

# Seed viability and re-growth of grasses used for mine waste rehabilitation

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Dissertation submitted in fulfillment of the requirements for the degree *Magister Scientiae* in **Environmental Sciences** at the Potchefstroom Campus of the North-West University

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November 2014

## Acknowledgements

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I am greatly indebted to the contributions and assistance of others to this study, and hereby express my gratitude:

- **My Heavenly Father**, Who carried me during every experience along the way.
- **My supervisors, Mr Piet van Deventer and Prof Klaus Kellner**, for their continued guidance and advice which are sincerely appreciated.
- **My parents, Mr Harry Muller and Mrs Irma Muller**, for selflessly providing me with the opportunity to attend university and for their unconditional love and encouragement.
- **Mr Stiaan van der Merwe, Miss Petra Muller, Miss Halcyone Muller, Miss Hermien Muller, and Mrs Christine Human**, for their support and encouragement.
- **Everyone who assisted with data monitoring and collecting:** N. Reynolds; S. Riekert; Z. Gagliano; G. Muller; H. Mayer; M. Ferreira; and M. du Plessis.
- **Mr Shawn Liebenberg** for his assistance with the statistical analyses.
- **Mrs Yolande van der Watt**, for her help with general and logistic aspects.
- To **Advance Seed**, a sincere gratitude for the generous financial contribution to make this study possible and for positive collaboration regarding this research.

## Abstract

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Sustainable rehabilitation can be compromised by the inability of vegetation to survive in hostile mine wastes on a long-term basis. The adverse chemical and physical properties of mine wastes, together with extreme pH conditions and lack of nutrients, provide poor growth conditions for vegetation during seed development and germination. This raises concern for the long-term survival of vegetation through means of seed production when under stress from the punitive properties of mine wastes.

Seed vigour is a function of a variety of factors to which the parent plant is subjected during seed formation and maturation. Environmental conditions experienced by the maternal plant during the growth season plays a significant role in determining subsequent germination rates in seeds. Traits of offspring seed depend on the abiotic environment attributed by the growth medium during seed development and maturation

The general aim of this study was to determine the viability of seed produced by a previous generation of grass species established in eight different mine wastes and two soils (namely: gypsum wastes; gold tailings with low pyrite content; gold tailings with high pyrite content; platinum tailings; kimberlite mine waste; fluorspar mine waste; andalusite mine waste; coal discard; red soil; and vertic soil) in order to identify suitable species for specific mine wastes to ensure long-term survival through means of seed production. The species selected included: *Eragrostis curvula*; *Eragrostis tef*; *Cenchrus ciliaris*; *Eragrostis curvula*; *Digitaria eriantha*; *Cynodon dactylon*; *Chloris gayana*; *Hyparrhenia hirta*; and *Sorghum bicolor*.

The progeny seed's viability and ability to germinate were determined through a pot trial study and additional germination testing at the laboratory of Advance Seed (Pty) Ltd. (AS). The germination results were correlated with the growth media analyses by statistical non-parametric correlations which indicated several significant correlations among the growth media properties themselves, and with the germination of the progeny seed. *C. gayana* (Rhodes grass) seed had poor germination percentages, especially seed harvested from Rhodes grass grown in acidic wastes. Seed harvested from each of the *E. curvula* grasses grown in various mine wastes, had excellent germination percentages.

According to the Repeated Measures ANOVA statistical analysis, there was a significant influence of the growth media in which the parent grass were grown as a variable on the

germination of the progeny seed batches from *S. bicolor*, *C. ciliaris*, *C. gayana*, and *D. eriantha*, indicating that the environmental factors as attributed by the growth media, i.e. the eight different mine waste materials and two soils, and experienced by the maternal plant, did indeed influence the germination of progeny seed. However, it was found that significant correlations between the properties of the growth media and the germination of the progeny seed, was species dependent.

The second general aim for this study was to evaluate above-ground re-growth of parent plants after cutting in the mine waste materials and soil types mentioned above. The ability of established grasses to re-grow after a cutting event was determined by cutting the above-ground biomass of the parent grasses, after which it was scored according observable above-ground growth in the following growth season. The measurement of re-growth was subjectively done by scoring the grasses according to observable above-ground biomass.

Re-growth was observed for all the perennial grass species. This can be ascribed to the grasses showing resilience to stress factors attributed by the growth media; or new grasses which emerged from seed that collected in the pots, being mistaken for re-growth; or new emerging grasses from the nodes of stolons and/or rhizomes being mistaken for re-growth. However, the emergence of new grasses was an indicator of good health, as biomass allocation to rhizomes and stolons is reduced under low nutrient availability and stress conditions. Therefore the emergence of new grasses is indicative that the plant is either tolerant to stress conditions or that the plant adapted to the restriction of growth due to the roots being bound to the size of the pot.

**Key words:** seed viability; mine wastes; sustainable rehabilitation; pH; re-growth; germination.

## Uittreksel

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Die volhoubaarheid van rehabilitasie projekte kan benadeel word deur die onvermoë van plantegroei om te oorleef in myn uitskot gronde oor 'n langtermyn tydperk. Die nadelige chemiese en fisiese eienskappe van myn uitskot gronde, tesame met uiterste pH toestande en 'n gebrek aan nutriënte, dra by tot verswakte groei toestande vir plantegroei tydens saad ontwikkeling en \_ontkieming. Dit wek kommer vir die langtermynoorlewing van plantegroei deur middel van saad produksie weens stress veroorsaak. deur mynuitskotgronde se eienskappe.

Saad se lewensvatbaarheid word grotendeels beïnvloed deur die vele omgewingsfaktore waaraan die ouerplant blootgestel is tydens saadontwikkeling. Die omgewingstoestande wat ervaar word deur die ouerplant tydens die groeiseisoen dra by tot die kieming sukses van die nageslag saad.

Die doel van die studie was om die kiemkragtigheid te bepaal van nageslag saad wat afkomstig is van grasse gevestig in agt verskillende mynuitskotgronde en twee gronde (naamlik: gips-; goud slik met 'n lae piriet inhoud; goud slik met 'n hoë piriet inhoud; platinum-; veldspaat-; andalusiet-; en steenkool mynuitskot; asook rooi grond; en vertiese grond). Asook om die geskiktheid van verskeie gras spesies in terme van langtermyn oorlewing deur middel van saad produksie vir mynuitskotgronde te bepaal. Die geselekteerde spesies sluit die volgende in: *Eragrostis curvula*; *Eragrostis tef*; *Cenchrus ciliaris*; *Eragrostis curvula*; *Digitaria eriantha*; *Cynodon dactylon*; *Chloris gayana*; *Hyparrhenia hirta*; en *Sorghum bicolor*.

Die nageslag saad se kiemkragtigheid en lewensvatbaarheid was bepaal deur middel van 'n pot proef studie, asook deur addisionele ontkiemings toetse wat deur die laboratorium van Advance Seed uitgevoer is. Statistiese nie-parametriese korrelasies tussen die groeimediums se eienskappe en die ontkiemings resultate van die nageslag sade het verskeie beduidende verwantskappe aangedui. Die nageslag saad van *C. gayana* het lae ontkiemings persentasies gehad, veral nageslag sade wat ge-oes is vanaf ouer plante wat gevestig was in suur mynuitskotgronde. *E. curvula* se nageslag saad, afkomstig van ouer plante wat in verskeie mynuitskotgronde gevestig was, het uitstekende ontkiemings persentasies gehad.

Volgens die statistiese ANOVA analyses, was daar 'n beduidende invloed van die groei medium waarin die ouer plant gevestig was, as 'n veranderlike op die ontkieming van die nageslag sade vir die verskeie spesies, naamlik, *S. bicolor*, *C. ciliaris*, *C. gayana*, en *D. eriantha*. Dit dui aan dat die groeimediums waarin die ouerplante gevestig was, die ontkieming van die nageslag

saad aansienlik beïnvloed het. Die beduidende korrelasies tussen die eienskappe van die groeimediums en die ontkieming van die nageslag sade, het egter verskil vir elke spesie.

Die tweede doel van die studie was om die bo-grondse hergroei van die ouerplante te evalueer nadat die gesnoei is. Die vermoë van gevestigde grasse om te her-groei, was bepaal deur die bo-grondse biomassa van die ouer plante te snoei, en na die verloop van die winter, was die bo-grondse biomasse ge-evalueer. Die her-groei was subjektief ge-evalueer deur die toekenning van punte volgens sigbare bo-grondse biomassa.

Her-groei is waargeneem vir al die meerjarige gras spesies. Dit kan egter toegeskryf word aan die grasse se veerkragtigheid teen stresfaktore; of aan die vestiging van nuwe grasse vanaf sade wat versamel het in die potte en verkeerdelik as her-groei ge-evalueer is; of aan die vestiging van nuwe grasse vanuit die nodes van stolons en/of risome wat verkeerdelik as her-groei ge-evalueer is. Ten spyte hiervan, word die vestiging van nuwe grasse aanvaar as 'n aanduiding dat die plant óf tolerant is vir sekere stres faktore, óf dat die plant aangepas het tot die beperkte ruimte vir die wortels as gevolg van die pot se bepaalde volume.

**Sleutelwoorde:** Saad lewensvatbaarheid; mynuitskotgronde; volhoubare rehabilitasie; pH; her-groei; ontkieming.

## Abbreviations

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AMD	:	Acid Mine Drainage
AS	:	Advance Seed (Pty) Ltd.
CARA	:	Conservation of Agricultural Resources Act
CEC	:	Cation Exchange Capacity
ECA	:	Environmental Conservation Act
EC	:	Electrical Conductivity
FAO	:	Food and Agriculture Organization of the United Nations
GEO LAB	:	<i>Grond- en Omgewingslaboratorium</i>
ISTA	:	International Seed Testing Association
LFA	:	Landscape Function Analyses
MA	:	Minerals Act
MPRDA	:	Mineral and Petroleum Resources Development Act
NEA	:	National Environmental Act
NEMA	:	National Environmental Management Act
NWU	:	North-West University
SAGEP	:	South African Guidelines for Environmental Protection
SER	:	Society for Ecological Restoration
SOM	:	Soil Organic Matter
TSF	:	Tailings Storage Facility
USEPA	:	United States Environmental Protection Agency

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## Introduction

### 1.1 Justification

South Africa gains economic growth from the extraction and mining of its abundant mineral resources. The exploitation of mineral resources and waste products from the mining industry often leads to the waning of environmental health by contributing to the loss of topsoil, seed banks and vegetation cover, resulting in loss of biodiversity, soil functions and stability within an ecosystem (Bradshaw, 1998:225; Grimshaw, 2007:295; Sutton & Weiersbye, 2007:92; Welsh *et al.*, 2007:175).

Most detrimental impacts of mine activities on the environment can be directly associated with pollution of contaminants and metals related to mine waste materials. Identified risks to environmental health associated with mine waste materials include the seepage and leaching of contaminants and salts into downstream and below surface water sources, thereby polluting and diminishing water quality (Grimshaw, 2007: 295; Sutton & Weiersbye, 2007: 92; Welsh *et al.*,2007:175). Erosion instability of mine waste can result in air pollution, surface runoff and, consequently, air and water pollution (Grimshaw, 2007:295; Welsh *et al.*, 2007:175). Latent and residual risks from mine waste materials are often mistakenly disregarded as they only become apparent long after mine closure; these impacts include acid generating potential, leaching and seepage of metals. This can result in contamination of ecosystems, water sources and consequently toxic levels of metals leading to potential bioaccumulation in biota and humans (Sutton & Weiersbye, 2007:92). Due to sulfide bearing minerals and metallurgic processes, extreme pH levels exist within gold mine waste materials, of which the status changes dynamically over short periods of time. Low pH levels in mine waste materials increase the solubility of metals, thereby making it bioavailable to vegetation which can result into bioaccumulation at toxic levels to human health, and the regression of vegetation cover (Wu *et al.*, 2011:788).

South African legislation, such as the Environmental Conservation Act (ECA) (1998) and the Mineral and Petroleum Resources Development Act (MPRDA) (Act 28 of 2002), requires developers to ecologically rehabilitate damaged and/or altered environments. The MPRDA (South Africa, 2002) in particular requires that environments affected by mining or prospecting

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operations must be, as far as reasonably practical, conducted towards its natural or a predetermined state, or an end land-use which conforms to the generally accepted principle of sustainable development. Furthermore, the Minerals Act (MA) (section 12 of 1991) placed the responsibility to protect and conserve the environment on the owner of the mining license until a certificate releasing the owner from the responsibility has been issued. Section 2(4)(a) of the National Environmental Management Act (NEMA) recognizes that some negative impact will occur during development, and it therefore calls for a risk-averse approach which anticipates negative impacts and tries to prevent or minimise and remedy these impacts. The costs of these preventative and remediate measures are specifically assigned to the party responsible and are referred to in section 2(4)(p) of NEMA, which is also known as the ‘polluter pays’ principle. This principle states that:

*The costs of remedying pollution , environmental degradation and consequent health effects and of preventing, controlling, or minimising further pollution, environmental damage or adverse health effects must be paid for by those responsible for harming the environment.*

According to the South African Guidelines for Environmental Protection (SAGEP) (South Africa, 1979) a combination of chemical amelioration of the medium and vegetative establishment is the most successful rehabilitation method. Some rehabilitation practices will stockpile overlain top soil during mining operations and will during rehabilitation when mining operations have ceased, cover the mine waste materials with the top soil, thus, providing a more suitable growth medium for vegetation. The top soil will lose structure and experience loss of nutrient status and microbial activity to a certain extent during stockpiling. It is therefore ameliorated accordingly to improve the nutrient status and pH levels, as well as ripped to reduce the effect of compaction. Other rehabilitation approaches used on mine waste materials include the use of caps or covers to control and contain pollution, in particular to prevent the contamination of water resources. Physical barriers, such as compacted clay layers, are used to cover tailings storage facilities (TSFs) and to minimize infiltration (Weiersbye, 2007:21).

Rehabilitation is the attempt to restore a degraded environment’s ecosystem functions and services to a sustainable and stable state similar to those of a natural or historical reference environment (Palmer *et al.*, 2006:2). A restored environment is described by the Society for Ecological Restoration (SER, 2004:7) as an ecosystem containing “sufficient biotic and abiotic resources to continue its development, sustaining itself structurally and functionally, and demonstrating resilience to normal ranges of environmental stress”. Van Deventer and Hattingh (2004:3) add to this description of a restored environment stating that it contains natural

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surviving vegetation which is self-sustainable. Furthermore, the common outcome for a successfully rehabilitated mine waste dump is that it is in a condition that is safe, stable, and non-impacting on the surrounding environment (Reichardt & Reichardt, 2007: 145).

Self-sustainable vegetation as an outcome relies on the selection and use of stress tolerant plants that are able to produce viable seed despite poor growth conditions (Weiersbye, 2007:21). Sustainable rehabilitation can be compromised by the inability of vegetation to survive in mine waste materials on a long-term basis. Another limiting factor includes the germination and establishment of plant species in hostile environments, such as native grass species in mine wastes (Brits, 2007:4; Van den Berg & Kellner, 2005:499). The selection of species based on their suitability with regard to certain environmental factors as attributed by disturbances is particularly difficult (Westcott, 2011:3). However, the use of local native species for rehabilitation has advantages, such as their inherent adaptation to prevailing climatic conditions, the provision of suitable habitat to native fauna and flora, and promoting biodiversity through facilitation of interactions between species and the inherent capacity of plant communities to breed and regenerate by seed (Vickers *et al.*, 2012:72; Weiersbye, 2007:14).

The adverse chemical and physical properties of mine wastes, along with severe pH conditions and poor nutrient status do not provide healthy growth conditions for vegetation (Bradshaw, 1998:256; Maboeta *et al.*, 2006:149; Weiersbye, 2007:20), and consequently provide a poor abiotic environment for the mother plant during seed development. In reality, mine waste materials are far from the ideal growth medium for vegetation, being contaminated with metals which can be toxic and detrimental to plant functioning at elevated levels (Weiersbye, 2007: 19). The pollution and contamination of wastes are related to the mineralogy of the host rock extraction methods, metallurgical treatments and are therefore very wastes specific. The extreme pH levels found in mine waste materials results in increased solubility, and consequently bioavailability, of metals while saline conditions are often found in mine waste materials due to the metallurgic processes (Weiersbye, 2007:19). There is no clear cut solution for these conditions, and when combined, which is mostly the case, they create a stressful growth environment for vegetation.

According to Roach and Wulf (1987:1152) maternal effects, such as poor nutrient status and abiotic stress, can physically change phenotypic attributes of the offspring (for example seed size and mineral content). Experimental studies have shown that maternal effects attributed by the growth medium affects seed both morphologically and physiologically, such as seed size,

germination timing and success (Schuler & Orrock, 2012:477). This raises concern for the long-term survival of vegetation through means of seed production when under strain from the harsh properties of mine waste. A lack of literature about seed viability and production under these conditions can be ascribed to lack of long-term vegetation monitoring of rehabilitation efforts.

In a review of South African legislation regarding mine closure Sutton and Weiersbye (2007: 89) found that the MPRDA (28 of 2002) does not adequately address the potential for environmental damage caused by mining activities to be irreversible, and assumes that all damage can be restored and rehabilitated. From an ecological perspective, degradation and damage from mining activities and wastes can last for many generations, if not at all irreversible (Sutton & Weiersbye, 2007: 93). Considering that self-sustainable and functional reproductive vegetation communities is a desirable outcome of rehabilitation, the lack of literature on this matter is contradicting and motivates the importance of this research.

### **1.2 Research Question**

The adverse properties of mine waste materials, such as extreme pH levels, and the lack of structure, organic matter and nutrients, do not provide favourable growth conditions for vegetation. Other than that, mine waste materials are sometimes contaminated with elevated levels of metals which will have a detrimental effect on plant growth and health. The milling and metallurgic processes that mine waste materials were subjected to, result in poor structure, homogenous texture, and slit sized particles. As a result it tends to crust and has poor water retaining properties. As such the long-term survival of vegetation is impaired and often re-seeding is required during rehabilitation processes. The adverse properties of mine waste materials are expected to reduce the seed viability and vigour, including the growth rate of grass. It is expected that the maternal environment attributed by the properties of mine waste materials will influence seed development, thus poor seed viability and potential sterility is anticipated for the offspring seed.

### 1.3 Project Overview

This project forms Phase 2 of a larger project that is carried out in collaboration with Advance Seed (Pty) Ltd. (AS)\*.

Phase 1 entailed the investigation of the difference in the germination and establishment rates between coated and non-coated selected grass seed types in different growth media, including mine waste. The Phase 1 project consisted of pot-trials, growth medium analysis and physiological assessment of the grass species. The grasses assessed included coated and non-coated seed types of *Chloris gayana* (Rhodes grass), *Cynodon dactylon* (Couch grass), *Digitaria eriantha* (Smuts Finger grass), *Eragrostis curvula* (Weeping love grass) and *Cenchrus ciliaris* (Buffalo grass) of which the seed were supplied by AS. Additional non-coated species included *Sorghum bicolor* (Sorghum), *Hyparrhenia hirta* (Common Thatching grass), *Lolium perenne* L. Synonym (Rye), and *Eragrostis tef* (Tef) (FAO, 2014).

During Phase 1 the seeds produced by these selected species, were harvested and sown for the commencement of Phase 2. Phase 2 investigated the germination and viability of seed harvested from the previous generation established during Phase 1.

### 1.4 Objectives

#### 1.4.1 General

The general aim of this study has been to determine the viability of seed produced by a previous generation of grass species established in eight different mine waste materials and two natural soils, in order to identify suitable species for specific mine waste materials to ensure long-term survival through means of seed production.

#### 1.4.2 Specific

Specific objectives for this project were to:

- Evaluate the viability and germination of progeny seed;
- Correlate the germination of progeny seed with properties of growth media in which the parent grass were grown in order to evaluate the effect the growth media had on the viability of the progeny seed;

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- Evaluate above-ground re-growth of parent plants after cutting in the follow-up growth season;
- Identify suitable species for specific mine wastes to achieve sustainability and ensure proper surface cover in a short period of time.

### **1.5 Dissertation structure and content**

The viability of seed from grasses grown in different growth media, which are eight different mine wastes and two natural soils, is the main subject matter continuous through this dissertation.

Chapter 2 discusses literature regarding mine rehabilitation and legislation, seed development and germination. The influence of a growth medium's properties on germination of grasses is discussed along with the review of maternal effects on seed viability and germination. Chapter 3 discusses the materials and methods used and executed in the components of the study.

Chapter 4 illustrates the results obtained during Phase 2 of the study for the objectives mentioned. Chapter 5 contains the discussion and conclusions of the results presented in Chapter 4 with regard to the objectives.

Chapter 6 discusses the knowledge gaps encountered during this study and recommendations for similar studies. A complete list of references and appendix is included at the end of the thesis.

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## Literature Review

### 2.1 Introduction

#### 2.1.1 Background

South Africa is rich in mineral resources and benefits greatly from its resulting mining sector. However, mining activities have resulted in the removal of top soil and loss of seed banks and vegetation which contributes to the degradation of ecosystems, resulting in a loss of functionality, sustainability, stability and biodiversity (Bradshaw, 1998:257; Grimshaw, 2007:295; Liebenberg *et al.*, 2013:734, Sutton & Weiersbye, 2007:89; Welsh *et al.*, 2007:175).

Most environmental threats resulting from mining activities are due to the large amount of waste rock and tailings material produced and deposited (Wong, 2003:775). Mine wastes are a primary component of mine waste and can be defined as finely ground solid waste rock from the milling and mineral extraction processes (Hossner & Sahandeh, 2006:154; Tordoff *et al.*, 2000:221). Its properties differ adversely from those of soil due to its anthropogenic origin. Soil is the result of weathering from parent rock material and consequently contains secondary minerals (Winegardener, 195:37), while mine wastes are essentially ground host rock which has never before been exposed to weathering by natural elements and consists of primary minerals. Even after it has been subjected to weathering elements, it takes a few years before natural weathering processes are activated.

Mining activities and associated waste products, of which mine wastes are the most problematic, impacts the environment adversely. Several of these environmental impacts become apparent only after mine closure and mining activities has ceased (Nel, 2008:24). Nel (2008:24) has identified three categories in which environmental impacts from mining can be divided: (1) degradation of the land surface; (2) degradation of water quality; and (3) degradation of air quality.

Mine wastes and wastes present challenges during phytoremediation and rehabilitation as it lacks organic material and nutrients supportive of biological growth (Tordoff *et al.*, 2000:221). Furthermore wastes materials are unstable, contain hazardous metals, may have a pH ranging anything from alkaline to highly acidic, and may be acid generating (Mendez & Maier, 2008: 48;

Tordoff *et al.*, 2000:221). This poses a dangerous threat to the surrounding environment in forms of dust pollution, metal poisoning, and leaching of products from mineral weathering into nearby water sources and atmosphere i.e. radiation (Lange *et al.*, 2012:908; Tordoff *et al.*, 2000:219).

Mine wastes and wastes presents challenges to the colonization by vegetation and the formation of a self-sustaining ecosystem (Cooke & Johnson, 2002:49). Due to the physical and chemical nature of the mine waste materials, particularly in the absence of a cover soil or material, rehabilitation can be extremely difficult in terms of establishing vegetation (Cooke & Johnson, 2002:49).

The design for TSFs adds further burden on the environment as the steep slopes accelerates surface erosion and dust pollution (Mendez & Maier, 2008:48). These above ground disposal dams for mine waste are problematic in semi-arid areas and become a source of air pollution in the form of particulate matter (Mendez & Maier, 2008:48). The design of TSFs should be geotechnically sound to reduce the risk of physical collapse, and resultant spillage (Rossouw, 2010: 2).

### **2.1.2 Legislation**

The Environmental Conservation Act (ECA) (73 of 1998), and Article 28 of the National Environmental Act (NEA) (73 of 1989) states that measures must be taken to minimise and rectify polluted and/or degraded environments of which the cause could not have been otherwise prevented (South Africa, 1998). This includes, but is not restricted to, the degradation of natural environments caused by mining activities.

In terms of the MPRDA (Act 28 of 2002), rehabilitation of environments affected by mining or prospecting operations must be, as far as reasonably practical, conducted towards its natural or a predetermined state, or a land-use which conforms to the generally accepted principle of sustainable development (South Africa, 2002). According to this Act, closure of mines is only granted if the mine complies with this requirement and the responsibility of protecting the environment is placed upon the owner of the mining rights, unless a certificate relieves this responsibility (South Africa, 2002). Furthermore, this Act also refers to the principles of Chapter 1 of NEMA (107 of 1998), which entails remediation of disturbed ecosystems, and consequential biodiversity loss as well as minimisation of pollution and degradation (South Africa, 1998). Additionally, the National Environmental Management Biodiversity Act (10 of 2004) (NEMBA)

(South Africa, 2004) provides for the management and conservation of South Africa's biodiversity within the framework of the NEMA act (107 of 1998). This includes the protection of species and ecosystems and the sustainable use of indigenous biological resources. According to the South African Guidelines for Environmental Protection (SAGEP) (South Africa, 1979) a combination of chemical amelioration of the medium and vegetative establishment is the most successful rehabilitation method for mine waste and areas affected thereby.

Additionally, the Conservation of Agricultural Resources Act (CARA) (Act 43 of 1983) forbids dispersal of seeds from species recognised as weeds in a region or “cause or permit the dispersal of any weed from any location in the Republic to any other location in the Republic” (South Africa, 1983). Seed of species with non-invasive potential for the specific region requiring revegetation, which is adapted to the specific environmental conditions of the disturbed area, may thus be included in the seed mixture for rehabilitation.

### **2.2 Sustainable Rehabilitation**

Rehabilitation is the attempt to restore a degraded environment's ecosystem functions and services to a stable state, not necessarily similar to a pre-existing state, but at least to an extent where it can yield self-sustaining ecosystems (Haagner, 2008:7). It differs from restoration which involves returning a degraded area to its original state; rehabilitation is mainly concerned with repairing ecosystems to the most functional state as governed by the biogeochemical potential of the area (Bradshaw, 1998:256; Haagner, 2008:7).

Rehabilitation is often required and used when an area or environment has been subjected to pollution and degradation to such extent that the recovery of ecosystem structure and functions similar to that of a reference site is unattainable. The objectives for successful rehabilitation includes the following: (1) surface stability; (2) appropriate and sustainable post-closure land use; (3) resistance to degradation and pollution; (4) long-term succession of plant communities; (5) the restoration of ecosystem functions (Mendez & Maier, 2008:48; Van Deventer *et al.*, 2008:25). Therefore, one of the main outcomes for successful rehabilitation of a TSF will be an established vegetation community which contributes to ecosystem functioning, and displays resistance to degradation.

In order to determine whether a rehabilitation project has met its objectives, the efficiency of the ecosystem functioning and sustainability thereof should be monitored. Landscape function analysis (LFA) is commonly used in rangeland monitoring, and has recently been applied for

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monitoring rehabilitated TSFs. LFA examines how well a landscape is functioning as a biophysical system, and uses visually assessed indicators of soil surface processes (Haagner, 2008:31; Tongway & Hindley, 2004:14).

The Society of Ecological Restoration International (SER) (2004) produced a Primer which lists attributes of a restored ecosystem which serve as a baseline for determining whether or not successful restoration has been accomplished (Ruiz-jaen & Aide. 2005:574; SER, 2004:3). The attributes include the following: the use of indigenous species where practically possible; restored functionality and resilience of ecosystems with regard to environmental stress; reduced potential threats to ecosystem health; and self-sustaining capabilities similar to that of a reference ecosystem (Ruiz-Jaen & Aide. 2005:574; SER, 2004:3).

In a review of restoration projects, Ruiz-Jaen and Aide (2005:574) found that most studies fail to measure all the attributes and that the three most common attributes measured in restoration projects were vegetation structure and density and ecological processes. According to Ruiz-Jaen and Aide (2005:574) these three attributes incorporates several of the SER Primer attributes, however, all three attributes are rarely measured in conjunction after restoration projects have ceased. Furthermore, Ruiz-Jaen and Aide (2005:574) found that very few restoration projects measured the self-sustainability of ecosystems or reproducing populations. Thus, the sustainability with regard to self-regenerative vegetation is poorly documented after restoration projects have ceased, and can be ascribed to the lack of long-term monitoring and data collection as required for each attribute. Therefore, long-term soil quality monitoring is essential, particularly after the restoration and rehabilitation projects are completed.

In the past, the outcome of rehabilitation projects were perceived as successful after a single season of good vegetation growth (Reichardt & Reichardt, 2007:147). Long-term monitoring of phytoremediation should be made priority since rehabilitation projects are long-term processes and requires several growing seasons depending on the level of contamination (Weiersbye, 2007:16).

The use of vegetation during rehabilitation is instrumental for achieving sustainable rehabilitation, as well as providing invaluable advantages, such as the recovery of autogenic processes, i.e. ecosystem functioning and services (SER, 2004:7). Vegetation aids the remediation of contaminated soils, contributes to surface stability, reduces the impact of rainfall on the soil surface, minimises surface erosion, prevents dust pollution and leaching, provides

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habitat to other biota, and enhances the aesthetic value of the landscape (Fourie, 2007:483; Hossner & Sahandeh, 2006:154; Lange *et al.*, 2012:908; Tordoff *et al.*, 2000:220). Furthermore, vegetation restores ecosystem services, such as carbon sequestration and ground water hydrology, as well as optimises the desired future end-land use (Lange *et al.*, 2012:908). In this context, the benefits of vegetation in rehabilitation projects directly relates to issues of environmental sustainability (Fourie, 2007:483).

According to the South African Guidelines for Environmental Protection (SAGEP) (South Africa, 1979) a combination of chemical amelioration and vegetative establishment is the most successful rehabilitation method. The use of indigenous vegetation for re-vegetation in semi-arid and arid regions provides a greater success rate as they already have survival mechanisms adapted to climatic conditions (Mendez & Maier, 2008:279). Another advantage is that the introduction of native vegetation avoids establishment of non-native and invasive species, while simultaneously complying with Act 43 of CARA (South Africa, 1983) which forbids the dispersal of seeds from species recognized as weeds.

When selecting suitable species for rehabilitation of mine waste, a variety of factors should be considered. These factors include the land-use, bio-physical environmental factors, and the physiological and morphological characteristics of the plants (Westcott, 2011:9). Site specific environmental factors influencing vegetation growth at mining sites include temperature extremes, dominant wind directions, surface and slope instability, and soil microbial levels (Tordoff *et al.*, 2000:221).

Differentiation in populations is partially driven by adaptations of species to local environments which result in an advantage for offspring in similar environments (Westcott, 2011:9; Van den Berg & Kellner, 2010:189). These species are ecotype species and have adapted to a certain environment over a period of time to bear specific phenological characteristics, enabling it to grow in environments with a specific set of environmental factors (Van den Berg & Kellner, 2010:189). Thus, theoretically, the use of local ecotype species adapted to the climatic environment of a specific site during rehabilitation, favours the establishment of vegetation and consequently the success thereof. However, the characteristics for mine waste materials will almost always differ from site to site and is unique regarding its geological origin, prevailing climatic conditions and metallurgic processes it is submitted to. Common characteristics of mine waste materials include slit-sized particles, elevated levels of heavy metals, and salinity (Weiersbye, 2007:19). These combined characteristics are rarely found in nature, and much less

so ecotype species adapted to these conditions. Thus, the ideal of selecting adapted ecotype species is often not practical and therefore not priority when selecting species particularly for rehabilitation.

### **2.3 Phytostabilization**

Phytostabilization is considered to be a phytoremediation method, as it uses plants as a tool in order to remediate organic and inorganic wastes (Jadia & Fulekar, 2009:924). The following definition for phytostabilization is provided by the US Environmental Protection Agency (USEPA) (2000:21):

*Immobilization of a contaminant in soil through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants, and the use of plants and plant roots to prevent contaminant migration via wind and water erosion, leaching, and soil dispersion.*

The process of phytostabilization entails that plants will minimise leaching through means of hydraulic control and facilitate the precipitations of metals to less soluble forms, absorb metals into root tissues, and consequently decreasing metal bioavailability and toxicity (Anawar *et al.*, 2013:731; Mendez & Maier, 2008:279). In order to support initial vegetation growth, soil amendments are often used during phytostabilization which in turn will aid the stabilization of soil (USEPA, 2000:22). Consideration should be given to the properties of the soil in question when selecting amendments. This is particularly important when attempting to stabilise mine wastes as they have adverse chemical and physical properties attributing to a hostile growth medium (Mendez & Maier, 2008:279). Plants considered for phytostabilization of mine wastes in semi-arid environments should be tolerant to salinity, acidity, nutrient deficiencies and drought (Mendez & Maier, 2008:276).

Phytostabilization is considered to be a cost effective and green phytoremediation method to remediate contaminated soils (Ahmadpour *et al.*, 2012:38; USEPA, 2000:21). This method entails several advantages which include: (1) the use of plants enhances ecosystem restoration; (2) soil removal is unnecessary; (3) it is applicable for different kinds of inorganic and organic pollutants; (4) it is aesthetically pleasing; (5) and the establishment of plants enhances microbial life (Ahmadpour *et al.*, 2012:38; Mendez & Maier, 2008:279; USEPA, 2000:21).

Using grass species for phytostabilization is common practice in South Africa, and for good reason. Some grass species are known to tolerate stress associated with mine waste materials,

while the rapid growth of grass is an attractive commodity with regard to obtaining surface stability as an outcome of rehabilitation. Surface erosion of TSFs is problematic, as it contributes to the migration of contaminants and consequently pollution.

