

# Inoculation of carbon and nitrogen in growth mediums to promote seed germination in mine rehabilitation

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*“Only one who devotes himself to a cause with his whole strength and soul can be a true master. For this reason mastery demands all of a person.” - **Albert Einstein***

*“Most of the fundamental ideas of science are essentially simple, and may, as a rule, be expressed in a language comprehensible to everyone.” - **Albert Einstein***

*“The more I study science the more I believe in God.” - **Albert Einstein***

*“The whole of science is nothing more than a refinement of everything.” - **Albert Einstein***

*“If we knew what it was we were doing, it would not be called research, would it?”  
- **Albert Einstein***

*“The most beautiful thing we can experience is the mysterious. It is the source of all true art and science.” - **Albert Einstein***

*“Out of clutter, find simplicity. From discord, find harmony. In the middle of difficulty lies opportunity”. - **Albert Einstein***

*“To raise new questions, new possibilities, to regard old problems from a new angle, require creative imagination and marks real advance in science.” - **Albert Einstein***

*“Not everything that can be counted counts, and not everything that counts can be counted.” - **Albert Einstein***

*"If you can't explain it simply, you don't understand it well enough."* - **Albert Einstein**

*"I think for months and years, ninety-nine times the conclusion is false. The hundredth time I am right."* - **Albert Einstein**

*"We cannot solve our problems with the same thinking we used when we created them."* - **Albert Einstein**

*"Education is not learning of facts, but the training of the mind to think."* - **Albert Einstein**

*"Anyone who has never made a mistake has never tried anything new."* - **Albert Einstein**

*"Data is not information, information is not knowledge, knowledge is not understanding, understanding is not wisdom."* - **Clifford Stoll**

*"Wisdom begins in Wonder"* - **Socrates**

### ***The Kindergarten Wall***

*Of all you learn here, remember this the best:*

*Don't hurt each other and clean up your mess.*

*Take a nap every day, wash before you eat,*

*Hold hands, stick together,*

*Look before you cross the street.*

*And remember the seed in the little paper cup:*

*First the root goes down,*

*and then the plant grows up!*

**-John McCutcheon**

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## Abstract

The mining industry has been a vital component in the development of South Africa. Activities related to the mining industry have resulted in major impacts, both environmental and social. The rehabilitation of mining-disturbed land involves the reversion of these areas to a usable and sustainable condition in the long-term, i.e. post-closure land use. However, rehabilitation can be inhibited through the inability of vegetation to survive in hostile mine waste materials. Unfavourable conditions such as extreme pH values, adverse chemical and physical properties, and the lack of nutrients in tailing, sub-soil and saprolite materials result in poor germination and growth conditions.

The focus of this research is to amend a selection of soils, sub-soils, saprolite and tailing materials so that it might be used as a suitable cover material ("topsoil") for rehabilitation purposes. The perfect carbon-nitrogen (C/N) ratio is between 25/1 to 35/1 according to literature. The incorrect C/N ratio and status has been identified as a major problem in germination percentages observed in undesired growth mediums. The current methodology to correct the C/N ratio and status is by adding compost (soil organic carbon) at high cost or in some cases topsoil if available. Compost is often very difficult to apply on the steep slopes of tailings storage facilities. The type of treatment necessary to improve sub-soil, saprolite and/or the horizon properties of fresh tailings without adding topsoil and/or large amounts of compost must be determined in order to achieve adequate germination percentages.

Three sets of experimental pot trails took place at the NWU nursery under controlled conditions with respect to water application. After the completion of amelioration application to substrates, sowing of seeds commenced in 1 L pots. Ten coated seeds (5 *Cynodon dactylon* seeds (Couch grass) and 5 *Chloris gayana* seeds (Rhodes grass)) were sowed in each pot. Germination percentages were subsequently determined. The first experiment illustrates the effect of the ameliorants on the substrates. The second experiment was done in order to manipulate the C/N ratio to obtain adequate germination percentages. Due to stunted growth observed in Experiment 1 and 2, the growth potential for grass was determined in Experiment 3. This was achieved by means of grass length

measurements.

The highest germination percentage was obtained in the gold tailings during Experiment 1. This is due to the high gypsum content that makes the substrate hygroscopic meaning that the moisture is absorbed from the air. The drainage potential of this specific gold growth medium was also moderate to good, which contributed to the good germination attained. No crust formation or compaction occurred in this growth medium. The substrate germination levels attained were mostly dependent on the physical characteristics.

The effect of ameliorants is substrate specific. Coated seeds are vital for rehabilitation practices, and need minimal additional amelioration to the substrate to prevent competition of nutrients between ameliorant and seed. The physical characteristics such as texture override the chemical characteristics in this experiment. However, chemical differences such as EC, CEC, pH and C/N ratio are still important in practice even though it was not considered in this experiment as statistically significant.

Furthermore, the second experiment revealed that the gold tailings performed the best with respect to the germination percentage attained. As previously mentioned, this is because of the high gypsum content that increased the water holding capacity (physical characteristic). The effective correction of this substrate's extremely low pH conditions (chemical characteristic) also played a role in the germination attained. In addition, in mine rehabilitation practices are the engineered soils (tailings) already stable prepared for vegetative growth. It was found that the Rhodes grass performed better during the experiments. Couch will thus act as climax species whereas Rhodes grass is a sub-climax species and will thus dominate initially. Rhodes grass begins to die off after three years when its life cycle is completed and the Couch grass will then take over by acting then as climax species. Thus, for Rhodes grass established in substrates based on the germination attained can be statistically summarised as: Gold Tailings is smaller than A – Horizon, which is equal to Potchefstroom Red Structured B, which is smaller than Platinum Tailings. Couch grass established in

substrates based on the germination attained can be statistically summarised as: Gold Tailings is smaller than Potchefstroom Red Structured B, which is equal to A – Horizon, which is equal to Platinum Tailings.

Water is the main influencing factor in germination. Thus the texture is more important (due to the water holding capacity) than additional nutrients for germination. The seeds also contain enough nutrients to sustain them for germination. Nutrients become more important for plant growth.

For experiment 3, an ANOVA statistical processing was done taking substrate, C/N ratio and grass length into consideration. It was concluded that the best growth condition is the A-Horizon with a C/N ratio of 12.5/1. The findings for best growth condition in the A-Horizon are validated by literature based on natural C/N ratios of 12.74/1 for South African dry lands and virgin soils.

The best growth observed, as per the third experiment, were the A-Horizon (351.96 mm); Platinum Tailings (326.93 mm), Gold Tailings (118.94 mm) and Potchefstroom Red Structured Soil (99.70 mm) based on mean grass length attained. The A–Horizon was the only substrate with sufficient phosphorus content (P: 17.4 mg/kg). This is followed by the platinum tailings (P: 6.0 mg/kg), gold tailings (P: 4.9 mg/kg) and Potchefstroom red structured B (P: 3.5 mg/kg). Thus, the higher the deficiency in phosphorus the lower the grass length attained. Phosphorus promotes root development which also supports this statement. There is also a general trend of low Mg resulting in poor germination. Furthermore, Ni and Zn toxicity is anticipated in the gold tailings consequentially resulting in stunted plant growth and development. Rhodes grass does not have a high tolerance against high magnesium content. The soil chemical nutrition plays a major role for plant growth.

There is no set default solution for rehabilitation practices. Each problem is site-specific and one has to integrate and investigate various technical aspects in order to succeed.

**Keywords:** Tailings, soil, sub-soil, topsoil, carbon, nitrogen, Couch grass, Rhodes grass, germination, experiments, ANOVA.



## **Uittreksel**

Die myn-industrie is 'n essensiële komponent in die finansiële ontwikkeling van Suid-Afrika. Aktiwiteite wat met die myn-industrie geassosieer word het 'n impak op beide omgewings- en sosiale aspekte. Rehabilitasie van hierdie versteurde grond moet sodanig gedoen word sodat die area weer bruikbaar en volhoubaar in die langtermyn kan wees, selfs nadat die myn gesluit is. Rehabilitasie kan misluk deur die onvermoë van plante om te oorleef in die ongunstige toestande van mynafvalmateriaal. Hierdie ongunstige toestande vir ontkieming sluit in ekstreme pH vlakke, nadelige chemiese en fisiese eienskappe, asook die tekort van nutriënte in mynslik, sub-grond en saproliet-materiaal.

Hierdie navorsing fokus op die ameliorasie van geselekteerde gronde, sub-gronde, saproliet en mynslikmateriale sodat dit gebruik kan word as 'n gepaste dekkingsmateriaal (boggrond) vir rehabilitasiedoeleindes. Die ideale koolstof-stikstof (C/N) verhouding word beskou as tussen 25/1 en 35/1 volgens literatuur. Die verkeerde C/N verhouding blyk die grootste probleem te wees met ontkiemingspersentasies wat bereik word in ongunstige groeimediums. Die huidige metode om die C/N verhouding reg te stel is deur addisionele kompos by te voeg teen 'n hoë koste, of in sommige gevalle boggrond indien dit beskikbaar is. Dit is dikwels moeilik om kompos toe te dien teen steil hellings van slikdamme. Die tipe behandeling wat die sub-grond, saproliet en horisoneienskappe van vars mynslik kan verbeter, sonder toevoeging van boggrond en groot hoeveelhede kompos, moet bepaal word om voldoende ontkiemingspersentasies te bereik.

Drie stelle eksperimentele potproewe is by die NWU-kwekery onder gekontroleerde omstandighede ten opsigte van watertoediening uitgevoer. Na die ameliorasie van die substrate voltooi is het die saai van sade 'n aanvang geneem in 1 L potte. Tien omhulde sade (5 *Cynodon dactylon* (kweek gras) sade en 5 *Chloris gayana* sade (Rhodes gras)) is gesaai in elke pot. Ontkiemingspersentasies is daarna ooreenkomstig bepaal. Die eerste eksperiment illustreer die effek van die ameliorante op die substrate. 'n Tweede eksperiment is gedoen om die C/N verhouding te manipuleer sodat voldoende ontkiemingspersentasies verkry word. As gevolg van die verhinderde groei wat geobserveer was in eksperiment 1 en 2, is die

groeipotensiaal van gras bepaal tydens eksperiment 3. Dit is bereik deur die lengtes van grasse te meet.

Die hoogste persentasie ontkieming is verkry in die goudmynslik tydens Eksperiment 1. Dit is as gevolg van die hoë gipsinhoud wat die substraat higroskopies maak wat beteken dat die vog geabsorbeer word vanuit die lug. Die dreinerings-potensiaal van die spesifieke goud groeimedium was middelmatig tot goed, wat dan bygedra het tot die ontkieming wat bereik was. Die substraat se ontkieming wat bereik is, is meestal afhanklik van die fisiese eienskappe.

Die effek van ameliorante is substraat-spesifiek. Omhulde saad is essensieel vir rehabilitasiedoeleindes en benodig derhalwe minimale addisionele ameliorasie aan die substraat om kompetisie vir nutriënte tussen die ameliorant en saad te voorkom. Die fisiese eienskappe soos byvoorbeeld tekstuur speel 'n groter rol as die chemiese eienskappe. Alhoewel die chemiese verskille in hierdie eksperiment nie statisties betekenisvol beskou was nie, speel EG, KUK, pH en C/N verhouding steeds 'n belangrike rol in die praktyk.

Voorts wys die tweede Eksperiment dat die gouduitskot die beste gevaar het met betrekking tot die ontkiemingspersentasies verkry. Soos voorheen gemeld, is die hoofsaaklik as gevolg van die hoë gipsinhoud wat die waterhouvermoë bevorder (fisiese eienskap). Die regstelling van die ekstreme lae pH toestand (chemiese eienskap) van hierdie substraat het ook 'n kleiner bydra gelewer in die ontkieming wat verkry was. Daarbenewens het kweekgras meer weerstand gebied as gevolg van beter ontkieming in ongunstige groeimediums. Verder, in mynrehabilitasiepraktyke, is die ingenieursgronde (mynuitskot) reeds stabiel voorberei vir vegetatiewe groei. Dit was bevind dat die Rhodes gras beter presteer het tydens die eksperimente. Kweekgras sal dus as klimaks-spesie optree terwyl Rhodes-gras as sub-klimaks spesie sal optree en dus aanvanklik sal oorheers. Rhodes-gras begin af te sterf ná drie jaar wanneer sy lewensiklus voltooi is en die kweekgras sal dan oorneem deurdat dit as klimaksspesie sal dien. Dus vir Rhodes gras wat gevestig is in substraat gebaseerd op die ontkieming bereik, kan dit

statisties opgesom word as: Goudslik kleiner as A–Horison, wat dan kleiner is as Potchefstroom Rooi Gestruktureerde B, wat dan kleiner is as Platimumslik. Kweek gras wat gevestig is in substrate gebaseerd op die ontkieming bereik, kan dit statisties opgesom word as: Goudslik kleiner as Potchefstroom Rooi Gestruktureerd B, wat gelyk is aan A – Horison, wat gelyk is aan Platimumslik.

Water is dus die hoof faktor wat ontkieming beïnvloed. Die tekstuur is dus belangriker (as gevolg van die waterhouvermoë) as die addisionele nutriënte vir ontkieming. Die saad bevat ook genoegsame nutriënte om die saad te onderhou tydens ontkieming. Nutriënte word toenemend belangriker vir die plant se groei.

Vir eksperiment 3, is 'n ANOVA statistiese prosessering wat die tipe substraat, C/N verhouding en graslengte in ag neem, gevolglik gedoen. Dit kan tot die gevolgtrekking lei dat die beste groeitoestand die apedale bogrond met 'n C/N verhouding van 12.5/1 is. Die bevinding vir die beste groeitoestand in die apedale bogrond is bevestig deur literatuur gebaseer op natuurlike C/N verhouding van 12.74/1 vir Suid-Afrikaanse droë-landtoestande en ongerepte grond.

