Technologies and Techniques. 15(2): 53-61.

Langella, F., Grawunder, A., Stark, R., Weist, A., Merten, D., Haferburg, G., Büchel, G. and Kothe, E. 2014. Microbially assisted phytoremediation approaches for two multi-element contaminated sites. Environmental Science and Pollution Research. 21(11): 6845-6858.

Mahar, A., Wang, P., Ali, A., Awasthi, M., Lahori, A., Wang, Q., Li, R. and Zhang, Z. 2016. Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicology and Environmental Safety. 126: 111-121. DOI: 10.1016/j.ecoenv.2015.12.023.

Pandey, V.C. and Maiti, D. 2020. Perennial grasses in phytoremediation-challenges and opportunities. Phytoremediation Potential of Perennial Grasses. p.1.

Pivetz, B.E. 2001. Phytoremediation of Contaminated Soil and Groundwater at Hazardous Waste sites. EPA/540/S-01/500. Washington, USA: United States Environmental Protection Agency.

Renault, S., Sailerova, E. and Fedikow, M.A.F. 2000. Phytoremediation and phytomining in Manitoba: Preliminary observations from an orientation survey at the Central Manitoba (Au) minesite (NTS 52L/13). Report of Activities 2000, Manitoba Industry, Trade and Mines, Manitoba Geological Survey, p.179-188.

Różanowski, B., Michałowski, M., Tora, B., Cablik, V. and Cernotova, L. 2012. Effectiveness of the use of willow tree (Salix viminalis) for wastewater treatment. AGH Journal of Mining and Geoengineering, 36(4):159-165.

Selvaraj, K., Sevugaperumal, R. and Ramasubramanian, V. 2015. Phytoextraction: Using Brassica as a Hyper Accumulator. Biochemistry and Physiology. 4(3): 174.

USEPA (United States Environmental Protection Agency). 2000. Introduction to phytoremediation. Office of Research and Development. Report No. EPA/600/R-99/107 [internet]. Washington DC. [cited 15 Sept 2022]. Available from: https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NRMRL&dirEntryId=63433
.

Chapter 5 Integrated conclusion and future prospects

5.1 Introduction

Topsoil is a scarce rehabilitation resource, and topsoil management should be improved or alternative options should be investigated to ameliorate contaminated substrates to a viable growth medium. Coal refuse materials are generally not considered a practicable growth medium, and the successful establishment of vegetation in these growth mediums has been cumbersome. Previously, the low soil pH and high levels of trace elements associated with coal mining received publicity as the cause for the ineffective establishment of vegetation in rehabilitation areas.

The subsections below provide a brief description of the concluding remarks from each chapter.

5.1.1 Chapter 1

Topsoil is becoming a scarce commodity for rehabilitation purposes, and it is necessary to investigate rehabilitation options that require less topsoil without compromising the quality of the rehabilitation performance. Innovative rehabilitation technologies also need to be explored where areas can be rehabilitated without topsoil, where topsoil sources are non-existent. Rehabilitation attempts should be holistic to consider plant species, climatic conditions, and landscape and be modified to the growth mediums' specific chemical and physical characteristics.

5.1.2 Chapter 2

Based on the results, the particle size distribution of AMD-contaminated soil and even coal spoils was not a limiting factor for plant growth. It was also visually observed that the successful establishment of vegetation improved the physical characteristics of the growth mediums. The chemical analyses confirmed that the AMD-polluted soil (EAMD) and coal spoil materials (ESP) were acidic and had low plant nutrient status.

Both the polluted growth mediums had elevated levels of trace elements like iron, closely followed by aluminium and barium, to name a few. Cadmium levels were not elevated; however, it had the highest availability percentage indicating all the cadmium was plant-available. Suppose the soil screening values (SSVs) are considered, which are used to determine whether the soil is contaminated or not. Some trace elements are known to be associated with low pH levels and cause phytotoxicity, like aluminium does not have an SVV threshold.

In that case, AMD-contaminated soil and coal spoil material are classified as not contaminated. Two deductions can be drawn from this: the growth mediums are not contaminated and should not pose difficulty in the establishment and sustainability of vegetation. Another alternative is to accept that the SSVs are inadequate to measure soil contamination and should be tailored to include constituents associated with coal with appropriate thresholds, or a separate screening tool should be developed to assess coal refuse material and soils polluted by coal mining.