Vegetation cover, when sufficient, is known to play an important role in reducing surface erosion (Morgan, 1986:61). The interception of raindrops by the above ground organs of plants reduces the kinetic energy thereof, thus, reducing the impact to the soil surface (Morgan 1986:75). Through the interception of raindrops, and the above ground plant organs that impart roughness to the flow, vegetation cover is able to slow down the velocity of surface runoff (Morgan 1986:75). The mechanism whereby vegetation cover reduces the velocity and impact of rain, and consequently surface runoff, also supports infiltration of water into the soil. Additionally, shading provided by the vegetation cover reduces evaporation thereby conserving moisture in the soil which affects the stability of soil aggregates in a positive manner (Morgan, 1986:77).

The concern of erosion stability on TSFs can be addressed mainly by the vegetation cover's ability to reduce surface runoff as well as through the mechanical reinforcement of the soil by the root system (Fourie, 2007:483; Holý, 1980:75). Additionally, the surface can be stabilized through rock materials, i.e. rock armour. This is applicable to wastes facilities where vegetation covers can serve as a mechanism to prevent saturation of soil and drainage of excess water from the wastes facility and potentially polluting other below surface water sources (Yunusa *et al.*, 2012:113).

### **2.4 The Maternal Environment and Seed Development**

Successful rehabilitation of TSFs can only be achieved when the objectives of rehabilitation has been met. Sustainable vegetation cover is one particular objective of successful rehabilitation with regard to phytostabilization. The establishment of sustainable vegetation cover can be compromised by the inability of vegetation to survive and produce viable seed when established on mine waste materials.

Seed production and quality are critical for species persistence. Bishaw *et al.* (2012:656) describes seed quality as the sum total of many aspects, among which are genetic, physical, physiological, and health quality and can be directly related to seed vigour. Seed vigour can be affected by various factors, namely genetics, physiological, cytological and pathological (Maguire, 1977:16).

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According to Maguire (1977:220) seed that is mechanically sound and capable of germinating promptly to produce developing seedlings which are able to emerge under favorable and unfavorable environmental conditions are vigorous. Seed vigour is a function of a variety of factors to which the parent plant is subjected to during seed formation and maturation (Mayer & Poljakoff-Mayber, 1989:43). These factors are referred to as maternal environmental factors and include: temperature; light; water availability; and nutrition.

According to Luzuriaga *et al.* (2005:164) effects occurring in the mother plant after fertilization, are dominant over those that occurred before fertilization. Therefore, effects detected during the early stages of development, such as seed mass, probability and rate of germination, are primarily contributed by the environment of the mother plant (Luzuriaga *et al.*, 2005:164). Additionally, maternal plants provide nourishment for seed, thus the effect of maternal plants on offspring fitness is great (Bischoff & Müller-Schärer, 2010:475; Galloway, 2004:93; Roach & Wulff, 1987:1152). The environmental conditions to which the maternal plant is directly subjected to, is known as the maternal environment and will influence seed traits such as vigour and viability. According to Roach and Wulff (1987:1152) maternal environmental factors, such as poor nutrient status and abiotic stress, can physically change phenotypic attributes or cause phenotypic variation in the offspring (for example seed size). Therefore, seed traits of the offspring, such as their seed provisioning and chemical arrangement (mineral resources), depend on the abiotic environment attributed by the growth medium during seed development and maturation (El-Keblawy *et al.*, 2009:11; Wang *et al.*, 2012:172).

It is widely accepted that post-zygotic effects on seed development, i.e. those occurring in the mother plant after fertilization, become dominant over pre-zygotic ones (Luzuriaga *et al.*, 1995:164). Thus, parental effects detected in early stages of plant development, such as seed mass, probability and rate of germination, are primarily the contribution of the mother plant environment. Such maternal effects in the earliest stages of plant life can persist, or even be enlarged in the mature plant, and eventually lead to differences in reproductive success.

Phenotypic plasticity is a mechanism which allows organisms to cope with environmental heterogeneity within the life-time of the organisms (Galloway, 2005:93; Terblanche & Kleynhans, 2009:1636). Galloway (2005:93) states that:

*Plasticity is a functionally appropriate adjustment of the phenotype that acts to enhance fitness under current environmental conditions.*

This means that phenotypic plasticity is the ability of a plant to change its morphology and/or physiology in response to growth variables. According to Volis *et al.* (2004:1121) environment specific phenotypic responses include phenological, vegetative or reproductive traits. Therefore, seed traits of offspring seed is able to change according to pressures of the maternal environmental factors it is subjected to.

### 2.4.1 Essential Nutrients and Seed Development

In order to successfully complete a life cycle, of which reproduction is a vital component, plants require a number of nutrient elements. A nutrient element is deemed essential when it forms part of a crucial plant constituent or metabolite and which without the plant is unable to complete a normal life cycle (Hopkins & Hürner, 2008:65). Essential elements are traditionally separated into two categories based on the relative concentrations required by plants, namely macronutrients: calcium (Ca), magnesium (Mg), phosphor (P), nitrogen (N), potassium (K) and sulphur (S); and micronutrients: copper (Cu), molybdenum (Mo), zinc (Zn) and nickel (Ni) (FSSA, 2007:82; Hopkins & Hürner, 2008:65).

Nutrient availability is greatly determined by the chemical and physical properties of soil. Some of the chemical constraints that can limit nutrient availability include salinity, acidity, and lack of soil organic matter (SOM). SOM maintains aggregation of colloids and improves water holding capacity and nutrient supply (Mills & Fey, 2004:388). Additionally SOM maintains exchangeable Potassium (K), Calcium (Ca), and Magnesium (Mg) through means of improved cation exchange capacity (CEC), furthermore, SOM also provides humic and fulvic acids which are essential for polysaccharides and other essential microbial activities (Duong *et al.*, 2012:197). Physical properties such as poor structure and water holding capacity can also reduce nutrient availability (Baligar *et al.*, 2001:926). These soil factors affect the mobility, mineralization, fixation, and adsorption mechanisms of nutrients and consequently the availability of nutrients (Baligar *et al.*, 2001:926).

Environmental factors as experienced by the mother plant, forms part of the influential environment for the growth potential of the offspring seeds and seedlings (Sills & Nienhuis, 1995:491). The nutrient status of soil is one of these environmental factors (Sills & Nienhuis, 1995:491).

Maternal effects as attributed by variant soil nutrient levels are manifested as variation in seed traits, such as size and mass (Roach & Wulff, 1987:1152). Nutrients and growth substances

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applied and available to the maternal plant may affect seed traits, especially if applied during seed development and maturation (Roach & Wulff, 1987:217). In a review by Roach and Wulff (1987:1152) about the effect of maternal resources on seed size, an increase in seed size was correlated with an increase in nutrient levels in many species.

Phosphorus (P) is an important macronutrient for plant growth and is vital for certain metabolic reactions, such as photosynthesis (Hopkins & Hürner, 2008:69; Sabrina *et al.*, 2013:75). It is mainly present in an insoluble form and on average only 2% of soil phosphorus is available to plants and its availability is mainly determined by soil pH (Sabrina *et al.*, 2013:75). Phosphorus is commonly the limiting element in soils, as it is unavailable to plants in its organic form and is highly immobile (Hopkins & Hürner, 2008:69).

According to Austin (1972:135) seed phosphorus reserves have been indicated to be of importance for obtaining vigorous seedlings. Severe phosphorus deficiency will affect seed size and composition, resulting in seed with decreased phosphorus content. Such seed will have a slower g, as well as a lower final percentage germination producing smaller seedlings.

The phosphorus reserves of seeds from a mature plant will vary with the availability of phosphorus in the soil (White & Veneklaas, 2012:2). Phosphorus reserves in seeds are the only resource of phosphorus sustaining initial growth of seedlings. During germination, phosphorus reserves are mobilized and translocated to the emerging root tissues, after it will be supplemented by phosphorus uptake from the roots (White & Veneklaas, 2012:1).

Nitrogen (N) is a vital macronutrient for plants and is used as a constituent for several proteins, hormones and chlorophyll (Hopkins & Hürner, 2008:68). Plants obtain nitrogen from the soil in the nitrate ( $\text{NO}_3^-$ ) or ammonium ( $\text{NH}_4^+$ ) form, however, nitrogen is limited in soil and plants have to compete with soil microorganisms for available nitrogen (Hopkins & Hürner, 2008:195). Several environmental factors influence the availability of nitrogen in soil, such as water status and pH, which influence the activity of microorganisms responsible for nitrogen fixation, nitrification and ammonification (Hopkins & Hürner, 2008:209). Nitrogen is mainly present in soil in the organic form which is unavailable for plant uptake and has to be converted to nitrate by a variety of microorganisms (FSSA, 2007:85). Plants under severe nitrogen stress were found to produce low yield of seed, much of which were abnormal (Austin, 1972:134). Symptoms of nitrogen deficiency in plants are slow growth and chlorosis of the leaves (Hopkins & Hürner, 2008:69).

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Potassium (K) is an essential macronutrient for plants as it is one of the most abundant cellular cations, is vital for protein synthesis, and is an activator for enzymes involved in photosynthesis (Hopkins & Hürner, 2008:69). Potassium serves as an osmoregulator for the osmotic potential in plant cells, and as such is a principle factor in plant movements, such as the closure and opening of the stomatal cells (Hopkins & Hürner, 2008:70). This function of potassium is vital in rehabilitation because of water deficiencies present in mine waste materials due to the lack of water retention in mine wastes (Van Deventer & Hattingh, 2014:62).

Awad *et al.* (2013:659) found that high rates of potassium application to soil resulted in an increased grain yield and weight per plant for Sudan grass compared to low rates of potassium application. These results can be attributed to the role of potassium in seed production as it is a nutrient that influences photosynthetic rates and carbon allocation (Awad *et al.*, 2013:656). This is because most mine waste materials contain very little exchangeable cations due to low levels of CEC. New standards should be developed to determine the most appropriate concentrations of essential cations. Moreover, experimental work indicated that the potassium should be at least 12% of the CEC, irrespective of the ration between calcium and magnesium (Van Deventer & Hattingh, 2014:62).

Other essential macronutrients for plants include hydrogen (H), carbon (C), oxygen (O), magnesium (Mg), calcium (Ca) and sulphur (S). Carbon, hydrogen and oxygen are required for the structural backbone of all organic molecules (Hopkins & Hürner, 2008:68). Deficiencies in carbon will result in the starvation of the plant, while water deficiencies will result to the desiccation of the plant.

Sulphur is taken up by plants as a divalent sulphate anion ( $\text{SO}_4^{2-}$ ) and is particularly important in the structure of proteins (Hopkins & Hürner, 2008:70). A deficiency in sulphur results in chlorosis due to reduced protein synthesis. Calcium (Ca) is taken up by plants as a divalent cation ( $\text{Ca}^{2+}$ ) and plays a vital role in cell division and is used in membranes. Due to its vital role in cell division, deficiency symptoms of calcium appear in the meristematic regions (Hopkins & Hürner, 2008:70). Magnesium is also taken up by plants as a divalent cation ( $\text{Mg}^{2+}$ ) and is critical for ATP reactions where it serves to link the ATP molecule to the active site of the enzyme (Hopkins & Hürner, 2008:71).

Plants are autotrophic organisms, taking their entire nutritional needs from their direct inorganic environment. Therefore, it is vital that each essential nutrient required by plants must be retained and used efficiently in order to complete a normal life cycle.

### **2.4.2 Metal Trace Elements and Plant Health**

The primary natural sources of metal trace elements are rocks, minerals, and atmospheric deposition (Fei *et al.*, 2014:33). Therefore, the weathering of parent rock and climatic conditions has a pre-dominant impact on the metal trace element status of soils (Kabata-Pendias, 2011:65). According to Kabata-Pendias (2011:65) the main soil properties involved in the processes of sorption and desorption of trace elements are: pH values; CEC; organic matter content; and microorganisms. Anthropogenic sources of metal trace elements include human activities, such as mining, the use of fossil fuels, emissions from motor vehicles, chemical fertilizers, and pesticides, which produce direct or indirect emissions of trace elements (Fei *et al.*, 2014:33).

Certain metal trace elements are required in small concentrations by plants in order to complete a healthy life cycle, and are therefore considered to be micronutrients (Hopkins & Hürner, 2008:65). According to Herselman (2007:5) metal trace elements are essential when a deficient supply thereof results in impaired biological functions, which can be reversed with supplementation. When essential metal trace elements are in deficient supply, plants will exhibit deficiency symptoms as a result of the malfunctioning of metabolic actions due to the absence of essential metal trace elements (Hopkins & Hürner, 2008:67).

The primary source of metal trace elements for plant is soil (Kabata-Pendias, 2011:95). The biological availability of metal trace elements in soils is associated with soil properties, especially pH and binding sites (Herselman, 2007:1; Kabata-Pendias, 2011:95). Soil pH is one of the main soil properties influencing the behaviour of metal trace elements. The  $H^+$  concentration of the soil solution is in dynamic equilibrium with the negatively charged surfaces of soil particles (Herselman, 2007:11). Thus, the negatively charged binding sites for cations is dependent on the soil pH, therefore an increase in pH promotes the sorption of metal trace elements (Herselman, 2007:11).

The low pH and elevated metal trace element concentrations of mine waste materials present particular challenges for vegetation germination and survival (Lottermoser *et al.*, 2009:243; Doronila *et al.*, 2014:62). Mine waste materials, particularly from gold mining activities, are associated with sulphide minerals (e.g. pyrite) which produces sulphuric acid when oxidized and

consequently results into the generation of acid mine drainage (AMD) and forms acid sulphate soils (Aucamp & Van Schalkwyk, 2003:123; Van Deventer *et al.*, 2008:26; Wu *et al.*, 2011:788). However, low pH levels in mine wastes can be ascribed to a number of occurrences or sources. The increased acidity of mine waste materials results in the mobilisation of metal trace elements and consequently toxicity thereof (Aucamp & Van Schalkwyk, 2003:123). Metal trace elements, such as copper (Cu), gold (Au), zinc (Zn), and arsenic (As) are chalcophile and geochemically associated with sulphide minerals, in particular pyrite, and after the gold has been extracted, these metal trace elements will remain part of the mine waste materials (Aucamp & Van Schalkwyk, 2003:124).

Essential trace elements most likely to cause problems in plants (through either deficiency or toxicity), are: copper (Cu), iron (Fe), zinc (Zn), boron (B), manganese (Mn), and nickel (Ni) (Herselman, 2007:5; Hopkins & Hürner, 2008:66). Copper (Cu) is available to plants as the divalent cupric ion ( $\text{Cu}^{2+}$ ) (Hopkins & Hürner, 2008:73). Copper is mostly immobile, because it is adsorbed by clay minerals and organic materials, and therefore it accumulates easily in the top soil (Herselman, 2007:9). However, its mobility increases in acidic conditions making it readily available to plants (Herselman, 2007:9). In plants, copper functions primarily as a cofactor for a variety of oxidative enzymes (Hopkins & Hürner, 2008:73). Additionally, copper is also a constituent of several enzymes in plants, and plays part in important physiological functions such as, photosynthesis, respiration, nitrate and carbohydrate metabolisms, and reproduction (Kabata-Pendias, 2011:262). Copper deficiency in plants are characterised by stunted growth and a distortion of young leaves (Hopkins & Hürner, 2008:73).

Of all the micronutrients, iron (Fe) is required by plants in the largest amount and is taken up as the ferric ( $\text{Fe}^{3+}$ ) or ferrous ( $\text{Fe}^{2+}$ ) ion (Hopkins & Hürner, 2008:71). Iron deficiency occurs in most instances due to soil factors that govern the mobility of iron (Kabata-Pendias, 2011:220). Iron is considered to be a key metal in energy transformations needed for syntheses and life processes of cells, and is also important for the synthesis of chlorophyll in plants (Hopkins & Hürner, 2008:71; Kabata-Pendias, 2011:220). Deficiencies of iron result in the simultaneous loss of chlorophyll and the degeneration of the chloroplast structure (Hopkins & Hürner, 2008:71). Iron deficiencies effect several physiological processes, and consequently will ultimately result in stunted plant growth and yield (Kabata-Pendias, 2011:221). Iron is very soluble in acidic soils which may promote iron toxicity in plants (Hopkins & Hürner, 2008:71;

Kabata-Pendias, 2011:221). The most common symptom of iron toxicity is necrotic spots on leaves, which indicates an accumulation of iron (Kabata-Pendias, 2011:221).

Zinc (Zn) is taken up by plants as the divalent cation ( $Zn^{2+}$ ) and is an activator and component of a variety of enzymes (Hopkins & Hürner, 2008:73; Kabata-Pendias, 2011:283). Zinc deficiency in plants will cause plants to have smaller leaves and shortened internodes (Hopkins & Hürner, 2008:73). Like copper, zinc is easily adsorbed by organic materials and clay minerals, consequently accumulating in the top horizon of most soils (Herselman, 2007:10; Kabata-Pendias, 2011:278). The immobilization of zinc is highly influenced by phosphorus and clay minerals in soils (Kabata-Pendias, 2011:278). Zinc becomes readily mobile at low pH levels and therefore zinc toxicity is associated with acidic soil conditions (Herselman, 2007:10; Kabata-Pendias, 2011:284). The most common symptoms of zinc toxicity are chlorosis in new leaves and stunted plant growth (Kabata-Pendias, 2011:284).

Boron (B) is present as boric acid in the soil solution and is preferred in the undissociated form for uptake by plant roots (Hopkins & Hürner, 2008:73). The role of boron in plant physiology is understood primarily on studies of what happens to plants during boron deficiencies and its role is still not well-understood (Hopkins & Hürner, 2008:73; Kabata-Pendias, 2011:320). Deficiency of Boron revealed impaired sexual reproduction and reduced seed yield (Kabata-Pendias, 2011:320). In response to boron deficiency, cell division and elongation in primary and secondary roots were inhibited (Hopkins & Hürner, 2008:73). According to Kabata-Pendias, (2011:322) the range between toxic and deficient boron levels is very narrow, and therefore some plants experience boron toxicity at levels elevated only slightly above the required amount of boron. Toxicity of boron will result in necrosis of leaves and the decay of growth points (Kabata-Pendias, 2011:321).

Manganese (Mn) is essential for photosynthesis due to its role in the electron transport system and also forms part of the oxygen-evolving complex, associated with photosystem II (Hopkins & Hürner, 2008:74; Kabata-Pendias, 2011:208; Oves et al., 2012:9). The mobility of manganese is dependent on the pH of a soil; therefore, the reactions most likely to control the mobility and behaviour of manganese are redox and hydrolysis (Kabata-Pendias, 2011:205). Manganese is mobile at low pH ranges, and immobile at high pH ranges, therefore, manganese deficiencies are more likely to occur in neutral and calcareous soils with higher pH values

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The manganese content of a plant is an effect of the bio-availability of manganese in soils. (Hopkins & Hürner, 2008:74; Kabata-Pendias, 2011:207). Deficiencies symptoms include browning of roots, and chlorosis in younger leaves, which can continue to become necrotic, reddish spots on leaves (Kabata-Pendias, 2011:208). Manganese deficient plants exhibit retarded growth and a decreased resistance to diseases (Kabata-Pendias, 2011:208). Toxicity of manganese in plants is related to acidic soils with high manganese concentrations (Hopkins & Hürner, 2008:74; Kabata-Pendias, 2011:205). Symptoms of manganese toxicity in plants include iron chlorosis, necrotic brown spots, and an uneven distribution of chlorophyll in leaves (Kabata-Pendias, 2011:208).

The distribution of nickel (Ni) in soils is closely related to the CEC and clay content of soil (Kabata-Pendias, 2011:239). Nickel accumulates in the soil in the form of compounds, such as nickel acetate, nickel carbonate and nickel oxide, and is relatively soluble at acidic pH levels (Sreekanth *et al.*, 2013:1130).

Nickel has only recently been regarded as an essential micronutrient and plays an essential role in plants with regard to metabolism, as nickel has been found to be a major component of plant enzymes, such as urease (Kabata-Pendias, 2011:239; Sreekanth *et al.*, 2013:1131). Nickel is also essential for its role in the nodulation of legumes (Kabata-Pendias, 2011:241). However, in excess, nickel can be toxic to plants and symptoms include stunted growth, impaired nutrient balance, and chlorosis due to disrupted iron uptake (Kabata-Pendias, 2011:241; Sreekanth *et al.*, 2013:1131).

Metal trace elements take part in important roles in the metabolic components and functioning of plants and therefore they are deemed essential for plants during the various life cycle stages (Oves *et al.*, 2012:6). Toxicity of metal trace elements occurs in plants when the required low concentrations thereof are exceeded, affecting plant growth at various life stages, in particular during germination and photosynthesis, due to its relative roles in these processes, such as transpiration, protein synthesis and enzyme activities (Wani *et al.*, 2012:53). Photosynthesis is arguably the most vital physiological process in plants and is adversely affected by metal trace element toxicity, as excess metal trace elements react with the photosynthetic apparatus at various levels (Wani *et al.*, 2012:53). These include alterations to the chloroplast membrane and consequently the reduction in photosynthetic efficiency (Wani *et al.*, 2012:53).

Another vital physiological process affected by the toxicity of metal trace elements is germination. Metal trace element toxicity is known to affect seed germination in two ways, first by their general toxicity and second, by the inhibition of water uptake (Kranner & Colville, 2011:96). Literature shows that most species experiences a concentration-dependant reduction in germination due to metal trace element treatment (Kranner & Colville, 2011:96). According to Kranner and Colville (2011:98) cadmium (Cd) toxicity resulted in the inhibition of seed imbibition, while in soybean, a reduced pod yield was found due to plant treatments with cadmium and nickel (Kranner & Colville, 2011:96). At lower concentrations of cadmium, soybean had a reduced seed size (Kranner & Colville, 2011:96). It has been found that seed viability and germination decreases when exposed to elevated concentrations of metal trace elements.

### **2.5 Germination of Seed in Anthropogenic Soils**

Germination is defined by the International Seed Testing Association (ISTA) (1996) as:

*The emergence and development of the seedling to a stage where the aspects of its essential structures indicate whether or not it is able to develop further into a plant under favourable conditions in the soil.*

Jann and Amen (1977:10) describe germination from a physiological perspective as the resumption of the metabolism and growth which were earlier depressed or suspended. Jann and Amen (1977:12) continue to state that imbibition of water is essential for the resumption of growth activities by a germinable seed. Simply put, imbibition is a physical process during which water enters the seed, causing it to swell, which consequently applies pressure on the seed coat resulting it to break, and ultimately enabling the embryo to emerge (Mayer & Poljakoff-Mayber, 1989:43).

Water uptake by seeds during germination can be categorized in three phases. The first phase represents initial rapid water uptake, which is followed by phase two during which water uptake by the seed has reached a plateau (Kestring et al., 2009:105). Phase three of water uptake is considered to be post-germination as it is characterised by the protrusion of the primary root and an increase of seed moisture content with only viable seeds reaching this phase (Kestring et al., 2009:105). However, imbibition can be inhibited by the decrease of water availability after initial water uptake during phase 1 (Mayer & Poljakoff-Mayber, 1989:43).

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Germination of seed in anthropogenic soils is influenced by various factors, such as seed quality, temperature, chemical and physical properties of the growth medium, burial depth, water availability, and salinity (Sayati & Hitchmough, 2013:28; Van den Berg & Kellner, 2005:188; Zehra *et al.*, 2011:122). Van den Berg and Kellner (2005:191) mentions the scale and grade of degradation as a factor influencing seed germination, as it will determine and contribute to specific abiotic stress factors, such as trace element toxicity, salinity and low moisture content.

According to Mayer and Poljakoff-Mayber (1989:43) the availability of water in soil at a given period of time is the determining factor for germination. Available water in soil is determined by various factors, such as osmotic potential, binding of water by soil colloids and soil texture (Mayer and Poljakoff-Mayber, 1989:43). Once germination commences, moisture availability is critical as moisture stress can delay and reduce seed germination (Sayati & Hitchmough, 2013:28; Tanveer *et al.*, 2012:446). Brevedan *et al.* (2013:4352) found that low moisture availability limited seed germination and resulted in a lower germination percentage.

Water availability for seed is partially determined by the salt concentration of the growth medium (Zehra *et al.*, 2011:122). A high soil salt concentration, also referred to as salinity, reduces the water potential of the imbibition medium, consequently inhibits it, and facilitates the entry of high amounts of ions into seeds which can result to toxicity (Patanè *et al.*, 2013:30; Tanveer *et al.*, 2012:446). Imbibition decreases as the concentration of solutes increases, largely due to osmotic effects.

The water potential of the seed, compared to that of the immediate vicinity, will determine the water-uptake ability of the seed. Under saline conditions, seed would therefore be physiologically desiccated, although adequate water is present in the near vicinity of the seed. The delay and extent of moisture absorption due to saline conditions varies with species, environmental conditions, and specific ions (Tanveer *et al.*, 2012:446).

According to Tanveer *et al.* (2012:446) when dominant factors, such as available moisture and salinity, are not limiting for germination, temperature is regarded as a determining factor for seed germination. Under favourable moisture conditions the time period for seed germination will be shortened during optimum temperatures (Brevedan *et al.*, 2012:4346). Brevedan *et al.* (2012:4351) found that the germination percentages for *D. eriantha* were optimum at constant temperatures rather than varying temperatures.

### 2.6 Seed maturation

Commercial seed is harvested mechanically once it has been established that the seed is ripe and ready for harvesting. According to Jacob *et al.* (2014:253) it is critical to harvest as soon as seed reach the physiological maturity point, in order to reduce potential qualitative and quantitative seed loss in order to ensure seed quality.

Seed development of higher plants can be distinguished into two main phases, namely embryo and endosperm development; and seed maturation (Baud *et al.*, 2002:151; Holdsworth *et al.*, 2008:35;). Embryo growth ends when the cell division in the embryo comes to an end. After this, seed maturation starts with a transition from maternal control to filial control (Holdsworth *et al.*, 2008:35). Early during the maturation phase, dormancy is initiated and only increases when the seed is fully developed (Holdsworth *et al.*, 2008:35). The seed maturation phase is completed once the storage compounds in the seed have accumulated; the water content has decreased, and after primary dormancy has commenced (Holdsworth *et al.*, 2008:35; Zerche & Ewald, 2005:574).

Seed harvested before it were able to reach physiological maturation, will have insufficient storage compounds, and have not yet to reached primary dormancy. Thus, it will not be able to germinate as successfully due to the insufficient storage compounds needed to support initial embryo development during germination. Such seed may also not be able to survive storage conditions as they were not able to successfully reach the primary dormant stage. The time of harvest for seed proves to be critical for seed merchants as this will influence the quality of seed they sell.

### 2.7 Selected Species

In semi-arid and arid environments the use of native vegetation adapted to the prevalent climatic conditions is beneficial for phytostabilization (Mendez & Maier, 2010:279). Additionally, the use of native species prevents the introduction of invasive and non-native species to a certain extent (Mendez & Maier, 2010:276). Furthermore, the use of native grass during rehabilitation of mine waste materials is supported by the Biodiversity Act (South Africa, 2004) which states that species selection for re-vegetation should comply with the standards determined by the regional biodiversity framework. The Conservation of Agricultural Resources Act (Act 43 of 1983) prohibits dispersal of seeds from species recognised as weeds (South Africa, 1983). Thus,

the use of native species is not only favourable in terms of climate adaptation, but also with regard to complying with local legislation.

Species selected for seed mixtures used for re-vegetation during rehabilitation practices should have adaptive traits suitable to survive the disturbed environment (Anawar *et al.*, 2012:739; Bradshaw, 2000:90). These traits, among others, include: life cycle (perennial or annual); seasonal growth form; seed production; and root development (Brits, 2007:5). Initially, after seeding, few plants, such as annuals, may dominate the ecosystem due to selective pressures, however, as succession progresses the presence and effort of less abundant species, such as perennials, will be vital for promoting a self-sustainable vegetation cover (Mendez & Maier, 2010:280).

According to Xia (2004:345) grasses has a set of characteristics (rapid growth, large biomass, strong resistance, and effective stabilization to soils) which makes them excellent candidates for vegetative rehabilitation. Grasses provide relative quick and effective ground cover compared to shrubs and trees making it an excellent facilitator for the temporarily stabilization of soils (Mendez & Maier, 2008:280; Xia, 2004:345). Furthermore, the growth form of grasses, namely dense stands, benefits soil structure by increased aggregation and improved cohesion of soil (Knootz *et al.*, 2013:2237). The establishment of pioneer species can improve edaphic conditions through increasing soil nutrient content and thus further facilitate the establishment of other plant species (Parraga-Aguado *et al.*, 2013:135). The facilitation of plant growth is critical for the restoration of degraded environments, especially in semi-arid climates (Parraga-Aguado *et al.*, 2013:135).

Surface stability as an outcome of rehabilitation is often one of the first objectives addressed during rehabilitation practices. Once surface stability on mine waste materials and TSF's has been achieved, the remainder objectives can be addressed with confidence. Surface stability eliminates other detrimental factors, such as surface runoff, erosion and crust formation, which can further influence the success with which other objectives are addressed. The use of grass, particular during rehabilitation, is substantial for meeting the criteria of the first objective of rehabilitation, namely surface stability, as grasses are fast growing and provides relative quick ground cover.

Direct seeding is regarded as an attractive re-vegetation method due to its low labour costs, and can increase the frequency, density and establishment of species (Brits, 2007:3; Mendez &

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Maier, 2010:280; Westcott, 2011:7). With added amelioration, direct seeding using agricultural seed mixtures is regarded to be an economical re-vegetation method (Tordoff *et al.*, 2000:219). Amelioration often entails added inorganic fertilizers and organic matter (compost, sewage sludge or mulch) to alleviate nutrient deficiencies of mine waste materials (Tordoff *et al.*, 2000:216).

The suppliers, AS, randomly selected five species for Phase 1 in order to evaluate the effect of an externally applied coating on the seed of the grass species that were used. During Phase 1 the seeds produced by these selected species, were harvested and sown for the commencement of Phase 2. The characteristics of the species relevant to this study, both Phase 1 and 2, are as follows (Van Oudtshoorn, 2009):

### *Eragrostis curvula* (Schrad.) Nees. (Weeping Love grass/*Oulandsgras*)

Characteristic features of this perennial, densely tufted grass species include long, loose hanging leaves which are often concentrated at the base of the plant. The inflorescences of Weeping Love grass are mostly an open panicle, and the spikelets are dark grey to dark olive green.

It is associated with regions with a high rainfall and grows well in overgrazed and trampled veld. It establishes well in disrupted areas and is often used to stabilize exposed soil. It is relatively palatable and is a very popular pasture particular in the inland Highveld regions of South Africa. Weeping Love grass establishes easily, reacts well to soil fertilizer and sprouts early in spring.

### *Cynodon dactylon* (L.) Pers. (Couch grass/*Kweekgras*)

Couch grass is a perennial and a characteristically short, creeping grass, with both stolons and rhizomes. The inflorescence is exclusively digitate, with flattened spikelets without awns. Couch grass is unique in that it grows in all types of soil, and is often found in disturbed places. It is therefore probably the most useful grass, as it serves as good pasture that can endure heavy grazing, stays green until late into winter and its root system makes it ideal for rehabilitation purposes.

### *Chloris gayana* Kunth. (Rhodes grass)

Rhodes grass is a perennial, tufted grass which spreads by means of stolons. The inflorescence is digitate, with loose fingers that typically curl when the spikelets have fallen off. The spikelets

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are brown with two awns. The leaf sheaths are compressed and the leaf blade is folded open and is smooth.

Rhodes grass is a good grazing grass and is easy to establish. It has a high nutritional value early in the season and can endure relatively intense grazing. Rhodes grass is generally used to stabilize exposed and disturbed soils.

### *Cenchrus ciliaris* L. (Buffalo grass/*Buffelsgras*)

Characteristics of this perennial, tufted grass include a shrub-like growth form and culms that have multiple branches. The inflorescences are a dense purple to straw coloured spike. The spikelets are surrounded by many wavy bristles which arise from a short stalk and the entire spikelet falls off when it reaches maturity.

Buffalo grass grows in dry, warm parts and favours sandy and well-drained soil types. It has a high leaf production and is a good grazing grass. It is highly palatable, which decreases as the plant matures. It is a hardy cultivated pasture with a deep root system and can endure trampling. It is, however, difficult to establish in clay soil, but once established, grows well.

### *Digitaria eriantha* Steud. (Smuts Finger grass/*Smutsvingergras*)

Smuts Finger grass is a perennial grass with both rhizomes and stolons. This tufted grass has inflorescences with semi-digitate or digitate with long, thin fingers (racemes). The lower part of the plant is usually hairy and often has long, hairy stolons. The culms are usually unbranched.

Smuts Finger grass grows in sandy and gravelly soil in the more arid parts, and favours damp soil in areas with a high rainfall. It mainly grows in undisturbed veld and its dominance in natural veld indicates good veld condition. It is a palatable grass and reacts well to fertilisation and can endure heavy grazing.

An additional four species which did not undergo any enhancement were selected for Phase 1, from which seed were also harvested and sown for the commencement of Phase 2. The characteristics of the species relevant to this study are as follow (Van Oudtshoorn, 2009):

### *Hyparrhenia hirta* (L.) Stapf. (Common Thatching grass/*Dekgras*)

Common Thatching grass is a perennial grass with rhizomes. Common Thatching grass is a relatively dense, erect, and tufted. Its spikelets are covered with white to grey hairs and each

raceme has four to seven hairy brown awns. Pairs of racemes are usually contracted and point upwards.