Die beste groei geobserveer per substraat was die A–Horison (351.96 mm); platimumuitskot (326.93 mm), gouduitskot (118.94 mm) en Potchefstroomse rooi gestruktureerde grond (99.70 mm), gebaseer op die gemiddelde graslengte. Die A–horison was die enigste substraat met genoegsame fosforinhoud (P: 17.4 mg/kg). Dit word gevolg deur die platimumuitskot (P: 6.0 mg/kg), gouduitskot (P: 4.9 mg/kg) en Potchefstroomse rooi gestruktureerde B (P: 3.5 mg/kg). Dus, hoe hoër die tekort aan die fosforinhoud is, hoe laer is die lengte van die gras. Fosfor bevoordeel ook die wortelontwikkeling van die plant, en ondersteun dus hierdie stelling. Daar is ook 'n algemene tendens dat lae Mg-inhoud swakker ontkieming laat geskied. Voorts was daar Ni en Zn toksisiteit ondervind in die goudslik wat gevolglik lei tot vertraagde groei en ontwikkeling van plante. Rhodes-gras het nie 'n hoë toleransie teen hoë magnesiuminhoud nie. Die grondchemiese voeding speel 'n belangrike rol in die groei van plante.

Daar is geen vasgestelde standaard-oplossing vir rehabilitasiepraktyke nie. Elke probleem is plek-spesifiek en mens moet verskeie tegniese aspekte integreer en ondersoek ten einde te slaag.

**Sleutelwoorde:** Mynslik, grond, sub-grond, bogrond, koolstof, stikstof, kweekgras, Rhodes-gras, ontkieming, eksperimente, ANOVA.

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## Abbreviations

• AMD	Acid Mine Drainage
• C	Carbon
• Ca	Calcium
• C/N	Carbon / Nitrogen ratio
• CEC	Cation Exchange Capacity
• EC	Electrical Conductivity
• IC	Ion Chromatography
• ICP-MS	Inductive Coupled Plasma Mass Spectrometry
• INT	2-(p-iodophenyl)-3- (p-nitrophenyl)- 5-phenyltetrazolium chloride
• K	Potassium
• MWS	Mine Waste Solutions
• Mg	Magnesium
• Mn	Manganese
• N	Nitrogen
• NWU	North-West University
• P	Phosphorus
• PAW	Plant Available Water
• pH	Negative logarithm of the hydrogen concentration (acid, neutral or alkali measurement)
• ppm	Parts per million
• PSD	Particle Size Distribution
• SOC	Soil Organic Carbon
• SOM	Soil organic matter
• TSF	Tailings Storage Facility (tailings dam)
• TTC	Triphenyltetrazolium chloride
• Tukey's HSD	Tukey's Honestly Significant Difference test

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# 1. Introduction

The mining industry has been a vital component in the development of South Africa's economy (Kumo *et al.*, 2014:3). Activities related to the mining industry such as mineral resource exploitation and waste product generation have resulted in major impacts (OECD, United Nations & OSAA, 2010:20), both environmental and social (Chamber of Mines of South Africa & Coaltech, 2007:3). Impacts include the weakening of environmental health (Darmondy *et al.*, 2009:265) via the loss of topsoil (Rai *et al.*, 2009:18), 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 of the environmental impacts are directly associated with pollution caused by metals and contaminants related to mine waste materials (Oelofse, 2008:1). Some risks to environmental health include seepage and leaching of contaminants and salts into surface and subsurface water resources decreasing the water quality (Grimshaw, 2007: 295; Sutton & Weiersbye, 2007:92; Welsh *et al.*, 2007:175). Air and water pollution as well as surface runoff is initiated by erosion instability of mine wastes (Grimshaw, 2007:295; Welsh *et al.*, 2007:175). A factor that is constantly disregarded is latent and residual risks from mine waste materials, for these only become obvious long after mine closure. Impacts resulting from this acidification process include acid generation, leaching and seepage of metals causing contamination of ecosystems, water sources and consequently toxic levels of metals leading to possible bioaccumulation in biota and humans (Van der Putten, 2005:256; Sutton & Weiersbye, 2007:92). Extreme pH conditions anticipated for gold mine waste materials are the result from the oxidation of sulphide bearing minerals (Uzarowicz, 2011:711) and metallurgical processes (Wu *et al.*, 2001). The solubility of metals increase dramatically in acidic pH conditions (Risk Assessment Forum, 2007:3-21) causing the metals to be bioavailable to vegetation, which causes bioaccumulation at levels toxic to human health (Wu *et al.*, 2001).

In order to preserve and protect our physical environment and minimize degradation, rehabilitation is therefore a necessity. The rehabilitation of mining-disturbed land involves the reversion of these areas to a usable and

sustainable condition in the long-term, i.e. post-closure land use. Various tools are used to achieve this objective, and attention to soil quality is critical for the success of rehabilitation. Different national legislations also require rehabilitation.

The Constitution is the ultimate law in South Africa that supersedes all laws in the country (South Africa Act 108, 1996). An example of this is the water law that must be subject to and consistent with the Constitution in all matters (South Africa Act 108, 1996). The Environmental Right involves that all South Africans have a right to an environment that is not harmful to their health or well-being as well as the right to have the environment protected for the benefit of the future generation (South Africa Act 108, 1996). The National Environmental Management Act involves the “polluter pays” principle, which 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”* (South Africa Act 107, 1998). Other acts that are applicable to rehabilitation are the MPRDA (South Africa Act 28, 2002) and the Minerals Act (South Africa Act 50, 1991). For a more comprehensive summary on legislation, refer to Appendix 3.

The most successful rehabilitation method is to apply chemical amelioration of the medium joined with vegetation establishment (SAGEP, 1979). Phytoremediation is influenced by the germination attained. The conservation of topsoil should be a priority for the mining industry due to the seed bank within this horizon. Topsoil provides a more stable growth medium for vegetation establishment due to the sustained nutrition necessary of vegetative growth. During mining operations topsoil is stockpiled for rehabilitation that will commence after operations have ceased. During topsoil stockpiling, the soil will experience loss of structure, nutrients content and microbial activity (Weiersbye, 2007:21).

The focus of the presented research is to propose ways to amend soil, sub-soil and tailing materials so that they can be utilized as a suitable cover material (“topsoil”) for rehabilitation purposes. The experiments took place at the NWU nursery under

controlled conditions with respect to water application. Physical characteristics that might influence germination such as crusting were eliminated due to the regular irrigation of samples. Amelioration was completed and planting commenced in 1 L pots. Ten coated seeds (Experiment 1) and 10 uncoated seeds (Experiment 2 and 3) (5 *Cynodon dactylon* seeds (couch grass) and 5 *Chloris gayana* seeds (Rhodes grass)) were sowed in each pot. Germination percentages were subsequently determined.

The first experiment illustrated the effects of different ameliorants on the germination of grass on the different substrates utilised. Although 5 ml of fertilizer was added to each pot, it was realised that the C/N ratios of the ameliorants range from six to 100 and could not statistically be included in the data analyses. The second experiment was completed in order to manipulate the C/N ratio by using a source with high C whilst applying different amounts of N. This enabled one to obtain various C/N ratios in order to investigate the effect of C/N ratios on the germination percentages. Due to stunted growth experienced during experiments 1 and 2, a third experiment was conducted to investigate the influence of C/N ratios applied and substrate on the growth potential. This project was therefore subdivided into three parts.

## **1.1 Background information on project**

Soil can be described as the unconsolidated minerals and organic material on the surface of the earth (Bardgett, 2005:2; Brady & Weil, 2008:926; Gobat *et al.*, 2004:11; Singer & Munns, 1992:7; Van der Watt & Van Rooyen, 1995:165). This material results from the interaction of weathering and biological activity on the parent material or underlying hard rock (Lindbo *et al.*, 2012:15; Singer & Munns, 1992:3; Rai *et al.*, 2009: 18). Soil serves as a growth medium that sustains plant growth (Brady & Weil, 2008:947; Gobat *et al.*, 2004:11; Larson & Pierce, 2013:71; Lindbo *et al.*, 2012:15; Singer & Munns, 1992:1; Soil Classification Working Group, 1991:240). A soil profile can be defined as a vertical section of the soil through all the horizons (A, B and C), and each of these horizons is parallel to the surface of



the land that comprises the solum (Brady & Weil, 2008:944; Bridges, 1990:4; NRCS, 2006:3; Soil Classification Working Group, 1991:236). A horizon can be described as a distinctive boundary within a soil profile which might differ in geophysical properties and can be grouped into master horizons and diagnostic horizons (with respect to particular properties) (Soil Classification Working Group, 1991:227; University of Oxford, 2013:282). Solum is the most weathered upper part of the soil profile; it is the A, B and E horizons respectively (Brady & Weil, 2008:949; Juma, 2013:21; Singer & Munns, 1992:6; Soil Classification Working Group, 1991:241) that reflects the results of soil-forming processes in the soil (Verheye, 2007). A profile can vary between 10 cm and several metres in thickness (NRCS, 2006:3) and is made up of various layers (horizons) (Van der Watt & Van Rooyen, 1995:176; Yong *et al.*, 2012:11), including topsoil and overburden layers (Juma, 2013:21).

The A-horizon is also referred to as topsoil because this fertile soil material refers to the first several centimetres ( $\pm 15$  cm) of the soil profile (Rai *et al.*, 2009:18; University of Oxford, 2013:594; Van der Watt & Van Rooyen, 1995:215). The A-horizon is therefore composed of a relatively balanced mixture of mineral and soil organic matter (SOM) (Bardgett, 2005:2; Brady & Weil, 2008:926; Singer & Munns, 1992:7); it is very important since it is the source of plant nutrients and the majority of plant roots are found here (Rai *et al.*, 2009:18; Singer & Munns, 1992:7). Because of this, topsoil must be correctly removed and stored in order to be utilised effectively in the implementation of rehabilitation measures (Rai *et al.*, 2009:18). The surface organic horizon (O-horizon) develops as the decomposing organic matter accumulates on the surface (Bardgett, 2005:2) or uppermost A-horizon (Van der Watt & Van Rooyen, 1995:175). When a surface soil is uncultivated, the top part of the A-horizon is composed of the L-, F- and H-layers (Samonil *et al.*, 2008:2599). These layers represent different degrees of decomposition of SOM (Van der Watt & Van Rooyen, 1995:176). The fresh litter (recognisable plant and animal remains), also known as the L-layer (Hoover & Lunt, 1952:369), lying directly on top of the surface was deposited during the previous annual cycle of plant growth (Bardgett, 2005:2; Van der Watt & Van Rooyen, 1995:175). According to Bardgett (2005:2), this layer is often overlooked in soil-sampling regimes, but is

perhaps the most active and functionally important zone of the soil profile. The fermentation layer (F-layer) situated below the L-layer comprises partially decomposed litter (Bardgett, 2005:3; Hoover & Lunt, 1952:369; Van der Watt & Van Rooyen, 1995:176). The humus layer (H-Layer) comprises humified material with little or no visible plant structures (well decomposed) often mixed with mineral material from below (Bardgett, 2005:3; Hoover & Lunt, 1952:369; Van der Watt & Van Rooyen, 1995:176).). The rest of the A-horizon follows below these layers (Bardgett, 2005:3; Van der Watt & Van Rooyen, 1995:176).

The A-horizon can either lie directly on the B-horizon (Soil Classification Working Group, 1991:11), as in the case of well-developed soils (Van der Watt & Van Rooyen, 1995:176), or on an intermediate leached horizon (E or A2) (Van Huyssteen, 2001:81). Due to leaching, the E- and A2-horizons are usually paler in colour than the horizons above and below (Soil Classification Working Group, 1991:9; Van Huyssteen, 2001:81). Relative to the A- and B-horizons, these intermediate horizons display a deficiency of trace elements and clay (Van der Watt & Van Rooyen, 1995:176). The B-horizon may be found beneath the A-horizon (Yong *et al.*, 2012:10), E-horizon (Brady & Weil, 2008:928) or O-horizon (Singer & Munns, 1992:7) and is generally used to identify soil types (Van der Watt & Van Rooyen, 1995:176). This horizon originates from the weathering of underlying rock (Lindbo *et al.*, 2012:15; Van Huyssteen, 2001:82; Yong *et al.*, 2012:10), and the weathering may be enhanced by the translocation of materials from overlying horizons (Van der Watt & Van Rooyen, 1995:177). The C-horizon (saprolite) that often comprises the parent material (Yong *et al.*, 2012:10) is found below the B-horizon (Brady & Weil, 2008:16; Van Huyssteen, 2001:82) and generally shows little change from the original parent material (Van der Watt & Van Rooyen, 1995:177; Yong *et al.*, 2012:10). The sub-soil material situated between the topsoil and the parent rock is known as the overburden (University of Oxford, 2013:417; Van der Watt & Van Rooyen, 1995:132).

Soil can be considered as a non-renewable resource (Meuser, 2013:173) for it is the most productive layer (Rai *et al.*, 2009:18) due to the high SOM concentration

(Defra, 2009:20; Yong *et al.*, 2012:10). Once soil is lost it takes many years for the environment to recover to its previous state and this gives emphasis to the importance of reusing stripped soil for successful rehabilitation (Chamber of Mines of South Africa & Coaltech, 2007:13). Before mining activities can commence, the topsoil and overburden must be removed and stored separately in order to prevent the mixing of soil layers (Chamber of Mines of South Africa & Coaltech, 2007:13; Rai *et al.*, 2009:18; Strohmayer, 1999:1). Although this process is much more costly in comparison with collective storage (Alcoa Inc., 2015), it preserves the seed bank and nutrient status of the topsoil necessary for rehabilitation (Rai *et al.*, 2009:18), since sub-soil layers do not contain the needed organic matter (Strohmayer, 1999:3). The topsoil can be used at rehabilitation sites and the stockpiled overburden returned to the mine pit (coal mining restored to original surface level) as soon as mining activities cease (Strohmayer, 1999:1). According to Goldfields (2005:4) and Meuser (2013:173), one can consider topsoil as a finite resource vital for successful rehabilitation. The collection and management of this resource must be used to the fullest extent for it often means the difference between rehabilitation success and failure (Goldfields, 2005:4).