5.1.3 Chapter 3

No growth inhibition due to metal toxicity was observed in the AMD-contaminated soil, which performed similarly, if not better, than the optimal control. Based on the SPAD meter readings, the chlorophyll content appears to be a function of plant maturity and growth stage rather than plant stress if the plants are grown in a controlled environment. The AMD-contaminated soil produced more dry material than the agricultural soils; however, in terms of fresh produce, EAGRI performed better.

The vegetation growing in ESP had the highest predicted protein percentage at the end of the growing season, and the plants from EAMD had the lowest predicted protein percentage. The predicted protein percentages in all the different growth mediums followed a similar trend throughout the growing season. This indicates that the protein content depends on the plant's maturity rather than plant stress. The forage quality of the grass grown in the polluted growth medium was better than the controls. Despite the contaminated growth medium outperforming the control mediums in terms of forage quality, all the forage was of good quality.

5.1.4 Chapter 4

Pasture species like *C. dactylon*, *C. zizanioides*, *C. gayana*, *M. sativa* and a mixture which included *C. dactylon, C. gayana, D. eriantha, M. sativa,* and *E. tef* can be applied for phytoremediation due to the hyperaccumulation properties the species have. The aforementioned species are also viable options for post-closure land use options ranging from grazing to essential oil production options. The accumulation rate depends on the plant species, including the elemental concentrations in the growth medium and the irrigation water quality.

A mass balance could not be calculated from the results obtained from the concentrations in the grass or selected plant species and the substrate after the growing season due to leachate losses and added concentration loads from the irrigation water.

5.2 Limitations

During the course of the project, numerous challenges and limitations were identified, and they are briefly mentioned below.

- The project was conducted within a controlled environment, and the plants were not subjected to extreme heat and drought conditions, which is the case on a mien rehabilitation site.
- The seepage from the pots was not collected to determine to what extent the trace elements were leached with water.
- Due to financial constraints, the growth medium's total and plant-available trace element concentrations were not analysed throughout the project, specifically with each harvest. The change in trace element concentration might have given significant insight into the mechanisms of phytoremediation.

  The concentrations of the trace elements in the plants were analysed by combining related materials from each harvest and were not assessed per harvest. A difference in the degree of phytoremediation or hyperaccumulation might have been observed during each individual harvest. If each harvest had been analysed separately, more insight could have been gained to determine the phytoremediation potential based on the plant's maturity.

- The trace element concentrations in the irrigation water were not considered during the project, and the potential load contribution in terms of concentration in the growth medium and in the plants. Irrigation water quality should be analysed continuously to determine the impacts on trace metal concentrations.

5.3 Recommendations

The findings from this study are based on a pot trial experimental setup and should be followed with field trials to substantiate results from this study. Field trials will fully quantify the growth medium's long-term effects on the plant's phytoremediation potential and sustainability. Field trials will also provide additional insight regarding the viability of phytoremediation under natural, uncontrolled conditions.

It is recommended that the pH of coal refuse material be continuously neutralised during rehabilitation practices. The pH levels proved to have significant correlations with other properties, such as Al solubility; therefore, other associated variables, such as Al toxicity, will theoretically improve when the pH is addressed. Additionally, plant growth will be optimum at a neutral pH level resulting in successful rehabilitation.

For the advancement in the field of phytoremediation, an integrated multidisciplinary approach should be followed that syndicates plant biology, soil science, microbiology, biochemistry and agriculture. Numerous studies exist for plant responses to specific metals; however, the combination of metals should be assessed to determine the plant responses to a multitude of elevated elements. Since phytoremediation and phytomining are both time-consuming, it might be worthwhile to investigate what effect the addition of chelates might have in terms of trace metal absorption.

5.4 Conclusion

The study suggests that the failure to establish vegetation in coal mine rehabilitation areas results from drought, insufficient aftercare and other extreme conditions rather than the chemical characteristics of the growth medium. It also means that plant performance can increase and be sustained with regular irrigation, defoliation and fertiliser applications until the vegetation has successfully been established. Pasture species currently used for mine rehabilitation purposes show great potential for hyperaccumulation and phytoremediation.