Common Thatching grass grows in well-drained soil and gravelly soil. This drought resistant grass is often found in disturbed places and protects the soil and stabilises hard, gravelly and eroded soil. It is used as thatching in South Africa and is grazed by livestock early in the growing season and after fires.

*Eragrostis tef* (Zucc.) Trotter. (Tef)

Tef is an annual, loosely tufted grass. Its inflorescence has loose branches and the leaf blade is hairless and relatively soft and thin. It is an exotic grass that only occurs in disturbed places. Tef grows in most types of soil and has become popular for re-vegetating exposed ground.

*Sorghum bicolor* (L.) Moench (Sorghum)

Sorghum is an annual or short-term perennial which culms up to 4 m high (FAO, 2014). Mature glumes of sessile spikelets are either red or reddish brown (FAO, 2014). Sorghum is adapted to a subtropical and temperate climate and grows in most soils that are not waterlogged (FAO, 2014). It provides good grazing and is not recommended for establishment in soils with acid saturation levels above 10%. Sorghum is very palatable, especially in the young and flowering stages (FAO, 2014).

### **2.8 Anthropogenic Soil Properties**

In order to evaluate the properties of soil that effects germination, knowledge of its basic definition and related processes must be understood. Soil is defined by Winegardner (1995:241) as:

*The unconsolidated mineral and organic matter found on the immediate surface of the earth that has been subjected to and influenced by genetic and environmental factors of parent material, climate, macro- and micro-organisms and topography, al acting over a period of time and producing a product – soil - that differs from the material from which it is derived in many physical, chemical, geological, and morphological properties and characteristics.*

Soil is a heterogeneous mixture of air, water, solids and micro-organisms and is one of the essential components of the ecosystem; controlling ecological processes through means of its physical, chemical and biological properties (Gobat *et al.*, 2004:6; Sparks, 2003:1).

Furthermore, soil could be considered as an ecosystem in itself, due to its properties and functions which are similar to those of whole ecosystems (Bradshaw, 1998:256).

Mining activities produce wastes and tailing materials deprived of these properties (Bradshaw, 1998:255). These mine wastes are regarded as anthropogenic soils due to its differences in terms of pedogenic origins and properties with conventional soils and is included as such in the soil taxonomy system of South Africa (Fey, 2010:143; Van Deventer & Hattingh, 2004:62). There is currently one soil form that caters for anthropogenic soil forms, namely the Witbank soil form (Fey, 2010:143). According to Fey (2010:143) the most extensive areas of anthropogenic soils in South Africa is resultant from the rehabilitation of mined land, including coal-, diamond-, gypsum-, and gold mining. Van Deventer and Hattingh (2004:62) state that the purpose of rehabilitation of anthropogenic soils is to improve its functions to such an extent, that it will imitate the functions of what is regarded as a natural soil.

The interaction between re-vegetated plants with soil is determinative for the persistence of vegetation on rehabilitated areas (Liebenberg *et al.*, 2013:734). Therefore the characterisation of soil should include properties representing soil quality as a whole (Liebenberg *et al.*, 2013:734).

Karlen *et al.* (1997:6) defines soil quality as:

the capacity of a soil to function, within ecosystem and land use boundaries, to sustain biological productivity, maintain environmental quality, and promote plant, animal and human health.

This definition implies that soil quality entails that soil has an inherent capacity for supporting crop growth and has a dynamic part as influenced by human activities (Van Deventer & Hattingh, 2004:64). Soil quality is concerned with the function that soils perform in ecosystems; i.e. accepting, storing, and releasing nutrients, water and energy; making soil quality inseparable from ecological functioning and, consequently, sustainability (Van Deventer & Hattingh, 2004:62).

Soil function as an indicator of soil quality; can be assessed by parameters, such as the physical and chemical properties of the soil in question (Karlen *et al.*, 1997:6). According to Van Deventer and Hattingh (2004:64) soil quality partially depends on the soil's inherent composition, which is a function of soil forming factors and geological materials. These

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inherent properties such as mineralogy and particle size distribution show little change over time (Van Deventer & Hattingh, 2004:64).

The use of soil is greatly affected by its physical properties (Foth, 1990:27). This is due to certain attributes, such as water holding capacity, root penetration ease, aeration and retention of nutrients, which are connected with the physical condition of the soil (Foth, 1990:27).

The physical and chemical disintegration of rocks and minerals, results in a wide range of particle sizes in conventional soil types (Gobat *et al.*, 2004:14). The relative size of the particles can be expressed as the soil's texture, which refers to the fineness or coarseness of the soil (Foth, 1990:27). Texture is determined by the relative proportions of clay-, slit- and sand-sized particles (Foth, 1990:27; Gobat *et al.*, 2004:45). Porosity, structure and the hydric regime of soil is directly controlled by its texture (Gobat *et al.*, 2004:46). Texture is a useful index to use for the purpose of soil classification, as it is an inherent property of soil changing little over time in conventional soils (Gobat *et al.*, 2004:46).

For conventional soils particle size distribution can range from 0.002 mm (clay size particles) to 2 mm (sand size particles) (Winegardener, 1995:19). Unlike conventional soils, mine waste materials has undergone excessive crushing and grinding, resulting in a fine, homogenous texture, and consequently having a small particle size distribution (Mendez & Maier, 2008:48; Rossouw, 2010:15).

Soil structure is a result of the geometric orientation of the soil grains with respect to each other (Winegardener, 1995:15). Soil structure can be described as the aggregation of soil particles into clusters of particles, which are separated from the adjoining aggregates (Foth, 1990:38). Structure directly depends on the mineralogy, texture, moisture content and organic matter of the soil and determines porosity, infiltration capacity, and root penetration (Gobat *et al.*, 2004:48; Winegardener, 1995:15). Conventional soils can be aggregated or apedal (Gobat *et al.*, 2004:48). The fine, homogenous texture of mine waste materials, together with its lack of organic material, results in a loss of structure, which makes compaction inevitable (Bradshaw, 1997:262). Compaction inhibits root development, attesting that vegetation establishment on such mine waste materials to be a challenging task (Bradshaw, 1997:262).

Mine waste materials not subjected to excessive milling, such as the coarse kimberlitic waste used during this study, will have a coarse texture which has its own set of problems. Coarse

wastes have difficulty in retaining and providing moisture to plant roots adding to the difficulty of establishing vegetation on these wastes (Bradshaw, 1997:262).

Clay minerals are naturally occurring, inorganic, secondary minerals which usually occurs in the clay-sized fraction (< 0.002 mm) of soil and is weathered from parent material (Foth, 1990:15; Gobat *et al.*, 2004:13; Winegardener, 1995:234). According to Sparks (2003:51) clay minerals are assemblages of tetrahedral and octahedral sheets; 1:1 clays is the result of one tetrahedral sheet bonded to one octahedral sheet, while 2:1 clays is two tetrahedral sheets coordinated to one octahedral sheet (Sparks, 2003:51). Clay minerals have a negative charge at the edges of the layers where unsatisfied negative valences exist, resulting in sites that retain ions (Sparks, 2003:54; Gobat *et al.*, 2004:20). Clay mineralogy therefore plays a central role in the chemical activity of soil, influencing its structure and its CEC (Gobat *et al.*, 2004:20).

Mine tailing materials, as a waste product from mining activities, originate from freshly ground rock and not from parent rock subjected to natural weathering (Maboeta *et al.*, 2006:150). Furthermore, unlike conventional soils which contain great amounts of secondary minerals, mine waste materials consist mainly of primary minerals, which contribute to the low buffering capacity of mine waste materials (Van Deventer & Hattingh, 2004:62). According to Van Deventer & Hattingh (2004:62), the clay fraction found in mine waste materials does not contain a charge which results in low CEC values and consequently lacks ion-exchange, which adds to the absence of a buffering capacity to counteract pH changes. Thus, based on the difference in clay particle content, significant differences in soil function and fertility between soils exist.

A soil's CEC is mostly attributed to its secondary clay mineral content (Sparks, 2003:64). The CEC is the total number of exchange sites, both on organic and mineral colloids, of a soil (Foth, 1990:171). Therefore, it is directly dependable on the charge of secondary clay minerals and the organic content of the soil, and is able to provide nutrients to plants as exchangeable cations (Foth, 1990:174).

The transport of nutrient elements from the soil solution to the plant is done by diffusion as a passive transport method (Gobat *et al.*, 2004:87; Hopkins & Hürner, 2008:41). Diffusion is the process whereby a nutrient element is transported from a high electrochemical potential to a low electrochemical potential (Gobat *et al.*, 2004:87). Natural uptake of minerals by plant roots involves the release of H<sup>+</sup> by the plant roots in order to displace the nutritional elements from the colloids. Anthropogenic soils, specifically mine waste materials, have poor nutrient availability

due to its low CEC value and lack of organic content and contain sulphuric acid produced by the oxidation of pyrite minerals associated with mine wastes (Van Deventer et al., 2008:27; Ye *et al.*, 2000:289). Additional acidity is produced by the oxidation products from ferrous sulphate minerals (Van Deventer et al., 2008:27). Acidification of soil involves the increase of free H<sup>+</sup> which influences the release of nutrient elements from the colloidal particles of soil (Hopkins & Hürner, 2008:41).

The pH of soil is indirectly responsible for the availability of nutrients to plants in soil, due to the exchangeability of ions and the electrochemical potential that takes place with changes in pH. Considering the extreme pH conditions and lack of organic matter associated with mine waste materials, conventional soils containing organic matter and nutrient elements will be a far better reservoir and growth promoter for vegetation.

Soil pH affects numerous soil chemical reactions and processes (Sparks, 2003:267). Low pH and soil acidity damages root cell membranes, inhibit the activity of beneficial microbes, and impair the physiological function of plants (Liu & Lal, 2013:1). Additionally, aluminium becomes soluble and bio-available to plants at low ranges of pH causing aluminium toxicity in plants (Sparks, 2003:267). When available to plants, aluminium causes stunted roots in susceptible plants (Sparks, 2003:267). Mine waste materials containing pyrite minerals, are often subjected to low pH conditions, due to the oxidation of pyrite (Sparks, 2003:270; Van Deventer et al., 2008:27). The complete oxidation of pyrite can be expressed as:



Due to the additional acidity produced by pyrite minerals in mine waste materials, the pH can become extremely low which in turn will promote the solubility and availability of aluminium to plants, causing aluminium toxicity.

Soil organic matter (SOM) is considered to be an attribute of soil quality, thus SOM is a property that reflects the capacity of a soil to generate and sustain plant growth (Van Deventer & Hattingh, 2004:5). Inherent soil properties, specifically soil mineralogy and particle size distribution, regulates the ability of a soil to preserve SOM (Carter, 2004:43). Anthropogenic soils subjected to degradation and mining activities experience a depletion of SOM and associated nutrients, such as nitrogen (Maboeta & Van Rensburg, 2003:265; Wong, 2003:776). A loss of SOM contributes to the loss of soil infiltrability and nutrient supply (Mills & Fey,

2004:388). However, a lack of SOM in mine wastes can be partially counteracted through addition of organic matter and fertilizers (Bradshaw, 2000:89; Schroeder *et al.*, 2005:318).

Added organic matter to anthropogenic soils can be both chemically and physically beneficial, and improves the chemical status of mine waste materials through the addition of nutrients in a slow-release organic form (Schroeder *et al.*, 2005:318). Additionally, physical attributes, such as water-holding capacity, infiltration and porosity, is improved by added organic matter in mine wastes (Schroeder *et al.*, 2005:318).

Metal trace elements occur at low concentrations in natural soils and originate from weathering and pedogenic processes of parent rock from which soils develop (Herselman, 2007:4). Metal trace elements are geochemically associated mainly with sulphide minerals in mine waste materials and in most cases occur at much higher concentrations than found in soils (Aucamp & Van Schalkwyk, 2002:124).

Essential metal trace elements are important for biological functions, and deficient intake of thereof can cause impairment of such biological functions (Herselman, 2007:6). The intake of metal trace elements is essential in small concentrations for healthy plant growth, although at higher concentrations they can be toxic. The mobility and accumulation of metal trace elements in soil and mine waste materials are influenced by several factors. According to Herselman (2007:16) soil pH is critical in controlling the bio-availability of metal trace elements. Generally the mobility of metal trace elements decreases with an increased pH, with the exception of arsenic (As), molybdenum (Mo), and chromium (Cr) which are more mobile under higher (alkaline) pH conditions (Herselman, 2007:16).

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## Methods and Materials

### 3.1 Introduction

In collaboration with AS a study was conducted during 2012 to 2014 and entailed two phases:

1. Phase 1 which entailed a pot trial study in order to compare the germination of coated and non-coated grass seed types in different growth media, which included eight mine waste materials and two control soils.
2. Phase 2 which investigated the viability of offspring seed from the grasses established in the different growth media (i.e. the eight mine waste materials and two soils used in this study) during Phase 1.

This project entails the second phase (Phase 2) which investigated the germination and viability of seed harvested from a previous generation of grasses which has established in different mine waste materials during the pot trial study of Phase 1. Succeeding Phase 1, Phase 2 included the same species used during Phase 1.

Phase 1 entailed the comparison and investigation of the germination and establishment rates of both coated and non-coated selected grass seed types in different growth media, including mine waste materials. The final results of Phase 1 can be found in the mini-dissertation submitted in partial fulfilment of the requirements for the degree *Bachelor of Science* (Honours) in Environmental Science at the Potchefstroom Campus of the North-West University, titled: 'Effect of seed coating on the germination of selected grass species in different mine wastes deposits: a nursery trial' (Muller, 2013).

### 3.2 Species Selected

Both Phase 1 and Phase 2 of this study were conducted at the nursery for Mine Rehabilitation on the premises of the North-West University (NWU) at Potchefstroom. The grass species selected by AS and used during Phase 2 included the following (Table 3.1): *E. curvula* (Weeping Love Grass); *C. dactylon* (Couch Grass); *C. gayana* (Rhodes Grass); *C. ciliaris* (Buffalo Grass); *D. eriantha* (Smuts Finger Grass); *S. bicolor* (Sorghum); *E. tef* (Tef) and *H. hirta* (Common Thatching Grass). A red soil was selected as the growth medium for the pot trial and was

ameliorated with fertilizer and compost. The motivation for the use of the sandy soil was to ensure a homogenous seed friendly growth medium and to minimize external factors such as acidity, and salinity. on germination.

**Table 3. 1: Species and applied coating selected for Phase 1 and 2 respectively.**

AS selected species	Phase 1	Phase 2
	Coated	Non-coated
<i>Eragrostis curvula</i>	✓	✓
<i>Cynodon dactylon</i>	✓	✓
<i>Chloris gayana</i>	✓	✓
<i>Cenchrus ciliaris</i>	✓	✓
<i>Digitaria eriantha</i>	✓	✓
Additional Selected species	Non-Coated	Non-coated
<i>Secale cereale</i>	✓	X
<i>Sorghum bicolor</i>	✓	✓
<i>Eragrostis tef</i>	✓	✓
<i>Hyparrhenia hirta</i>	✓	✓

Rye (*Secale cereale*) was one of the selected species used during Phase 1, however, Rye was excluded from trials during Phase 2 (Table 3.1). The main reason for this was that Rye grass is a winter perennial. All the other selected species flowered during summer, which is when the offspring seed were harvested for the use during Phase 2. Rye grass flowers in winter and thus no seed were harvested from Rye during seed harvesting which commenced in the summer. Due to Rye being a winter perennial, its growth regime differs from all the other selected species and for this reason Rye was excluded from the re-growth monitoring and consequently will not be further discussed or mentioned.

### 3.3 Origin of growth mediums: mine waste materials and soils

During Phase 1, the germination of coated and non-coated grass seed types in different growth media was compared. These grasses were allowed to grow and complete a life-cycle. Shortly after these grasses have reached the reproductive stage, the seeds were harvested. These seeds were planted in the follow-up season with the commencement of Phase 2 in 2014. Due to the

growth media's hostile environment as experienced by the grasses during seed development, it was deemed applicable to describe the origin of the mine waste materials used.

**Table 3. 2: Growth media in which parent grasses were grown (during Phase 1) from which progeny seed were harvested for use in Phase 2.**

No	GROWTH MEDIUM	SOURCE
T1	Gypsum wastes	Potchefstroom fertilizer factory wastes
T2	Gold Mine tailings with <1% pyrite content	Stilfontein No 4 TSF
T3	Gold Mine tailings with > 2 % pyrite content	Stilfontein No 5 TSF
T4	Platinum tailings	Paardekraal, Rustenburg: Marensky reef
T5	Kimberlite Mine Wastes	Cullinan mine (old Premier mine)
T7	Fluorspar Mine Wastes	Fluorspar mine – Zeerust – North West
T8	Andalusite Mine Wastes	Andalusite mine – Groot Marico – North West
T9	Fine Coal Discard	Coal fields – Witbank area
TC	Soil: Hutton soil form: A & B horizon	Potchefstroom area
TV	Soil: Vertic soil: Arcadia soil form	Potchefstroom area

The gypsum wastes (T1) were collected from a fertilizer factory in the Potchefstroom area. The gypsum used in this study is not of natural origin, but was generated as a by-product during the manufacture of fertilizer and contains large amounts of calcium and sulphate ions (Rodríguez-Jordá *et al.*, 2010:763).

The gold waste types (T2 & T3) were collected respectively from two different TSF's at Stilfontein. Dolomites of the Malmani Subgroup outcrop in the Stilfontein area and is underlain by the Witwatersrand Super Group within which the ore bodies is found (McCarthy & Rubidge, 2005:194). One of the main ore bodies of the Central Rand Group is the Vaal Reef which is a pebbly, quartz arenite bed (Antrobus *et al.*, 1986:561). Another main ore body for gold is the Ventersdorp Contact Reef which is found at the base of the Ventersdorp Super Group (Antrobus *et al.*, 1986:561). The Ventersdorp Contact Reef is an auriferous, uraniferous, pyritic, oligomictic conglomerate (Antrobus *et al.*, 1986:566). According to Antrobus *et al.* (1986:566) twelve minerals constitute up to 99 % of the ore minerals present in the reef. These include among others: gold, pyrite, carbon, uraninite and pyrrhotite, with pyrite being the most predominate mineral (Antrobus *et al.*, 1986:566).

The platinum wastes (T4) were collected at a platinum mine from the Rustenburg area. Platinum is mined from the Merensky Reef which is a sheet-like, composite body within the Bushveld Igneous Complex (Leeb-du Toit, 1986:1101). The Merensky Reef has a high concentration of platinum group elements within the pegmatoidal feldspathic pyroxenite as indicated by mineralogical studies (Viljoen & Hieber, 1986:1121). The most abundant platinum mineral groups in the Merensky Reef are associated with platinum/palladium sulphides, namely braggite and cooperite (Viljoen & Hieber, 1986:1121). These platinum group minerals are closely associated with base-metal sulphides (Viljoen & Hieber, 1986:1122).

The kimberlite mine wastes (T5) were from the Cullinan (old Premier) mine. The Cullinan mine extracts diamonds from a kimberlite pipe which is the largest ever found in South Africa and intruded the Transvaal Super Group and the Bushveld Igneous Complex (Field *et al.*, 2008:40). Kimberlite is an ultrabasic igneous rock and occurs mainly as small volcanic dykes, pipes and sills (Field *et al.*, 2008:40). The matrix contains primary ground mass constituents or phenocrystals, and olivine (Field *et al.*, 2008:40). This pipe contains diamonds as xenocrysts and other crustal xenoliths (Field *et al.*, 2008:41).

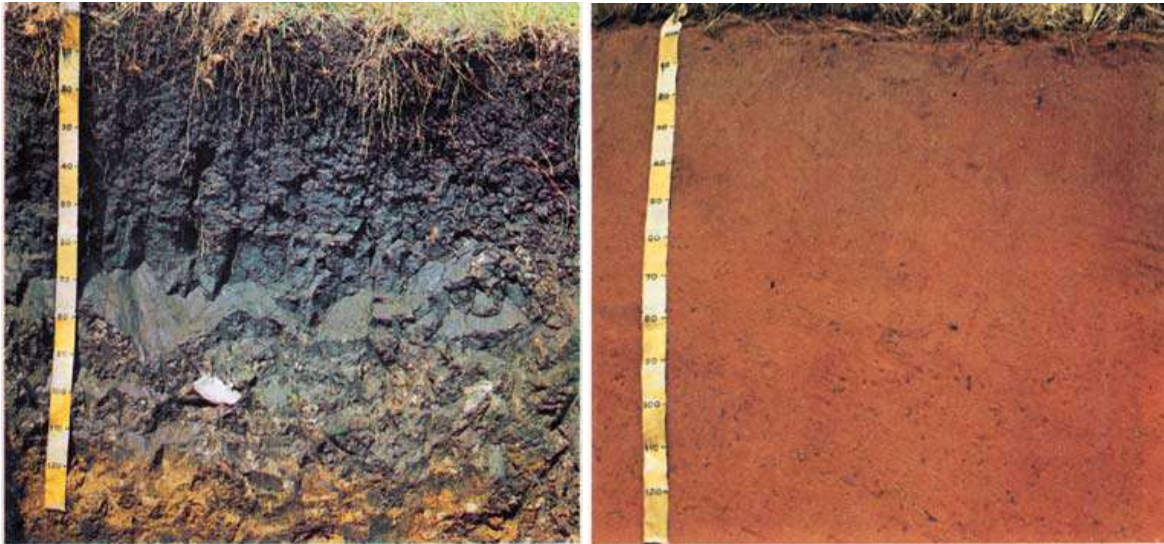
The andalusite wastes (T7) were collected at an andalusite mine in the Groot-Marico district which mines andalusite from metamorphosed shales found in the Bushveld Igneous Complex (Overbreek, 1989:70). According to Overbreek (1989:70) South Africa possesses the largest portion of the world's known deposits of andalusite. Andalusite occurs in hornfels and schists in the metamorphosed shales which are from the Daspoort Stage of the Bushveld Igneous Complex (Overbreek, 1989:70).

Fluorspar mine wastes (T8) were collected from a fluorspar mine located near Zeerust. Fluorspar mineralization occurs in the Frisco Formation from the Malmani Subgroup (dolomites) of the Transvaal Super Group (Bear, 1986:855). These rocks were subjected to thermal metamorphism during the emplacement of the Bushveld Igneous Complex (Bear, 1986:855). The thermal metamorphism of the dolomites resulted in the development of silicates, quartz and water (Ryan, 1986:844). Fluorspar is associated with calcite, talc, sulphides and quartz (Bear, 1986:855). Replacement of calcium carbonate by the precipitation of fluorine from mineralizing fluids resulted in the mineralization of fluorspar and sulphide in the dolomite and chert host rock (Ryan, 1986:844).

The fine discard coal (T9) was produced as a result from washing plants at coal mines from the Springs-Witbank Coalfield. Five major coal seams are present in the Springs-Witbank Coalfield (Smith & Whittaker, 1986:1971). These seams were deposited in glacio-fluvial, deltaic, and coastal plain settings and are locally displaced and bent by Karoo dolerite sills and dykes (Smith & Whittaker, 1986:1971). The five coal seams (numbered 1 to 5 from the base up) are contained within a 70 m succession and the parting thickness between these seams remains relatively constant over most of the field (Smith & Whittaker, 1986:1972). The coal seams occur in a sedimentary sequence comprised of, from the base up, a diamictite, siltstone, pebbly mudstone, gravel and conglomerate, which is overlain by swamp, fluviodeltaic, and shoreline deposits (Smith & Whittaker, 1986:1972). The Springs-Witbank Coalfield is part of the coal-bearing Ecca Group of the Karoo Super Group (Smith & Whittaker, 1986:1972).

The vertic soil (TV) used in this study is from an Arcadia soil form from the Potchefstroom area (Figure 3.1). The properties of vertic soils are a function of their high clay content, most of which are smectite clay (Fey, 2010:36). Consequently vertic soils have a high cation exchange capacity (CEC) and a base saturation close to 100% (Fey, 2010:37). Despite of what the black colour of vertic soils suggests, the organic carbon content is low. The colour is due to the dispersed nature of the humic material that combined with calcium to form a black calcium-humate complex (Fey, 2010:37). Due to the high surface area and CEC, vertic soils have an excellent capacity for plant nutrient retention, metal sorption and acid buffering (Fey, 2010:37). Vertic soils have a tendency to shrink and swell with changes in water content due to the high smectite content during which characteristic slickensides occur (Figure 3.1) (Fey, 2010:37). During periods of shrinkage cracks appears allowing infiltration of water, during swelling of the soils the cracks seals limiting water infiltration drastically (Fey, 2010:37).

The red soil (TC) obtained from the Hutton soil form in the Potchefstroom area (collected from the A horizon) is an oxidic soil that is uniformly coloured with red oxides of iron (Figure 3.1) (Fey, 2010:105). The apedal soil is characterised by relatively low CEC which reflects the oxidic mineralogy and dominant kaolinite clay mineral content. The iron (Fe) content of this soil horizon is low due to the strong pigmenting effect of iron oxides. Consequently properties linked to high iron content, such as (P) fixation and anion exchange capacity, are not always strongly expressed. The B horizon is well drained and aerated (Fey, 2010:106).



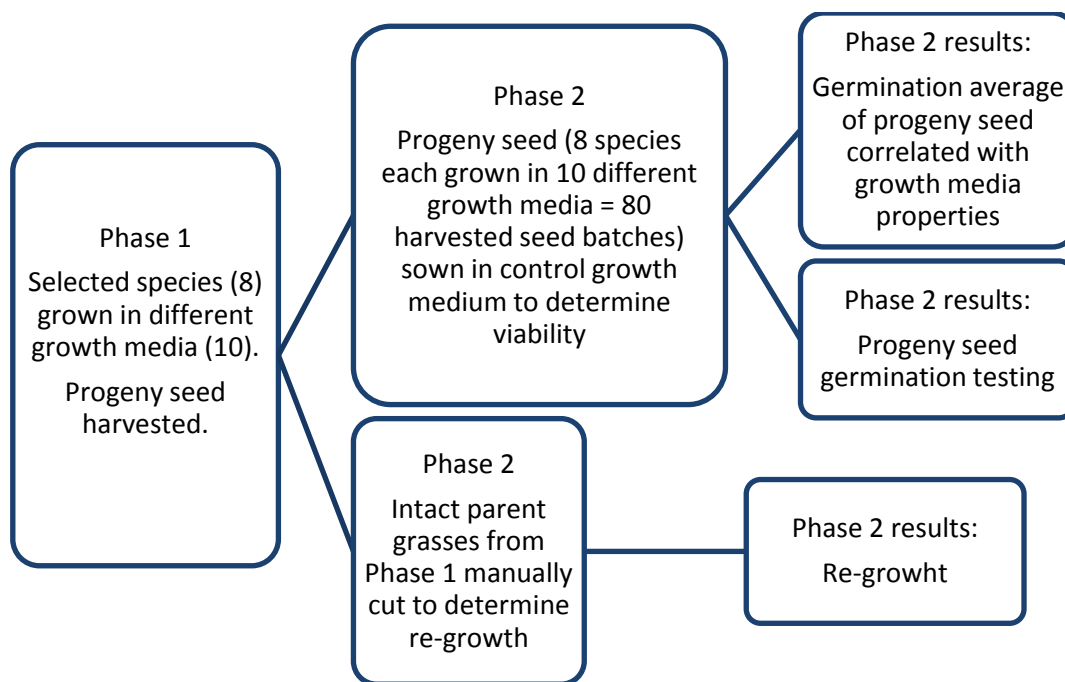
**Figure 3. 1: Arcadia soil form with a Vertic A horizon (left); and the Hutton soil form with a red apedal B horizon (right) (Fanourakis, 2012:118).**

### **3.4 Experimental Design**

This project; Phase 2 of the study, was conducted at the nursery for Mine Rehabilitation on the premises of the North-West University (NWU) at Potchefstroom. The grass species, selected by AS, include the following: Weeping Love grass; Couch grass; Rhodes Grass; Buffalo grass; Smuts Finger grass; Sorghum; Tef and Common Thatching grass. Only the parent grasses were planted in different mine waste materials during Phase 1, and the seeds harvested from these grasses was sown in only one growth medium, namely a red soil, during Phase 2 (Figure 3.2). The motivation for the use of the sandy soil was to ensure a homogenous seed friendly growth medium and to minimize external factors such as acidity, and salinity on germination. Thus, the red soil used in Phase 2 is regarded as a control, and not a variable.

The progeny seed were harvested from the plumes of each grass species (excluding Rye) grown in each growth medium (Figure 3.1). Thus for every species, ten seed batches were harvested according to the ten different growth media (namely eight different mine waste materials and two soils) in which the parent grasses were planted in. Seedling emergence was monitored within a particular species based on the growth medium the preceding generation was grown in, and as such will be referred to as a seed batch. After drying for three weeks at room temperature, the seed from the different seed batches were sown separately and replicated four times with ten seeds per replicate in the red soil, and was labelled according to the original growth medium and species. Rules prescribed by ISTA (1985) required a minimum amount of three replicates for each seed type during the pot trial. The position of each replicate in the pot

trail layout was determined by a chance-factor in order to ensure randomization of replicates of all seed types. The seedling emergence was monitored every second day after for a period of 31 days, which commenced after the progeny seed were sown.



**Figure 3. 2: Diagram of experimental design for Phase 2 which originated from Phase 1.**

The existing parent grasses, replicated in each of the ten growth media, were cut manually and above the growth medium's surface, six months after they were planted during Phase 1 (April 2013). Irrigation continued regularly after the harvesting. The grasses were allowed to re-grow after they were cut. Six months after the cutting event, (November 2013) the re-growth was evaluated according to the observable above-ground biomass. Each replicate of every species grown in each of the ten growth media, were evaluated six months after the cutting event (November 2013) according to the observable above-ground biomass and classified accordingly into two groups; for either observable re-growth that occurred after the cutting event, or for no observable re-growth that occurred after the cutting event.

### 3.5 Germination

#### 3.5.1 Pot trial experiments

Seed were harvested from each species grown in each growth medium during Phase 1 during January 2013. The seed were harvested manually from the plumes of the grasses after it had reached the reproductive stage. The seed were labelled and kept separate and according to the

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species and every growth medium in which the grasses were established. The seed were allowed to dry for approximately three weeks at room temperature after harvesting, after which it was kept in storage.

Phase 2 of the pot trial study commenced in January 2014 during which the harvested seed were sown. Plastic containers with a one litre capacity were filled with the ameliorated red soil. The soil was kept moist by means of irrigation with a sprinkler system. The harvested seeds were sown by hand in the moist soil allowing the seed to be slightly covered by the soil. Four replicates containing ten seed each were made for each seed batch. One plastic container was used per replicate and one replicate contained ten seeds harvested from one species grown in a specific growth medium. The trial was subjected to natural conditions, and was irrigated regularly (Figure 3.3).

Germination monitoring commenced seven days after the seed were sown and seedling emergence was recorded every second day thereafter for 32 days in order to obtain the cumulative seedling emergence for the duration of the trial period. Every emerging seedling was recorded. A germination average was calculated from the sum of the four replicates per seed batch.

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(a) Plastic containers filled with red soil, ready for planting



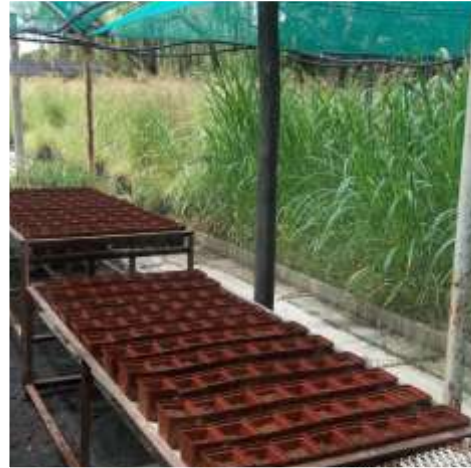
(b) Seeds were sown in containers filled with soil and labelled accordingly



(c) Wetting the soil after seed were sown



(d) Layout of pots in nursery: all replicates were placed under irrigation and shade



**Figure 3. 3: Steps taken during the sowing of the seed.**

### 3.5.2 Seed Germination Tests

Germination tests according to the seed testing standards set by ISTA were conducted at the laboratories of AS in a controlled environment. Three planting methods prescribed by ISTA (1985) were used for the germination tests, namely: between paper (BP) for Sorghum; sand (S) for Buffalo grass; and on top of paper (TP) for the remainder species (Table 3.1). Controlled temperatures were maintained at 20° C at night and 30° C at day respectively. The minimum and maximum number of days at which the germination percentages were recorded was species specific and was stipulated by the ISTA (1985) rules.

### 3.6 Re-growth

During Phase 1, the germination of coated and non-coated selected grass seed types in different growth media were compared. After the grasses reached the reproductive stage, the established grasses were cut at the base of the plant. All the grass species replicated in each growth medium, were cut manually and approximately 10 cm above the growth medium's surface to remove most of the above-ground biomass during April 2013 (six months after planting). Irrigation continued regularly and grasses were allowed to re-grow during the winter season.

In November 2013, six months after the cutting event, the grasses were classified in a subjective manner; indicative of whether or not it has shown observable signs of growth, i.e. observable above-ground biomass (leaves and stems). The grasses were classified into two groups based on observations of the above-ground biomass. The first group indicated that there was visual evidence of above-ground biomass since it has been cut. The second group indicated that there was visual evidence that the roots and base is still intact, but no visual evidence was found that any above-ground re-growth occurred. Ten replicates were planted for each species in every growth medium, therefore, the counts per growth medium adds up to ten.

### 3.7 Growth Medium Analysis

The growth media in which the grasses were planted during Phase 1 was analysed as to obtain information about its' physical and chemical properties. The results from these analyses were used during Phase 2 to determine the specifics of the maternal abiotic environment during seed development.