A “direct return” of topsoil involves the removal of topsoil from an area that is prepared for mining towards an area in need of top soil for rehabilitation (Goldfields, 2005:6; Alcoa Inc., 2014). Because the soil used in this method is not stockpiled, the double-handling of soil is minimized (Alcoa Inc., 2015) and this ensures the preservation of important nutrients, organic matter and microbes in the soil (Goldfields, 2005:6). The standard method of soil removal and return involves a bottom dumper (Goldfields, 2005:6), while trucks and loaders are used to transfer soil from stockpiles (Alcoa Inc., 2015). Dozers and graders (heavy equipment (Strohmayer, 1999:1)) are used to level the soil across the landscape surface after the relocation (Alcoa Inc., 2015). Scattering of topsoil on rehabilitation sites should be kept to a minimum depth of 100 mm and a maximum of 200 mm (Goldfields, 2005:4).

Although one makes use of nutrient-rich topsoil during the rehabilitation process, germination still often tends to be poor to non-existent. The poor germination might be related to either the incorrect balance of the carbon to nitrogen ratio or the low C and N status. Even though the C/N ratio is within the threshold range of 25/1 (Groot-Nibbelink *et al.*, 2009:1), the concentration of each element relative to volume may be too low. Sub-soil, saprolite and fresh tailings typically have low C and N concentrations.

## **1.2 Scope of project**

The scope of this research project is the germination establishment of two grass species by determining the success of different substrates subjected to several amelioration treatments with different C/N ratios and statuses. Two grass species, *Cynodon dactylon* (couch grass) and *Chloris gayana* (Rhodes grass), were selected for all three of the experiments. The substrates used in the trials (indicated in Table 1) included saprolite, sub-soil and tailing mediums.

## **1.3 Justification**

Rehabilitation specialists struggle to germinate seeds on TSFs efficiently, which resulted in the inspiration for this project. This project should provide insight into rehabilitation via the incremental rate of seeds germinating.

Procedures such as the removal, transport and storage of topsoil, the amelioration of soils or tailings, and the establishment of plant species are essential. This is costly but yet a necessity in order to ensure successful rehabilitation of mining-impacted sites. The costs associated with the application of additional topsoil are compensated by the success in vegetation establishment (Australian Government, 2006:38). As such, knowledge of the soil quality indicators of different ameliorated substrates for plant establishment can play a critical role in the reduction of soil and tailings degradation. If this project succeeds providing information on soil dynamics, it will result in a substantial cost reduction of mine rehabilitation. It might even

provide insight into agronomic systems and processes ensuring acceptable germination for efficient food production.

Photos 1 & 2 illustrate the A-horizon after strip-mining took place. They only reshaped the B-horizon without applying proper amelioration, which resulted in the poor vegetation cover (Van Deventer & Ferreira, 2013:158).



**Photo 1:        Unsuccessful soil redistribution at a mine site in the Mpumalanga area emphasises the need for the current research. Credit: Piet van Deventer**



**Photo 2:        Unsuccessful soil redistribution at a mine site in the North West Province emphasises the need for the current research. Credit: Piet van Deventer**

## **1.4 Problem statement**

Inadequate concentrations of C and N status, or an imbalance in the C/N ratio of substrates such as sub-soil, saprolite and tailings materials, can result in poor to no seed germination. The current methodology to correct the soil C and nutrient status involves the addition of compost (soil organic matter (SOM)) and/or topsoil, depending on availability and quality. However, these rectifications are both costly and are often difficult to apply on steep slopes. Topsoil in many cases is not available, and therefore an alternative surface material must be used for vegetation establishment. This study consists of three experiments. The first experiment indicated the significance of the effect of various amendments to different substrate types. The difficulty lies within the fact that there is no default rectification, but it is rather site and material specific. The second experiment was a follow-up to illustrate that the C/N ratio is much more important in mine rehabilitation than previously thought. This experiment was done to find a more generalised rectification method. A third experiment was done to investigate the growth potential of grass germinated in the second trail. This was done by measuring the grass lengths.

## **1.5 Aim and objectives**

**The aim of this project was to:**

- Determine which types of inoculation treatments, as applied to different substrates (sub-soil, saprolite and tailings), will improve quality in order to facilitate adequate germination percentages without adding topsoil and/or large amounts of compost. These individualised inoculation treatments can then be applied to infertile substrates prior to the use of alternative topsoil.
- Determine which C/N ratio treatments applied to different substrates (sub-soil, saprolite and tailings) will improve germination percentages. The individualised C/N ratios can then be applied to infertile substrates. Growth potential of grass should be incorporated by means of grass length achieved.

**The objectives of this project were to:**

- a. Identify the most suitable amelioration method per substrate to be used as topsoil (Experiment 1);
- b. Distinguish between the most suitable C/N ratio and/or status for various substrates based on germination (Experiment 2).
- c. Incorporate growth potential of grass by means of grass length acquired simultaneously as the C/N ratios (Experiment 3).

## **1.6 Hypothesis**

Restoration of the C/N ratio of various growth mediums will improve germination and possibly sustained growth of *Cynodon dactylon* (couch grass) and *Chloris gayana* (Rhodes grass) in previously unsuitable materials to ease the demand of topsoil in rehabilitation practices of the mining industry.

## **1.7 Climate**

Only the climate of Potchefstroom is discussed due to the experiments being conducted in the NWU nursery. All the substrates were subjected to the same climatic conditions. The Potchefstroom region, in the central part of the North West Province, receives summer rainfall, with the highest rainfall events occurring mid-summer (Odendaal *et al.*, 2008; SA Explorer, 2011). The average annual rainfall is  $\pm 507$  mm with the lowest average rainfall (0 mm) per month occurring in June and the highest (97 mm) in January (SA Explorer, 2011). The average midday temperatures in Potchefstroom range between 17.9°C in June to 29°C in January (SA Explorer, 2011). The coldest temperatures in this region are experienced in July, with an average night-time temperature of 0°C (SA Explorer, 2011). Hailstorms, a result of convective storms, occur sporadically in the summer - approximately 1-3 times per year (De Villiers & Mangold, 2002). The flash density from lightning strikes is around 5-6 flashes/km<sup>2</sup>/yr in the central parts which is a major source of veld fires (De Villiers & Mangold, 2002). Seasonal fluctuations in

mean temperatures between the warmest and the coldest months are between 12°C and 15°C (De Villiers & Mangold, 2002). The windy months occur between August and November mainly from a northern direction (De Villiers & Mangold, 2002). Heavy frost occurs in the region approximately 31-60 days on average (De Villiers & Mangold, 2002). The humidity is approximately between 28-30% for the region, with the highest humidity ranging between 64-66 % during February (De Villiers & Mangold, 2002). The humidity affects the resident flora of the region due to the high-potential evapotranspiration rates (De Villiers & Mangold, 2002).



## **2. Literature study**

### **2.1 What is topsoil**

Topsoil (a fertile soil material) refers to the first several centimetres ( $\pm 15$  cm) of the soil profile (Rai *et al.*, 2009: 18; University of Oxford, 2013:594; Van der Watt & Van Rooyen, 1995:215) and contains a large quantity of seeds and nutrients (Brady & Weil, 2008:950) vital to the success of mine rehabilitation. Because of this, topsoil must be correctly removed and stored in order to be utilised effectively in the implementation of rehabilitation measures (Rai *et al.*, 2009: 18). Topsoil is defined in some cases as a cover of organic material that serves as a growth medium for vegetation (Rai *et al.*, 2009: 18; Van der Watt & Van Rooyen, 1995:215), the nutritional content of which (N, P & K) must be well-balanced (Gouvernement du Québec, 1997:61). Goldfields (2005:7) mentioned that topsoil varies from deep loamy soil in low-lying areas to heavy vegetation and rocky outcrops due to the topographical location. Useful topsoil is considered as fertile surface material up to a depth of 300 mm (Rai *et al.*, 2009: 18)

Topsoil can aid in numerous functions such as providing seeds and other propagules, macro- and micro-nutrients, micro-organisms, ground-cover development, as well as the amelioration of adverse constituents in the underlying mine waste (Australian Government, 2006:38). Soils have in general fewer problems associated with respect to plant establishment in comparison with mine waste. The costs associated with the application of additional topsoil are compensated for by the success in vegetation establishment (Australian Government, 2006:38).

It is recommended that the topsoil and subsurface horizons should be stripped and replaced separately (Goldfields, 2005:6) due to the undesirable characteristics associated with the subsoil such as high salinity and sodicity, extreme acidity and associated aluminium toxicity, or calcium deficiencies (Australian Government, 2006:38). The double-stripping will guarantee that the horizon containing the

nutrients, microbes and seeds is returned to the surface (Australian Government, 2006:38).

The topsoil thickness stripped is dependent on depth below the surface and/or a distinct colour change (Defra, 2009:24). In the case of stripping too deeply, one might reduce the fertility of the topsoil now being consequentially mixed with unfertile sub-soil (Defra, 2009:24). During the collection of vegetation and topsoil, in areas consisting of dense vegetation, it will be more practical if the vegetation is pushed or grubbed into separate stockpiles (Goldfields, 2005:4). This is recommended due to the vegetation causing damage to scrapers on recovery (Goldfields, 2005:5). Factors such as the desired vegetation, the quantity and quality of the surface and sub-soil available and the nature of the underlying material will influence the total depth of the topsoil replaced on the tailing, waste rock or spoil (Australian Government, 2006:38).

The topsoil-handling plan must take into account that the root zone should be supplied with sufficient water throughout the driest season (Australian Government, 2006:38). Goldfields (2005:6) recommends a spreading of topsoil to a minimum of 100 mm and a maximum of 200 mm and Meuser (2013:172) recommended a mechanically compacted thin layer of 5-10 cm (Meuser, 2013:172). Topsoil placement should be allocated first to priority areas if inadequate amounts of topsoil are available (Goldfields, 2005:6). The root zone supplying objective is attained by using a material with the capacity of high availability of water that increases as the depth of the topsoil does (Australian Government, 2006:38). Boldt-Leppin *et al.* (2000) stated that it is necessary to have the know-how of the micro-climate at the site of the waste material as well as the geotechnical/soil mechanical information on the materials used for construction. The topsoil properties differ over the vegetation period due to the tillage operations, plant cover development and changing soil moisture conditions (Jakab *et al.*, 2013:147). Soil interactions between the soil system and rainfall experienced will change the soil system (Jakab *et al.*, 2013:147). Defra (2009:25) mentioned that the subsoil is also a vital component of soil, for it stores moisture by means of transmitting rainfall to deeper layers or

watercourses which in turn enable deep root systems of trees, scrubs and grasses. The subsoil aids in the reduction of surface water runoff and erosion, controlling waterlogging of the surface layers helping the crops withstand summer droughts and providing anchorage for trees (Defra, 2009:25).

According to the Australian Government (2006:38) topsoil cover ought to be designed so that it reduces rainfall percolation into underlying tailings, and in doing so, also decreases the seepage from mine tailings (Rykaart & Caldwell, 2006:2). Topsoil covers should also limit the entry of oxygen to the tailings (Australian Government, 2006:38). According to Defra (2009:27), the main aim of temporary stockpiling is to “maintain soil quality and minimise damage to the soil’s physical condition so that it can be easily reinstated once respreads as a cap” (Weiersbye, 2007:21). The microbial activity decreases in depth and time as the stockpiling period is prolonged during the mining operations (Sheoran *et al.*, 2010:7).

Covers are constructed for tailing impounds, heap leach pads, waste rock dumps, sludge ponds and landfills (Van Deventer, 2009:7). Varieties of caps exist worldwide and are dictated by the environment (ITRC, 2010:1; Van Deventer, 2009:7), of the mine, waste covered, climate and the governing legislations (Rykaart & Caldwell, 2006:2). Topsoil used as a phytocap can be very effective if used in the appropriate setting with the applicable design (ITRC, 2010:1). Although phytocaps are aesthetically acceptable, it might not be more effective or sustainable than other cover types such as rock cladding (Van Deventer, 2009:8). However, in terms of end land use functionality, phytocaps might be the more sustainable choice. The evapo-transpiration cap can be described as a vegetated soil layer (Van Deventer, 2009:7). This is a very economical rehabilitation option and is easily constructed and maintained. This principle is based on the soil’s water-holding capacity until it is removed via evapo-transpiration. The capacity to store precipitation effectively prevents any percolation of water past the cover (ITRC, 2010:2).

Vegetation cover is by far the most popular method for metalliferous tailings surface stabilisation in South Africa. Vegetation is used as a secondary stabilisation method in more recent times (Van Deventer, 2009:7). A vegetative cover comprises soil that is sufficient for proper root support and moisture storage, as well as a vegetative layer (Sylvia *et al.*, 2005: 176). The vegetative layer will consist of growth media as well as adjustments with macro- and micro-nutrients to support growth (ITRC, 2010:2). This cover will serve as a protection against erosion caused by gullyng and buffing by the wind and surface water (Van Deventer, 2009:7). It is recommended that indigenous species (Goldfields, 2005:7) with shallow root systems must be established so that the cap's integrity stays intact (ITRC, 2010:2). Some advantages of caps include minimization of liquid migration and promoting drainage while controlling erosion. These advantages can be permanent and cost effective (ITRC, 2010:2; U.S. Department of Energy, 2000:6). Both the acute and chronic risks to human health and ecological receptors are addressed as dust emissions (ITRC, 2010:2).

During the topsoil stripping process one must minimise the dust emissions (Van Deventer, 2009:11) resulting from stripping activities (Ferris *et al.*, 1996:11). Equipment and vehicles' movement on undisturbed topsoil must be kept to a minimum (Van Deventer, 2009:7) in order to eliminate topsoil contamination (Ferris *et al.*, 1996:11). The removal of topsoil is done in such a manner as to guarantee drainage flow from the disturbed areas to sediment control structures (Ferris *et al.*, 1996:11). In order to preserve the topsoil and minimising any possible erosion, a topsoil ditch is made at the toe of the stockpile (Ferris *et al.*, 1996:11). The slope at which the stockpile is to be stacked is a 2H:1V slope angle (Ferris *et al.*, 1996:12). However, this angle is too steep and is therefore not considered as the ideal in South Africa. The natural angle of repose of most soil materials is approximately 3:1 to 4:1 (Van Deventer, 2015: Personal correspondence). The entire area is bladed at which topsoil is collected to gather any additional topsoil that can be utilized (Ferris *et al.*, 1996:12). One of the main problems associated with unsuccessful rehabilitation is the steepness of slopes of tailings disposal facilities (TDF) (Minerals Council of Australia, 1998:19).

Slopes steeper than 27 degrees will reduce top soil adherence (Minerals Council of Australia, 1998:19). However, the angle of repose differs with various materials. To maximise top soil retention on the tailings slope, the slope angle should be approximately 19 degrees; this will, however, be dependent on the soil properties and the specific site variables of the TDF (Minerals Council of Australia, 1998:19).

The utilization of topsoil material as part of TDF rehabilitation has both advantages and disadvantages. When selecting the suitable topsoil material, one should keep in mind that no single type of topsoil will be suitable for all situations (ITRC, 2010:1; Van Deventer, 2009:7). Some quality criteria with respect to topsoil are of importance. It is necessary for a topsoil to have the capacity to control infiltration and air entry, resist erosion by wind and water, and keep stable and in the long term it should not creep or slide down the sides of the TDF (Rykaart & Caldwell, 2006:2). It should also be able to support vegetation and biomass, and must be sustainable over a defined period of time (Rykaart & Caldwell, 2006:2).

Some advantages of topsoil covers on the slopes of TDFs include:

- assists with the reduction of runoff on the slope (Australian Government, 2006:38);
- reduces wind and water erosion (Van Deventer, 2009:7);
- lowers the angle of the slope (ITRC, 2010:2);
- serves as a growth medium (Gouvernement du Québec, 1997:61);
- reduces seepage and leaching (Rykaart & Caldwell, 2006:2); and
- and stabilizes the TDF (Rykaart & Caldwell, 2006:2).

Topsoil application for vegetation establishment purposes can only be beneficial, yet variations might occur and are site-specific (ITRC, 2010:1; Van Deventer, 2009:7).

Some disadvantages of topsoil on the slopes of TDFs include:

- topsoil must be moved and reshaped (Defra, 2009:27);

- movement of equipment as well as the moisture content of the soil increases the soils ability to compact and breakdown drastically (Australian Government, 2006:39);
- The process of applying top soil to TDF can be very prolonged;
- There is a substantial cost impact when applying top soil to TDF; and
- There will be a maintenance requirement throughout the topsoil's lifecycle.

## **2.2 How is topsoil (substrate) quality defined to serve as a suitable germination and growth medium**

According to Hattingh and van Deventer (2001:1), the importance of soil type categorisation and soil variables or properties with respect to land or soil use must be recognised.

The concerns about soil degradation are emphasised in the modern society (Nkonya *et al.*, 2011:11) due to the absence of proper management practices (Hattingh & Van Deventer, 2001:1). When considering the functionality of healthy soils in an ecosystem, the cause for concerns regarding soil degradation is evident (Brady & Weil, 2008:30; Hattingh & Van Deventer, 2001:1). The ability of the substrate to serve as a medium for plant and biological production (Weil & Magdoff, 2004:1) serves as a buffer and filter; serves as a promoter of health (faunal, floral and human); serves to store and release water, gases and nutrients are some of the vital functions (Doran & Zeiss 2000:6, Hattingh & van Deventer, 2001:1; Weil & Magdoff, 2004:1). According to Hattingh and van Deventer (2001:2); soil quality can be effectively addressed on a smaller scale in current practice.

According to Doran and Zeiss (2000:4) and the Soil Science Society of America (1995), soil quality refers to the capacity of a soil to function within natural or managed ecosystem boundaries. Soil quality therefore has a profound effect on the health and productivity of a given ecosystem and the environment related to it (Adeboye *et al.*, 2011:34; Doran & Zeiss 2000:4; Weil & Magdoff, 2004:1). General factors that can influence the nature of the soil and its quality vary over a period and

were identified as climate, topography, biota, parent material, time and anthropogenic factors such as mining (Bardgett, 2005:6; Krull *et al.*, 2010:5; Singer & Munns, 1992:288; Van Deventer & Ferreira, 2013:38). Soil quality can therefore be defined as the capacity of a soil to accept, store and recycle water, nutrients and energy (Anderson & Gregorich, 1984); and to sustain biological productivity, maintain environmental quality (water and air) (Krull *et al.*, 2010:6; Larson & Pierce, 2013:71), and promote plant, animal and human health (Doran & Zeiss, 2000:4). According to Doran and Zeiss (2000:4), Larson and Pierce (2013:71) and Weil and Magdoff (2004:2), soil quality refers to a soil's fitness for a specific use.

Soil health refers to the self-regulation, stability, resilience (Doran & Zeiss, 2000:4; Weil & Magdoff, 2004:2), and lack of stress symptoms in soil as an ecosystem (Hattingh & Van Deventer, 2001:3). The soil health describes the soil community's biological integrity (Weil & Magdoff, 2004:2). The integrity can be viewed as the balance among organisms within a soil and between soil organisms and their environment (Brady & Weil, 2008:30).

Both inherent and dynamic soil properties influence the soil quality (Doran & Zeiss, 2000:4; Hattingh & Van Deventer, 2001:2; Weil & Magdoff, 2004:2). Most soil quality investigation was previously done on the dynamic soil properties and how they change in relation to the inherent features of the soil (Weil & Magdoff, 2004:2). The intrinsic features are related to the soil's inherent capacity for crop growth whereas the dynamic features are influenced by the soil user or manager (Hattingh & Van Deventer, 2001:2).

The inherent soil properties are related to the pedogenic processes (Larson & Pierce, 2013:69). It is known as a static property and may include soil texture, depth of bedrock, and type of clay, cation exchangeable capacity (CEC), and drainage class. Both time and extrinsic factors influence this type of quality (Weil & Magdoff, 2004:2). Soil management has mainly three broad functions: plant productivity, assimilation and recycling of waste materials, and environmental protection of water and air quality (Brady & Weil, 2008:30). Soil quality therefore describes the

properties of soil that make it suitable to perform specific functions such as the support of plant growth (Weil & Magdoff, 2004:2). Anthropogenic factors usually influence the dynamic soil properties (Brady & Weil, 2008:30). Dynamic properties are subjected to change over a relative short period of time (Weil & Magdoff, 2004:2). Dynamic properties include SOM, soil structure, infiltration rate, bulk density, and water nutrient holding capacity and pH. Both the management practices and the inherent properties of the soil influence the dynamic properties (Brady & Weil, 2008:30; Weil & Magdoff, 2004:2).

### **2.2.1 Soil organic matter (SOM)**

The decay of plant tissue results in the formation of large complex organic molecules (resistant by-products) in the soil (Brady & Weil, 2008:510; Singer & Munns, 1992:3). These organic colloids (humus) are responsible for the dark colours (brown to black) in the soil (Cooperband, 2002:1; Soil Classification Working Group, 1991:233; Singer & Munns, 1992:41). Microbial activities break down the plant material under suitable conditions of the soil to produce humus as a resistant, but slowly decomposing end-product (Brady & Weil, 2008:511; Singer & Munns, 1992:41). Humus is therefore either a product synthesized by microorganisms or a product of the break down and decomposition of other organic compounds (Brady & Weil, 2008:511; Cooperband, 2002:1; Fenton *et al.*, 2008:1; Singer & Munns, 1992:41). Only the part of matter unidentifiable as tissue is properly referred to as soil humus (USDA, 2009). Some of the organic matter decomposition products are soluble in water and will therefore vanish rapidly (Brady & Weil, 2008:513; Singer & Munns, 1992:41). In order to form new compounds with a higher molecular weight, clays combine with organic compounds to form new compounds which will decompose slowly (Brady & Weil, 2008:513; Singer & Munns, 1992:41). SOM aids in the promotion of water retention and porosity serves as a chemical buffer and contributes to maintaining soil structure (Howard & Howard, 1990:306), store and transition of air and water and improvement in plant nutrient retention (N, P and K). SOM also improves the soils' resistance to compaction, provides a source of energy for soil microbes and cohesion properties of soil (Brady & Weil, 2008:515; Cooperband, 2002:2; Chan, 2008:2; Fenton *et al.*,



2008:1; Marcel Dekker Inc., 1985:3; Singer & Munns, 1992:3). It acts as a binding agent between minerals to form aggregates in the soil (Cooperband, 2002:2; Singer & Munns, 1992:41). Humus is highly charged and has a large surface area per unit mass, similar to and in some cases higher than that of clay minerals, which is the reason for the high reactivity in soil (Brady & Weil, 2008:513; Singer & Munns, 1992:41).

Soil Organic Matter (SOM) can be defined as the organic fraction (including the micro-organisms - Cooperband, 2002:1; Fenton *et al.*, 2008:1) of the soil and indicates animal and plant residues at various stages of decomposition (Brady & Weil, 2008:948; Chan, 2008:1; Krull *et al.*, 2010:5). It includes cells and tissues of soil organisms and substances synthesized by the soil population (Brady & Weil, 2008:948). SOM is composed of carbon (C), hydrogen (H), oxygen (O), phosphorus (P), nitrogen (N), and sulphur (S) (Krull *et al.*, 2010:5; Singer & Munns, 1992:41). SOM is the main source of N and S in unfertilized soils, while P, N and S are essential elements for general plant growth (Chan, 2008:2; Singer & Munns, 1992:41). SOM is commonly determined as the amount of organic material within the soil that can pass through the 2 mm sieve (Brady & Weil, 2008: 510).

Due to the prominent role of elemental carbon (C) in the chemical structure of all organic substances (Weil & Magdoff, 2004:4), it is no surprise that the term *soil organic carbon* (SOC) is often used (Brady & Weil, 2008:510). SOC describes the C component of the soil organic matter (Brady & Weil, 2008:510; Chan, 2008:1) and is expressed as % carbon by weight (i.e. g C per 100 g of soil). SOM actually measure the C in the material and use a conversion factor (1.72, assuming average of 58 % C in SOM in soils (Chan, 2008:6)) to estimate the organic matter and therefor SOC is appropriate for quantitative discussions (USDA, 2009). Organic matter commonly contain approximately half carbon by weight (50 % C), therefor one can estimate SOM as two times the organic C ( $SOM = 2 \times SOC$ ). In comparing SOM and SOC values caution must be applied, for C content of SOM does vary (Brady & Weil, 2008:510; Krull *et al.*, 2010:8).

SOM concentrations are associated with the major indicators of soil quality (Krull *et al.*, 2010:7). The balance of gains and losses of organic carbon assists in determining the level which organic matter will accumulate in soil. The organic C is chiefly managed by the types and amounts of residues added to the soil (USDA, 2011:1). During tillage practices, the loss of soil productivity and erosion has a major impact on the soil organic matter (Iowa State University, 2005:1). Although the typical carbon content of dry plant matter is about 40 % (Mayland, 1986:659); and the ideal carbon content with respect to the soil fertility is no more than 5% SOM by weight (10% by volume) (Weil & Magdoff, 2004:4). The threshold value of SOC in most soils is at 2 % SOC (Weil & Magdoff, 2004:10) (equivalent to 3.4 % SOM); below it most soils are prone to structural destabilisation and crop yields are reduced (Krull *et al.*, 2010:7). There are similar relationships between the SOC content and aggregate stability (Krull *et al.*, 2010:7). Soil aggregates are unstable at SOC <2 %, moderately stable at 2-2.5 %, and very stable at SOC contents >2.5 % (Krull *et al.*, 2010:7).

When SOM is added to soil it mineralizes to humus. Humus consists of humin (a black humic substance that is not soluble in water), humic acids and fluvic acids that play an important role (Bot & Benites, 2005:8; MVSA Bemestingshandleiding, 2007:30). SOM can also be described by means of its reaction with acids and bases (Brady & Weil, 2008:511; Singer & Munns, 1992:51; University of Oxford, 2013:285). The term insoluble humin refers to the SOM that is insoluble in a strong base such as NaOH (Brady & Weil, 2008:511; Singer & Munns, 1992:51). Humic acid can be extracted from the soil with dilute alkali and precipitated by acidification to pH 1.0-2.0 (Brady & Weil, 2008:511; Soil Classification Working Group, 1991:227; University of Oxford, 2013:285). Humic acid is therefore soluble in a base (NaOH) and insoluble in a strong acid such as HCl. The SOM that is soluble in both the acid and base are fluvic acids (Brady & Weil, 2008:511; Singer & Munns, 1992:51; Soil Classification Working Group, 1991:224; University of Oxford, 2013:285).