The analyses of representative samples (Figure 3.2) were carried out by the laboratories of *Grond en Omgewings Laboratorium* (GEO LAB), Potchefstroom. The amount of exchangeable cations (EC) retained on the reactive colloidal surfaces of the soil types was determined by the ammonium acetate method, as described by Schollenberger and Simon (1945) and Rhoades (1982) respectively. The EC was measured by the use of an EC-meter from the liquid fraction, after centrifuged to a saturated paste.

The CEC of each growth medium was measured to indicate the total capacity of each growth medium to hold exchangeable cations, and the capacity for exchange of cations between the soil and the soil solution (Gobat *et al.*, 2004:70). The CEC was measured using 1 M Ammonium acetate (pH 7) (Rhoades, 1982).

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\*P.O. Box 5546, Kockspark, Potchefstroom, 2523.

The exchange acidity pH values ( $\text{pH}_{\text{KCl}}$ ) of all the growth media were measured on a mass basis, using KCl as exchanging salt in a 1:2,5 soil: liquid ratio suspension. A pH-meter was used to record measurements of the solution-fraction of the suspensions, four hours after mixing the soil samples with KCl (Rhoades, 1982).

The actual pH ( $\text{pH}_{\text{water}}$ ) was measured on mass basis by placing growth medium samples in distilled water in a 1:2,5 soil:water ratio suspension. This method allows the electrode to only measure the protons in solution, resulting in higher  $\text{pH}_{\text{water}}$  measurements compared to  $\text{pH}_{\text{KCl}}$  measurements of the same sample (Rhoades, 1982).

The soluble Phosphate content of the growth media was determined by using the p-Bray 1-method as described by Bray and Kurtz (1945). The P-Bray 1-solution, containing Ammonium Fluoride and hydrochloric acid, was added to each growth media sample in a 1:7,5 soil:extraction agent ratio, together with a flocculent agent. An auto-analyser was used to determine the Phosphate content of each growth medium sample.

Particle size distribution was analysed by sieving the soil samples through a 2000, 1000, 500, 250, 100 and 53  $\mu\text{m}$  sieve separately. The fraction left on each size sieve was weighed and recorded. Smaller fractions were determined by means of the hydrometer method.

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## Results

### 4.1 Introduction

This chapter presents the results and outcomes from the components of Phase 2 with regard to the aims of the study described in Chapter 1. The results of the pot trial and growth medium analysis will be reported on while the discussion thereof will follow in Chapter 5.

Additionally the seed germination tests conducted at the laboratories of AS provided insight on the vigour of offspring seed from each species grown in every growth medium during Phase 1. No comparison is made among species for both the pot trial and seed viability test. The results from the pot trial and germination tests of the progeny seed were compared to the growth medium analysis.

### 4.2 Germination

This section addresses the first objective, namely to assess the viability and germination of the progeny seed, which were harvested from grasses grown in different growth media, including eight different mine waste materials and two control soils. The results from the germination tests conducted at the laboratories of AS will be reviewed briefly, after which the germination results from the pot trial will be presented and compared with the germination tests results, which follows hereafter.

Germination tests (Figure 4.1), according to ISTA guidelines, were conducted at the laboratories of AS in order to evaluate the potential germinability and viability of the progeny seed. The objective of these tests were to supplement the germination results from the pot trial and to determine any irregularities regarding the maturity of the progeny seed, as immature (green) seed would not be able to germinate.

As mentioned in section 3.4.2, three planting methods tests were used for the germination tests, namely: between paper (BP); on top of sand (S), and on top of paper (TP). The minimum and maximum number of days at which germination percentages were recorded was species specific and was stipulated by the ISTA (1985) rules.

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The first and final count days are also species specific as prescribed by ISTA rules (1985). The first count serves as an indication of the vigour of the seed batch, while the final count provides the germination percentage of the seed batch.

Green seeds with empty caryopsis were observed for the progeny seed of Common Thatching grass, particularly seed batches from the gold tailings with high pyrite content (T3), the kimberlite mine wastes (T5), the fluorspar mine wastes (T7), and the vertic soil (TV) (Table 4.1). Extremely low germination percentages (1 % - 6 %) were observed for seed batches from the remainder growth media.

Green (and potentially immature) seeds were found in the fluorspar seed batch (T7) of Sorghum. This accounts for the zero germination percentage result for this seed batch (Table 4.1). Seed from all the seed batches for each species were sown and replicated in the pot trial experiment (section 4.2.2), regardless of green seeds found in seed batches of Common Thatching grass and Sorghum.

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Table 4. 1: The first and final count germination percentages of progeny seed from each species, grown in each growth medium.

Growth Media	Sorghum (S1)		Cenchrus ciliaris (S3)		Eragrostis curvula (S5)		Cynodon dactylon (S7)		Chloris gayana (S9)		Digitaria eriantha (S10)		Eragrostis tef (S12)		Hyparrhenia hirta (S13)	
	1st count 4 days %	Final 10 days %	1st count 6 days %	Final 28 days %	1st count 4 days %	Final 10 days %	1st count 6 days %	Final 21 days %	1st count 5 days %	Final 14 days %	1st count 5 days %	Final 14 days %	1st count 3 days %	Final 10 days %	1st count 5 days %	Final 21 days %
T1 - Gypsum	97	99	1	4	64	81	6	13	2	2	25	60	89	89	5	6
T2 - Gold < 1 % pyrite	82	86	16	23	79	82	2	10	3	3	23	40	85	86	1	1
T3 - Gold > 2 % pyrite	97	99	11	20	74	77	3	21	4	4	21	38	94	95	*	*
T4 - Platinum	93	98	7	9	86	87	15	27	19	19	26	49	96	97	2	2
T5 - Kimberlite	93	95	10	17	83	89	3	14	0	0	7	13	92	94	*	*
T7 - Fluorspar	0	0	29	33	77	67	14	77	0	0	54	58	93	93	*	*
T8 - Andalusite	92	98	24	35	60	88	68	77	18	19	7	44	96	96	1	1
T9 - Coal	80	99	13	19	71	73	4	38	0	0	30	51	94	95	1	1
TC - Red soil	70	94	5	16	60	79	5	54	0	0	36	50	91	92	4	5
TV - Vertic soil	86	93	21	32	65	72	2	16	0	0	16	31	77	82	*	*
Planting Method	BP – 20/30°C		S – 20/30°C		TP – 20/30°C		TP – 20/30°C		TP – 20/30°C		TP – 20/30°C		TP – 20/30°C		TP – 20/30°C	

(\* Seed are green with empty caryopsis)

#### 4.2.1 Pot trial germination results

The cumulative germination data were obtained over a trail period of 32 days which commenced seven days after the progeny seed were sown. The cumulative germination data is presented as a percentage of the total 40 hand sown seeds from each seed batch (i.e. seed from one particular species grown a specific growth medium). The data obtained for every species during the pot trail are discussed separately for each species, presented by the differences in the trends of the germination rates for the different seed batches, i.e. progeny seed of a particular species harvested from a preceding generation grown in the ten different growth media. The maximum germination referred to in the results of the pot trial; only reflect the maximum emergence percentage reached during the 32 day trial period, irrespective of any further emergence after this trial. No comparison was made between the species. Seedling emergence was monitored within a particular species based on the growth medium the preceding generation was grown in, and as such will be referred to as a seed batch.

Due to the cumulative nature of the germination data, dependency exists between each of the germination counts during the 26 day monitoring trail for every replicate. In other words, the germination count at day 9 is dependent of the count at day 7, because the count at day 9 will include seedlings that were already counted at day 7. Thus, the statistical model used for analysis of variance was repeated measures analysis of variance (Repeated Measures ANOVA) which takes the dependency within a variable (in this study the germination) into account.

##### (a) *Eragrostis curvula* (Schrad.) Nees (Weeping Love grass)

The initial germination percentages for the progeny seed of Weeping Love grass differs slightly from the final germination percentages, particularly for seed batches harvested from the gypsum wastes, the andalusite mine wastes, the coal discard and the vertic soil (Table 4.2). The final germination percentage for the vertic soil seed batch were recorded only on day 32 during the pot trial experiment, while for the andalusite seed batch the final germination percentage were recorded on day 23 (Figure 4.1). This delay in germination indicates poor seed vigour for the vertic soil- and the andalusite seed batches.

The final germination percentages differs for the germination tests and the pot trial experiment with regards to the gypsum wastes, gold tailings with a low pyrite content, gold tailings with a

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high pyrite content, the andalusite mine wastes, coal discard, and the fluorspar mine wastes seed batches (Table 4.2). An higher final germination percentage were recorded on day 10 when compared to day 32 during the pot trial experiment for the andalusite mine wastes-, platinum tailings-, kimberlite mine wastes-, red soil-, and the vertic soil seed batches (Table 4.2). The opposite was found for the gypsum wastes-, the gold wastes with low pyrite content-, gold wastes with high pyrite content-, the fluorspar mine wastes-, and the coal discard seed batches which had a higher recorder germination percentage on day 32 during the pot trail experiment compared to day 10 during the germination tests. This might be due to the longer trial period of the pot trial experiment, which exceeds the trial period of the germination tests with 22 days, allowing more time for the progeny seed of Weeping Love grass to germinate and consequently recording higher germination rates. However, it does not explain the higher germination percentages recorded on day 10 rather than day 32 for the remainder seed batches.

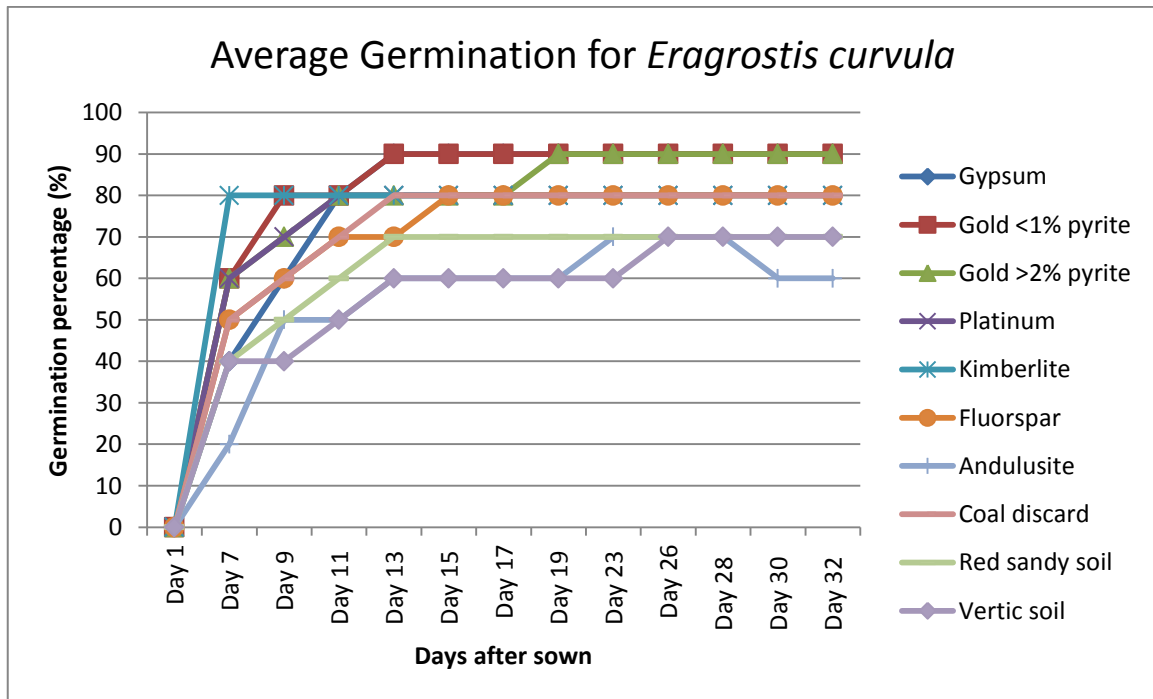
The highest final germination percentage recorded was 90 % for the gypsum wastes, the gold wastes with low pyrite content, and the gold wastes with high pyrite content seed batches during the pot trial experiment (Table 4.2). Alternatively, the lowest germination percentage recorded was 60 % for the andalusite mine wastes seed batch during the pot trial experiment (Table 4.2). This is significant as it indicates that the progeny seed of the Weeping Love grass were highly germinable with regard to the lowest recorded germination percentage having a relatively high numerical value.

The germination percentages varied slightly, ranging from 60 % (lowest germination percentage) to 90 % (greatest germination percentage). The Repeated Measures ANOVA statistical analysis indicated no significant difference between the germination of the different seed batches for Weeping Love grass ( $p = 0.1366$ ;  $f = 1.69$ ).

## Chapter 4 - Results

**Table 4. 2: Results of the average percentages (%) for the first count (4days) and final day (10 days) of germination for *Eragrostis curvula* (Weeping Love grass) seed batches and pot trial germination (%) after 32 days.**

		T1 - Gypsum	T2 - Gold <1% pyrite content	T3 - Gold >2% pyrite content	T4 - Platinum	T5 - Kimberlite	T7 - Fluorspar	T8 - Andalusite	T9 - Coal discard	TC - Red	TV - Vertic Clayey
Germination tests (on top of paper planting method)	1st count 4 days %	64	79	74	86	83	77	60	71	60	65
	Final 10 days %	81	82	77	87	89	67	88	73	79	72
Pot trial germination	Final 32 days %	90	90	90	80	80	80	60	80	70	70



**Figure 4. 1: Average germination percentage (%) over a 32 day period for progeny seed harvested from *Eragrostis curvula* (Weeping Love grass) grown in the different growth media.**

**(b) *Cynodon dactylon* (L.) Pers. (Couch grass)**

There is a significant difference between the initial (day 6) and the final (day 21 and 32 respectively) germination percentage for the progeny seed of Couch grass (Table 4.3). This excludes the andalusite seed batch which had a germination percentage of 68 % on day 6, and a final germination percentage of 70 % on day 32. The initial low germination rates indicates poor seed vigour for the progeny seed of Couch grass, except for the andalusite seed batch which had a significantly high initial germination percentage (Table 4.3)

Despite the difference between the initial and final germination rates, the final germination percentages varied for the different seed batches (Table 4.3). For the pot trial experiment the final germination percentages ranged from 10 % for the coal discard and red soil seed batches to 70 % for the andalusite seed batch.

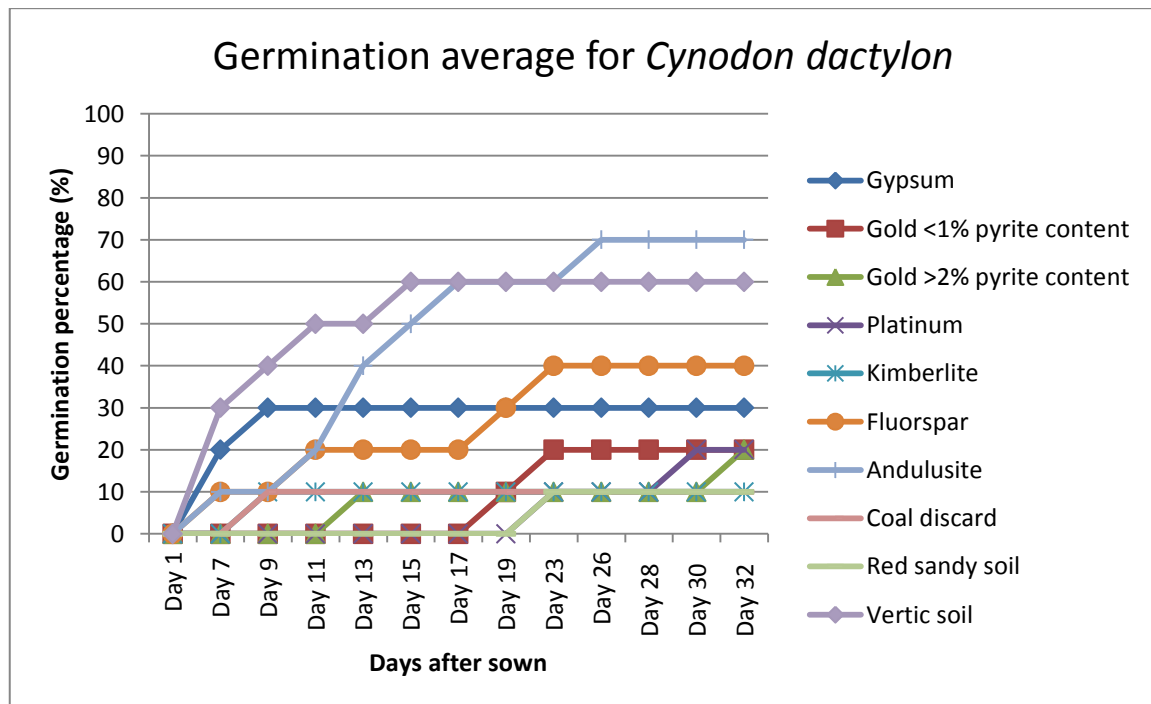
The andalusite seed batch had the greatest germination percentage (70 % from the pot trial experiment and 77 % from the germination test) as well as the greatest initial germination percentage (68 % on day 6) (Table 4.3 & Figure 4.2). The likelihood exists that the andalusite seed batch is vigorous and germinates easily.

The Repeated Measures ANOVA statistical analysis indicated that there was a significant influence of the growth media in which the parent grasses were grown as a variable on the germination of Couch grass progeny seed ( $p = 0.001$ ,  $f = 5.84$ ), which was anticipated due to the great variance in germination percentages between the seed batches (Figure 4.2).

## Chapter 4 - Results

**Table 4. 3: Results of the average percentages (%) for the first count (4days) and final day (21 days) of germination for *Cynodon dactylon* (Couch grass) seed batches and pot trial germination (%) after 32 days.**

		T1 - Gypsum	T2 - Gold <1% pyrite content	T3 - Gold >2% pyrite content	T4 - Platinum	T5 - Kimberlite	T7 - Fluorspar	T8 - Andalusite	T9 - Coal discard	TC - Red	TV - Vertic Clayey
Germination tests (top of paper planting method)	1st count 6 days %	6	2	3	15	3	14	68	4	5	2
	Final 21 days %	13	10	21	27	14	77	77	38	54	16
Pot trial germination	Final 32 days %	30	20	20	20	10	40	70	10	10	60



**Figure 4. 2: Average germination percentage (%) over a 32 day period for progeny seed harvested from *Cynodon dactylon* (Couch grass) grown in the different growth media.**

**(c) *Chloris gayana* Kunth. (Rhodes grass)**

Progeny seed of Rhodes grass from the kimberlite, fluorspar, coal discard, vertic soil, and the red soil seed batches did not germinate during the germination tests (Table 4.4). However, seed from these seed batches did germinate during the pot trial experiment (Table 4.4). This difference might be due to the different sowing methods. During the germination tests the seed were placed on top of paper, while during the pot trial experiment the seed were sown in and slightly covered with ameliorated red soil.

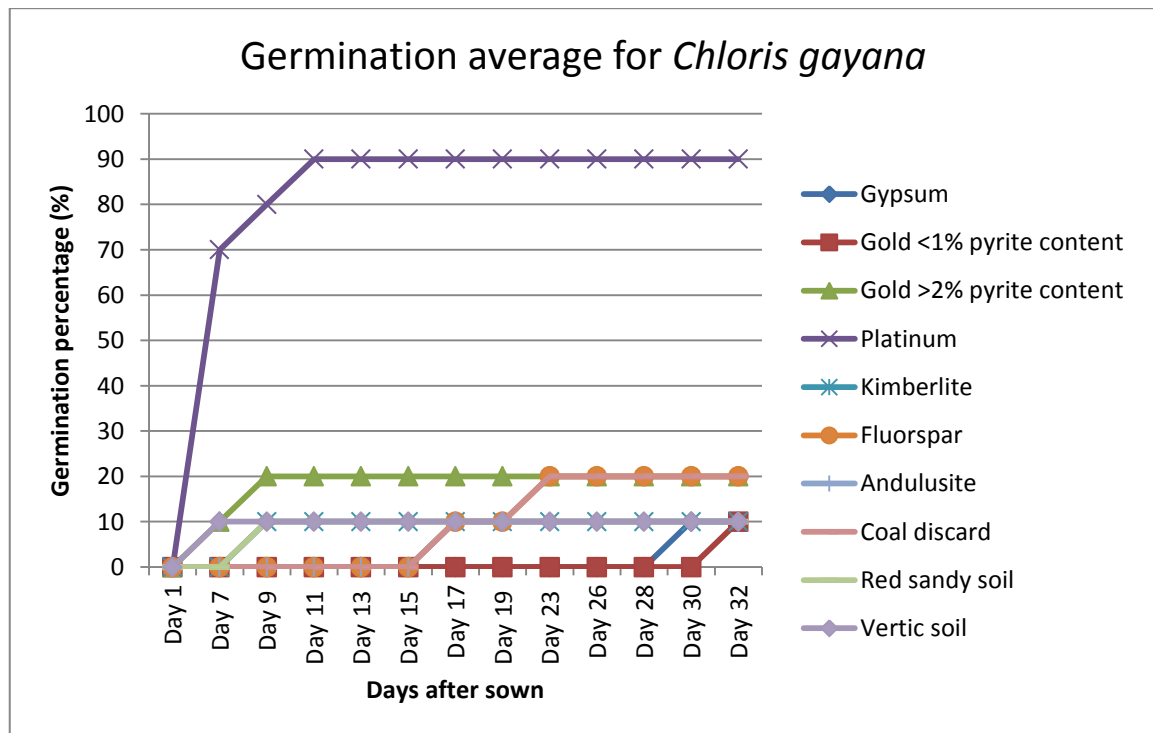
Excluding the platinum and andalusite seed batches, the initial germination percentages of the seed batches were significantly low (Table 4.4). The final germination percentages on day 14 from the germination tests differed very little with the initial germination percentages on day 5 (Table 4.4). On day 32 from the pot trial experiment, the germination percentages had increased, although it remained relatively low with the exception of the platinum seed batch for which a 90 % germination rate was recorded (Table 4.4 & Figure 4.3).

The low initial and final germination percentages (excluding those recorded for the platinum seed batch) indicates that the progeny seed from Rhodes grasses grown in different growth media has poor vigour and is not able to germinate. The progeny seed of the platinum seed batch germinated exceedingly well compared to progeny seed from the other seed batches. Due to the good germination results from the platinum seed batch and the poor germination results of the remainder of the seed batches, variance is anticipated. The Repeated Measures ANOVA statistical analysis indicated that there was a significant influence of the growth media in which the parent grass were grown as a variable on the germination of Rhodes grass progeny seed ( $p = 0.001$ ,  $f = 38.47$ ).

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**Table 4. 4: Results of the average percentages (%) for the first count (4days) and final day (14 days) of germination for *Chloris gayana* (Rhodes grass) seed batches and pot trial germination (%) after 32 days.**

		T1 - Gypsum	T2 - Gold <1% pyrite content	T3 - Gold >2% pyrite content	T4 - Platinum	T5 - Kimberlite	T7 - Fluorspar	T8 - Andalusite	T9 - Coal discard	TC - Red	TV - Vertic Clayey
Germination tests (top of paper planting method)	1st count 5 days %	2	3	4	19	0	0	18	0	0	0
	Final 14 days %	2	3	4	19	0	0	19	0	0	0
Pot trial germination	Final 32 days %	10	10	20	90	10	20	10	20	10	10



**Figure 4. 3: Average germination percentage (%) over a 32 day period for progeny seed harvested from *Chloris gayana* (Rhodes grass) grown in the different growth media.**

**(d) *Cenchrus ciliaris* L. (Buffalo grass)**

The initial germination percentages for Buffalo grass progeny seed were low (Figure 4.4 & Table 4.5). Seed from the kimberlite and the red soil seed batches were still germinating as late as day 30 during the pot trial experiment (Figure 4.4). From Figure 4.4 it is evident that progeny seed of Buffalo grass experienced a lag phase during germination and that the time interval thereof differed for the different seed batches. Despite the lag phase, the germination percentages recorded on day 28 during the germination test were still low for the gypsum and platinum seed batches (Table 4.5). This could indicate poor vigour for these seed batches; however, the platinum seed batch also had the highest recorded germination (50 %) during the pot trial experiment. Thus, the poor initial germination results could most likely be attributed by the lag phase that the progeny seed of Buffalo grass experienced.

There was very little difference between the initial- and final germination percentages for the vertic soil, andalusite, and the fluorspar seed batches (Table 4.5). For the remainder seed batches the difference between initial and final germination percentages were great.

The final germination percentages recorded during the pot trial experiment (day 32) ranged from 20 to 50 % for the different seed batches (Table 4.5). During the pot trial experiment the gypsum and the red soil seed batches had the greatest final germination percentages (50 %) while the lowest final germination percentage was recorded for the platinum seed batch (20 %).

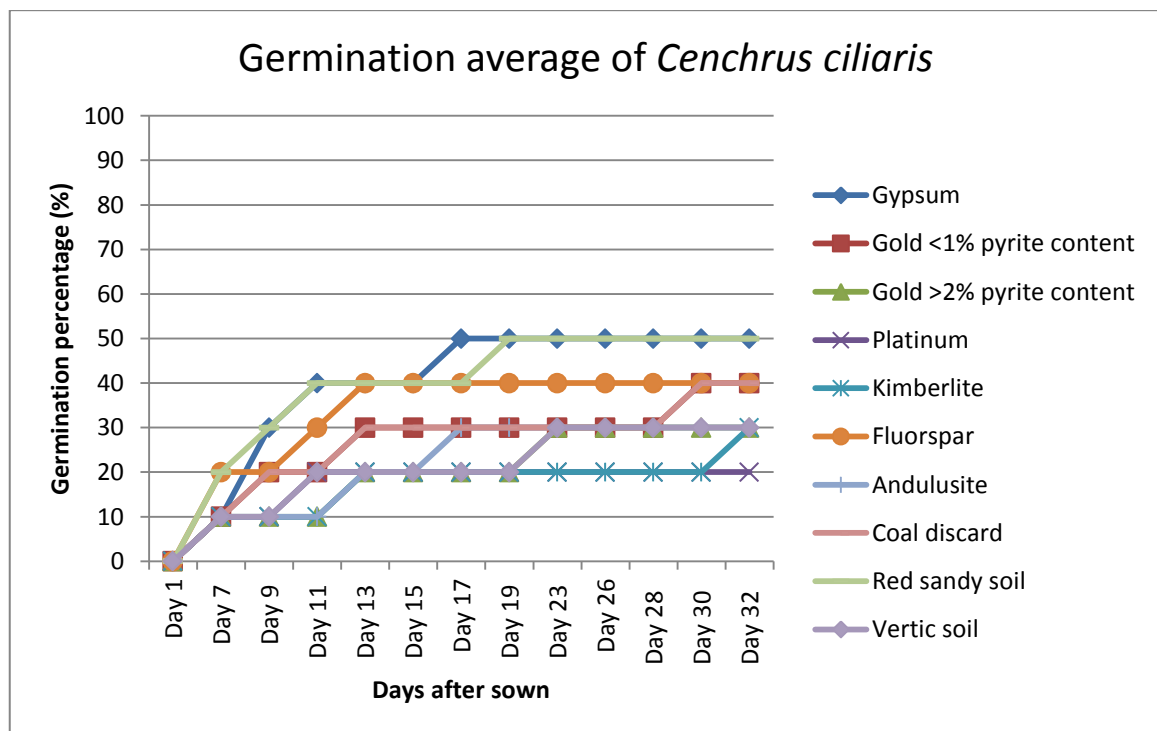
From Figure 4.4 it is evident that a great deal of variation in the germination percentages occurred among the different seed batches. The variation could simply have resulted from the short lag phase that the seed batches experienced during germination. Also, both the germination test and the pot trial experiment indicated that the lowest and greatest final germination percentages differed only with 30 % (Table 4.5).

The Repeated Measures ANOVA statistical analysis indicated no significant difference between the germination of the different seed batches for Buffalo grass ( $p = 0.0586$ ;  $f = 2.21$ ). However a variable is considered to have a significant influence on the outcome (in this instance the germination of the progeny seed) when the  $p$  value is less than 0.05. Thus, the  $p$  value of 0.0586 will be treated as a borderline case, and therefore it will be assumed that the growth media in which the parent Buffalo grasses were grown in, did have a significant influence in the germination of the different seed batches.

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**Table 4. 5: Results of the average percentages (%) for the first count (4days) and final day (28 days) of germination for *Cenchrus ciliaris* (Buffalo grass) seed batches and pot trial germination (%) after 32 days.**

		T1 - Gypsum	T2 - Gold <1% pyrite content	T3 - Gold >2% pyrite content	T4 - Platinum	T5 - Kimberlite	T7 - Fluorspar	T8 - Andulusiet	T9 - Coal discard	TC - Red	TV - Vertic Clayey
Germination tests (planted in sand)	1st count 6 days %	1	16	11	7	10	29	24	13	5	21
	Final 28 days %	4	23	20	9	17	33	35	19	16	32
Pot trial germination	Final 32 days %	50	40	30	20	30	40	30	40	50	30



**Figure 4. 4: Average germination percentage (%) over a 32 day period for progeny seed harvested from *Cenchrus ciliaris* (Buffalo grass) grown in the different growth media.**

**(e) *Digitaria eriantha* Steudel (Smuts Finger grass)**

The initial germination percentages for the progeny seed of Smuts Finger grass differs from the final germination percentages (Table 4.6). Some seed batches of Smuts Finger grass, in particular the kimberlite, coal discard and vertic soil seed batches, only reached a final germination percentage after day 15 during the pot trial experiment (Figure 4.5). The great difference between the first and final germination percentages as well as the low initial germination percentages, indicates poor seed vigour, especially for the gypsum, gold wastes with a high pyrite content, platinum, fluorspar, and coal discard seed batches, which had the greatest differences between the first and final germination percentages (Table 4.6).

The highest germination percentages from both the germination tests (which used the on top of paper planting method) and the pot trial experiment were recorded respectively for the fluorspar- and gypsum seed batches (Table 4.6). The kimberlite seed batch had the lowest germination percentage with only 13 % on day 14 during the germination test and 20 % on day 32 for the pot trial experiment (Table 4.6). The second lowest germination percentage was recorded for the vertic soil seed batch with 31 % on day 14 during the germination test and 10 % on day 32 during the pot trial experiment (Table 4.6).

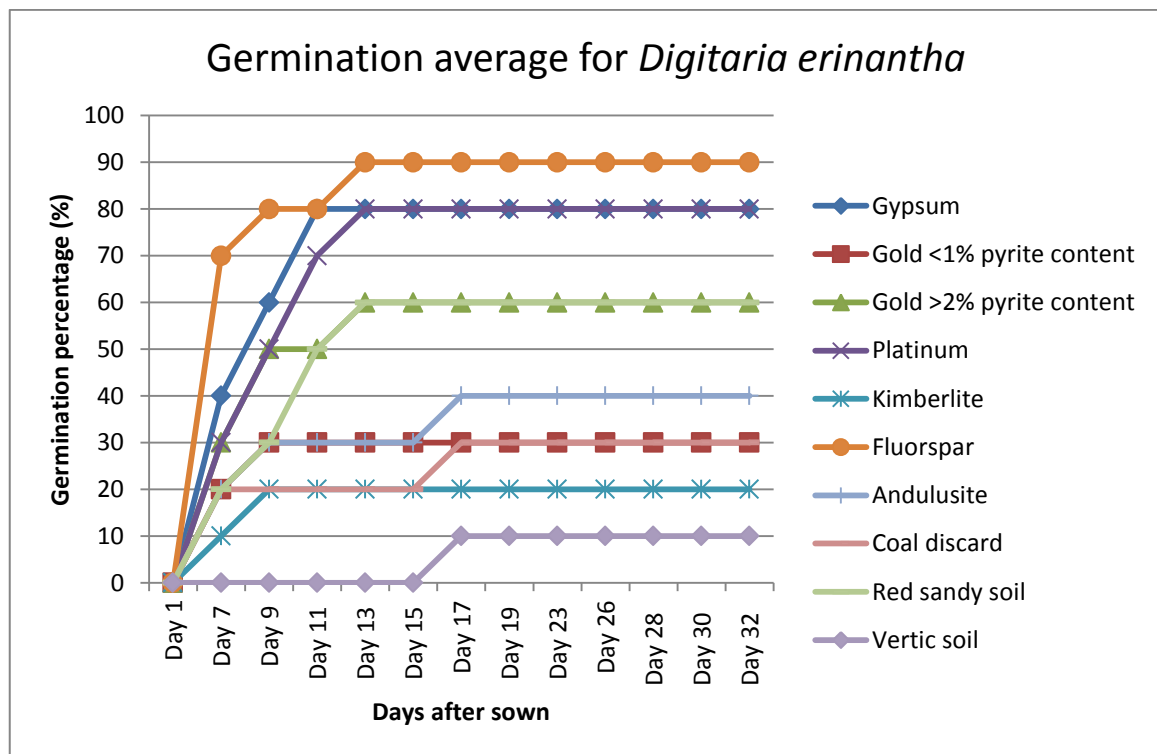
The final germination percentages (recorded on day 14) from the germination tests were lower compared to the final germination percentages (recorded on day 32) from the pot trial experiment for the gypsum, gold with a high pyrite content, platinum, fluorspar, and red soil seed batches. This might be due to the longer trial period of the pot trial experiment, which exceeds the trial period of the germination tests with 18 days, allowing more time for the progeny seed of Smuts Finger grass to germinate and consequently recording higher germination rates.