### 2.2.2 Plant nutrients

Vegetation establishment on mine waste poses challenges to phytoremediation because the substrate (sub-soil and mine waste) lacks organic carbon (Tordoff *et al.*, 2000:221). According to Verheye (2007:123), the required nutrients for plant growth are taken from the soil (MVSA Bemestingshandleiding, 2007:82; Stevenson, 1986:66) by means of the root system (Hopkins & Hüner, 2009:39). The root systems of most plants are extensive (combinations of primary roots, secondary and tertiary branches, and root hairs) and penetrate large volumes of the soil in order to obtain the required nutrients and water (Hopkins & Hüner, 2009:39). The nutrients are obtained from the parent rock material (Hopkins & Hüner, 2009:39) as well as the mineralization of organic matter (humus) (Stevenson, 1986:66; Verheye, 2007:123) in various stages of decomposition (Hopkins & Hüner, 2009:39). The primary sources of nutrient elements are the mineral particles in the solid phase (Hopkins & Hüner, 2009:39). The various elements are released into the soil solution during the weathering process, which becomes the immediate source of nutrients (Hopkins & Hüner, 2009:39). The soil colloids (clay and humus) maintain the soil reservoir of soluble nutrients by means of retaining the nutrients and releasing them into the soil solution making it available for plant uptake (Hopkins & Hüner, 2009:40). According to Stevenson (1986:66), humus (a carbonaceous residue (Hopkins & Hüner, 2009:40)) contributes to the soil fertility (ability of the soil to make plant nutrients available to the plant (MVSA Bemestingshandleiding, 2007:1)) or productivity of the soil due to the positive effects of it on the physical (Weil & Magdoff, 2004:4), chemical and biological properties of the soil (Stevenson, 1986:66). Humus serves as a reservoir for N, P and S necessary for plant growth, promote good soil structure and serves as a source of energy for microorganisms (Stevenson, 1986:66). The organic N, P and S can be converted through the activity of microorganisms into available mineral forms including  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$  (Stevenson, 1986:67). These conversions are affected by the factors influencing microbial activity such as temperature and pH as well as the C/N, C/P, and C/S ratios of decomposing plant residues (Stevenson, 1986:67). Ecosystems tend to have a balance between the removal (plant uptake and erosion) and the release (weathering and mineralization) of nutrients (Verheye, 2007:123). An imbalance in the nutrient cycle of a normal managed ecosystem (e.g. farm, forest

and garden) is the result of the human interference (Verheye, 2007:123).

The so-called structural elements (carbon (C), Hydrogen (H) and oxygen (O)) (Stevenson, 1986:2) are the building blocks of many organic compounds and structures that plants consist of (MVSA Bemestingshandleiding, 2007:90). The plant tissue comprises C, H, O, N, P, S, Ca, Mg, K, Cl, Fe, Mn, Zn, Cu, B and Mo (Verheye, 2007:123). However, C, H and O are excluded from the mineral element nutrient group, because they are provided to the plant in the form of carbon dioxide and soil water (Hopkins & Hüner, 2009:62; Verheye, 2007:123). These three elements (C, H and O) form the greatest part (Krull *et al.*, 2010:5) of the plant's biomass and are responsible for the life and growth processes of the plant (MVSA Bemestingshandleiding, 2007:90).

The mineral elements found in much smaller quantities in plants join the functions of the organic compounds (MVSA Bemestingshandleiding, 2007:90). In some cases they form part of the compounds and structures, and in other cases have specific stimulating and regulatory roles in the processes in plants (MVSA Bemestingshandleiding, 2007:90).

When elements are required in larger quantities they are referred to as macro nutrients (Krull *et al.*, 2010:5) which include: H, C, O, N, P, S, Ca, Mg, and K (Hopkins & Hüner, 2009:66; Krull *et al.*, 2010:5; MVSA Bemestingshandleiding, 2007:90; Verheye, 2007:123). Micronutrients (trace elements) such as Fe, Mn, Zn, Cu, B, Cl, Ni and Mo are needed in much smaller quantities (MVSA Bemestingshandleiding, 2007:87; Verheye, 2007:123). The distinction between macro- and micronutrients is the relative concentrations found in tissue or required in nutrient solutions (Hopkins & Hüner, 2009:65). A comprehensive overview of each macro-element is given below.

### **2.2.2.1      Calcium ( $\text{Ca}^{2+}$ )**

According to Hopkins & Hüner (2009:70), calcium is taken up by the plant in the form of a divalent cation ( $\text{Ca}^{2+}$ ). Deficiencies of  $\text{Ca}^{2+}$  under natural conditions are unlikely due to abundant concentrations in the soil (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:91; Verheye, 2007:126). However, if deficiencies develop they will occur due to the immobility of the  $\text{Ca}^{2+}$ -ion (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:91).

Calcium is essential for the division of cells for two main reasons. Firstly, it plays a role in the mitotic spindle during cell division; and secondly forming Ca-pectate in the middle lamella of the cell plate that forms between daughter cells (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:91). It is also essential in the physical integrity and normal functioning of membranes (Hopkins & Hüner, 2009:70). According to Hopkins and Hüner (2009:70), calcium is a second messenger in a variety of hormonal and environmental responses. When acting as a second messenger in protein phosphorylation,  $\text{Ca}^{2+}$  is essential for the regulation of activities of a number of enzymes (Hopkins & Hüner, 2009:70). Calcium promotes the formation of proteins and is necessary for cell growth (MVSA Bemestingshandleiding, 2007:91). Calcium and magnesium both occur as exchangeable bases in the soil and can therefore be leached out (Verheye, 2007:126).

Symptoms appear in the meristematic regions where cell division occurs and new cell walls are being laid down (Hopkins & Hüner, 2009:70). Symptoms of Ca-deficiency are initially visible on young leaves (youngest tissue) which have a gel-like appearance, slow to unfold and tips may stick together (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:93). Young leaves deform and become yellow (chlorosis), followed by necrosis on the leaf margins (MVSA Bemestingshandleiding, 2007:93) and in extreme cases death of the meristem (Hopkins & Hüner, 2009:70). Due to the deterioration of the middle lamella, deficiencies of solution cultures will result in discolouring and poor growth as well as a slippery appearance (Hopkins & Hüner, 2009:70).

#### **2.2.2.2 Potassium ( $K^+$ )**

Plants need large quantities of potassium ( $K^+$ ), most abundant cellular cation in comparison to other elements (Hopkins & Hüner, 2009:66,69; MVSA Bemestingshandleiding, 2007:90). Potassium is provided as potash (potassium carbonate  $K_2CO_3$  and potassium chloride KCl) in agricultural practices (Hopkins & Hüner, 2009:69).

According to Hopkins and Hüner (2009:69), potassium is a common deficiency in sandy soils due to the high solubility as well as the ease with which  $K^+$  leaches out. Unlike P and N, K does not occur in the organic compounds and structures of plants, but rather in the cell sap (MVSA Bemestingshandleiding, 2007:90).

It plays a role in the transportation of N in the plant, the translocation of starch, and as an activator for a number of enzymes involved in photosynthesis and respiration (Hopkins & Hüner, 2009:69; MVSA Bemestingshandleiding, 2007:90; Uchida, 2000:31). Potassium also promotes the fibre strength and contributes significant to regulating the osmotic potential (Hopkins & Hüner, 2009:70). Potassium is a principal factor in plant movements such as the opening and closing of stomatal guard cells as well as the daily changes in the orientation of leaves (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:90). It also aids in the stability of plant stems (especially in grains), drought, frost tolerance and fruit quality (MVSA Bemestingshandleiding, 2007:90). According to Verheye (2007:125),  $K^+$  promotes the synthesis and translocation of carbohydrates which enhance the thickening of cell walls and stalk strength. It also promotes the formation of protein, sugar, starch and oils (Verheye, 2007:125). Potassium serves as a charge balancer of both diffusible and non-diffusible anions due to its high mobility (Hopkins & Hüner, 2009:70). Mica and feldspar minerals are a source of potassium in soils which can be absorbed on the exchange complex in soil, leached out or be fixed in the interlayer spaces of mica minerals (Verheye, 2007:125).

Potassium is very mobile in plants (MVSA Bemestingshandleiding, 2007:90) and therefore not structurally bound (Hopkins & Hüner, 2009:70); K-fertilizer is thus added to the soil as a single application during planting (Verheye, 2007:125). Deficiencies appear first in the older leaves (Uchida, 2000:31) as mottling or chlorosis, followed by necrotic lesions (spots and dead tissue) (Hopkins & Hüner, 2009:70) and eventually death of leaf margins (Verheye, 2007:125). Deficiencies are usually visible in older plants (MVSA Bemestingshandleiding, 2007:93) and can also result in shortened and weakened stems that are more susceptible to root-rotting fungi (Hopkins & Hüner, 2009:70).

### **2.2.2.3      *Magnesium ( $Mg^{2+}$ )***

Magnesium is taken up by plants as a divalent cation ( $Mg^{2+}$ ). According to Hopkins and Hüner (2009:70), magnesium is required in relatively large amounts in plants. However, it is also less abundant in soils than calcium. Magnesium forms the core of the complex chlorophyll molecule and deficiencies can occur in acidic sandy soils (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:91; Verheye, 2007:126).

The largest proportion of  $Mg^{2+}$  is found in the porphyrin moiety of the chlorophyll molecule (Hopkins & Hüner, 2009:70). It serves as a ribosome structure stabilizer as well as an activator of numerous essential enzymes (Hopkins & Hüner, 2009:70). Photosynthesis cannot take place in the absence of the chlorophyll molecule and plants will die in such an instance (MVSA Bemestingshandleiding, 2007:91). Magnesium is involved in the translocation of phosphorus (P) and is very mobile in plants (MVSA Bemestingshandleiding, 2007:91). According to Hopkins & Hüner (2009:71), it serves to link ATP molecules to the active sites of the enzymes.

Deficiency symptoms initiate in older leaflets as chlorosis (Verheye, 2007:126) due to the breakdown of chlorophyll in the lamina of the leaf that lies between the veins (Hopkins & Hüner, 2009:71); which might develop into necrotic spots later. The so-called “beads-effect” between the leaf veins is characteristic of this deficiency in

some plants (MVSA Bemestingshandleiding, 2007:93). The chloroplasts in the region of the veins retain chlorophyll much longer and are more prone to magnesium deficiencies (Hopkins & Hüner, 2009:71). According to Hopkins and Hüner (2009:71),  $Mg^{2+}$  is withdrawn from the older leaves effortlessly (due to its mobility) and transported to the younger ones that are more actively growing and synthesising chlorophyll.

#### **2.2.2.4 Nitrogen (N)**

Nitrogen is available in excess in the atmosphere (Hopkins & Hüner, 2009:68), yet it is absorbed from the soil and is one of the most limiting nutrients for food production (Verheye, 2007:124). After it is taken up mainly in the form of an inorganic nitrate ion ( $NO_3^-$ ), and in a few cases ammonium ion ( $NH_4^+$ ) (Hopkins & Hüner, 2009:68), most nitrogen is reduced to the amine ( $-NH_2$ ) form (MVSA Bemestingshandleiding, 2007:90). Amine occurs in the plant in organic compounds such as phospholipid and chlorophyll (Verheye, 2007:124), amino acids, proteins, hormones (indole-3-acetic acid and cytokinin) (Hopkins & Hüner, 2009:68), adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), and therefore in structures such as nuclei, cell walls and chromosomes (Uchida, 2000:32). Once the  $NO_3^-$  is in the plant, it is reduced to  $NH_4^+$  before it can be incorporated into amino acids, proteins and other nitrogenous organic molecules (Hopkins & Hüner, 2009:68).

It is clear that life processes such as photosynthesis, growth, reproduction and maintenance of genetic identities are co-determined by nitrogen (MVSA Bemestingshandleiding, 2007:90). The obvious effect of this is that the characteristic green colour of leaves and the vegetative growth of plants are associated with nitrogen (Hopkins & Hüner, 2009:69; Uchida, 2000:32; Verheye, 2007:124).

Common deficiency symptoms are chlorosis and slow, stunted growth of the leaves (Hopkins & Hüner, 2009:69; Uchida, 2000:32; Verheye, 2007:124). Nitrogen is



highly mobile in plants and as the older leaves yellow and die, nitrogen is mobilized largely in the form of amines and amides to the younger ones (Hopkins & Hüner, 2009:69; MVSA Bemestingshandleiding, 2007:90). Yellowing usually forms an inverted v-pattern in the mid vein (MVSA Bemestingshandleiding, 2007:92); and as the severity of the deficiency increases, symptoms will occur on younger leaves whilst the older leaves turn completely yellow or brown and fall off the plant (Hopkins & Hüner, 2009:69).

Nitrogen stress will lead to the accumulation of anthocyanin pigments in some species that signifies the purplish colour of the stems, petioles, and underside of leaves (Hopkins & Hüner, 2009:69). The cause of anthocyanin accumulation in plants is not known, however might be related to the overproduction of carbon structures that cannot be utilized to produce amino acids and other nitrogen-containing compounds in the absence of nitrogen (Hopkins & Hüner, 2009:69). Deficiency symptoms must not be confused with the herbicide damage or red flower damage (MVSA Bemestingshandleiding, 2007:92). According to Verheye (2007:124), the vegetation has a reduced resistance to diseases and insects due to the plants tenderness caused by nitrogen deficiencies.

Nitrogen can either be leached from the soil or denitrified; and the crop yield determines the necessary amount of fertilizer being applied (in two applications) to the soil (Verheye, 2007:124). The first application (two-thirds) will be applied during the planting and the other one-third will be applied later during the growing season as a top dressing (Verheye, 2007:124). If excess nitrogen is applied it will stimulate abundant growth of the shoot system (favouring a high shoot/root ratio) which results in a delayed onset flowering of agricultural and horticultural crops (deficiencies of N will result in reduced shoot growth and early flowering) (Hopkins & Hüner, 2009:69).