The results from the germination tests and the pot trial experiments showed that the germination percentages of the different seed batches differs significantly, with the difference between final recorded germination percentages ranging from 10 % (vertic seed batch on day 32) to 90 % (fluorspar seed batch on day 32). The Repeated Measures ANOVA statistical analysis indicated that there was a significant influence of the growth media in which the parent grass were grown as a variable on the germination of Smuts Finger progeny seed ( $p = 0.001$ ,  $f = 14.49$ ), which was anticipated due to the great variance in germination percentages between the seed batches.

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**Table 4. 6: Results of the average percentages (%) for the first count (4days) and final day (14 days) of germination for *Digitaria eriantha* (Smuts Finger grass) seed batches and pot trial germination (%) after 32 days.**

		T1 - Gypsum	T2 - Gold <1% pyrite content	T3 - Gold >2% pyrite content	T4 - Platinum	T5 - Kimberlite	T7 - Fluorspar	T8 - Andulusiet	T9 - Coal discard	TC - Red	TV - Vertic Clayey
Germination tests	1st count 5 days %	25	23	21	26	7	54	7	30	36	16
	Final 14 days %	60	40	38	49	13	58	44	51	50	31
Pot trial germination	Final 32 days %	80	30	60	80	20	90	40	30	60	10



**Figure 4. 5: Average germination percentage (%) over a 32 day period for progeny seed harvested from *Digitaria eriantha* (Smuts Finger grass) grown in the different growth media.**

**(f) *Sorghum bicolor* (L.) Moench (Sorghum)**

The initial germination percentages for the Sorghum progeny seed differs very little from the final germination percentages (Figure 4.6). This is verified by the results obtained from the germination tests which also showed little difference between the first count and final count of germination percentages (Figure 4.6). The results from the germination tests indicated that the progeny seed from Sorghum plants which grew in fluorspar mine wastes were green, and no germination for this seed batch were observed and recorded in either the germination tests or the germination pot trial (Figure 4.6).

The highest germination percentages (97 % – 99 %) were recorded for progeny seed from gypsum wastes for both the germination tests and the germination pot trial (Figure 4.1 & Figure 4.6). Compared to the germination tests, the pot trial presented observable lower germination percentages for the remainder seed batches (Table 4.7).

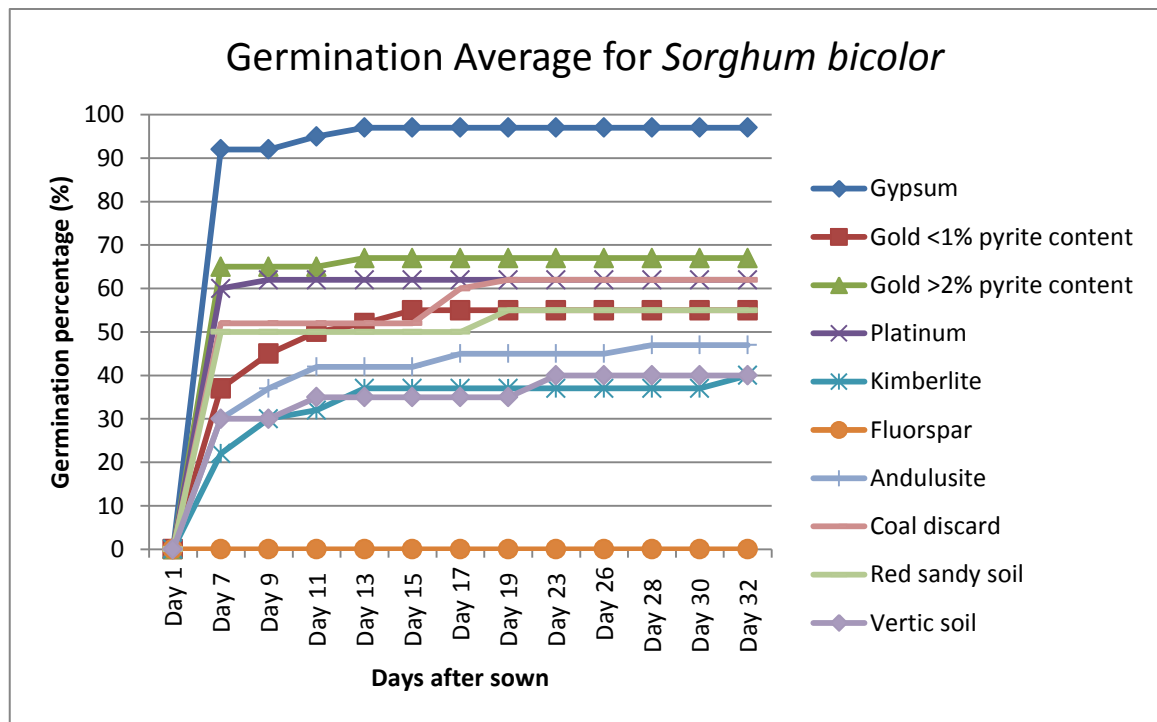
Excluding the fluorspar seed batch, which contained green and potentially immature seed, the lowest germination percentages were recorded for the kimberlitic and vertic soil seed batches during the pot trial with an average of 40 % each. However, these seed batches scored an germination percentage significantly higher during the germination tests which made use of a between paper planting method (95 % germination average for the kimberlite seed batch; 93 % germination average for the vertic soil seed batch) (Table 4.7).

According to the Repeated Measures ANOVA statistical analysis, there was a significant influence of the growth media in which the parent grass were grown as a variable on the germination of Sorghum progeny seed ( $p = 0.0138$ ;  $f = 2.89$ ).

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**Table 4. 7: Results of the average percentages (%) for the first count (4days) and final day (10 days) of germination for *Sorghum bicolor* (Sorghum) seed batches and pot trial germination (%) after 32 days.**

		T1 - Gypsum	T2 - Gold <1% pyrite content	T3 - Gold >2% pyrite content	T4 - Platinum	T5 - Kimberlite	T7 - Fluorspar	T8 - Andalusite	T9 - Coal discard	TC - Red	TV - Vertic Soil
Germination tests (between paper planting method)	1st count 4 days %	97	82	97	93	93	0	92	80	70	86
	Final 10 days %	99	86	99	98	95	0	98	99	94	93
Pot trial germination	Final 32 days %	97	55	67	62	40	0	47	62	55	40



**Figure 4. 6: Average germination percentage (%) over a 32 day period for progeny seed harvested from *Sorghum bicolor* (Sorghum) grown in the different growth media.**

**(g) Eragrostis tef (Zucc.) Trotter (Tef)**

The initial germination percentages for the Tef progeny seed batches differ very little from the final germination percentages (Table 4.8 & Figure 4.7). This is verified by the results obtained from the germination tests which also showed little difference between the first count and final count of germination percentages (Table 4.8).

The germination average for the progeny seed of Tef grass was overall high ranging from 70 % for the andalusite seed batch to 100 % for the gold, kimberlite, fluorspar, and coal seed batches. All seed batches germinated during both the germination tests and the pot trial, indicating that all progeny seed of Tef grown in all the growth media are matured and able to germinate. Thus the assumption can be made that the time period and method of harvest (done manually by cutting the plumes) did not influence the germination results obtained.

The lowest germination percentage was recorded from the pot trial results for the andalusite seed batch with a germination average of 70 %. Using the on top of paper planting method, the germination tests indicated an alternatively higher germination average (96 %) for the same andalusite seed batch. Furthermore, the Repeated Measures ANOVA statistical analysis indicated no significant difference between the germination of the different seed batches for Tef ( $p = 0.2957$ ;  $f = 1.26$ ).

**Table 4. 8: Results of the average percentages (%) for the first count (4days) and final day (10 days) of germination for *Eragrostis tef* (Tef grass) seed batches and pot trial germination (%) after 32 days.**

		T1 - Gypsum	T2 - Gold <1% pyrite content	T3 - Gold >2% pyrite content	T4 - Platinum	T5 - Kimberlite	T7 - Fluorspar	T8 - Andalusite	T9 - Coal discard	TC - Red	TV - Vertic Clayey
Germination tests (on top of paper planting method)	1st count 3 days %	89	85	94	96	92	93	96	94	91	77
	Final 10 days %	89	86	95	97	94	93	96	95	92	82
Pot trial germination	Final 32 days %	80	90	100	90	100	100	70	100	90	90

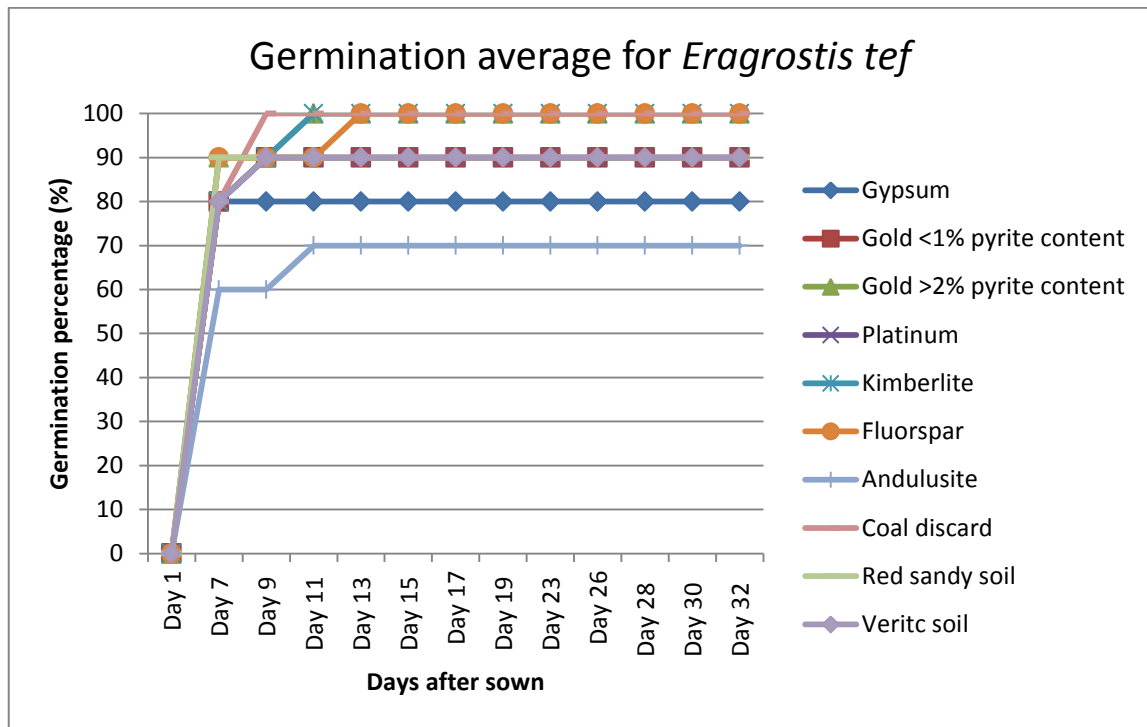


Figure 4. 7: Average germination percentage (%) over a 32 day period for progeny seed harvested from *Eragrostis tef* (Tef grass) grown in the different growth media.

#### (h) *Hyparrhenia hirta* (Common Thatching grass)

Overall very low germination averages were recorded on the final count day for both the germination tests and the pot trial (Table 4.9 & Figure 4.8). During the pot trial some seed batches (veritic soil, coal discard and the kimberlite seed batches) only germinated as late as 30 days into the trail period (Figure 4.8). The highest germination averages recorded was from the pot trial and never exceeded 10 % (Table 4.9). The low germination percentages recorded during the first count is indicative of poor seed vigour for the progeny seed of Common Thatching grass.

Green seed with empty caryopsis were found in several of the seed batches during the germination testing (Table 4.9). The green seed found in the seed batches accounts for the low germination rates of the progeny seed from Common Thatching grass.

It is highly probable that the time of harvesting did not accord with the time period during which intact seeds matures and dries. The assumption is that the seed were harvested mistakenly while it was still green and immature.

The green and potentially immature seed found in the seed batches of Common Thatching grass accounts for the low germination results from both the pot trial experiment and the germination

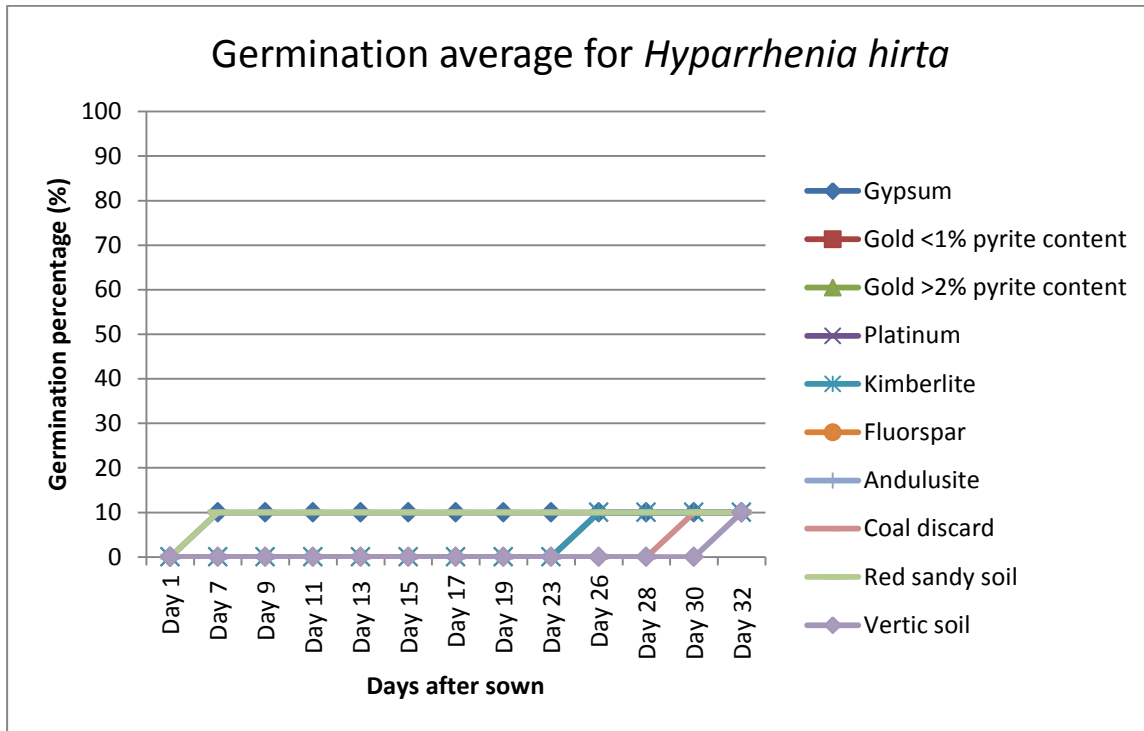
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tests. The Repeated Measures ANOVA statistical analysis indicated that there is no significant difference between the germination results for the different seed batches for Common Thatching grass ( $p = 0.0833$ ,  $f = 1.94$ ), which is expected with regard to the low germination rates and green seed found in the seed batches.

**Table 4. 9: Results of the average percentages (%) for the first count (4days) and final day (21 days) of germination for *Hyparrhenia hirta* (Common Thatching grass) seed batches and pot trial germination (%) after 32 days.**

		T1 - Gypsum	T2 - Gold <1% pyrite content	T3 - Gold >2% pyrite content	T4 - Platinum	T5 - Kimberlite	T7 - Fluorspar	T8 - Andalusite	T9 - Coal discard	TC - Red	TV - Vertic Clayey
Germination tests (on top of paper planting method)	1st count 5 days %	5	1	x*	2	x*	x*	1	1	4	x*
	Final 21 days %	6	1	x*	2	x*	x*	1	1	5	x*
Pot trial germination	Final 32 days %	10	0	0	10	10	0	0	10	10	10

\*Seeds are green with empty caryopsis



**Figure 4. 8: Average germination percentage (%) over a 32 day period for progeny seed harvested from *Hyparrhenia hirta* (Common Thatching grass) grown in the different growth media.**

### 4.3 Re-growth

The re-growth of parent grasses, after cutting the above-ground biomass, was determined in order to evaluate the ability of established grasses to re-grow after an incident such as harvesting (similar to the cutting event). Vegetation cover on TSF's is not isolated from such incidents and can be regarded as an anticipated risk. Therefore, during phytostabilization, it is critical to select species with the ability to grow after a cutting event.

The re-growth of parent grasses was classified into two groups (re-growth and no re-growth respectively) according to observable above-ground biomass, six months after it was cut. This evaluation of the re-growth from the parent grasses was done intentionally to replicate current rehabilitation monitoring practices. Ruiz-Jean and Aide (2005:574) reviewed restoration studies and found that most failed to measure vegetation structure and density, and that very few projects continued to monitor restoration success after three years. Thus, the assumption is that vegetation growth is not adequately measured according to height, cover, and biomass. Therefore, the method chosen for measuring the re-growth was based on the assumption and probability that current monitoring practices are insufficient and lacks coherence with scientific measurements.

The manner in which the re-growth was evaluated was subjective, and therefore the results will be used in isolation from the other objectives for this study. The re-growth data was not statistically analysed due to its lack in objectivity. Ten replicates were planted for each species in every growth medium, therefore, the counts per growth medium adds up to ten.

Sorghum is an annual species, i.e. it completes its life cycle, from germination to the production of seed, within one year, after which it dies. The manual cutting was done after the parent grasses reached the reproductive stage. Thus, when the manual cutting commenced, the sorghum plants had already completed its life cycle. Due to this, the sorghum plants were excluded from the re-growth experiments as it was anticipated that no re-growth would occur after the manual cutting as the sorghum plants have already completed its life cycle.

#### (a) *Eragrostis curvula* (Schrad.) Nees. (Weeping Love grass)

Table 4.10 presents the count of Weeping Love grass replicates from each growth medium that had shown observable re-growth after the cutting event. Re-growth, in the form of above-ground biomass, was observed for the majority replicates in all of the growth media (Table 4.10). This

was anticipated as Weeping Love grass is a perennial grass that establishes easily in disturbed areas and a wide range of soils.

**Table 4. 10: Re-growth counts from ten replicates for each growth medium for *Eragrostis curvula* (Weeping Love grass).**

	Re-growth	No re-growth
T1 - Gypsum	9	1
T2 - Gold <1% pyrite content	10	
T3 - Gold >2% pyrite content	9	1
T4 - Platinum	9	1
T5 - Kimberlite	9	1
T7 - Fluorspar	8	2
T8 - Andalusite	9	1
T9 - Coal discard	9	1
TC - Red	9	1
TV - Vertic Clayey	9	1

**(b) *Cynodon dactylon* (L.) Pers. (Couch grass)**

Couch grass creeps with both stolons (surface creepers) and rhizomes (underground creepers). Thus, it is highly possible that observed re-growth could have occurred through the emergence of new plants at the nodes of stolons and rhizomes. During the re-growth monitoring there was no distinction made between re-growth of cut grass and the emergence of new plants from the nodes of stolons and rhizomes. Due to the hostile conditions of the growth media (with special reference to the mine wastes materials) growth in any form, whether from the established plants that were cut or from new emerging plants at the nodes of stolons and rhizomes, is regarded as an indicator of good growth vigour.

Couch grass replicates showed observable re-growth in all the growth media (Table 4.11). The lowest re-growth count (6) of couch grass replicates were recorded from the gold wastes with high pyrite content, the kimberlite wastes, and the vertic clay soil (Table 4.11).

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**Table 4. 11: Re-growth counts from ten replicates for each growth medium for *Cynodon dactylon* (Couch grass).**

	Re-growth	No re-growth
T1 - Gypsum	8	2
T2 - Gold <1% pyrite content	7	3
T3 - Gold >2% pyrite content	6	4
T4 - Platinum	10	
T5 - Kimberlite	6	4
T7 - Fluorspar	10	
T8 - Andalusite	9	1
T9 - Coal discard	9	1
TC - Red	8	2
TV - Vertic Clayey	6	4

**(c) *Chloris gayana* Kunth. (Rhodes grass)**

Rhodes is a stoloniferous perennial grass, therefore, re-growth of replicates from all the growth media were anticipated, whether the growth occurred from new plants that emerged from the nodes of stolons, or from re-growth of the existing cut grasses.

Rhodes grass replicates showed excellent observable re-growth in all the growth media (Table 4.12). The lowest re-growth count (5) was recorded from the coal discard wastes and the vertic clay soil (Table 4.12).

**Table 4. 12: Re-growth counts from ten replicates for each growth medium for *Chloris gayana* (Rhodes grass).**

	Re-growth	No re-growth
T1 - Gypsum	6	4
T2 - Gold <1% pyrite content	8	2
T3 - Gold >2% pyrite content	9	1
T4 - Platinum	8	2
T5 - Kimberlite	6	4
T7 - Fluorspar	7	3
T8 - Andalusite	10	
T9 - Coal discard	5	5
TC - Red	9	1
TV - Vertic Clayey	5	5

**(d) *Cenchrus ciliaris* L. (Buffalo Grass)**

Table 4.13 presents the count of Buffalo grass replicates from each growth medium that had shown observable re-growth after the cutting event. Although re-growth was observed from replicates in each growth medium, the re-growth counts were lower than anticipated for this perennial grass (Table 4.13). The lowest count for observable re-growth from a single growth medium was 1 and was recorded for the gypsum wastes (Table 4.13). The majority replicates had no observable re-growth occur after the grasses were cut (Table 4.13).

**Table 4. 13: Re-growth counts from ten replicates for each growth medium for *Cenchrus ciliaris* (Buffalo grass).**

	Re-growth	No re-growth
T1 - Gypsum	1	9
T2 - Gold <1% pyrite content	4	6
T3 - Gold >2% pyrite content	2	8
T4 - Platinum	6	4
T5 - Kimberlite	3	7
T7 - Fluorspar	7	3
T8 - Andalusite	3	7
T9 - Coal discard	3	7
TC - Red	5	5
TV - Vertic Clayey	4	6

**(e) *Digitaria eriantha* Steudel (Smuts Finger Grass)**

Table 4.14 presents the count of Smuts Finger grass replicates from each growth medium that had shown observable re-growth after the cutting event. Re-growth, in the form of above-ground biomass, was observed for the majority replicates in all of the growth media (Table 4.14). This was anticipated as Smuts Finger grass is a perennial grass. The lowest count of observable re-growth was recorded for replicates from the andaluiste growth medium (Table 4.14).

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**Table 4. 14: Re-growth counts from ten replicates for each growth medium for *Digitaria eriantha* (Smuts Finger grass).**

	Re-growth	No re-growth
T1 - Gypsum	6	4
T2 - Gold <1% pyrite content	9	1
T3 - Gold >2% pyrite content	8	2
T4 - Platinum	8	2
T5 - Kimberlite	6	4
T7 - Fluorspar	10	
T8 - Andalusite	5	5
T9 - Coal discard	9	1
TC - Red	8	2
TV - Vertic Clayey	7	3

**(f) *Eragrostis tef* (Zucc.) Trotter (Tef)**

Table 4.15 presents the count of Tef grass replicates from each growth medium that had shown observable re-growth after the cutting event. Tef is an annual grass, thus no observable re-growth was anticipated as Tef had already completed its life cycle when the grasses were cut. Despite this, observable re-growth did occur for some replicates in all of the growth media excluding gypsum, and kimberlite wastes (Table 4.15) from which no replicates indicated any re-growth. The re-growth count per growth medium never exceeded 3, which is relatively low, but nonetheless good for an annual grass species such as Tef.

**Table 4. 15: Re-growth counts from ten replicates for each growth medium for *Eragrostis tef* (Tef).**

	Re-growth	No re-growth
T1 - Gypsum		10
T2 - Gold <1% pyrite content	3	7
T3 - Gold >2% pyrite content	1	9
T4 - Platinum	2	8
T5 - Kimberlite	1	9
T7 - Fluorspar		10
T8 - Andalusite	2	8
T9 - Coal discard	1	9
TC - Red	3	7
TV - Vertic Clayey	2	8

**(g) *Hyparrhenia hirta* L. (Satpf.) (Common Thatching grass)**

Table 4.16 presents the count of Common Thatching grass replicates from each growth medium that had shown observable re-growth after the cutting event. Re-growth, in the form of above-ground biomass, was observed for the majority replicates in all of the growth media (Table 4.16). This was anticipated as Common Thatching grass is a perennial grass. The lowest count (2) of observable re-growth was recorded for replicates from the kimberlite wastes growth medium (Table 4.16).

**Table 4. 16: Re-growth counts from ten replicates for each growth medium for *Hyparrhenia hirta* (Common Thatching grass).**

	Re-growth	No re-growth
T1 - Gypsum	7	3
T2 - Gold <1% pyrite content	5	5
T3 - Gold >2% pyrite content	8	2
T4 - Platinum	6	4
T5 - Kimberlite	2	8
T7 - Fluorspar	7	3
T8 - Andalusite	5	5
T9 - Coal discard	7	3
TC - Red	5	5
TV - Vertic Clayey	4	6

#### 4.4 Growth Media Analyses

This section addresses the second objective, namely to correlate the germination of the progeny seed with the properties of growth media in which the parent grass were grown, in order to evaluate the effect the growth media had on the viability of the progeny seed. As discussed in Chapter 3, representative samples from each growth medium were collected for analysis. The results from the growth media analyses will be reviewed briefly, thereafter the correlation between the germination of the progeny seed (discussed in section 4.2.1) and the growth media will be presented and reviewed.

##### 4.4.1 Properties of growth media

Soil is a complex medium (Winegardener, 1995:10). The inorganic solid phase is derived from parent rock that is degraded by weathering processes. Mine waste materials, is classified as an anthropogenic soil as it is not an inherent product from weathering of parent rock. All growth media samples were analysed according to the method described in section 3.7. The results will be discussed separately for each growth medium with reference to Table 4.17.

**Table 4. 17: Chemical analysis results for the ten different growth media in which the parent grasses were planted.**

Growth Media	pH (KCl)	EC mS/m	Al mg/kg	NO3- mg/l	F mg/l	Ca:Mg ratio	C:N ratio	K % of total CEC	Nett acid potential (ton/ha lime)	Total S %
Gypsum wastes	4.1	199	16.86	18.0	27.5	198	0.07:1	22.339	5	13.43
Gold tailings < 1 % pyrite	5.3	193	1.07	13.8	0.1	12	0.09:1	6.0352	15	0.26
Gold tailings > 2 % pyrite	6.4	422	0.01	168.6	0.1	5	3.28:1	13.811	116	1.03
Platinum tailings	8.0	205	0.01	48.5	0.0	5	0.00:1	6.6658	-32	0.03
Kimberlite mine wastes	7.6	121	0.01	7.4	1.2	5	2.32:1	19.390	-121	0.00
Fluorspar mine wastes	7.9	146	0.00	1.7	14.9	8	303:1	5.7978	-139	0.38
Andalusite mine wastes	5.5	11	0.01	16.0	0.1	1	4.79:1 42.44:	6.1987	3	0.00
Coal discard	3.8	390	574.50	13.3	0.0	6	1	8.1680	255	1.37
Red soil	4.1	13	8.36	48.8	0.0	1	3.37:1 52.02:	11.593	6	0.00
Vertic soil	7.6	55	0.19	4.4	1.0	0	1	1.2386	3	0.00

**(a) Gypsum Wastes**

Chemical analyses results are shown in Table 4.17.

The gypsum wastes collected from a fertilizer factory in the Potchefstroom area contains large amounts of calcium and sulphate ions. The gypsum used in this study is not of natural origin, but was generated as a by-product during the manufacturing of fertilizer from rock phosphate (Rodríguez-Jordá *et al.*, 2010:763).

The analyses of the gypsum wastes indicated a low pH (KCl) value of 4.1, however, the nett acid potential indicated a similar low value (5 ton/ha lime). The analyses indicated high levels of extractable Al which can be ascribed to the low pH value of the gypsum wastes as the mobility of Al increases with decreasing pH values. The saturated EC of the gypsum wastes were 199 mS/m<sup>-1</sup>. The ratio of Ca to Mg was significantly high due to the elevated amounts of calcium ions in the gypsum wastes which are essentially calcium sulphates. In contrast, the ratio of C to N was small, suggesting that gypsum wastes are deprived of organic carbon.

**(b) Gold tailings with low (< 1 %) pyrite content**

Chemical analyses results are shown in Table 4.17.

The ore rock, from which gold is mined, contains pyrite which is a sulphide mineral. This gold tailing materials has pyrite content of less than 1 %.

The growth medium analyses of gold tailings with low pyrite content indicated low pH (KCl) levels (5.3) with a slightly elevated saturated EC of 193 mS/m<sup>-1</sup>. The analyses showed low levels of extractable Al and similarly low levels of nett acid potential (15 ton/ha lime). Oxidation of pyrite produces sulphuric acid, however, this particular tailings contains less than 1 % pyrite, ultimately lowering the net acid generating potential thereof. The ratio of Ca to Mg was high (12:1), while the C to N ratio was low due to lack of organic carbon in the tailings.

**(c) Gold tailings with high (> 2 %) pyrite content**

Chemical analyses results are shown in Table 4.17.

This particular gold tailings material had a higher percentage (> 2%) of pyrite content compared to the previous discussed gold wastes. Due to the potential of oxidation of the pyrite, which is high in content, the net acid potential was exceedingly elevated (116 ton/ha lime) compared to the gold tailings with a low pyrite content. The pH is higher than that of the gold tailings with low pyrite content (T2) which is an indication that all the pyrite has not yet oxidised.

The pH (KCl) levels were slightly lower than neutral (6.4), but not to entirely acidic. In coherence with the pH levels, the extractable Al was low as well illustrating that at decreased pH levels, the solubility of Al increases. The saturated EC of the gold wastes with high pyrite content was elevated (422 mS/m<sup>-1</sup>) to levels associated with saline soils.

### **(d) Platinum tailings**

Chemical analyses results are shown in Table 4.17.

The ore from which platinum is mined, is associated with base metals, therefore the tailings will have a high base saturation. The platinum tailings had the highest pH (KCl) (8) for all the growth media. The saturated EC for the platinum tailings was 205 mS/m<sup>-1</sup> and a nett acid potential of -32 (ton/ha lime). This indicates that the platinum tailings will not be subjected to acidic conditions.

The carbon content of the platinum tailings is practically non-existent with a C to N ration of 0.00. The Ca to Mg ratio, however, is 5 indicating no severe deficiencies of Ca. The extractable Al was low (0.01 mg/kg) which may be due to the decreased mobility at the high levels of pH measured for the platinum tailings.

### **(e) Kimberlite mine wastes**

Chemical analyses results are shown in Table 4.17.

The kimberlite mine wastes are a waste product from kimberlitic ore which is mined for diamonds. The pH (KCl) measured for the kimerlite mine wastes is border line to neutral pH levels (7.6). Due to the neutral pH levels of the kimberlite mine wastes, the measured extractable Al was low (0.01 mg/kg). The saturated EC was also low (121 mS/m<sup>-1</sup>), as well as the C to N ratio which was 2.32. The Ca to Mg ratio, however, was 5 and slightly elevated compared to the C:N ratio.

Considering the pH and saturated EC, the kimberlite mine wastes do not seem to be as hostile to plant growth. With added amelioration to improve the carbon content, one expects plants to grow and establish easily in this wastes. The physical properties, however, contradicts this with the kimberlite mine wastes being a very coarse gravelled medium with poor moisture retention.

### **(f) Fluorspar mine wastes**

Chemical analyses results are shown in Table 4.17.

Fluorspar mine wastes are the waste product from Fluor extraction. The pH (KCl) for the fluorspar wastes were 7.9 which is slightly more alkaline. This alkalinity is reflected in the excessively low nett acid potential (-139 ton/ha lime) for the fluorspar mine wastes. It is clear that the fluorspar mine wastes are not acid generating and in accordance the extractable Al was practically non-existent (0.00 mg/kg). The C:N ratio was significant for the fluorspar mine wastes (303:1). The saturated EC was 143 mS/m<sup>-1</sup>.

### **(g) Andalusite mine wastes**

Chemical analyses results are shown in Table 4.17.

Andalusite mine wastes are a by-product from aluminium mining. The pH (KCl) for the andalusite mine wastes were low (5.5), but not entirely acidic or acid generating as the nett acid potential was only 3 ton/ha lime. The saturated EC (11 mS/m<sup>-1</sup>) as well as the extractable Al (0.001 mg/kg) were low (Table 4.17). The C:N ratio (4.79) was greater than the Ca:Mg (1) ratio.

### **(h) Coal discard**

Chemical analyses results are shown in Table 4.17.

The coal discard is naturally rich in carbon, which explains the high C:N ratio of 42.44. The pH (KCl) of the coal discard material was significantly low (3.8) indicating acidic conditions. The nett acid potential was high for the coal discard material (255 ton/ha lime) indicating that is acid generating. The low pH and the potential generation of acid can be ascribed to sulphide minerals associated with coal seams. The coal discard materials had the highest amount of extractable Al (574.50 mg/kg). The saturated EC was elevated at 390 mS/m<sup>-1</sup> which is at levels similar to saline soils.

### **(i) Red soil**

Chemical analyses results are shown in Table 4.17.

The measured pH (KCl) (4.1) and saturated EC ( $13 \text{ mS/m}^{-1}$ ) for the red soil was low, which is indicative of the prevalent oxidizing conditions of this soil. The low EC is characteristic of this apedal soil. It had a low Ca: Mg ratio of 1, and a C: N ratio of 3.37. The extractable Al was 8.38 mg/kg. It had a nett acid potential of 6 ton/ha lime.

### (j) Vertic clayey soil

Chemical analyses results shown in Table 4.17.