#### **2.2.2.5      *Phosphorus (P)***

Phosphorus content of natural soils is dependent on both the P-content of the parent material and the soil pH (Verheye, 2007:125). Phosphorus is available in the soil solution as forms of polyprotic phosphoric acid ( $\text{H}_3\text{PO}_4$ ) (a polyprotic acid contains more than one proton each with a different dissociation constant) (Hopkins & Hüner, 2009:69). The monovalent soluble orthophosphate ( $\text{H}_2\text{PO}_4^-$ ) anion is readily taken up by roots (MVSA Bemestingshandleiding, 2007:90; Uchida, 2000:32) at a soil pH of 6.8; whereas  $\text{HPO}_4^{2-}$  occurs predominantly at a soil pH between 6.8 and 7.2 which is less readily absorbed (Hopkins & Hüner, 2009:69). Phosphorus in alkaline soils (pH greater than 7.2) cannot be absorbed by plants due to its trivalent  $\text{PO}_4^{3-}$  form in which it will occur (Hopkins & Hüner, 2009:69). Hopkins and Hüner (2009:69) state that phosphorus will form insoluble complexes with aluminium and iron at neutral pH conditions, whilst calcium and magnesium complexes will precipitate the phosphorus in basic soil conditions. Phosphorus is generally limited in highly calcareous conditions, for insoluble phosphate is very slowly released into the soil solution (Hopkins & Hüner, 2009:69). According to Verheye (2007:125), the optimum pH is 6.5 for phosphorus availability.

It is essential for organic phosphorus to be converted into an inorganic form either by means of micro-organisms or through phosphatase enzymes released by the roots before it is available for plant uptake (Hopkins & Hüner, 2009:69). Plants also compete with the soil micro-flora for the small amount of available phosphorus (Hopkins & Hüner, 2009:69). Phosphorus is therefore more likely to be the limiting element in natural ecosystems than nitrogen (Hopkins & Hüner, 2009:69). Plants increase their phosphorus uptake by means of the intimate associations between the roots and soil fungi (mycorrhiza) (Hopkins & Hüner, 2009:69). According to Hopkins and Hüner (2009:69), phosphorus is primarily in the form of phosphate esters (sugar-phosphates) that play a vital role in photosynthesis and metabolism (Hopkins & Hüner, 2009:69). The most important organic compounds and plant structures that build orthophosphate into their chemical structure are ATP, ADP, DNA, phospholipids and phytins (MVSA Bemestingshandleiding, 2007:90). According to Verheye (2007:125) and Hopkins and Hüner (2009:69), phosphorus in

the form of nucleotides such as adenosine triphosphate (ATP) and ADP, as well as inorganic phosphate ( $P_i$ ), phosphorylated sugars and phosphorylated organic acids plays an important role in providing biochemical energy to all living cells. As in the case of N, P plays a role in the photosynthesis, growth, reproduction and maintenance of genetic identities of plants and respiration (MVSA Bemestingshandleiding, 2007:90; Uchida, 2000:32). Phosphorus is associated with cellular divisions, flowering and ripening (MVSA Bemestingshandleiding, 2007:90).

The main function of P is to stimulate root growth in new plants (MVSA Bemestingshandleiding, 2007:90). Phosphorus is relatively immobile in both the plant and soil (MVSA Bemestingshandleiding, 2007:90) at high and low pH values (Verheye, 2007:125). The phosphorus content in South African soils is naturally low and is therefore adjusted to an optimal state of 15 to 25 mg P kg<sup>-1</sup> (Verheye, 2007:125).

Deficiencies cause an abnormally dark green (even purplish) colour in the leaves of young plants (Hopkins & Hüner, 2009:69; MVSA Bemestingshandleiding, 2007:92; Uchida, 2000:32). Growth is hindered by this deficiency (Verheye, 2007:125) and can lead to short internodes and thin stems in grains (Hopkins & Hüner, 2009:69), and hinder node formation in maize (MVSA Bemestingshandleiding, 2007:92). The phosphorus deficiency symptoms will first occur on the older bottom leaves (Verheye, 2007:125) as a dark greenish-purple colour (anthocyanin pigments accumulation), and later as the severity increases the leaves might become malformed and exhibit necrotic spots (Hopkins & Hüner, 2009:69). The purple colour must not be confused with anthocyanin accumulation (MVSA Bemestingshandleiding, 2007:92). In the case of excess phosphorus content the growth of roots over shoots will be stimulated which results in a shoot/root ratio reduction (Hopkins & Hüner, 2009:69).

#### **2.2.2.6 Sulphur (S)**

Sulphur occurs in several forms in most soils such as iron sulphates and elemental sulphur (Hopkins & Hüner, 2009:70). The uptake of S, in the form of a divalent

sulphate anion ( $\text{SO}_4^{2-}$ ) (Hopkins & Hüner, 2009:70; Uchida, 2000:49), in plants occurs in similar quantities to that of P (MVSA Bemestingshandleiding, 2007:91).

Sulphur deficiencies are not common in soils due to the capability of micro-organisms to oxidise sulphates or decomposing organic sulphur compounds (Hopkins & Hüner, 2009:70). Sulphur deficiencies are hard to demonstrate in greenhouses located in these areas, as there are high concentrations of airborne sulphur (Hopkins & Hüner, 2009:70).

Sulphate is reduced to the SH-form, which makes up part of the amino acids such as methionine, thiamine and biotin, which are the building blocks of proteins (MVSA Bemestingshandleiding, 2007:91). Coenzyme A (containing sulphur) is also an important component in respiration and fatty acid metabolism (Hopkins & Hüner, 2009:70). According to Hopkins and Hüner (2009:70), sulphur in the form of iron-sulphur proteins (i.e. ferredoxin) play a vital role in the electron transfer reactions of photosynthesis and nitrogen fixation. It promotes the formation of chlorophyll as well as nodules on the roots of legumes. Sulphur is relatively immobile in most plant species (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:91).

Sulphur deficiencies cause chlorosis of the leaf (Uchida, 2000:49), specifically the tissue surrounding the vascular bundles (Hopkins & Hüner, 2009:70) and causes reduced growth (MVSA Bemestingshandleiding, 2007:93). Chlorosis occurrence is the result of reduced protein synthesis rather than the direct impairment of chlorophyll synthesis (Hopkins & Hüner, 2009:70). The chlorophyll is stabilized by the binding to protein in the chloroplast membranes (Hopkins & Hüner, 2009:70). The deficiency symptoms are first visible in younger leaves (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:93; Uchida, 2000:49).

### 2.2.3 C/N ratio and status

The general relationship between SOM and nitrogen of soil is that the higher the SOM content the higher the nitrogen content within the soil (Stevenson, 1986:164). The conversion of C and N into microbial tissue is associated with the decay of organic residues in soil (Hoorman & Islam, 2010:3; Stevenson, 1986:164), whilst some of the C is converted to CO<sub>2</sub> during the decaying process (Krull *et al.*, 2010:5; USDA, 2011:1). According to Brady and Weil (2008:504) and Mayland (1968:659), the carbon content of dry plant matter is approximately 42%. The nitrogen content is much lower within these plant residues (Mayland, 1986:659; Mary *et al.*, 1996:74) and varies widely (from <1 to >6 %) (Brady & Weil, 2008:504). Carbon provides the energy and nitrogen builds tissue for the plants (Miller, 2000:20). The carbon to nitrogen ratio in soil is important for three reasons, as (1) it helps to determine the rate of decay; and (2) the rate at which nitrogen is made available to plants; (3) as well as the fact that intense competition amongst micro-organisms exists for the available soil nitrogen due to the addition of residues to the soil that consist of high C/N ratios (Brady & Weil, 2008:504). During decomposition of organic matter, carbon dissipates more rapidly than nitrogen which in turn lowers the carbon to nitrogen ratio (Miller, 2000:20). The C/N ratio (by weight) ranges between 8/1 to 15/1 with the median as 10/1 or 12/1 (Brady & Weil, 2008:504; Miller, 2000:20; Mayland, 1986:569).

Miller (2000:20) stated that the ratio of composted manure is 20/1; cornstalks 60/1; straw 80/1; sawdust 400/1, and alfalfa hay 12/1 (Hoorman & Islam, 2010:4). Brady and Weil (2008:504) stated that the C/N ratio in plant residue ranges from between 10/1, and 30/1 in legumes (Stevenson, 1986:165) and young green leaves to as high as 600:1 in sawdust. As plants mature, the proportion of protein in their tissues declines, whilst the proportion of lignin and cellulose, and the C/N ratio increase (Brady & Weil, 2008:504). The sawdust is considered to be highly carbonaceous due to the high carbon content in comparison with the low nitrogen content (Miller, 2000:20). If adequate amounts of sawdust are added to the soil, there will be enough nitrogen to serve as food for bacteria and fungi to aid in the decomposition of material (Miller, 2000:20). These organisms will ingest the soil and create a

nitrogen deficiency, thereby decreasing the crop yield (Miller, 2000:20). The C/N ratios are less variable in micro-organisms than that of plants ranging between 5/1 and 10/1 (Brady & Weil, 2008:504). Bacteria are generally richer in protein than fungi resulting in a lower C/N ratio (Brady & Weil, 2008:504). Miller (2000:20) stated that legumes are highly desirable, for they comprise the highest amounts of nitrogen and have a low C/N ratio (Miller, 2000:20). A good C/N ratio for a composting pile ranges between 20/1 and 35/1, with the optimum ratio considered as 30/1 (Jenkins, 2005:33).

The C/N ratios range from 8/1 to 15/1 (median of 12/1) within the organic matter of arable (cultivated) surface horizons (Brady & Weil, 2008:505; Du Toit *et al.*, 1994:75). Surface layers usually contain a higher ratio than sub-soils in the soil profile. Little variation occurs in the C/N ratio that is similarly managed in a given climatic region. The rate of decay and release of nutrients to the soil is dependent on temperature (high temperature equals low C/N ration). The C/N ratio is relatively high (30/1) in humid regions that are more severely leached, acidic A horizons; whilst in calcium-rich soils of semi-arid grasslands the C/N ratio is relatively low. Forest O horizons commonly have C/N ratios of 30/1 to 40/1 and if these soils are cultivated and limed (to increase the soil pH and calcium content) the C/N ratio lowers to approximately 12/1 due to the enhanced decomposition (Brady & Weil, 2008:505).

Soil microbes require a balance of nutrients in order to extract energy and to build their cells (Brady & Weil, 2008:506; Miller, 2000:20; Stevenson, 1986:164). According to Brady and Weil (2008:506), soil organisms metabolise carbonaceous material in order to utilise the carbon for building essential organic compounds (Jenkins, 2005:33). Organisms do not need carbon alone to multiply and grow, but also sufficient nitrogen (Jenkins, 2005:33) to synthesize nitrogen containing cellular compounds such as amino acids, enzymes and DNA (Brady & Weil, 2008:506). Soil microbes (C/N ratio 6/1 - 12/1 (Stevenson, 1986:165)) need approximately eight parts of carbon for every one part of nitrogen (Brady & Weil, 2008:506; USDA, 2011:1). Miller (2000:20) mentioned that the C/N ratio of soil microbes ranges from

4/1 to 10/1. Only one-third of carbon that is metabolized by the microbes is incorporated into the cells (Brady & Weil, 2008:506) and the remainder is respired (USDA, 2011:1) and lost as CO<sub>2</sub> (Stevenson, 1986:164). The microbes therefore require 1 g of N for every 24 g of C in their nutrition (Brady & Weil, 2008:506). In order to obtain enough C and N to stay alive, i.e. for maintenance purposes and energy, a diet is required of 24/1 where sixteen parts of C are utilised for energy and eight parts for maintenance (USDA, 2011:1).

The soil microbes will have to search the soil solution to obtain sufficient N if the C/N ratio exceeds approximately 25/1 (Brady & Weil, 2008:506; USDA, 2011:1). Decomposition slows down when the N is used up in the presence of too much C, resulting in the death of some organisms. Other organisms will utilise the once stored N of the decomposing organisms to create new cell walls. C/N ratios higher than 35/1 result in microbial immobilization of available nitrogen (nitrate and ammonium) (Stevenson, 1986:164) in the soil. The incorporation of high C/N residues will therefore deplete the supply of soluble N to the soil (Brady & Weil, 2008:506) resulting in an N deficiency for higher plants (Miller, 2000:20). Additional fertilizers of N are required if the C/N ratio exceeds 35/1 in order to compensate for the N immobilization by soil organisms. Residues with C/N ratios ranging between 25/1 and 35/1, usually have a short-term effect on the nitrate and ammonia of the soil. During the initial stages of decomposition a tie-up of mineralized nitrogen (USDA, 2011:1) occurs which will be released at a later stage. The nitrogen in their bodies will be released back into the soil as the organisms die and decompose (USDA, 2011:1). The decay of organic material can also be delayed if there is not a sufficient amount of nitrogen present in the soil (undergoing decomposition) (USDA, 2011:1) or soil solution to support the microbial growth (Brady & Weil, 2008:506). In the case of C/N ratios less than 25/1 part of the nitrogen will be released as ammonia during the rotting stage.

The practical importance of the C/N ratio becomes obvious as one compares the changes that occur in the soil (Brady & Weil, 2008:506) when residues of either high or low C/N ratio are added (USDA, 2011:1). Low carbon-dioxide production

validates that the general-purpose organisms are responsible for low-level activities in the soil (Brady & Weil, 2008:506). The level of nitrates would very slowly increase as the native soil organic matter decays (Brady & Weil, 2008:506) resulting in nitrogen loss (as in the case of low carbon, leading to nitrogen loss as ammonia) or uptake by the plants (Brady & Weil, 2008:506). The ratio of available carbon to available nitrogen is of importance due to some carbon being very resistant to biological breakdown resulting in the insignificant presence of the nutrients.

If large amounts of readily decomposable organic material are added to low nitrogen content soils (Brady & Weil, 2008:506), the microbial communities will respond to the new food supplies resulting in the heterotrophic (consumers of preformed organic materials) zymogenous (an opportunistic organism found in soils immediately following addition of readily decomposable organic substrate) micro-organisms becoming active (Brady & Weil, 2008:506). These zymogenous organisms will multiply rapidly and yield large amounts of carbon dioxide (Brady & Weil, 2008:506). Little or no mineral nitrogen ( $\text{NH}_4^+$  or  $\text{NO}_3^-$  (Stevenson, 1986:165)) is available to higher plants due to the high demand for nitrogen by the micro-organisms during this period (Brady & Weil, 2008:506). Both cellulose- and lignin-decomposing fungi will increase as well as the actinomycetes and bacteria during the decomposition of SOM. It is not possible for the bacteria to decompose lignin, however, bacteria can break down cellulose. On the other hand, fungi can break down both the lignin and cellulose. Actinomycetes have an effect on cellulose break-down. Some types of soil fauna have the ability to digest cellulose and hemicellulose.

Plant cell walls comprise lignin, cellulose and hemicellulose. Crude protein, hemicellulose, cellulose, lignin, fat and wax will decompose slowly, whereas sugar, starch and simple proteins will decompose rapidly. The different rates of decomposition are: proteins > carbohydrates > cellulose and hemicellulose > lignin (Stevenson, 1986:167).

According to Brady and Weil (2008:506), the nitrate depression period persists in



the soil until the activities of the decay organisms gradually subside due to the lack of carbon. The carbon dioxide formation descends as the number of microorganisms do, ensuing in a less acute demand for nitrogen (Brady & Weil, 2008:506). Because carbon is lost (by respiration) and nitrogen is conserved (by incorporation into microbial cells) during the decay process (Brady & Weil, 2008:506; Miller, 2000:20; Stevenson, 1986:164), the remaining amount of plant material decreases (Brady & Weil, 2008:506). If the C/N ratio of remaining material drops below 20 (Stevenson, 1986:165) it is likely that mineral nitrogen will be released (Brady & Weil, 2008:506). The original conditions succeed (nitrates appear again) with the exception of the soil being richer in both humus and nitrogen (Brady & Weil, 2008:506). This depression period may last for several months (USDA, 2011:1; Brady & Weil, 2008:506).

If the added residues with either a higher C/N ratio that are easily decomposed (Stevenson, 1986:165) or large quantities of residues (USDA, 2011:1) are added to the soil, a longer and more severe nitrate depression occurs (Brady & Weil, 2008:506). Planting should be delayed until after the depression period or additional nitrogen can be applied to satisfy the nutritional requirements of both the microbes and plants, in order to avoid producing stunted, chlorate and nitrogen-starved seedlings (Brady & Weil, 2008:506). If the C/N ratio of the organic materials is low, there is more than enough nitrogen present to satisfy the needs of the decomposing organisms (Brady & Weil, 2008:507). Some of the nitrogen from organic compounds (USDA, 2011:1) is therefore released into the soil solution, amplifying the level of soluble nitrogen available for plant uptake (Brady & Weil, 2008:507) soon after the decomposition is initiated. Nitrogen-rich materials decompose quickly, resulting in an intense microbial activity and growth period (Brady & Weil, 2008:507).

Nitrogen mineralization involves not only the saprophytic bacteria and fungi but the entire food web (Brady & Weil, 2008:507). A large biomass of bacterial and fungal cells (containing much of the original nitrogen) is produced as soon as the organic residues are added to the soil (Brady & Weil, 2008:507). As the C/N ratio decreases

the microbial tissues are attacked by a synthesis of new biomass and some of the immobilized N is released via the net mineralization (Stevenson, 1986:164). The nitrogen is immobilized and only becomes available for plant uptake if the microbial biomass begins to die off (Brady & Weil, 2008:507).

A healthy soil ecosystem contains certain nematodes, protozoa, and earthworms that feed on bacteria and fungi (Brady & Weil, 2008:507). These micro and meso-fauna make use of only a small fraction of the carbon to produce eggs and grow, whilst they respire most of the carbon in the microbial cells (Brady & Weil, 2008:508). The animals ingest more nitrogen than they can use and most of the carbon is converted to CO<sub>2</sub> (Stevenson, 1986:165) by respiration (USDA, 2011:1). The C/N ratio of these animals does not differ much from that of their microbial food (Brady & Weil, 2008:508). The excess nitrogen is then excreted as NH<sub>4</sub><sup>+</sup> (Stevenson, 1986:165) into the soil solution as plant-available mineral nitrogen (Brady & Weil, 2008:508). The rate of nitrogen mineralization may increase by as much as 100% for bacterial-feeding nematodes due to the microbial feeding activity of soil animals (Brady & Weil, 2008:508). It seems that the bacterial-feeding nematodes seem to have less effect on the nitrogen mineralization when the C/N ratio of residues is very high (Brady & Weil, 2008:508). This phenomenon is due to the bacteria that quickly remobilize the nitrogen (Stevenson, 1986:167) excreted by the nematodes because the bacteria have plenty of carbon but little nitrogen to grow on (Brady & Weil, 2008:508). The cycling and efficient use of nitrogen and other nutrients can be enhanced by management practices (USDA, 2011:1) that favour a complex food web with many trophic levels (Brady & Weil, 2008:508).

#### **2.2.4 Soil micro-organisms**

According to Rai *et al.* (2010:5), a soil-integrated system consists of various interdependent physical, chemical and biological processes that are significantly influenced by environmental factors. The soil-microbial community is organised in complex food webs which stabilize various soil processes such as the biogeochemical processes (Rai *et al.*, 2010:5). According to Gobat *et al.*

(2004:105), microbes were previously considered as spreaders of disease, which is true in the minority of cases. Microbes include both eukaryotic (fungi, protozoa, algae, plants and animals) and prokaryotic (bacteria which are the smallest soil organisms and physiologically most diverse) types of the cell structure (Singer & Munns, 1992:144). Microbes are a collective term referring to all the microscopic organisms and can therefore be defined as single cell organisms that are too small to see with the naked eye and include bacteria, viruses, Achaea (bacteria-like creatures), fungi and protists (include primitive algae, amoebas, slime molds and protozoa) (Van der Watt & Van Rooyen, 1995:120). Microbes live either as individuals or in clusters together in communities and are considered as the oldest life form on earth dating back more than 3.5 billion years (Bardgett, 2005:25). Their roles involve the transformation of plant and animal wastes (Bardgett, 2005:26); oxidation, reduction, precipitation and solubilisation of mineral ions; fixation of molecular di-nitrogen; control of bio-element cycles in particular those of carbon, nitrogen, sulphur and iron; and transformation of parent material (Gobat *et al.*, 2004:105). According to Subhani *et al.* (2001:333) and Kumar *et al.* (2013:896), the metabolic functions of microorganisms regulate the turnover and mineralization of organic substances, nutrient transformations as well as the cycling of organic wastes in soil ecosystems.

There are various species of varying sizes that live in the soil such as micro-fauna (body width <0.1 mm; e.g. protozoa and nematodes), meso-fauna (body width 0.1-2.0 mm; e.g. micro-arthropods and enchytraeids) and macro-fauna (body width >2 mm; e.g. earthworms, termites and millipedes) (Bardgett, 2005:25; Singer & Munns, 1992:152, 153, 155). These fauna are usually allocated to functional groups based on their feeding habits. These soil animals appear to feed across different trophic levels (Bardgett, 2005:26). Microbes such as bacteria, fungi, actinomycetes and algae are primary consumers of the soil food web. Microbes are the most numerically abundant (thousands of species present in the soil) and diverse members of the soil food web of which the most abundant groups are bacteria and fungi (Bardgett, 2005:26). Soil microbes contribute to the plant growth in various ways (Doi & Ranamukhaarachchi, 2009:223).

According to Batra and Manna (2009:296) and Subhani *et al.* (2001:333), the microbial activity supports a number of fundamental soil properties such as structure and fertility, and is therefore vital in soil productive sustainability. Soil microbial activity can be defined as the overall metabolic activity of all the micro-organisms that occupy the soil (Subhani *et al.*, 2001:333) and can be predicted by means of measuring the dehydrogenase activity (Batra & Manna, 2009:296; Casida, 1977:130; Doi & Ranamukhaarachchi, 2009:223; Klein *et al.*, 1971; Subhani *et al.*, 2001:333). Doi and Ranamukhaarachchi (2009:223) stated that soil dehydrogenase activity is considered to be a good indicator of overall microbial activity since dehydrogenases are not as active as extracellular enzymes in soil. The physical, chemical, biological and microbial properties (Batra & Manna, 2009:296) influence the soil quality (including plant growth (Doi & Ranamukhaarachchi, 2009:223)) and its degradation; of which the biological and microbial activities are the most sensitive (Subhani *et al.*, 2001:333), for they respond rapidly to change (Kumar *et al.*, 2013:898). The role of microbial activity is significant in the soil ecosystem's development, functioning (Subhani *et al.*, 2001:333), stability and fertility (Kumar *et al.*, 2013:898). Batra and Manna (2009:296) stated that the microbial biomass represents an important reservoir of nutrients in soil and is essential for long-term fertility of soils. The C, N, microbial biomass and dehydrogenase activity are quick to respond to soil pH and could be increased either by means of lime addition to acid soils or gypsum to alkaline soils (Batra & Manna, 2009:269). According to Kumar *et al.* (2013:898), enzyme activities have a rapid response to the natural or anthropogenic (management) disturbances induced and are therefore considered as an effective soil quality indicator.

Subani *et al.* (2001:333) mentioned that there is considerable interest in the study of enzyme activity for it reflects the potential capacity of a soil to perform certain biological transformations associated with soil fertility. Enzymes facilitate the development of plant cover and establish the biochemical cycles that provide insight into the changes that the below-ground functioning system experiences while the plant community develops (Kumar *et al.*, 2013:898). Both the accumulated enzyme activity and the enzymatic activity of increasing microorganisms result in

the enzyme activity found in the soil (Kumar *et al.*, 2013:898). Enzymes are associated with viable proliferating cells, yet it can originate from either a dead cell into the solution or excreted from a living cell (Kumar *et al.*, 2013:898). Enzyme studies provide information on the release of nutrients due to microbial activities and SOM degradation (Kumar *et al.*, 2013:898) as well as indicators of ecological change (Subhani *et al.*, 2001:333). One can establish correlations between soil fertilization, microbial activity, biochemical cycling of various elements in soil and degree of pollution (heavy metals) by means of enzyme analyses (Doi & Ranamukhaarachchi, 2009:223; Kumar *et al.*, 2013:898). It will also enable one to assess the succession stage of an ecosystem (Kumar *et al.*, 2013:898).

Dehydrogenase enzymes are a vital component of the enzymatic activities that participate in ensuring the correct sequence of all the biochemical routes in soil biogeochemical cycles (Kumar *et al.*, 2013:899). Doi and Ranamukhaarachchi (2009:223) and Subhani *et al.* (2001:333), stated that soil dehydrogenase activity reflects the workings of a group of potential non-specific intracellular enzymes, which take part in various metabolic reactions involved in oxidative energy transfer, that are present in living soil microbes. Doi and Ranamukhaarachchi (2009:223) also mentioned that the dehydrogenase activity is affected by wet and dry seasonal cycles that are exhibited by the air-dried samples that experience an increased dehydrogenase activity (depending on the soil makeup). Dehydrogenase enzymatic activity (DHA) methods are widely used due to its simplicity on comparison to other quantitative methods (Kumar *et al.*, 2013:899). The change in soil enzyme activities may provide a useful index of changes in soil quality (Kumar *et al.*, 2013:899). This method therefore provides information on how the biological activity and microbial populations in soil correlate (Kumar *et al.*, 2013:899).

The DHA represents the immediate metabolic activities of soil microorganisms present in the material at the time of the test (Kumar *et al.*, 2013:899). This method is an oxidative degradation process (oxido-reductases) which involves the dehydrogenation of organic matter by means of transferring hydrogen (protons) and electrons from substrates to acceptors (Kumar *et al.*, 2013:899). The preferred

oxidants are water-soluble tetrazolium salts (Kumar *et al.*, 2013:899), as they form water-insoluble coloured formazans (Casida, 1977:630) which one can measure spectrophotometrically. The oxidation of organic compounds is catalysed by the intracellular dehydrogenase enzymes (the oxidoreductases process) that separates two-H atoms (Kumar *et al.*, 2013:899; Subhani *et al.*, 2001:333). Some of the specific dehydrogenases types act as a transfer of the separated H atom to either nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate (Kumar *et al.*, 2013:899; Subhani *et al.*, 2001:333). Separated H atoms take part in the reductive process of biosynthesis by means of the coenzymes formed (Kumar *et al.*, 2013:899; Subhani *et al.*, 2001:333). The overall dehydrogenase activity of soil depends on the activities of various dehydrogenases (Kumar *et al.*, 2013:899; Subhani *et al.*, 2001:333). These various dehydrogenases are considered to be the fundamental part of the enzyme system of all microorganisms including the enzymes of the respiratory metabolism, the citrate cycle and N metabolism (Kumar *et al.*, 2013:899; Subhani *et al.*, 2001:333). The dehydrogenase activity serves as an indicator of the microbiological redox systems and can thus be considered as a good measure of the microbial oxidative activities in soils (Kumar *et al.*, 2013:899; Subhani *et al.*, 2001:333). The hydrogen can be transferred to the soluble tetrazolium salts (TTC, INT) with the formation of red formazans. This can be determined calorimetrically after the extraction with a solvent took place (Kumar *et al.*, 2013:899; Subhani *et al.*, 2001:333).