The pH (KCl) for the vertic soil was relatively neutral at a value of 7.6. The saturated EC was low at  $55 \text{ mS/m}^{-1}$ . The Ca:Mg ratio was small, which was unexpected as the black colour of this soil can be ascribe to calcium-humate complexes that form from the combination of calcium with humic material (Fey, 2010:37). The C:N ratio was elevated at 52. In accordance with the neutral pH, the nett acid potential of the vertic soil was low (3 ton/ha lime).

### 4.4.2 Particle Size Distribution of Growth Media

Particle size distribution is probably the most basic tool for soil description as it measures the size range of mineral components of a soil. It is an inherent property of soil, and together with soil mineralogy, it regulates a soil's ability to preserve SOM and retain moisture (Gobat *et al.*, 2004:48; Winegardener, 1995:15).

The growth media analyses revealed that in terms of particle size distribution, for particles < 2mm, the kimberlite mine wastes differ greatly from any other growth media used in this study. The very coarse sand (34.6 %) and coarse sand (33.2 %) fractions of the kimberlite mine wastes make up most of this growth medium's particle composition and also made it the most coarse growth medium used during this study (Table 4.18).

The andalusite mine wastes showed similarities with the coal discard in terms of particle size distribution for particles < 2mm (Table 4.18). The largest silt content was found in the andalusite mine wastes (68.6 %), which also had a relatively large clay percentage (15.2 %). Similarly, the coal discard had a relatively large silt (20.9 %) and clay (19.7 %) percentage.

Little difference was found between the two gold tailings in terms of particle size distribution. Both had high percentages for the fine sand, very fine sand, and silt size classes (Table 4.18).

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According to the particle size distribution analysis, gypsum wastes had the largest percentage for one size class (76.6% very fine sand) giving it the smallest particle size distribution and the most homogenous texture. In reality this is not a true reflection of the general behaviour of gypsum wastes, because most of the material is soluble with severe hydro-physical disturbance.

The apedal properties of the red soil was confirmed by the particle distribution analysis which found that the red soil had the most equal particle distribution between the sand size classes (Table 4.18) which makes it a poorly sorted soil.

The platinum tailings and fluorspar mine wastes showed similarities in the particle distribution analysis. Both had a high percentage in the fine sand and very fine sand size classes (Table 4.18), indicating that both wastes are well sorted. The vertic soil had the largest percentage particles allocated in the clay fraction, indicating that it has high levels of clay content.

**Table 4. 18: Particle size distribution for the various growth media**

Growth Media		Very coarse sand %	Coarse sand %	Medium sand %	Fine sand %	Very fine sand %	Silt %	Clay %
Gypsum	T1	0.0	0.3	0.5	0.9	76.6	14.7	6.9
Gold < 1 % Pyrite	T2	0.2	0.6	2.9	33.9	33.5	24.2	4.6
Gold > 2 % Pyrite	T3	0.1	0.6	6.5	45.7	33.2	11.8	2.1
Platinum	T4	0.0	0.3	8.7	48.0	33.4	7.4	2.1
Kimberlite	T5	34.6	33.2	13.7	7.7	3.6	2.9	4.3
Fluorspar	T7	0.1	2.3	16.0	36.0	31.3	12.2	2.2
Andalusite	T8	2.2	4.0	3.4	2.4	4.1	68.6	15.2
Coal	T9	1.1	6.0	13.1	21.2	18.0	20.9	19.7
Red Soil	TC	0.5	4.1	25.3	38.2	24.5	2.9	4.3
Vertic Soil	TV	1.9	3.5	9.8	15.9	17.7	13.3	37.9

#### 4.5 Correlation between germination results and growth media analyses

This section addresses the first two objectives, namely to evaluate the viability and germination of progeny seed, and to correlate it with the properties from the growth media in which the parent grass were grown, in order to evaluate the effect the growth media had on the viability of the progeny seed. As mentioned in Chapter 3 (section 3.3), the progeny seed were harvested from the parent grasses which grew in different growth media (mine wastes materials and two control soils). This section will review the results from non-parametric correlation statistical analyses between the germination results of the progeny seed from the pot trial and the analyses of the growth media in which the parent grasses were grown in.

It is anticipated that the maternal environment attributed by the properties of mine waste will influence seed development, thus poor seed viability and potential sterility is anticipated for the offspring seed from grasses grown in mine waste materials. To determine the relationship between the properties of the growth media and the germination of progeny seed, non-parametric correlations were done.

The Spearman's correlation is a non-parametric measure of the strength and direction of association that exists between two variables measured on at least an ordinal scale. A correlation coefficient ( $\rho$ ) of +1 indicates that the two variables are perfectly positively correlated; i.e. if one variable increases, the other increases by a proportionate amount. Conversely, a coefficient of -1 indicates a perfect negative relationship: if one variable increases, the other decreases by a proportionate amount (Field, 2013:266).

An effect size measures the size of an effect, such as the strength of a relationship between two variables (Field, 2013:79). Spearman's correlation ( $\rho$ ) quantifies the relationship between two variables i.e. it quantifies the effect of one variable on the outcome of another variable; thus it can also be interpreted as an effect size.

An effect size of  $\rho = .10$  indicates that the effect explains 1 % of the total variance, thus it indicates a small effect. An effect size of  $\rho = .3$  indicates that the effect explains 9 % of the total variance, thus it indicates a medium effect. An effect size of  $\rho = .5$  indicates that the effect accounts for 25 % of the variance, thus it indicates a large effect (Field, 2013:82).

The germination of progeny seed from each species were correlated with selected properties of each of the growth media. A brief review of the results from the non-parametric correlations will follow.

The non-parametric correlations found that significant relationships existed between the growth media properties themselves. The pH (KCl) was significantly and negatively related to the Al content ( $p = 0.001$ ;  $\rho = -.908$ ), which simply put indicates that a decrease in pH levels will be accompanied by a proportionate increase of Al content. This confirms that at low pH levels, the mobility of Al increases. The large effect size ( $< .5$ ) indicates a strong relationship between pH and Al. Similarly the pH had a negative ( $\rho = -.728$ ) and significant ( $p = 0.026$ ), relationship with the nett acid potential, indicating that a decrease in pH values would result in a increase in the nett acid potential. The saturated EC was positively ( $\rho = .736$ ) and significantly ( $p = 0.017$ ) related with the total S (sulphide). An increase in the EC would be followed by a proportionate increase of total S.

### **4.5.1 *Eragrostis curvula* (Schrad.) Nees (Weeping Love grass)**

There was no significant relationship found between the germination of progeny seed from Weeping Love grass and the properties of the growth media. Some properties of the growth media did, however, have a large size effect on the germination of the progeny seed. For instance the saturated EC had a positive relationship with the germination of progeny seed and had a large effect size ( $\rho = .596$ ). This indicates that the germination of the progeny seed increased proportionally to an increase in the saturated EC of the growth medium. Similarly, the total S (Sulphur) also had a large effect size ( $\rho = .519$ ) on the germination of the progeny seed, but the positive relationship between these variables were not significant.

### **4.5.2 *Cynodon dactylon* (L.) Pers. (Couch grass)**

There was no significant relationship found between the germination of progeny seed from Couch grass and the properties of the growth media. Some properties of the growth media did, however, have a medium size effect on the germination of the progeny seed.

The F content was not significantly related to the germination, however, it did have a positive large size effect on the germination of progeny seed from Couch grass ( $\rho = .537$ ). This indicates that the germination of the progeny seed increased proportionally to an increase in the F content of the growth medium. Other properties found to have a negative medium size effect

on the germination of Couch grass progeny seed, were Al content ( $\rho = -.317$ ), K (potassium) content ( $\rho = -.468$ ), and the nett acid potential ( $\rho = -.349$ ). A decrease in these properties would result in a proportionate, increase in the germination of Couch grass progeny seed.

#### **4.5.3 *Chloris gayana* Kunth. (Rhodes grass)**

Only the saturated EC had a significant ( $p = 0.04$ ) positive relationship with the germination of Rhodes progeny seed with a correlation coefficient of .688. Thus, an increase of EC would be met with a proportional increase in the germination of Rhodes grass progeny seed.

No significant relationships were found between the germination of progeny seed and the remainder growth media properties. Some properties of the growth media did, however, have a medium size effect on the germination of the progeny seed.

The F content was not significantly related to the germination, however, it did have a negative medium size effect on the germination of progeny seed from Couch grass ( $\rho = -.427$ ). This indicates that the germination of the progeny seed increased proportionally to a decrease in the F content of the growth medium. Both the pH ( $\rho = .332$ ) and the total S content ( $\rho = .364$ ) properties, had a positive medium sized effect on the germination of Rhodes grass progeny seed.

#### **4.5.4 *Cenchrus ciliaris* L. (Buffalo grass)**

The saturated EC had a significant ( $p = 0.029$ ) positive relationship with the germination of progeny seed from Buffalo grass with a correlation coefficient of .759. Thus, an increase of EC would be met with a proportional increase in the germination of Buffalo grass progeny seed.

The total S content was the only other property that had a significant ( $p = 0.006$ ) positive relationship with the germination of progeny seed with a correlation coefficient of .864. This indicates that an increase of total S content would be met with a proportional increase in the germination of Buffalo grass progeny seed.

Though not significant, the F content did have a large size effect ( $\rho = .530$ ) on the germination of progeny seed. This indicates that the germination of the progeny seed increased proportionally to an increase in the F content of the growth medium.

#### **4.5.5 *Digitaria eriantha* Steudel (Smuts Finger grass)**

There was no significant relationship found between the germination of progeny seed from Smuts Finger grass and the properties of the growth media. Some properties of the growth media did, however, have a medium size effect on the germination of the progeny seed.

The F content was not significantly related to the germination of the progeny seed, however, it did have a positive medium size effect on the germination of progeny seed from Smuts Finger grass ( $\rho = .328$ ). This indicates that the germination of the progeny seed increased proportionally to an increase in the F content of the growth medium. Other properties found to have a medium size effect on the germination of Smuts Finger grass progeny seed, were pH ( $\rho = .326$ ), and the total S content ( $\rho = .373$ ). A decrease in these properties would result in a proportionate, decrease in the germination of the Smuts Finger grass progeny seed.

Another medium size effect was indicated for the nett acid potential, which also had a negative relationship ( $\rho = -.333$ ) with the germination of progeny seed. Although not significant, a negative medium size effect indicates that the nett acid potential accounts for a medium size of the variance in the germination of progeny seed from Smuts Finger grass.

#### **4.5.6 *Sorghum bicolor* (L.) Moench (Sorghum)**

A significant negative relationship was found between the germination of Sorghum progeny seed and the pH ( $p = 0.024$ ;  $\rho = -.734$ ). This indicates that a decrease in pH would be met by a proportionate increase of germination in Sorghum progeny seed.

Contradictive to the negative association between the germination of Sorghum progeny seed and the pH, a significant positive relationship was found between the germination of progeny seed and Al content ( $p = 0.019$ ;  $\rho = .755$ ). Therefore an increase in Al would be met by a proportionate increase in germination of Sorghum progeny seed.

#### **4.5.7 *Eragrostis tef* (Zucc.) Trotter (Tef)**

There was no significant relationship found between the germination of progeny seed from Tef and the properties of the growth media. The nitrate (NO<sub>3</sub><sup>-</sup>) content of the growth media did, however, have a large size effect on the germination of the progeny seed.

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The nitrate content was not significantly related to the germination, however, it did have a positive large size effect on the germination of progeny seed from Tef ( $\rho = .502$ ). This indicates that the germination of the progeny seed increased proportionally to an increase in the nitrate content of the growth medium.

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## Discussion and Conclusion

### 5.1 Introduction

Seed production and quality are critical for species persistence (Mayer & Poljakoff-Mayber, 1989:43). Bishaw *et al.* (2012:656) describe seed quality as the sum total of many aspects, among which are genetic, physical, physiological, and health quality. Seed vigour, which is directly related to seed quality, can be affected by various factors, namely genetics, physiological, cytological and pathological factors (Maguire, 1977:216). According to Maguire (1977:220) seed that is mechanically sound and capable of germinating promptly to produce developing seedlings able to emerge under favorable and unfavorable environmental conditions are vigorous. Seed vigour is a function of a variety of factors to which the parent plant is subjected to during seed formation and maturation (Mayer & Poljakoff-Mayber, 1989:43).

The effects of the maternal environment may be induced by nutrient supply, light availability and quality, or competitive interaction; and may affect seed, as well as seedling and adult traits (Wulff *et al.*, 1994:763). Environmental conditions experienced by the maternal plant during the growth season plays a significant role in determining subsequent germination rates in seeds (El-Keblawy *et al.*, 2009:11). These induced environmental maternal effects can be mediated through to the embryo via storage reserves, hormones, enzymes, and toxins in seeds (Latzel *et al.*, 2009:1669).

Mine waste materials originate from freshly ground rock and not from parent rocks which has been subjected to natural weathering. Furthermore, such mine waste materials consist mainly of primary minerals, unlike conventional soils which contain great amounts of secondary minerals (Van Deventer & Hattingh, 2004:64). Due to mine waste materials being subjected to milling and metallurgic processes, it is characterized by elevated concentrations of metals, extreme pH conditions, lack of organic content, nutrients and microbial activity (Mendez & Maier, 2008:278). As such the combined characteristics of mine waste materials are rarely found in natural conditions, and are an unhealthy growth medium for vegetation. Thus, the characteristics of mine waste material can potentially influence the viability of seed still intact on the mother plant by contributing to environmental-induced maternal effects.

The environmental conditions to which the parent plant is directly subjected to, is known as the maternal environment and will influence seed traits such as vigour and viability. Soil, as a growth medium for plants, accounts for some of the maternal environmental factors experienced by the parent plant. Traits of offspring seed depend on the abiotic environment attributed by the growth medium during seed development and maturation (El-Keblawy *et al.*, 2009:11; Wang *et al.*, 2012:170). Certain properties of soil are known to influence and affect plant growth, and consequently seed production and the quality thereof. These prevailing properties include the nutrient status of the soil, salinity, the pH value of the soil, contaminants in the soil and potential toxicity thereof, and its ability to retain and release moisture to the plant.

The aim of this study was to determine the viability of seed produced by a previous generation of grass species established in eight different mine waste materials and two soils, in order to identify suitable species for specific mine waste materials to ensure long-term survival through means of seed production. This chapter will discuss and review the results reported in Chapter 4.

### **5.2 Interaction between properties of the growth media**

Soil quality is an inherent function of its properties, and can be assessed by parameters, such as the physical and chemical properties of the soil (Karlen *et al.*, 1997:6; Moussa. 2007:3). The quality of a soil is partially determined by its inherent composition which is a function of soil forming factors and geological materials (Van Deventer & Hattingh, 2004:64).

Due to its origin, mine waste materials consist mainly of milled ore rock, and consequently it lacks these inherent properties derived from soil forming factors and geological materials. Additionally, it has adverse properties, such as lack of organic matter and nutrients, extreme pH conditions, and poor structure. Therefore, the capacity of mine waste materials to support and maintain plant growth and consequently ecosystem functioning is greatly diminished.

This motivated the second objective of this study which was to correlate the germination of the progeny seed with properties of the growth media (which included eight different mine waste materials and two soils) in which the parent grass were grown in order to evaluate the effect that the growth media had on the viability of the progeny seed.

The non-parametric correlation found that significant relationships existed between the growth media properties themselves. The pH (KCl) was significantly and negatively related to the aluminium (Al) content ( $p = 0.001$ ;  $\rho = -.908$ ), which simply put indicates that a decrease in

pH levels will be accompanied by a proportionate increase of Al content. Similarly the pH had a negative ( $\rho = -0.728$ ) and significant ( $p = 0.026$ ), relationship with the net acid potential, indicating that a decrease in pH values would result in an increase in the net acid potential. Low pH levels are associated with acidic soil conditions, explaining the negative relationship between the pH and the net acid potential.

Mine waste materials containing ferrous sulphate minerals are inclined to produce acidity by means of oxidation of the sulphate bearing minerals (Van Deventer et al., 2008:27). Acid reacts with silicate minerals that contain aluminium (Al), and at low pH levels, Al rather than hydrogen would be the main exchangeable cation (Winegardener, 1995:180). Acidification of soil involves the increase of free  $H^+$  which influences the release of nutrient elements from the colloidal particles of soil (Hopkins & Hürner, 2008:41). Thus the low pH values of mine wastes increases the solubility of toxic metals, namely Al, and elevated concentrations of these metals can impair the physiological functions of plants and essentially interfere with nutrient uptake (Liu & Lal, 2013:8). This explains the negative significant relationship that was found between the pH and the Al content, and also proves that low pH values may be a predominant factor affecting the quality of mine waste materials and success of vegetation establishment on these mine waste materials.

A significant ( $p = 0.017$ ) and positive ( $\rho = 0.763$ ) relationship was found between the saturated EC and the total sulphur (S) content, i.e. an increase in EC would be met by an proportionate increase in the total S content. The EC represents the total ionic charge of the adsorption complex active in the adsorption of ions, and is based on the concept that the electrical current carried by a salt solution under standard conditions increases as the salt concentration of the solution increases (Sparks, 2003:45; Winegardner, 1995:31). Thus the EC represents the electrical current caused by soluble ions such as  $Cl^-$ ,  $SO_4^{2-}$ ,  $HCO_3^-$ ,  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  (Sparks, 2003:45). Theoretically, an increase in the S content would result in an increase of ions in the soil solution, which would increase the electrical current of thereof, and consequently increase the saturated EC.

Sparks (2003:45) describes soil pH as the master variable of soils. Soil pH greatly affects and influences numerous soil processes, reactions, and microbial life forms. The significant relationships between the growth media properties with the soil pH emphasize the necessity for monitoring and maintaining pH levels, not only for soil quality and agricultural purposes, but also with regard to rehabilitation of contaminated soils and mine wastes.

### 5.3 Germination of Progeny Seed

#### 5.3.1 Germination of Progeny Seed from *Eragrostis curvula* (Schrad.) Nees. (Weeping Love grass)

*Eragrostis curvula* (Weeping Love grass) is a perennial grass sown as a pasture species in South Africa and establishes well in disrupted areas (Russel *et al.*, 1990; Van Oudtshoorn, 2009). Weeping Love grass is often used for rehabilitation practices to stabilize exposed soils with great success.

The progeny seed of Weeping Love grass used in this study were harvested separately from each of the parent grasses grown in every growth media, including eight different mine waste materials and two control soils, and is referred to as seed batches, i.e. each seed batch was harvested from a parent plant grown in a particular growth medium. The germination rate and percentages of the progeny seed were then correlated with the growth media properties using a statistical non-parametric correlation model.

Overall the progeny seed batches of Weeping Love grass germinated successfully (Table 4.2). The germination percentages were extraordinarily good, with average germination percentages as high as 90 % for seed batches from the gypsum wastes, gold wastes with high pyrite content, and gold wastes with low pyrite content. The results indicate that the progeny seed was viable and had excellent vigour, which is characteristic of Weeping Love grass and probably the reason it is used in rehabilitation practices (FAO, 2014).

Repeated Measures ANOVA statistical analysis indicated that there was no significant difference between the average germination percentages of the progeny seed harvested from parent plants grown in different growth media. Similarly the statistical non-parametric correlations found no significant relationship between the germination of the progeny seed harvested from parent grasses grown in each of the growth media and the properties of the growth media.

Despite none of the growth media properties having a significant relationship with the germination of the progeny seed, some properties were found to have a large size effect on the germination, i.e. it accounted for 25 % of the total variance in the germination of the progeny seed. Both the EC and the total S had a large effect size on the germination of the progeny seed. This implies that these properties contributed, to some extent to the variance in the germination of progeny seed.

The EC represents the total ionic charge of the adsorption complex active in the adsorption of ions and is based on the concept that the electrical current carried by a salt solution under standard conditions increases as the salt concentration of the solution increases (Sparks, 2003:290; Winegardner, 1995:236). Thus an elevated EC indicates a high electrical current due to increased salt concentrations, i.e. soluble ions such as  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  (Sparks, 2003:297).

Salinity affects plant growth in various ways (Sparks, 2003:290). Seed germination can be effected by salinity stress through osmotic effects which can prevent or delay germination, or can reduce the viability of seeds through ion toxicity (Wang *et al.*, 2013:355; Sparks, 2003:297).

EC levels higher than  $400 \text{ mS.m}^{-1}$  is regarded to be saline; however, plant growth can be affected at much lower concentrations (Morgenthal, 2003:13). Several guidelines suggest that EC for soil should be preferably lower than  $200 \text{ mS.m}^{-1}$  and should not exceed  $400 \text{ mS.m}^{-1}$  (Morgenthal, 2003:13). Although an EC higher than  $400 \text{ mS.m}^{-1}$  is regarded to be saline, the productivity of Weeping Love grass can already be reduced at  $200 \text{ mS.m}^{-1}$  according to Morgenthal (2003:13). In contrast to this, the results indicated that the EC was related positive to, and had a large size effect on the variation of the germination of Weeping Love grass progeny seed. More significant than this, the progeny seed that had the highest germination average (90 %), were harvested from parent grasses grown in growth media with elevated EC concentrations; namely the gypsum wastes ( $199 \text{ mS.m}^{-1}$ ), gold wastes with low pyrite content ( $193 \text{ mS.m}^{-1}$ ), and gold wastes with high pyrite content ( $422 \text{ mS.m}^{-1}$ ).

With regard to the excellent germination results, despite the salinity of some growth media and the fact that none of the growth media properties were found to have a statistical significant effect on the germination of the progeny seed, it can be concluded that the progeny seed of Weeping Love grass was not affected by the maternal environmental factors as experienced by the parent grasses grown in each of the different growth media. The fact that the progeny seed of Weeping Love grass was found to be viable and vigorous when harvested from parent grasses established in different mine waste materials, will add to its popularity and continuous use in future mine rehabilitation practices.

### **5.3.2 Germination of Progeny Seed from *Cynodon Dactylon* (Schrad.) Nees. (Couch grass)**

The progeny seed of *Cynodon dactylon* (Couch grass) used in this study were harvested separately from each of the parent grasses grown in every growth media, including eight

different mine waste materials and two control soils, and is referred to as seed batches, i.e. each seed batch was harvested from a parent plant grown in a particular growth medium. The germination rate and percentages of the progeny seed were then correlated with the growth media properties using a statistical non-parametric correlation model.

Couch grass is a perennial and characteristically short, creeping grass, with both above-ground stolons and underground rhizomes (Russel *et al.*, 1990:97; Van Oudtshoorn, 2009:139). The seed production of couch grass is generally sparse and it reproduces mainly vegetatively by means of rhizome and stolon growth (Horowitz, 1996:307). It is considered to be a weed in many countries; however, it is often used in rehabilitation practices to initially stabilise exposed surfaces (Singh *et al.*, 2013:32).

All the progeny seed batches of Couch grass germinated during the pot trial, and were found to be viable during the laboratory tests (Table 4.3). The germination averages between the different seed batches varied, and according to the Repeated Measures ANOVA statistical analysis there was a significant influence of the growth media in which the parent grass were grown as a variable on the germination of the progeny seed ( $p = 0.001$ ,  $f = 5.84$ ).

Progeny seed from the andalusite seed batch germinated exceptionally well (70 % average germination). The likelihood exists that the viability of this seed batch was positively influenced by the andalusite mine wastes. The non-parametric correlations, however, did not find any significant relationship between the properties of the growth media and the germination of the progeny seed batches, and therefore the particular influence of the andalusite mine wastes on the viability of the seed batch cannot be explained.

Although not being significantly related to the germination of the seed batches, the F content, did have a positive large size effect on the germination of the progeny seed ( $\rho = .537$ ). A large size effect accounts for 25% of the variance (Field, 2013:82). Thus, the F content of the growth media was to some extent, but not significantly so, responsible for the variation in germination between the seed batches. The positive correlation coefficient indicates a positive interaction between the F content and the germination of the seed batches. This means that an increase in the F content was, met with a proportionate increase in the germination of the seed batches, which is peculiar as the toxicity of F, particularly when airborne, is well known. Airborne F is considered to be phytotoxic and hazardous to plants, and effects plant metabolism with regard to oxygen uptake, respiratory disorders and assimilation (Kabata-Pendias, 2011:390).

It has been indicated that plants readily take up F from polluted soils, however, the bio-availability of soil F is insignificant compared to that of airborne F compounds (Kabata-Pendias, 2011:390). The toxicity of F, when this element is absorbed by roots, is not common and its requirement to plants and essential role in plant metabolism is not yet known (Kabata-Pendias, 2011:390).

Despite the large size effect of F, it does not account for the exceptional germination of the andulasite seed batch with regard to the F content of each separate growth medium. Andulasite has a low F content (0.1 mg/l) compared to other growth media with lower germination averages, such as the gypsum wastes which had a large F content (27.5 mg/l). Thus, it cannot be concluded that the F content of the growth media was either beneficial or detrimental to the viability of the offspring seed. It, however, only contributed to the variance in the germination averages between the different seed batches, and not even significantly so.

Similarly to a large size effect, a medium size effect accounts for 9% of the total variance (Field, 2013:82). Negative medium size effects were attributed by the Al content ( $\rho = -.317$ ), K (potassium) content ( $\rho = -.468$ ), and the nett acid potential ( $\rho = -.349$ ), each accounting for 9% variance in the germination of the seed batches.

The negative relationship between the Al content and the germination indicates that an increase in Al content would be met with a proportionate decrease in germination and was reflective of the detrimental effects Al toxicity has on plant health. Al toxicity is closely related to acidic soil conditions as the  $\text{Al}^{3+}$  ion is soluble and bio-available at low (4-5) pH conditions (Kabata-Pendias, 2011:327). In these acidic soils Al is regarded as the limiting factor for plant growth (Kabata-Pendias, 2011:327; Rout *et al.*, 2001:4). Al toxicity has several detrimental effects on plants, including: reduced root growth; impaired nutrient uptake and transport by plants; imbalanced ratio of cations to anions; and impaired cell division (Kabata-Pendias, 2011:327; Rout *et al.*, 2001:4).

The negative association between the nett acid potential and the germination of the offspring seed indicates that an increase in the nett acid potential will be met with a proportionate decrease in the germination of the offspring seed. This phenomenon can be attributed to the increased bio-availability and resulting phytotoxicity of Al associated with acidic soil conditions.

### 5.3.3 Germination of Progeny Seed from *Chloris gayana* Kunth. (Rhodes grass)

*Chloris gayana* (Rhodes) is a perennial grass which spreads by means of stolons (Morgenthal & Van Rensburg, 2004:62; Russel *et al.*, 1990:84; Van Oudtshoorn, 2009:154). Rhodes is often used in rehabilitation practices and to stabilize exposed and disturbed soils due to its extensive root system and tolerance to soils with poor nutrient status, salinity, and low pH (Russel *et al.*, 1990:84; Keeling & Werren, 2005:57; Van Oudtshoorn, 2009:154). It is used mainly to stabilize slopes and to minimize erosion (Keeling & Werren, 2005:57).

The overall germination, excluding the platinum seed batch which had a germination average of 90 %, was very low and indicates that the progeny seed of Rhodes grass lacks vigour (Table 4.4). The Repeated Measures ANOVA statistical analysis indicated that the growth media, as experienced by the parent grasses did have a significant influence on the variance of the germination of the progeny seed.

The exceptional germination average of the platinum seed batch can be ascribed to the high pH value of the platinum wastes, as the pH is the main property that sets this growth medium apart from the other growth media, and also because it had the highest pH (KCl) for all the growth media (see Table 4.17 in Section 4.4.1). The high pH of the platinum tailings can be attributed to its high base saturation which is due to its geological origin; platinum ore is associated with base metals. The high pH and base saturation accounts for the slight alkali conditions of the platinum tailings and therefore the platinum tailings are not prone to become acidic.

It is likely that the high pH levels of the platinum tailings accounted for the excellent vigour and germination average of the platinum seed batch, as the other seed batches from the remainder growth media, all of which had lower pH levels, had poor germination averages ( $\leq 20$  %). Low pH and soil acidity is known to damage root cells, inhibit beneficial microbial activity, and to impair the physiological function of plants (Liu & Lal, 2013:31). Additionally numerous heavy metals, such as Cu and Al, become soluble and bio-available to plants at acidic soil conditions causing toxicity in plants due to the elevated levels thereof (Sheldon & Menzies, 2005:341; Sparks, 2003:144). Keeling and Werren (2005:57) found that at elevated concentrations of heavy metals associated with low pH levels; the growth of Rhodes grass is significantly reduced. The detrimental effects of low pH levels, together with elevated levels and consequently toxicity of heavy metals to plants, could have potentially influenced and reduced the viability and germination averages of the progeny seed from Rhodes grass. However, the statistical non-

parametric correlations indicated no significant relationships between any of the properties of the growth media, as experienced by the parent grasses and the germination of the progeny seed batches.

Progeny seed produced by Rhodes grass will most likely be viable and able to germinate when used for rehabilitation practices on mine waste materials with high pH levels. From the germination results the conclusion can be drawn that progeny seed is less viable and likely to germinate when harvested from parent grasses grown in mine waste materials with low pH levels. Therefore, Rhodes grass is not recommended as a suitable species for rehabilitation of acidic mine wastes with regard to establishing a long-term sustainable vegetation cover.

### **5.3.4 Germination of Progeny Seed from *Cenchrus ciliaris* L. (Buffalo Grass)**

*Cenchrus ciliaris* (Buffalo grass) is a perennial grass and is commonly used for mine site rehabilitation as well as erosion control (Marshall *et al.*, 2012). Buffalo grass is suited for this purpose as it has a high tolerance to drought and can tolerate soils with low levels of nutrients (Marshall *et al.*, 2012).

The Repeated Measures ANOVA statistical analysis indicated that the growth media in which the parent Buffalo grass were grown in, did have a significant influence in the germination variance of the different seed batches. The greatest germination percentages (50 %) were recorded for the red soil seed batch and the gypsum seed batch, while the lowest final germination percentage was recorded for the platinum seed batch (20 %) (Table 4.5). The germination results indicates that progeny seed of Buffalo grass are viable and germinable when harvested from plants grown in mine waste materials with adverse properties, such as extreme pH conditions, salinity and poor nutrient status.

The statistical non-parametric correlations between the properties of the growth media in which the parent grasses grew and the germination of the progeny seed produced by these grasses, indicated that the saturated EC had a significant ( $p = 0.029$ ) positive relationship with the germination of progeny seed from Buffalo grass with a correlation coefficient of .759; i.e. an increase of saturated EC would be met with a proportional increase in the germination of the progeny seed.

The EC represents the total ionic charge of the adsorption complex active in the adsorption of ions (Sparks, 2003:290; Winegardner, 1995:236). Thus, an elevated EC indicates a high

electrical current due to increased ion concentrations (Sparks, 2003:297). Mine waste materials commonly lack organic material, secondary minerals and nutrients, making it a poor growth medium for plants (Ye *et al.*, 2000:289). Therefore, it is expected that an increase in the saturated EC will be associated with greater germination percentages due to the improved nutrient status that accompanies an increased EC.

In addition, the statistical non-parametric correlation indicated a positive significant relationship between the total S content and the germination of Buffalo progeny seed. Thus, an increase of total S content would be met with a proportional increase in the germination of Buffalo grass progeny seed. An increase in germination of the progeny seed proportionate to an increase in total sulphur content is to be expected, because sulphur is an essential nutrient for plant growth and development (Jones, 2012:81; Nazar *et al.*, 2011:82;). Sulphur is required for protein synthesis, forms part of amino acids, and is also significant for N assimilation (Jones, 2012:81; Nazar *et al.*, 2011:82). Furthermore, sulphur is considered to be essential as it is regarded to be functionally convergent with the assimilatory pathway of N (Nazar *et al.*, 2011:82).

The statistical non-parametric correlations and the germination results Buffalo grass showed that nutrition is essential for producing viable seed. An improved EC status and S content will most likely result in Buffalo grasses producing more viable offspring seed. The germination of the progeny seed never exceeded 50 %, and this could be indicative of seed remaining intact for long time periods. It was found that the Buffalo grass seed could remain intact for as long as five years on Gypsum wastes, and that environmental conditions, such as air humidity, temperature, soil pH, and salinity, influences Buffalo grass germination (Van Deventer & Hattingh, 2004:62). The progeny seed of Buffalo grass did germinate, and with attentive care with regard to nutrient and EC status, this species will most likely persist over the extent of one generation when grown on mine wastes.

### **5.3.5 Germination of Progeny Seed from *Digitaria eriantha* Steudel (Smuts Finger grass)**

*Digitaria eriantha* (Smuts Finger grass) is a perennial with both rhizomes and stolons (Russel *et al.*, 1990:110; Van Oudtshoorn, 2009:181). This drought resistant grass is popular for rehabilitation practices in South Africa.

The germination results from the pot trial indicated observable variation among the different seed batches of Smuts Finger Grass progeny seed (Table 4.6). This variation among the germination averages ranged from 10 % (vertic seed batch) to 90 % (fluorspar seed batch). The

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Repeated Measures ANOVA analyses indicated that the growth media, in which the parent grasses grew, did have a significant influence on the variance of the germination between the different seed batches. Thus, the growth media does influence the viability of the offspring seed.

The statistical non-parametric correlations showed no significant relationships between the properties of the growth media, in which the parent grasses grew, and the germination of the progeny seed batches. Thus, the variation in germination of the progeny seed could be accounted for by the growth media, as determined by the statistical Repeated Measures ANOVA, but the mechanism responsible for this variation could not be accounted for by means of the statistical non-parametric correlations.

Despite no significant relationships being found between the growth media properties and the germination of the progeny seed, some medium size effects attributed by the growth media properties were found. A medium size effect indicates that the property in question accounts for 9 % of the total variation of the germination (Field, 2013:83).

The F content had a positive medium size effect on the germination of the Smuts Finger grass progeny seed. Thus, the F content of the growth media was to some extent, but not significantly so, responsible for the variation in germination between the seed batches. The positive correlation coefficient indicates that an increase in the F content was met with a proportionate increase in the germination of the seed batches. However, the toxicity of F is well known and is not considered to be an essential trace element. Airborne F is considered to be phytotoxic and hazardous to plants, and effects plant metabolism with regard to oxygen uptake, respiratory disorders and assimilation (Kabata-Pendias, 2011:327).

The pH had a positive medium size effect on the germination of Smuts Finger grass progeny seed. The positive nature of the medium size effect indicates that an increase in pH levels would, to some extent, be met with an increase in the germination of progeny seed. Low pH and soil acidity damages root cell membranes, inhibit the activity of beneficial microbes, and impair the physiological function of plants (Liu & Lal, 2013:31). Soil pH will determine the solubility of heavy metals and consequently the potential for phyto-toxicity. (Morgenthal, 2003:13; Sparks, 2003:267). Therefore the positive correlation, i.e. an increase in pH will be favoured by the progeny seed resulting in an increase in germination, was expected as optimum plant growth is between pH (KCL) values of 4.5 and 6 (Morgenthal, 2003:13).

The statistical non-parametric correlations found that the nett acid potential had a negative medium size effect on the variation of the germination from the Smuts Finger Grass progeny seed. This indicates that an increase in the nett acid potential would account for a decrease in the germination of the progeny seed. In accordance with the positive correlation between the pH and the germination of progeny seed, the negative correlation between the nett acid potential and the germination of the progeny seed shows that plant growth is adversely influenced by acidity and low pH levels and that optimum plant health will be at pH (KCl) levels ranging between 5 and 6 (Morgenthal, 2003:13). This confirms the fact that the pH and acidity respectively contributes to the variation in germination of progeny seed, and that the pH levels of a growth medium is likely to influence the viability and germination percentages of offspring seed.

Despite the significant influence that the growth media had on the germination of the progeny seed, the correlation between the growth media properties and the germination of the offspring seed did not indicate significant relationships. The progeny seed of Smuts Finger Grass did germinate proving to be viable, although great variation was found between the different seed batches from the different growth media. The variation was accounted for by the growth media as indicated by statistical Repeated Measures ANOVA, however, the mechanism responsible for this variation could not be accounted for by means of the statistical non-parametric correlations. Smuts Finger Grass is a feasible option for the use in rehabilitation practices as the progeny seed produced by grasses grown in various mine wastes is viable and able to germinate.

### **5.3.6 Germination of Progeny Seed from *Sorghum bicolor* (L.) Moench (Sorghum)**

Sorghum is an annual which culms up to 4 m high (FAO, 2014). Sorghum is adapted to a subtropical and temperate climate and grows in most soils that are not waterlogged (FAO, 2014).

The germination tests, which were conducted by AS according to rules prescribed by ISTA, indicated that progeny seed from the fluorspar seed batch were potentially immature as it contained green seed. Similarly the germination results obtained from the pot trial study indicated abnormal seed as no germination was recorded for any of the replicates containing progeny seed from the fluorspar seed batch (Table 4.7). Alternatively, progeny seed from the gypsum seed batch did germinate, indicating that the high F content found in both the fluorspar and gypsum wastes was not solely responsible for the abnormalities found for the progeny seed from the fluorspar seed batch.

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High germination percentages were recorded for the remainder seed batches, with the gypsum wastes seed batch having the highest germination percentages. According to the Repeated Measures ANOVA statistical analysis, there was a significant influence of the growth media in which the parent grass were grown as a variable on the germination of Sorghum progeny seed ( $p=0.0138$ ;  $f=2.89$ ) which indicates that the environmental factors as attributed by the growth medium and experienced by the maternal plant, did influence the germination of progeny seed.

A significant negative relationship was found between the germination of Sorghum progeny seed and the pH ( $p = 0.024$ ;  $\rho = -.734$ ). This negative association indicates that a decrease in pH would be met by a proportionate decrease of germination in Sorghum progeny seed. Soil acidity can cause damage to root cell membranes, inhibit the activity of beneficial microbes, and impair the physiological function of plants (Liu & Lal, 2013:31; Sparks, 2003:267). However, the negative relationship is indicative that Sorghum is able to grow and produce viable seeds in acidic growth conditions.

The statistical non-parametric correlations indicated a significant positive relationship between the germination of progeny seed and the Al content of the growth media in which the maternal plants grew. The positive relationship between the Al content and the germination of Sorghum progeny seed indicates that an increase in Al content would be met with a proportionate increase in germination.

Al toxicity is regarded as a limiting factor for plant growth and is commonly known for its detrimental effects on plants (Kabata-Pendias, 2011:327; Rout *et al.*, 2001:4). The positive response in germination of the progeny seed from Sorghum to an increased Al content contradicts the statement above and suggests that Sorghum is tolerant to Al toxicity (Neumann & Nieden, 2001:685; Yang *et al.*, 2013:4).

According to Yang *et al.* (2013:4) Al tolerant species, which includes Sorghum, have mechanisms for detoxifying Al externally such as the secretion of Al-chelating substances. It is suggested that Sorghum is able to secrete organic acid anions from the roots when exposed to Al, which forms stable nontoxic complexes with Al in the rhizosphere (Yang *et al.*, 2013:5). This prevents the binding of Al to cellular components and consequently potential Al toxicity (Yang *et al.*, 2013:5).

Another suggested Al tolerant mechanism of Sorghum is through silicon (Si) amelioration (Neumann & Nieden, 2001:685). The proposed mechanism suggests that Si plays a role in the

transportation and deposition of Al in the cytoplasm or the vacuole of the plant (Neumann & Nieden, 2001:685). Also, it was found that the formation of insoluble Al/Si compounds outside plant cells was responsible for the Al tolerance mechanism of Sorghum (Neumann & Nieden, 2001:685).

Excluding the green seed found in the fluorspar seed batch, the progeny seed of Sorghum germinated well. The positive significant relationship of Al with the germination of the progeny seed indicated that the progeny seed of Sorghum is tolerant to Al, and potentially other heavy metals; and can be used for re-vegetation of mine wastes, particularly those who are prone to elevated levels of metal trace elements.

### **5.3.7 Germination of Progeny Seed from *Eragrostis tef* (Zucc.) Trotter (Tef)**

*Eragrostis tef* (Tef) is an annual grass (Russel *et al.*, 1990:162; Van Oudtshoorn, 2009:139). It grows in most types of soil and has become popular for re-vegetating exposed ground and disturbed areas (Russel *et al.*, 1990:162; Van Oudtshoorn, 2009:139).

The progeny seed of Tef used in this study were harvested separately from each of the parent grasses grown in every growth media, including eight different mine waste materials and two control soils, and is referred to as seed batches, i.e. each seed batch was harvested from a parent plant grown in a particular growth medium. The germination rate and percentages of the progeny seed were then correlated with the growth media properties using a statistical non-parametric correlation model.

Overall high germination averages, ranging from 70 to 100 %, were recorded for offspring seed from all the seed batches during both the germination tests and the pot trial experiment (Table 4.8). This is a strong indication that the progeny seed for all seed batches were viable and not affected by the environmental factors contributed by the growth media during seed maturation.

The Repeated Measures ANOVA statistical analysis indicated no significant difference between the germination for the different seed batches of Tef ( $p=0.2957$ ;  $f=1.26$ ). This reflects the fact that progeny seed of Tef were not significantly affected by the growth media with regard to seed maturation and therefore the progeny seed of Tef were viable and able to germinate.

There was no significant relationship between the germination of progeny seed from Tef and the properties of the growth media. The nitrate ( $\text{NO}_3^-$ ) content of the growth media did, however,

have a large size effect on the variation between the germination of the seed batches from Tef ( $\rho = .502$ ). This indicates that the germination of the progeny seed increased proportionally to an increase in the nitrate content of the growth medium.

The positive large size effect that the nitrate ( $\text{NO}_3^-$ ) content had on the variation between the germination of seed batches from Tef is self-explanatory as nitrogen is one of the primary essential macronutrients for plants and is taken up as nitrate ( $\text{NO}_3^-$ ) by the root system (Farago & Mehra, 1994:41; Hopkins & Hürner, 2008:68). Nitrogen is an essential constituent of many important molecules including proteins, nucleic acid, hormones and chlorophyll (Hopkins & Hürner, 2008).

According to Hilhorst and Karssen (2001) seeds may take up nitrogen during development on the mother plant, and that, for some species, the nitrate content of seeds on the mother plant is directly related to soil nitrate levels. The positive association between the nitrate content of the growth media and the variance in germination of the Tef seed batches resembles this, indicating that to some extent, the nitrate content in the growth medium in which the mother plant grows, does relate positively to the germination success and viability of offspring seed.

### **5.3.8 Germination of Progeny Seed from *Hyparrhenia hirta* (Common Thatching grass)**

*Hyparrhenia hirta* (Common Thatching grass) is a perennial, rhizomatous grass (Russel et al., 1990:185; Van Oudtshoorn, 2009:189). Common Thatching grass does not spread well by seed and it is a poor and erratic seeder and sheds its seed readily (FAO, 2014). It is often found in disturbed places and protects the soil and stabilises hard, gravelly and eroded soil.

During the germination tests conducted in the laboratories of AS it was noted that the harvested offspring seed were green with empty caryopsis in all the seed batches. Similarly all the seed batches had low germination averages, if any at all, for both the pot trial and the germination tests. The empty caryopsis and green colour, along with the lack of seedling emergence, proved to be evident that the time at which the offspring seed of Common Thatching grass were harvested, did not accord with the time period during which intact seed matures and dries while still on the parent grass. Currently, Common Thatching grass is an unlisted grass in South Africa and no guideline exists with regard to when seed have reached maturity for harvesting, therefore the harvesting of immature seed was not unforeseen and the time of harvesting was an estimated guess.

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According to Jacob *et al.* (2014:253) it is critical to harvest as soon as seed reached the physiological maturity point, in order to reduce potential qualitative and quantitative seed loss and to ensure seed quality. During the maturation process, seed are susceptible to adverse environmental conditions, which can accelerate the deterioration thereof (Bedane *et al.*, 2007). Harvesting seed prematurely will result in insufficient storage compounds, and prevents seed from reaching primary dormancy (Bedane *et al.*, 2007). Such seed will not be viable and able to germinate as compounds supportive of initial embryo development during germination will be deficient.

From observations during the pot trial and germination tests it is highly probable that the offspring seed of Common Thatching grass were harvested prematurely, and by doing so, the viability of the seed were reduced immensely. Thus, the germination averages for most seed batches were low or nothing at all. For this reason no conclusions will be drawn, other than that the progeny seed were harvested prematurely which resulted in little seedling emergence and therefore the effect of the growth media on the viability of the progeny seed could not be further investigated. An executive decision was made not to further investigate the correlation between the properties of the growth media and the germination of the progeny seed.

## 5.4 Conclusions

### 5.4.1 Viability of Progeny Seed

The general aim of this study was to determine the viability of seed produced by a previous generation of grass species established in eight different mine wastes and two natural soils, in order to identify suitable species for specific mine waste materials to ensure long-term survival through means of seed production. Seed vigour can be described as the function of a variety of factors to which the parent plant is subjected to during seed maturation (Mayer & Poljakoff-Mayber, 1989:43). Mine waste materials are known to be hostile for sustaining plant growth and characteristics such as extreme pH levels, salinity and elevated levels of metal trace elements can detrimentally influence the viability of offspring seed.

The viability and germinability of the progeny seed were determined through a pot trial study and additional germination testing at the laboratory of AS. The germination results were correlated with the growth media analyses by statistical non-parametric correlations which indicated several significant correlations among the growth media properties themselves, and with the germination results from the progeny seed.

According to the Repeated Measures ANOVA statistical analysis, there was a significant influence of the growth media in which the parent grass were grown as a variable on the germination averages of the progeny seed batches from Sorghum, Buffalo grass, Rhodes grass, and Smuts Finger grass, indicating that the environmental factors as attributed by the growth media, i.e. the eight different mine waste materials and two soils, and experienced by the maternal plant, did indeed influence the germination of progeny seed. However, it was found that significant correlations between the properties of the growth media and the germination of the progeny seed, was species dependent.

**Table 5. 1: The significant influence of the growth media on the variation of the germination of the progeny seed; and the significant correlations between the properties of the growth media and the germination of the progeny seed**

Significant relationships between the properties of the growth media and germination (statistical non-parametric correlations) (“+” positive relationship) (“-“ negative relationship)					
Species	Significant influence of growth media as a variable on germination (ANOVA)	pH (KCl)	Total Al content	Saturated EC	Total S content
Weeping Love grass ( <i>E. curvula</i> )	No				
Couch grass ( <i>C. dactylon</i> )	No				
Rhodes grass ( <i>C. gayana</i> )	Yes			X (+)	
Buffalo grass ( <i>C. ciliaris</i> )	Yes			X (+)	X (+)
Smuts Finger grass ( <i>D. eriantha</i> )	Yes				
Sorghum ( <i>S. bicolor</i> )	Yes	X (-)	X (+)		
Tef ( <i>E. tef</i> )	No				

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Weeping Love grass was not affected by the maternal environmental factors as experienced by the parent grasses grown in each of the different growth media (Table 5.1). The fact that the progeny seed of Weeping Love grass was found to be viable and vigorous when harvested from parent grasses established in different mine wastes materials, suggests that it is tolerant or at the least resilient to stress factors, such as extreme pH levels, salinity, and elevated levels of metal trace elements.

All the progeny seed batches of Couch grass germinated during the pot trial, and were found to be viable during the germination tests conducted at the laboratory of AS. The Repeated Measures ANOVA statistical analysis indicated that there was a significant influence of the growth media in which the parent grass were grown as a variable on the germination (Table 5.1). Progeny seed from the andalusite seed batch germinated exceptionally well and the likelihood exists that the viability of this seed batch was positively influenced by the andalusite mine wastes. The non-parametric correlations, however, did not find any significant relationship between the properties of the growth media and the germination of the progeny seed batches, and therefore the particular influence of the andalusite mine wastes on the viability of the seed batch cannot be explained.

The germination results suggest that progeny seed of Rhodes grass is less viable and likely to germinate when harvested from parent grasses grown in acidic mine waste materials. Therefore, Rhodes grass is not recommended as a suitable species for rehabilitation of acidic mine wastes with regard to establishing a long-term sustainable vegetation cover.

The progeny seed of Buffalo grass did germinate, and it was found that the growth media did account significantly for the variation in the germination of the progeny seed (Table 5.1). With attentive care in regard with nutrient and EC status, this species will most likely persist over the extent of one generation when grown on mine waste materials and TSF's.

The variation in the germination between the different seed batches of Finger grass was accounted for by the growth media as indicated by the statistical Repeated Measures ANOVA analysis (Table 5.1). The non-parametric correlations found no significant correlations between the germination of the progeny seed and any of the properties from the growth media, i.e. no property was found to have a significant influence on the viability of the progeny seed (Table 5.1). However, the germination results indicated that the progeny seed from all the seed batches did germinate and is therefore regarded as viable. Thus, the mine waste materials did account

for the variation in the germination of the different seed batches; however, the progeny seed were still viable and able to germinate concluding that Finger grass is suitable for the use in rehabilitation practices of mine waste materials with regard to sustainability, i.e. surviving and producing viable offspring seed.

The progeny seed of Smuts Finger grass did germinate proving to be viable, although great variation was found between the different seed batches from the different growth media. The variation was accounted for by the growth media as indicated by statistical Repeated Measures ANOVA, however, the specific property from the growth media responsible for this variation could not be accounted for by means of the statistical non-parametric correlations. Smuts Finger Grass is a feasible option for the use in rehabilitation practices as the progeny seed produced by grasses grown in various mine wastes is viable and able to germinate.

The negative significant relationships of Al and pH respectively with the germination of the progeny seed, indicated that the progeny seed of Sorghum is tolerant to Al, and potentially other heavy metals, as well as acidic soil conditions, and can be used for re-vegetation of mine waste materials, particularly those who are prone to acidity and heavy metal contamination.

The Repeated Measures ANOVA statistical analysis indicated no significant difference between the germination for the different seed batches of Tef. Thus, progeny seed of Tef were not significantly affected by the growth media with regard to seed maturation and therefore the progeny seed of Tef were viable and able to germinate, and can be recommended for use in rehabilitation of several mine wastes.

It was found that progeny seed of Common Thatching grass were green with empty caryopsis. For this reason no conclusions will be drawn, other than that the progeny seed were most likely harvested prematurely which resulted in little seedling emergence and therefore the effect of the growth media on the viability of the progeny seed could not be further investigated.

For some species the viability of offspring seed was less susceptible for the influence of the growth media, these species are: Tef (*E. tef*); Weeping Love grass (*E. curvula*); and Couch grass (*C. dactylon*). The Repeated Measures ANOVA statistical analyses found no significant influence of the growth media as a variable on the germination of the progeny seed for these species, and therefore, the viability of offspring seed will most likely not be detrimentally affected by the mine wastes. Consequently, these species will remain vigorous in terms of seed

production and germination of offspring seed, thereby contributing to the sustainability of vegetation covers on TSF's.

The viability of progeny seed for some species was found to be susceptible for the influence of mine waste materials, i.e. the viability was affected by the characteristics of the mine wastes. These species are: Rhodes grass (*C. gayana*); Buffalo grass (*C. ciliaris*); Sorghum (*S. bicolor*); and Smuts Finger grass (*D. eriantha*). Significant correlations between the properties of the growth media and the germination of progeny seed indicated which properties most likely influenced the seed viability. In one instance, as was the case with Rhodes grass, the viability of the progeny seed was reduced and the ANOVA statistical analyses did indicate that the growth media significantly influenced the germination of the progeny seed, however, the statistical non-parametric correlations did not indicate any significant correlations between the growth media properties and the germination of the progeny seed; thus, the mechanisms responsible for influencing and reducing the viability of the progeny seed is still unknown. These species is less likely to produce viable offspring seed and in the long-term will not contribute greatly to the sustainability of vegetation covers on TSF's; however, the management of properties of the mine wastes, such as low pH levels, can reduce the risk of grasses producing non-viable offspring seed.

As mentioned above, the germination tests conducted at the AS laboratory was done according to the ISTA prescribed rules. These standardized germination tests allows for seed merchants to made data of different seed lots and species comparable, such as for marketing and research (Hampton, 1993:106). Generally seed testing in accordance with the ISTSA rules is used for quality control during seed handling, of which the results are submitted to customers as documentation on seed quality (Hampton, 1993:107). The standardized seed tests should be done in an advanced seed laboratory, particularly for germination tests which requires chambers with temperature, light and moisture control (Hampton, 1993:107). According to Hampton (1993:107) seed testing as prescribed by ISTA, implies a standardized procedure which is most likely to be subjected to statistical analysis.

During the pot trial, the seed were subjected to fluctuating temperature, light and moisture conditions and were sown in a red soil which served as the growth medium. The standardized methods followed during the germination tests at AS included the use of paper and sand as a growth medium, which allows one to easily monitor and measure the shoot to root ratio, which is often used as an indicator of normal and abnormal seedlings. Due to the different methodologies

and circumstances under which the pot trial was subjected to, the germination results obtained from the pot trial cannot be compared to the results obtained from the germination tests conducted at AS according to ISTA rules. However, the germination tests conducted at AS did indicate abnormalities found in some seed batches. The progeny seed of Common Thatching grass were green and had empty caryopsis, as well as the fluorspar seed batch of Sorghum, which were also green. It is likely that these seed were not yet matured at the time of harvesting.

### **5.5 Re-growth of grass species after cutting**

A suggested outcome for an established vegetation cover in a rehabilitated area is that it should be resilient to natural disturbances (Ruiz-Jean & Aide, 2005:574). This includes, but is not limited to, grazing and harvesting through means of cutting. Additionally, a healthy vegetation cover minimizes surface runoff and erosion, and assists surface stability on slopes by mechanically reinforcing the soil through the root system (Fourie, 2007:483; Morgan, 1986:61). One of the objectives for this study was to evaluate above-ground re-growth of parent plants after cutting. In order to determine the ability of established grasses to re-grow after an incident such as harvesting or grazing the above-ground biomass of the parent grasses were cut, and measured for observable above-ground growth in the following growth season. The measurement of re-growth was subjectively done by scoring the grasses according to observable above-ground biomass.

Tef (*E. tef*) had poor observable growth, however, Tef is an annual grass, and therefore no re-growth was anticipated as it had already completed its life cycle when the cutting event commenced. Despite this, some observable re-growth did occur in some growth media, but the re-growth could have been mistaken for new emerging plants from seed that collected in the pots during the reproductive stage and seed harvesting event.

Weeping Love grass is a perennial and as anticipated it did show observable re-growth after the previous cutting event. However, it is likely that some new grasses that emerged from seed which collected in the pots could have been mistakenly recorded as re-growth.

Re-growth was observed for Buffalo grass from each growth medium. Although re-growth was observed from each growth medium, the re-growth counts were lower than anticipated for this perennial grass. The poor re-growth count per replicate can be ascribed to the roots of the grasses that most likely became root bound during the pot trial. The cutting event took place 6 months after the grasses were planted in the pots, and the re-growth monitoring took place

twelve months after the grasses were planted. Thus, the possibility exists that the Buffalo grasses experienced additional stress due to the roots being restricted in regard of growth.

After the cutting event, Common Thatching grass showed good observable re-growth in the majority of the growth media. In the kimberlite mine wastes Common Thatching grass showed the least observable re-growth. This can be attributed by the poor water retention that the kimberlite mine wastes have, which could have resulted in additional water stress experienced by the grasses planted in this growth medium. Additionally, the high pH value of the kimberlite mine wastes could have attributed to the poor observable re-growth of Common Thatching grass. It is commonly known that Common Thatching grass does not grow well in soils with high pH values.

Smuts Finger grass is a perennial with both rhizomes and stolons (Van Oudtshoorn, 2009:193). Re-growth, in the form of above-ground biomass, was observed for all the growth media. This was anticipated due to the possibility that new grasses emerging from the rhizomes and stolons could have been recorded mistakenly as re-growth from the existing grasses. During the re-growth monitoring there was no distinction made between re-growth of cut grass and emergence of new plants from the nodes of stolons and rhizomes. However, the emergence of new grasses is still an indicator of good health, as biomass allocation to rhizomes and stolons is reduced under low nutrient availability and stress conditions (Dong & De Kroon, 1994:103). Therefore the emergence of new grasses is indicative that the plant is either tolerant to stress conditions or that the plant adapted to the restriction of growth due to the roots being bound to the size of the pot.

Couch grass creeps with both stolons and rhizomes. Thus it is highly possible that observed re-growth could have occurred through the emergence of new plants at the nodes of stolons and rhizomes. Due to the hostile conditions of the growth media (with special reference to the mine waste materials) growth in any form, whether from the established plants that were cut or from new emerging plants at the nodes of stolons and rhizomes, is regarded as an indicator of good growth vigour.

Rhodes is a perennial grass which spreads by means of stolons (Van Oudtshoorn, 2009:154; Russel *et al.*, 1990:84; Morgenthal & Van Rensburg, 2004:62). Therefore good re-growth of replicates from all the growth media was anticipated, whether the growth occurred from new

plants that emerged from the nodes of stolons, or from re-growth of the existing cut grasses. The cut Rhodes grasses showed excellent observable re-growth in all of the growth media.

The method, with which the re-growth was monitored and accordingly scored, was not an objective one. The results indicated that the grasses did show observable re-growth after they were cut, excluding Tef which is an annual grass and showed poor observable re-growth. The monitoring of re-growth did not distinguish between re-growth from existing grasses, or the emergence of new grasses from seed or the nodes of stolons and rhizomes. Therefore the re-growth could not be statistically analysed, however, the re-growth was visually observable and recorded accordingly.

### **5.5.1 Re-growth of Grasses**

One of the objectives for this study was to evaluate above-ground re-growth of parent plants after cutting. The ability of established grasses to re-grow after a cutting event was determined by cutting the above-ground biomass of the parent grasses, after which it was scored according to observable above-ground growth in the following growth season. The measurement of re-growth was subjectively done by scoring the grasses according to observable above-ground biomass. Sorghum is an annual grain species and was excluded from the re-growth trial.

Tef was the only species which had poor observable re-growth after the cutting event. This poor growth vigour can be ascribed to the fact that Tef is an annual species, and had already completed its life cycle once the cutting event commenced.

Re-growth was observed for all the remainder species. This can be ascribed to the grasses showing resilience to stress factors attributed by the growth media; or new grasses which emerged from seed that collected in the pots, being mistaken for re-growth; or new emerging grasses from the nodes of stolons and/or rhizomes being mistaken for re-growth. However, the emergence of new grasses is still an indicator of good health, as biomass allocation to rhizomes and stolons is reduced under low nutrient availability and stress conditions (Dong & De Kroon, 1994:103). Therefore, the emergence of new grasses is indicative that the plant is either tolerant to stress conditions or that the plant adapted to the restriction of growth due to the roots being bound to the size of the pot.

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### Recommendations

#### 6.1 Introduction

Vegetation and soil are generally considered to be the most important aspects during rehabilitation of TSF's (Morgenthal & Van Rensburg, 2004:62). Rehabilitation success is often ascribed to ground cover and biomass production due to the importance of vegetation cover as a soil erosion factor (Morgenthal & Van Rensburg, 2004:62). In more recent years, self-sustainable vegetation has been considered as an important outcome of rehabilitation projects; however, sustainable rehabilitation can be compromised by the inability of vegetation to survive in mine waste materials on a long-term basis.

The lack of nutrients, organic matter and extreme pH levels collaboratively contribute to the poor quality of mine wastes as a growth medium for the parent grasses during seed production (Bradshaw, 1998:256; Maboeta *et al.*, 2006:31; Weiersbye, 2007:89). Environmental factors that influence seed production (i.e. maternal environmental factors), includes nutrient availability, temperature, and rainfall. Seed vigour can be described as a function of a variety of factors to which the parent plant was subjected to during seed production (Schuler & Orrock, 2012:477). Therefore, it was argued that the maternal environmental factors attributed by the growth medium, such as metal toxicity, acidity and salinity, can affect and potentially reduce germination success of offspring seed. This motivated the general aim of this study, which was to determine the viability of seed produced by a previous generation of grass species established in eight different mine waste materials and two natural soils, in order to identify suitable species for specific mine waste materials to ensure long-term survival through means of seed production.

#### 6.2 Knowledge Gaps encountered during this study

It was found that the growth media, as a variable, did significantly influence the germination of the progeny seed for the following species: Sorghum (*S. bicolor*); Buffalo grass (*C. ciliaris*); Rhodes grass (*C. gayana*); and Smuts Finger grass (*D. eriantha*). Thus, the viability of these species is affected by the mine wastes as a growth medium; however, some species did show resilience to stress factors. For instance, Sorghum had a positive germination response to increases Al content and decreased pH levels, indicating that it produces viable offspring seed in

acidic growth media. Despite the significant influence that the growth media had on the germination of the progeny seed, the correlation between the growth media properties and the germination of the offspring seed did not always indicate significant relationships, as was the case for Smuts Finger grass (*D. eriantha*). The variation in germination of the progeny seed could be accounted for by the growth media, as determined by the statistical repeated measures ANOVA, but the mechanism responsible for this variation could not be accounted for by means of the statistical non-parametric correlations. Therefore, the exact mechanisms and growth medium properties responsible for influencing the viability of progeny seed is still unknown.

Seed traits that have high adaptive implications for survival include: seed mass; dormancy level; and germination rate (Luzuriaga *et al.*, 2005:164). These traits depend both on the environmental conditions under which seed germinate and grow, and the environmental conditions to which the maternal generation was subjected to (Luzuriaga *et al.*, 2005:164; Wulff *et al.*, 1994:736). The environmental conditions include nutrient availability, light, moisture conditions, and temperature (Luzuriaga *et al.*, 2005:165; Wulff *et al.*, 1994:763). Even though the growth media, as a variable, did significantly influence the viability of the progeny seed, several other environmental factors, such as light and temperature, not accounted for in this study could have contributed to variation in the viability of the progeny seed.

The negative significant relationships of Al and pH respectively with the germination of the progeny seed indicated that the progeny seed of Sorghum is tolerant to Al, and potentially other heavy metals, as well as acidic soil conditions. The suggested mechanisms for Al tolerance in higher plants include the detoxification of Al externally at the roots by secretions of organic acid anions which form stable complexes with Al (Yang *et al.*, 2013:5). Additionally, Si is suggested to play a role in the transportation and deposition of Al in the cytoplasm or the vacuole of the plant, and that Si forms insoluble compounds with Al outside plant cells (Neumann & Nieden, 2001:685). It is likely that an array of mechanisms is responsible for the observed tolerance of Sorghum to Al and acidity collectively, and the exact understanding of these mechanisms is still unclear. However, the formation of stable, non-toxic complexes with Al at the rhizosphere will most likely prevent Al accumulation in the plant and the seed, thereby minimizing the exposure and associated risks of Al toxicity to the embryo.

During the germination tests, it was found that the progeny seed of Common Thatching grass (*H. hirta*) was green with an empty caryopsis. The conclusion was drawn that the intact seed was still immature when harvesting thereof commenced. Common Thatching grass is currently not

listed in the Plant Improvement Act (Act 53 of 1976) and therefore there are no requirements, such as minimum germination percentages, to which it has to adhere to. This is most likely why no guideline is available with regard to harvesting time in order to ensure that mature seed is harvested. This is a knowledge gap that needs to be addressed as the use of Common Thatching grass is not uncommon in rehabilitation practices and the seed is often harvested.

### **6.3 Recommendations for future studies**

These include:

- 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 further fully quantify the long-term effects of the growth media on the viability of progeny seed over several generations. Field trials will also provide additional insight regarding the viability of progeny seed with regard to seed banks and dormancy, as well as the ecological importance of viable seed during rehabilitation projects.
- The variation in germination of the progeny seed could be accounted for by the growth media, as determined by the statistical Repeated Measures ANOVA, but the mechanism responsible for this variation could not always be accounted for by means of the statistical non-parametric correlations. Therefore, the exact mechanisms and growth medium properties responsible for influencing the viability of progeny seed is still fairly unknown.
- From observations during the pot trial and germination tests it is highly probable that the offspring seed of Common Thatching grass were harvested prematurely, and by doing so, the viability of the seed were drastically reduced. An alternative method for harvesting seed is suggested, namely the use of a cone placed under the plume and around the stem which will collect seed as it reaches maturity. This method will ensure greater quantities of seed per harvest, and will also prevent the harvesting of immature, green seed, as it will still be intact and attached to the plant and not be able to collect in the cone.
- The measurement of re-growth was subjectively done by scoring the grasses according to observable above-ground biomass, six months after it has been cut and left to re-grow. During the measurement of re-growth, no distinction has been made between new grasses

that emerged from nodes of stolons and rhizomes and existing grasses. It is proposed that alternative methods that measures re-growth objectively should be explored and used; for example the dry weight, of biomass yield, and that distinction should be made between the re-growth of existing plants and new plants that emerged from reseeded or at the nodes of stolons and/or rhizomes.

- The importance of drawing up an experimental design in collaboration with statistical procedures must be emphasized, particularly for pot trial experiments in order to minimize outside variables (for example shading), and to obtain sound statistical data. Statistical analyses were challenging due to nature of germination monitoring. Monitoring of seedling emergence was done every second day, and counting every new emerging seedling, resulted in great quantities of data sets, much of which contained several 'zero' scores due to some days having no new emerging seedlings. Due to this, the statistical analyses of the data were challenging.
- It is recommended that the pH of mine wastes should be, if practically possible, neutralized during rehabilitation practices. The pH levels proved to be significantly correlated with other properties, such as Al solubility, therefore when the pH of soil or mine wastes is addressed, other associated variables, such as Al toxicity, will theoretically improve. Additionally, plant growth and germination will be optimum at a neutral pH level, contributing to the success of rehabilitation.
- Germination test were conducted at AS according to the ISTA seed testing rules, and used three planting methods (between paper, on top of paper and in sand), none of which used soil as a medium. These results did indicate that some seed batches were green and had empty caryopsis (*H. hirta*), however, due to the planting methods that differs from the method used during the pot trial, which used red soil as a growth medium, the germination percentages in this regard should not be compared. It is suggested that, for the purposes of pot and field trials in particular, the ISTA rules should only be regarded as a guideline, and then only with regard to the first and final seedling count days which are specific for each species. The ISTA rules are set for laboratory measures in order to determine a seed batch's vigour and quality under optimal conditions. The planting measures for these quality tests prescribed by ISTA does not entail soil as a growth

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medium, and therefore the rules of ISTA cannot be complied in the case of field trails or pot trials which uses soil as a growth medium. ISTA guidelines test the quality of a seed batch regardless of the growth medium/soil in which the seed will ultimately be sown.

## References

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AS (Advance Seed). 2014. Advance Seed Website: AgriCote Enhanced Seeds. <http://www.advanceseed.com/site/agricote>. Date of access: 1 July 2013.

Ahmadpour, P., Ahmadpour, F., Mahmud, T.M.M., Abdu, A., Soleimani, M. & Tayefeh, F.H. 2012. Phytoremediation of heavy metals: a green technology. *African Journal of Biotechnology*. 11(76): 36-43.

Anawar, H.M., Canha, N., Santa-Regina, I. & Freitas, M.C. 2013. Adaptation, tolerance, and evolution of plant species in a pyrite mine in response to contamination level and properties of mine wastes: sustainable rehabilitation. *Journal of Soils & Sediments*. 13:730-741.

Antrobus, E.S.A.; Brink, W.C.J.; Brink, M.C.; Caulkin, J.; Hutchinson, R.I.; THOMAS, D.E.; Van Graan, J.A. & Viljoen, J.J. The Klerksdorp Goldfield. 1986. (In Anhaeusser, C.R. & Maske, S, eds. 1986. Mineral Deposits of Southern Africa: Volume 1. Geological Society of South Africa: Johannesburg. p. 549-598).

Austin, R.B. 1972. Effects of environment before harvesting on viability. (In Roberts, E.H., ed. 1972. Viability of seeds. London: Chapman and Hall Ltd. p. 114-149).

Aucamp, P. & Van Schalkwyk, A. 2002. Trace element pollution of soils by abandoned gold mine wastes, near Potchefstroom, South Africa. *Bulletin of Engineering Geology and the Environment*. 62:123-134.

Awad, A., Hafiz, S., Hammada, M.S., Ei-Nouby, A. & El-Hendawy, S. 2013. Grain yield production of Sudan grass (*Sorghum sudanense* (Piper) Stapf) as influenced by cutting numbers, potassium rates, and intra row spacing in a semiarid environment. *Turkish Journal of Agriculture and Forestry*. 37:657-664.

Baligar, V.C., Fageria, N.K. & He, Z.L. 2001. Nutrient use efficiency in plants. *Communications in Soil Science and Plant Analysis*. 32(7 & 8):921-950.

## References

- Baud, S., Boutin, J., Miquel, M., Lepiniec, L. & Rochat, C. 2002. An integrated overview of seed development in *Arabidopsis thaliana* ecotype WS. *Plant Physiology and Biochemistry*. 40:151-160.
- Bear, L.M. 1986. Note on the Bulhoek Fluorspar Mine, Groot-Marico district. 1986. (In Anhaeusser, C.R. & Maske, S., eds. 1986. Mineral Deposits of Southern Africa: Volume 1. Geological Society of South Africa: Johannesburg. p. 851.).
- Bedane, G.M., Gupta, M.L. & George, D.L. 2006. Optimum harvest maturity for guayule seed. *Industrial Crops and Products*. 24:26-33.
- Biodiversity Act *see* South Africa.
- Bischoff, A. & Müller-Schärer, H. 2010. Testing population differentiation in plant species – how important are environmental maternal effects. *Oikos*. 119: 445-454.
- Bishaw, Z., Struik, P.C. & Van Gastel, J.G. 2012. Farmers' seed sources and seed quality: 1. Physical and physiological quality. *Journal of Crop Improvement*. 26(5):655-692.
- Bradshaw, A.D. 1998. Restoration of mined lands – using natural processes. *Ecological engineering*. 8:255- 269.
- Bradshaw, A.D. 2000. The use of natural processes in reclamation – advantages and difficulties. *Journal of Landscape and urban planning*. 51:89 – 100.
- Bray, R.H. & Kurtz, L.T. 1945. Determination of the total, organic and available forms of phosphorus in soils. *Soil Science*. 59:39-45.
- Bredan, R.E., Busso, C.A., Fioretti, M.N., Toribio, M.B.M., Baioni, S.S., Torres, Y.A., Fernandez, O.A., Giorgetti, H.D., Bentivegna, D., Entio, J., Ithurrart, L., Montenegro, O., Mujica, M.M., Rodriguez, G. & Tucac, G. 2013. Water stress and temperature effects on germination and early seedling growth of *Digitaria eriantha*. *African Journal of Agricultural Research*. 8(32):4345-4353.

## References

- Brits, Y. 2007. A comparison of selected enhanced (coated) and nonenhanced grass seed types for re-seeding of disturbed areas. Potchefstroom: North-West University. (Dissertation – MSc.) p. 159.
- Brits, Y. & Kellner, K. 2009. Comparing enhanced and non-enhanced grass seed types used in re-seeding rehabilitation practices. *Grassroots: Newsletter of the Grassland Society of Southern Africa*. 9(2):12-18
- Doronila, A.I., Maddox, L.E., Reichman, S.M., King, D.J., Kolev, S.D. & Woodrow, I.E. 2014. Vegetation response of Australian native grass species redgrass (*Bothriochloa macra* (Steudel) S.T. Blake) and spider grass (*Enteropogon acicularis* (Lindl.) Lazarides) in saline and arsenic contaminated gold mine wastes: a glasshouse study. *Minerals Engineering*. 56:61-69.
- Carter, M.R. 2004. Soil quality for sustainable land management: organic matter and aggregation interactions that maintain soil functions. *Agronomy Journal*. 94:38-48.
- Cooke, J.A. & Johnson, M.S. 2002. Ecological restoration of land with particular reference to the mining of metals and industrial minerals: a review of theory and practice. *Environmental Reviews*. 10:41-71.
- Conservation of Agricultural Resources Act *see* South Africa.
- Dong, M. & De Kroon, H. 1994. Plasticity in morphology and biomass allocation in *Cynodon dactylon*, a grass species forming stolons and rhizomes. *Oikos*. 70(1):99-106.
- Duong, T.T.T., Penfold, C. & Marschner, P. 2012. Amending soils of different texture with six compost types: impact on soil nutrient availability, plant growth and nutrient uptake. *Plant and Soil Journal*. 354:197-209.
- El-Keblawy, A., Al-Sodany, Y.M. & Al-Hadad, F.A. 2009. Effects of time of seed maturation on dormancy and germination requirements of *Sporobolus spicatus* (Vahl) Kunth, a native desert grass of the United Arab Emirates. *Grassland Science Journal*. 55:11-17.
- Environmental Conservation Act *see* South Africa.

## References

- FAO, (Food and Agriculture Organization of the United Nations). 2014. FAO Website. www.fao.org. Date of access: 25 Feb. 2014.
- FANOURAKIS, G.C. 2012. Estimation soil plasticity properties from pedological data. *Journal of the South African Institution of Civil Engineering*. 54(2):117-125.
- Farago, M.E & Mehra, A. 1994. Metal Ions and Plant Nutrition. (In Farago, M.E., ed. 1994. Plants and the Chemical Environment: Biochemistry, uptake, tolerance and toxicity. New York: VCH Publishers. p. 31-66.).
- Fei, Q., Hongbing, J., Qian, L., Xinyue, G., Lei, T. & Jinguo, F. 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.
- Fey, M., ed. 2010. Soils of South Africa. Cape Town: Cambridge University Press. p.287.
- Field, A., ed. 2013. Discovering statistics using IBM SPSS statistics. 4<sup>th</sup> Ed. London: Sage Publications Ltd. p.899.
- Field, M.; Stiefenhofer, J.; Robey, J. & Kurszlaukis, S. 2008. Kimberlite-hosted diamond deposits of southern Africa: a review. *Ore Geology Reviews*. 34:33-75.
- Foth, H.D.. ed. 1990. Fundamentals of soil science. 8<sup>th</sup> Ed. New York:John Wiley & Sons. p.353.
- Fourie, A.B. 2007. The engineering contribution of vegetation to the stability of cover systems. (In Mine Closure. 2007. Santiago, Chile: Salviat Impresores. 1<sup>st</sup> Ed. p. 483-494).
- FSSA (Fertilizer Society of South Africa). 2007. Fertilizer handbook. Lynwood Ridge, South Africa: MVSA. 6<sup>th</sup> rev. ed. p.313.
- Galloway, L. 2004. Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytologist*. 166:93-100.
- Gobat, J.M.; Arango, M. & Matthey, W., eds. 2004. The living soil. Enfield, NH, USA: Science Publishers. p.602.

## References

- Grimshaw, S. 2007. Planning for closure as part of project approval. (*In Mine Closure 2007. Santiago, Chile: Salviat Impresores. 1<sup>st</sup> Ed. p 289-300.*).
- Haagner, A.S.H. 2008. The role of vegetation in characterising landscape function on rehabilitating gold wastes. Potchefstroom: North-West University. (Dissertation – MSc.). p.223.
- Hampton, J.G. 1993. The ISTA perspective of seed vigour testing. *Journal of Seed Technology*. 17(2):105-109
- Herselman, J.E. 2007. The concentration of selected trace metals in South African soils. Stellenbosch: University of Stellenbosch. (Dissertation – PHD). p.199.
- Hilhorst, H.W.M. & Karssen, C.M. 2001. Effect of chemical environment on seed germination. (*In Fenner, M., ed. 2001. Seeds: The ecology of regeneration in plant communities. 2<sup>nd</sup> Ed. San Diego, California: Academic Press. p.291-308.*).
- Holdsworth, M.J., Bentsink, L. & Soppe, W.J.J. 2008. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. *New Phytologist*. 179:33-54.
- Hopkins, W.G. & Hürner, N.P.A., eds. 2008. Introduction to plant physiology. 4<sup>th</sup> Ed. New York, USA: Wiley Publishers. p.503.
- Horowitz, M. 1996. Bermudagrass (*Cynodon dactylon*): a history of the weed and its control in Israel. *Phytoparasitica*. 24(4):305-320.
- Hossner, L.R. & Shahendeh, H. 2006. Rehabilitation of minerals processing residue (wastes). (*In, Taylor & Francis, eds. Encyclopedia of Soil Science, 2<sup>nd</sup> Ed. New York. p.1450-1455.*).
- ISTA. (International Seed Testing Association). 2009. International Rules for Seed Testing. Basserdorf, Switzerland: ISTA. ISBN:2310-3655.
- Jadia, C.D. & Fulekar, M.H. 2009. Phytoremediation of heavy metals: recent techniques. *African Journal of Biotechnology*. 8(6):921-928.

## References

- Jann, R.C. & Amen, R.D. 1977. What is germination? (In Kahn, A.A., Ed. 1977. The physiology and biochemistry of seed dormancy and germination. Amsterdam: North-Holloand Biomedical Press. p.7-28.).
- Jacob, E.A.J., Mertz, L.M., Henning, F.A., Peske, S.T., Villela, F.A. & Labbé, L.M.B. 2014. Ideal seeds harvest moment of different maize hybrids. *Ciência Rural*. 44(2):253-260.
- Jones, J.B., ed. 2012. Plant nutrition and soil fertility manual. 2<sup>nd</sup> Ed. New York, USA: CRC Press. p.282.
- Kabata-Pendias, A., ed. 2011. Trace elements in soils and plants. 4<sup>th</sup> Ed. New York: CRC Press: p.520.
- Karlen, D.L., Mausbach, M.L.; Doran, J.W., Cline, R.G., Harris, R.F. & Schuman, G.E. 1997. Soil quality: a concept, definition, and framework for evaluation. *Soil Science Society*. 61:4-10.
- Keeling, S.M. & Werren, G. 2005. Phytoremediation: the uptake of metals and metalloids by Rhodes grass grown on metal-contaminated soil. *Remediation*. 2005 (Spring):53-61.
- Kestring, D., Klein, J., De Menezes, L.C.C.R. & Rossi, M.N. 2009. Imbibition phases and germination response of *Mimosa bimucronata* (Fabaceae: Mimosoideae) to water submersion. *Aquatic Botany*. 91:105-109.
- Koontz, M.B., Knootz, J.M., Pezeshiki, S.R. & Moore, M. 2013. Nutrient and growth responses of *Leersia oryzoides*, rice cutgrass, to varying degrees of soil saturation and water nitrogen concentration. *Journal of Plant Nutrition*, 36(14):2236-2258.
- Kranner, I. & Colville, L. 2011. Metals and seeds: Biochemical and molecular implications and their significance for seed germination. *Environmental and Experimental Botany*. 72:93-105.
- Lange, C.A.; Kotte, K.; Smit, M.; Van Deventer, P.W. & Van Rensburg, L. 2012. Effects of different soil ameliorants on karee trees (*Searsia lancea*) growing on mine wastes dump soil – part 1: pot trails. *International Journal of Phytoremediation*. 14(9):908-924.

## References

- Latzel, V., Hájek, T., Klimešová, J. & Gómez, S. 2009. Nutrients and disturbance history in two *Plantago* species: maternal effects as a clue for observed dichotomy between resprouting and seeding strategies. *Oikos*. 118:1669-1678.
- Leeb-Du Toit, A. 1986. The Impala Platinum Mines. (In Anhaeusser, C.R. & Maske, S., eds. 1986. Mineral Deposits of Southern Africa: Volume 11. Johannesburg: Geological Society of South Africa: p. 1091-1106.).
- Liebenberg, D., Claassens, S. & Van Rensburg, L. 2013. Insights and lessons learned from the long-term rehabilitation of an iron ore mine. *International Journal of Environmental Restoration*. 7 (3): 733-744.
- Liu, R. & Lal, R. 2013. A laboratory study on amending mine soil quality. *Water Air and Soil Pollution*. 224(1679):1-17
- Lottermosser, B.G., Munksgaard, N.C. & Daniell, M. 2009. Trace element uptake by Mitchell grasses grown on mine wastes, Cannington Ag-Pb-Zn Mine, Australia: implications for mined land reclamation. *Water, Air and Soil Pollution*. 203:243-259.
- Luzuriaga, A.L., Escudero, A. & Pérez-García, F. 2005. Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). *Weed Research*. 46:163-174.
- Maboeta, M.S., Claassens, S., Van Rensburg, L. & Jansen van Rensburg, P.J. 2006. The effects of platinum mining on the environment from a soil microbial perspective. *Water, Air, and Soil Pollution*. 175:149-161.
- Maguire, J.D. 1977. Seed quality and germination. (In Khan, A.A., ed. 1977. The physiology and biochemistry of seed dormancy and germination. Amsterdam, Netherlands: North-Holland Biomedical Press. p. 219-233.).
- Marshall, V.M., Lewis, M.M. & Ostendorf, L.B. 2012. Buffel grass (*Cenchrus ciliaris*) as an invader and threat to biodiversity in arid environments: a review. *Journal of Arid Environments*. 78(2012):1-12.

## References

- Mayer, A.M. & Poljakopp-Mayber, A., eds. 1989. The germination of seeds. Oxford: Pergamon Press. p.265.
- McCarthy, T. & Rubidge, B., eds. 2005. The story of Earth and life: a Southern African perspective on a 4.6-billion year journey. Cape Town, South Africa: Struik Nature. p.333.
- Mendez, M.O. & Maier, R.M. 2008. Phytoremediation of mine wastes in temperate and arid environments. *Review of Environmental Sciences of Biotechnologies*. 7:47-59.
- Mendez, M.O. & Maier, R.M. 2010. Phytostabilization of mine wastes in arid and semiarid environments – an emerging remediation technology. *Environmental Health Perspectives*. 116(3):278-283.
- Minerals Act *see* South Africa.
- Mineral and Petroleum Resources Development Act *see* South Africa.
- Morgan, R. P. C. 1986. Soil Erosion and Conservation (*Ed.* Davidson, D. A). Essex, England: Longman. p. 295.
- Morgenthal, T.L. 2003. The assessment of topsoil degradation on rehabilitated coal discard dumps. Potchefstroom: North-West University. (Mini-dissertation – MSc.). p.123.
- Morgenthal, T.L. & Van Rensburg, L. 2004. Ecosystem development on seven rehabilitated discard dumps. *African Journal of Range and Forage Science*. 21(1):57-66.
- Moussa, A.S. 2007. Evaluation of selected soil properties in semi-arid communal rangelands in the Western Bophirima District, South Africa. Potchefstroom: North-West University. (Thesis – PHD.) p.194.
- Muller, I. 2013. Effect of seed coating on the germination of selected grass species in different mine wastes deposits: a nursery trial. Potchefstroom: North-West University. (Mini-dissertation – Hons. BSc.). p.82.
- National Environmental Act *see* South Africa.

## References

National Environmental Management Biodiversity Act *see* South Africa.

Nazar, R., Iqbal, N., Masood, A., Syeed, S. & Khan, N.A. 2011. Understanding the significance of sulfur in improving salinity tolerance in plants. *Environmental and Experimental Botany*. 70:80-87.

Nel, J.H. 2008. An investigation of mine closure: gold mine case studies on the East Rand in South Africa. Potchefstroom: North-West University. (Dissertation – MSc.). p.72.

Neumann, D. & Zur Nieden, U. 2001. Silicon and heavy metal tolerance of higher plants. *Phytochemistry*. 56:685-692.

Overbreek, P.W. 1989. Andalusite in South Africa. *Journal of the South African Institute of Mining and Metallurgy*. 89(6):157-171.

Oves, M., Khan, M.S., Zaidi, A. & Ahmad, E. 2012. Soil contamination, nutritive value, and human health risk assessment of heavy metals: an overview. (In Zaidi, A. Wani, P.A. & Khan, M.A., eds. 2012. Toxicity of Heavy Metals to Legumes and Bioremediation. New York, USA: Springer. p. 1-27.).

Palmer, M.A., Falk, D.A. & Zedler, J.B. 2006. Ecological theory and restoration ecology. (In Palmer, M.A., Falk, D.A. & Zedler, J.B., eds. Foundations of Restoration Ecology. Washington, D.C.: Island Press. p. 1-10.).

Parraga-Aguado, I., Querejeta, J., Conzález, M., Jiménez-Cárceles, F.J. & Conesa, H.M. 2014. Usefulness of pioneer vegetation for the phytomanagement of metal(loid)s enriched wastes: grasses vs. shrubs vs. trees. *Journal of Environmental Management*. 133:51-58.

Patanè, C., Saita, A. & Sortino, O. 2013. Comparative effects of salt and water stress on seed germination and early embryo growth in two cultivars of sweet sorghum. *Journal of Agronomy and Crop Science*. 199:30-37.

Plant Improvement Act *see* South Africa.

## References

- Reichardt, M.T. & Reichardt, C.L. 2007. Vandals or entrepreneurs? Acknowledging and managing the human factor during mine closure in the developing world context. (*In Mine Closure*. 2007. Santiago, Chile: Salviat Impresores. 1<sup>st</sup> Ed. p. 145-154.).
- Resulović, H. & Čustović, H. 2007. Technosols – Development, classification and use. *Agriculturae Conspectus Scientificus*. 72(1):13-16.
- Rhoades, J.D. 1982. Soluble Salts. (*In PAGE, A.L., MILLER, H. and KEENEY, D.R., eds.* 1982. *Methods of Soil Analysis: Part 2 – Chemical and Microbiological Properties*. 2<sup>nd</sup> Ed. p. 167-181.).
- Roach, D.A. & Wulff, R.D. 1987. Maternal effects in plants. *Annual Review of Ecological Systems*. 18:209–235.
- Rodriguez-Jordá, M.P., Garrido, F. & Garcia-González, M.T. 2010. Potential use of gypsum and lime rich industrial by-products for induced reduction of Pb, Zn and Ni leachability in an acid soil. *Journal of Hazardous Materials*. 175:762-769.
- Rossouw, A.S. 2010. Functional evaluation of a gold mine wastes rehabilitation project. Johannesburg: University of Johannesburg. (Dissertation – MSc.) p.192.
- Rout, G.R., Samantaray, S. & Das, P. 2001. Aluminium toxicity in plants: a review. *Agronomie*. 21:3-21.
- Ruiz-Jaen, M.C. & Aide, T.M. 2005. Restoration success: how is it being measured? *Restoration Ecology*. 13 (3):569-577.
- Russel, G.E.G, Watson, L., Koekemoer, M., Smook, L., Barker, N.P., Anderson, H.M. & Dallwitz, M.J. 1990. Grasses of Southern Africa: an identification manual with keys, descriptions, distributions, classification and automated identification and information retrieval from computerized data, ed. Leistner, O.A. Cape Town: National Botanic Gardens. p.437.
- Ryan, P.J. 1986. The Witkop fluorspar mine near Zeerust, Western Transvaal. (*In Anhaeusser, C.R. & Maske, S., eds.* 1986. *Mineral Deposits of Southern Africa: Volume 1*. Johannesburg: Geological Society of South Africa. p.843-849.).

## References

- Sabrina, D.T., Hanafi, M.M., Gandahi, A.W., Mohamed, M.T.M. & 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.
- SAGEP (South African Guidelines for Environmental Protection). 1979. Handbook of Guidelines for Environmental Protection. *Chamber of Mines of South Africa: The Design, Operation and Closure of Residue Deposits*. Pretoria: The Government Printer.
- Sayuti, Z. & Hitchmough, J.D. 2013. Effect of sowing time on field emergence and growth of South African grassland species. *South African Journal of Botany*. 88:28-35.
- Schollenberger, C.J. & Simon, R.H. 1945. Determination of Exchange Capacity and Exchangeable Bases in Soil-Ammonium Acetate Method. *Soil Science*. 1:13-24.
- Schroeder, K., Rufaut, C.G., Smith, C., Mains, D. & Craw, D. 2005. Rapid plant-cover establishment on gold mine wastes in Southern New Zealand: glasshouse screening trials. *International Journal of Phytoremediation*. 7:307-322.
- Schuler, M.S. & Orrock, J.L. 2012. The maladaptive significance of maternal effects for plants in anthropogenically modified environments. *Evolutionary Ecology*. 26:475-481.
- SER (Society for Ecological Restoration). 2004. *The SER International Primer on Ecological Restoration*. International Science & Policy Working Group. (available from <http://www.ser.org>). Tucson, Arizona: SER. p.15.
- Sheldon, A.R. & Menzies, N.W. 2005. The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Kunth.) in resin buffered solution culture. *Plant and Soil*. 278:341-349.
- Singh, K., Pandey, V.C. & Singh, R.P. 2013. *Cynodon dactylon*: an efficient perennial grass to revegetate sodic lands. *Ecological Engineering*. 54:32-38.
- Sills, G.R. & Nienhuis, J. 1995. Maternal phenotypic effects due to soil nutrient levels and sink removal in *Arabidopsis thaliana* (*Brassicaceae*). *American Journal of Botany*. 82(4):491-495.

## References

Smith, D.A.M. & Whittaker, R.R.L.G. 1986. The Springs-Witbank Coalfield. (*In* Anhaeusser, C.R. & Maske, S., *eds.* 1986. Mineral Deposits of Southern Africa: Volume 11. : Johannesburg: Geological Society of South Africa. p.1969-1984.).

South Africa. 2004. Biodiversity Act 10 of 2004. Pretoria: Government Printer.

South Africa. 1983. Conservation of Agricultural Resources Act 43 of 1983. Pretoria: Government Printer.

South Africa. 1989. Environmental Conservation Act 73 of 1989. Pretoria: Government Printer.

South Africa. 1991. Minerals Act 50 of 1991. Pretoria: Government Printer.

South Africa. 2002. Mineral and Petroleum Resources Development Act 28 of 2002. Pretoria: Government Printer.

South Africa. 2004. National Environmental Management Biodiversity Act 10 of 2004. Pretoria: Government Printer.

South Africa. 1998. National Environmental Act 107 of 1998. Pretoria: Government Printer.

South Africa. 1976. Plant Improvement Act 53 of 1976. Pretoria: Government Printer.

Sparks, D.L., *ed.* 2003. Environmental Soil Chemistry. Amsterdam; Boston: Academic Press. 2<sup>nd</sup> Ed. p. 352.

Sreekanth, T.V.M., Nagajyothi, P.C., Lee, K.D. & 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.

Sutton, M.W. & Weiersbye, I.M. 2007. South African legislation pertinent to gold mine closure and residual risk. (*In* Mine Closure 2007. Santiago, Chile: Salviat Impresores. 1<sup>st</sup> Ed. p.89-102.).

## References

- Tanveer, A., Arshad, M.S., Ayub, M., Javaid, M.M. & Yaseen, M. 2012. Effect of temperature, light, salinity, drought stress and seeding depth on germination of *Cucumis melo* var. *agresitis*. *Pakistan Journal of Weed Science Research*. 18(4):445-459.
- Terblanche, J.S. & Kleynhans, E. 2009. Phenotypic plasticity of desiccation resistance in *Glossina pupariae*: are there ecotype constraints on acclimation responses? *Journal of Evolutionary Biology*. 22:1636-1648.
- Tongway, D.J. & Hindley, N.L., eds. 2004. Landscape Function Analysis: Procedures for Monitoring and Assessing Landscapes with Special Reference to Minesites and Rangelands. Australia: CSIRO. p.80.
- Tordoff, G.M.; Baker, A.J.M. & Willis, A.J. 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. *Chemosphere*. 41:219-228.
- USEPA (United States Environmental Protection Agency). 2000. Introduction to phytoremediation. Office of Research and Development. Report No. EPA/600/R-99/107, Washington DC. p.105.
- Van den Berg, L. & Kellner, K. 2005. Restoring degraded patches in a semi-arid rangeland of South Africa. *Journal of Arid Environments*. 61:467-511.
- Van den Berg, L. & Kellner, K. 2010. Important factors for ecotype selection in restoration application. *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie*. 29(4):186-196.
- Van Deventer, P.W., Hattingh, J. & Bloem, D. 2008. Some rehabilitation issues on Witwatersrand gold wastes dam. *Civil Engineering*. August:25-29.
- Van Deventer, P.W. & Hattingh, J. 2004. The effect of the chemical properties of wastes and water application on the establishment of a vegetative cover on gold wastes dams: report to the Water Research Commission. Gezina, South Africa: Water Research Commission. February 2004. p.162.
- Van Oudtshoorn, F., ed. 1999. Guide to grasses of Southern Africa. Pretoria: Briza Publications. 1<sup>st</sup> Ed. p.289.

## References

- Vickers, H., Gillespie, M. & Gravina, A. 2012. Assessing the development of rehabilitated grasslands on post-mined landforms in north west Queensland, Australia. *Agriculture, Ecosystems and Environments*. 160:72-84.
- Viljoen, M.J. & Hieber, R. 1986. The Rustenburg section of Rustenburg Platinum Mines Limited, with reference to the Merensky Reef. (In Anhaeusser, C.R. & Maske, S., eds. Mineral Deposits of Southern Africa: Volume 1I. Johannesburg: Geological Society of South Africa. p.1107-1134.).
- Volis, S., Verhoeven, K.J.F., Mendlinger, S. & Wards, D. 2004. Phenotypic selection and regulation of reproduction in different environments in wild barley. *Journal of Evolutionary Biology*. 17:1121-1131.
- Wang, L., Baskin, J.M., Baskin, C.C., Cornelissen, J.H., Dong, M. & Huang, Z. 2012. Seed dimorphism, nutrients and salinity differentially affect seed traits of the desert halophyte *Suaeda aralocaspica* via multiple maternal effects. *BMC Plant Biology*. 12:170-181.
- Wang, Y., Jiang, G.Q., Han, Y.N. & Liu, M.M. 2013. Effects of salt, alkali and salt-alkali mixed stresses on seed germination of the halophyte *Salsola ferganica* (Chenopodiaceae). *Acta Ecologica Sinica*. 33:354-360.
- Wani, P.A., Khan, M.S. & Zaidi, A. 2012. Toxic effects of heavy metals on germination and physiological processes of plants. (In Zaidi, A. Wani, P.A. & Khan, M.A., eds. 2012. Toxicity of Heavy Metals to Legumes and Bioremediation. New York, USA: Springer. p. 45-66.).
- Weiersbye, I.M. 2007. Global review and cost comparison of conventional and phytotechnologies for mine closure. (In Mine Closure 2007. Santiago, Chile: Salviat Impresores. 1<sup>st</sup> Ed. p. 13-30.).
- Welsh, D., Bianco, A. & 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. 1<sup>st</sup> Ed. p.167-178.).

## References

- Westcott, M. 2011. An evaluation of the germination and establishment of three selected coated grass species in different soil types for rehabilitation. Potchefstroom: North-West University. (Dissertation – MSc.). p. 137.
- White, P.J. & Veneklaas, E.J. 2012. Nature and nurture: the importance of seed phosphorus content. *Plant and Soil Journal*. 357:1-8.
- Winegardener, D.L., ed. 1995. An introduction to soil science for environmental professionals. Boca Raton, Florida, USA: Lewis Publishers. p.271.
- Wong, M.H. 2003. Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere*. 50:775-780.
- Wu, Q., Wang, S., Thangavel, P., Li, Q., Zheng, H., Bai, J. & Qiu, R. 2011. Phytostabilization potential of *Jatropha curcas* L. in polymetallic acid mine wastes. *International Journal of Phytoremediation*. 13:788-804.
- Wulff, R.D., Cáceres, A. & Schmitt, J. 1994. Seed and seedling responses to maternal and offspring environments in *Plantago lanceolata*. *Functional Ecology*. 8(6):763-769.
- Xia, H.P. 2002. Ecological rehabilitation and phytoremediation with four grasses in oil shale mined land. *Chemosphere*. 54(2004):345-353.
- Yang, L., Qi, Y., Jiang, H. & Chen, L. 2013. Roles of organic acid anion secretion in aluminium tolerance of higher plants. *BioMed Research International: Biotechnology*. 2013:1-16.
- Ye, Z.H., Wong, J.W.C. & Wong, M.H. 2000. Vegetation response to lime and manure compost amendments on acid lead/zinc mine wastes: a greenhouse study. *Restoration Ecology*. 8(3):289-295.
- Yunusa, I.A.M., Zolghar, S., Zeppel, M.J.B., Li, Z., Palmer, A.R. & Eamus, D. 2012. Fine root biomass and its relationship to evapotranspiration in woody and grassy vegetation covers for ecological restoration of waste storage and mining landscapes. *Ecosystems*. 15:113-127.

## References

Zehra, A., Gul, B., Ansari, R. & Khan, M.A. 2011. Role of calcium in alleviating effect of salinity on germination of *Phragmites karka* seeds. *South African Journal of Botany*. 78:122-128.

Zerche, S. & Ewald, A. 2005. Seed potassium concentration decline during maturation is inversely related to subsequent germination of primrose. *Journal of Plant Nutrition*. 28:573-603.

## Appendices

### Appendix 1

Table A 1 Metal content (mg/kg) for each growth medium. .... 127

Table A 2: Results from soil analyses for the different growth media. .... 128

**Table A 1 Metal content (mg/kg) for each growth medium.**

	Ammonium NO3 extractable metals mg/kg									
	Al	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	U
<b>T1</b>	16.86	1.21	1.18	21.18	0.05	0.17	0.42	0.71	0.09	0.01
<b>T2</b>	1.07	1.13	14.90	10.53	0.34	1.20	0.39	1.58	0.05	0.01
<b>T3</b>	0.01	1.09	1.84	10.25	0.19	0.26	0.10	0.20	0.06	0.01
<b>T4</b>	0.01	1.09	0.26	1.33	0.02	0.17	0.15	0.09	0.02	0.01
<b>T5</b>	0.01	1.04	0.15	4.55	0.02	0.09	0.07	0.08	0.04	0.01
<b>T7</b>	0.00	0.95	7.25	2.68	0.01	0.01	0.04	0.11	0.02	0.01
<b>T8</b>	0.01	1.06	0.39	1.51	0.02	0.03	0.05	0.15	0.02	0.01
<b>T9</b>	574.50	0.99	28.40	220.33	2.33	3.20	1.15	5.04	0.08	0.05
<b>TC</b>	8.36	0.89	2.38	0.41	0.04	0.83	0.04	0.32	0.02	0.01
<b>TV</b>	0.19	0.05	0.09	0.06	0.06	0.19	0.04	0.00	0.00	0.01

## Appendices

**Table A 2: Results from soil analyses for the different growth media.**

Sample no	pH(KCl)	pH(H <sub>2</sub> O)	EC	P(Bray 1)	K		Ca		Mg		Ca:Mg	Na		CEC	K	Total S	Organic C	Total N	C:N ratio
			mS m <sup>-1</sup>	mg kg <sup>-1</sup>	cmol kg <sup>-1</sup>	mg kg <sup>-1</sup>	cmol kg <sup>-1</sup>	mg kg <sup>-1</sup>	cmol kg <sup>-1</sup>	mg kg <sup>-1</sup>	cmol kg <sup>-1</sup>	mg kg <sup>-1</sup>	cmol kg <sup>-1</sup>	cmol kg <sup>-1</sup>	mg kg <sup>-1</sup>	cmol kg <sup>-1</sup>	%	%	%
T1	4.1	3.9	199	252	0.051	20	27.976	5595	0.142	17	198	0.050	12	0.2	22.33964	13.43	0.00	0.05	0.07
T2	5.3	5.4	193	1	0.076	30	8.965	1793	0.779	94	12	0.039	9	1.3	6.035286	0.26	0.00	0.04	0.09
T3	6.4	6.1	422	0	0.115	45	10.213	2043	2.158	261	5	0.510	117	0.8	13.81171	1.03	0.12	0.04	3.28
T4	8.0	6.6	205	0	0.079	31	1.964	393	0.407	49	5	0.297	68	1.2	6.665813	0.03	0.00	0.02	0.00
T5	7.6	9.7	121	5	1.846	720	9.753	1951	1.934	234	5	3.746	862	9.5	19.39019	0.00	0.09	0.04	2.32
T7	7.9	5.7	146	1	0.061	24	10.114	2023	1.325	160	8	0.199	46	1.1	5.797838	0.38	12.05	0.04	303.04
T8	5.5	6.0	11	3	0.222	87	1.958	392	3.352	406	1	0.086	20	3.6	6.198715	0.00	0.28	0.06	4.79
T9	3.8	2.5	390	1	0.064	25	11.186	2237	1.747	211	6	0.012	3	0.8	8.168044	1.37	54.34	1.28	42.44
TC	4.1	4.1	13	4	0.160	62	0.441	88	0.406	49	1	0.004	1	1.4	11.59368	0.00	0.17	0.05	3.37
TV	7.6	8.7	55	5	0.49	191	20.54	4117	19.66	2389	0.4	1	186	39.5	1.23863	0.00	0.92	0.02	52.02