There are two main methods of dehydrogenase activity, i.e. the use of tetrazolium salts and the use of P-iodonitrotetrazolium violet or INT salts (Kumar *et al.*, 2013:899). The tetrazolium salts method to determine the microbial activity in the soil is based on the reduction of 2, 3, 5- triphenyltetrazolium chloride (TTC) to the creaming red-coloured formazan (TPF) (Kumar *et al.*, 2013:899). According to Kumar *et al.* (2013:899) and Subhani *et al.* (2001:333), water-soluble tetrazolium salts are preferred, for they form water-insoluble coloured formazans which can be measured spectrophotometrically. Substrates must be prepared in CaCO<sub>3</sub> prior to the testing (Kumar *et al.*, 2013:899). The second method involves the use of INT 2-(p-iodophenyl)-3- (p-nitrophenyl) - 5-phenyltetrazolium chloride (p-iodonitrotetrazolium violet, or INT). These salts act as an artificial electron acceptor

in the substrate in order to determine the DHA in the soil (Kumar *et al.*, 2013:900). The INT proved to be more suitable for the determination of the dehydrogenase activity in soils in comparison with the TTC (Kumar *et al.*, 2013:900). The high electron affinity of the INT resulted in the more accurate estimation of DHA for it competes more effectively with oxygen for the free electrons (not necessary to carry out the determinations under anaerobic conditions) (Kumar *et al.*, 2013:900). According to Kumar *et al.*, (2013:900), under both the anaerobic and aerobic conditions more INT is reduced in comparison with TTC which results in an improved sensitivity (Kumar *et al.*, 2013:900; Subhani *et al.*, 2001:333). The O<sub>2</sub> does not interfere with the INT reduction (reduction from INT to INTF [iodonitrotetrazolium formazan]) resulting in a more exact measurement of DHA in soil extracts (Kumar *et al.*, 2013:900). Kumar *et al.* (2013:900) state that the INT reduction in both the aerobic and anaerobic conditions produces more formazan than the TTC method.

The soil microbial activity might be influenced by numerous factors such as activity of incubation time and temperature, pre-incubation of soil, soil aeration, the soil moisture content (Kumar *et al.*, 2013:900), soil pH, nutrient availability, organic matter, addition of pesticide, heavy metals and manure (Subhani *et al.*, 2001:337). According to Casida (1977:630), the dehydrogenase activity (TTC) method were developed for variations in microbial numbers, oxygen consumption, CO<sub>2</sub> evolution, soil C/N ratios, soil humus content, activity of other enzymes in soil, plant growth and yields, seasons, soil particle size fractions and soil sampling depth.

## **2.3 Factors affecting germination**

Germination is defined as “the process of a seed’s sprouting in which the first event is protrusion of the radicle or embryonic root through the seed coat. In a more general sense, germination is the beginning of active growth of a spore or seed” (Bewley *et al.*, 2013:133; Chastain, 2012:2; Nabors, 2004:590). According to Starr and Taggart (1998:530) germination of resting spores and seeds is “a resumption of activity following a period of arrested development”. Cassell’s Thesaurus defines

germination as “to sprout or bud, burgeon, develop, generate, grow, pollute, shoot, spring up, sprout, start growing or to take root” (Cassell & Co, 2000:276). Yazdi *et al.* (2013) stated that “seed germination is a key event in plants’ life cycle”.

There are numerous environmental factors that affect seed germination and viability (Bowden *et al.*, 2007:11; Jerrett & Gillen, 2015). These factors include temperature (Bowden *et al.*, 2007:11; Yazdi *et al.*, 2013:1), sunlight, pH, seed burial depth (Tanveer *et al.*, 2013:40), soil moisture (Bowden *et al.*, 2007:11; Yazdi *et al.*, 2013:1), salinity (Van den Berg & Kellner, 2005:191), oxygen and air (Jerrett & Gillen, 2015) (gasses) and growth medium composition (Campbell, 2005:9) (substrate (Hattingh, 2012:1) Dormant seeds slowly respire and utilize food reserves within and will initiate germination as soon as the ideal environment occurs (Jerrett & Gillen, 2015).

Hattingh (2012:1) and Bowden *et al.* (2007:11) stated that water is the elementary requirement for germination, for it determines the speed of germination. Soil moisture is considered as the most important environmental factor influencing seed germination (Yazdi *et al.*, 2013:1), for the seed requires water for the embryo to swell (Jerrett & Gillen, 2015) (enlarge) and break through the covering structures. The available water in the soil lies between field capacity (-0.03 MPa) and permanent wilting point (-1.5 MPa) (Tanveer *et al.*, 2013:40). Tanveer *et al.* (2013:40) stated that wet soil ranges between 0.0 MPa to -0.03 MPa, moist soil between -0.03 MPa to -0.75 MPa, and semi-arid and arid soils between -0.75 MPa to -1.5 MPa. Mature seeds are usually weighed dry (5 % - 20 % water by weight (Nabors, 2004:135)) and need to absorb a large amount of water (Jerrett & Gillen, 2015) relative to the dry weight of the seed (Hattingh, 2012) via the process of imbibition (Nabors, 2004:135). Imbibition is a passive process (Nabors, 2004:135) in which water molecules move into the seed rapidly (Uruc & Demirezen Yilmaz, 2008:2), mainly towards the proteins that are hydrophilic stored inside the endosperm or cotyledon (Starr & Taggart, 1998:530).



Germination occurs in most seeds within a few hours after the completion of imbibition (Nabors, 2004:135). Dormant seeds (10-15 % of water) (Jerrett & Gillen, 2015)) can germinate under low soil-moisture conditions and the initial stages might proceed (Bowden *et al.*, 2007:11; Hattingh, 2012:1). These conditions are generally not favourable in allowing the seed to perform at its full genetic potential. Excess soil moisture will also inhibit germination due to the restriction of oxygen availability (Hattingh, 2012:1).

Enzymes that break down stored food reserves in the seed to metabolic useful chemicals are activated as soon as the seed imbibes water (De Carvalho *et al.*, 2001:139; Hartmann *et al.*, 2011:104; Hattingh, 2012:1; Jerrett & Gillen, 2014; Smith, 2011:1; Wiwart *et al.*, 2006:328). As the seed grows too big for the shell, the seed coat will break and germination is initiated (Jerrett & Gillen, 2014; Starr & Taggart, 1998:530). More oxygen will reach the embryo as soon as the seed coat splits and aerobic respiration conditions occur (Chastain, 2012:4). Subsequently, a rapid elongated division of the meristem will commence, resulting in growth of the primary root (first root of seedling's sporophyte). As soon as the seedling's primary root breaks through the seed coat, germination is considered to be over (Starr & Taggart, 1998:530).

One will observe the leaves or the cotyledons (embryonic leaf in seed-bearing plants) after the germination (Chastain, 2012:14; Jerrett & Gillen, 2014). The seedling's food reserves are drained as soon as the seedling emerges and continued growth is dependent on photosynthesis to provide the energy (Chastain, 2012:17; Hattingh, 2012:1). Photosynthesis will only commence as soon as the true leaves are developed (Jerrett & Gillen, 2015). The plant will require continuous supplies of water (Van den Berg & Kellner, 2005:188); nutrients and light at the emerging stage (Hattingh, 2012:1). If rainfall and irrigation occur too soon after the seed is planted in a dry soil, a crust can form that inhibits the seedling's emergence (Asgrow, 2014:2).

Tanveer *et al.* (2013:40) and Pons (200:2370) considered temperature and sunlight as the most important environment signals regulating germination, species distribution and ecological interaction. Seeds can respond differently with respect to temperature and sunlight (Edwards, 2008:1). Germination takes place under various temperatures (4 °C - 37 °C (Bowden *et al.*, 2007:12; Hattingh, 2012:1) and are species, populations or ecotypes and even for a single seed collection, specific (Jerrett & Gillen, 2015). One of the main functions of temperature is to control the metabolic processes (Burke & Oliver, 1993:295). Seed can germinate in soil temperatures as low as 12.8 °C to 13.2 °C (De Carvalho *et al.*, 2001:139). Soil temperature plays a significant role in the germination rate (Hattingh, 2012:2), emergence is slow and seedling vitality can be reduced (De Carvalho *et al.*, 2001:139) at too low soil temperatures (Asgrow, 2014:2). The optimum temperature is the condition that allows the fastest germination (De Carvalho *et al.*, 2001:139). Hattingh (2012:1) stated that the optimum germination temperature for most crops ranges between 12°C-25°C. Problems associated with imbibition can be prevented by keeping the seeds above water in a sealed container for 1-2 days at ambient temperatures (that is, 20°C-25°C) prior to sowing. The seed might not sprout if the soil is too cold or hot (Jerrett & Gillen, 2015). Temperature affects the diffusion of respiratory gases, the rate of water absorption or imbibition (Hattingh, 2012:1), and the rate of chemical reactions involved in the metabolism of the seed. The seasonal changes experienced in natural control of the physiological changes in some species and seed can become dormant (Propert, 2000:274). Cold soils can result in dormant seed (Propert, 2000:273) that might become more vulnerable to wildlife digging up the seed, insects and seed or seedling diseases (Asgrow, 2014:2). Sub-optimal temperatures result in longer germination periods and lower success rates. Hattingh (2012:1) mentioned that temperatures that are too high can reduce enzyme efficiency which eventually will result in denatured protein and the seed being killed. Winter species will require cool temperatures and summer species warm temperatures to trigger germination processes (Propert, 2000:262).

Germination, sprouting and growth factors are all affected by light in some species (Campbell, 2005:12). According to Jerrett and Gillen (2014), light is a requirement for some seeds to germinate and can hinder others. However, Bewley and Black

(1982:276) mentioned that most seeds will germinate equally well in light and darkness, yet many seeds germinate only in the light and only a few will germinate in the dark. The fact is that there can be varieties (Pons, 2000:237) within a single batch resulting in one seed being insensitive at one temperature but requiring light at another. Seed becomes more sensitive to light during imbibition (De Carvalho *et al.*, 2001:139). The modern vegetable crops either prefer light or are not affected by it and should be planted shallow, for it will allow small amounts of light to filter through the soil (Jerrett & Gillen, 2015). Some wild species as well as herbs prefer darkness and should therefore be planted deep within the soil (Jerrett & Gillen, 2015). The response of the seed is dependent on the intensity, duration and light quality as well as temperature (Frankland & Smith, 1967:354). The phytochromes control the light in the seeds in all the cases that affect the seed response to light (Pons, 2000:237).

The gaseous environment that surrounds the seed is important (Smith, 2011:2). Success of germination rests on the metabolic activity involved (De Carvalho *et al.*, 2001:139). Seed will generally germinate well in air of mixed gases comprising 20% oxygen, 0.03% carbon dioxide and 80% nitrogen (Hattingh, 2012:1). Hartmann *et al.* (2011:107) stated that a general rule of thumb is that the lowering of O<sub>2</sub> or an increase in CO<sub>2</sub> will result in a lower germination than anticipated. Oxygen is required by the seed for aerobic respiration that is the main source of energy for the seed prior to photosynthesis (growing of leaves) (Bowden *et al.*, 2007:13; Hattingh, 2012:1). Jerrett and Gillen (2014) mentioned that dormant seeds require a minimal amount of oxygen, whereas germination requires a large amount of oxygen (Hartmann *et al.*, 2011:107). It is most likely that seed germination will be poor or fail completely in oxygen-deprived environments (anaerobic, Jerrett & Gillen, 2015)) Hartmann *et al.*, 2011:104 such as waterlogged and tightly compacted soils (Hattingh, 2012:1) (this will reduce water availability and oxygen required for germination, root and plant growth, and nutrient uptake (Asgrow, 2014:2)). Jerrett and Gillen (2014) stated that seeds obtain their oxygen from dissolved oxygen in water and air within the soil. In the case of soil pores being filled with water, the amount of oxygen required by the seed reduces (Asgrow, 2014:2).

The availability of moisture, temperature and light exposure (Yazdi *et al.*, 2013:6) influences seed germination (Tanveer *et al.*, 2013:40) and has an indirect effect on the burial depth of a seed (Bowden *et al.*, 2007:16; Edwards, 2008:1; Yazdi *et al.*, 2013:6). The seed size is a good suggestion of how deeply the seed must be planted and matches with the amount of light required. The seed must be buried at least as deep as the seed is long (Jerrett & Gillen, 2015).

### **2.3.1 Crusting**

Crusting can be defined as a hard crust with a higher bulk-density, higher shear strength, and lower hydraulic conductivity and porosity (USDA, 2001:1) than the rest of the soil profile (Brady & Weil, 2008:931; University of Oxford, 2013:144; Van der Watt & Van Rooyen, 1995:173; Zejun *et al.*, 2002:456). The hard surface soil layer is lightly cemented with calcium carbonate, silica or iron oxide which is harder and more compact than the soil below (University of Oxford, 2013:144). The compaction layer thickness can range between a few millimetres to tens of millimetres (Belnap *et al.*, 2001:7; Brady & Weil, 2008:931; Sylvia *et al.*, 2005: 176; USDA, 2008:1; Van der Watt & Van Rooyen, 1995:173) and is hard and sometimes brittle when dry (Brady & Weil, 2008:931; University of Oxford, 2013:144; Van der Watt & Van Rooyen, 1995:173).

Crusts form commonly by means of mechanical action or pedogenesis (University of Oxford, 2013:144) in arid environments (Zejun *et al.*, 2002:456). It is less unusual that they are relict or fossil features exhumed on the soil surface (University of Oxford, 2013:144). According to USDA (2001:1), the crusts can be defined by their strength and air-dry rupture resistance (USDA, 2008:1). Decline of soil structure (Lal & Stewart, 1992:2), compaction, disintegration, detachment, entrainment and deposition are all results of the interaction between the soils and water generated from rain (Jakab *et al.*, 2013:147). These interactions result in the formation of crusts (Jakab *et al.*, 2013:147). Examples of crusts may include limonite ( $\text{FeO}(\text{OH}) \cdot n\text{H}_2\text{O}$  (University of Oxford, 2013:342)), hematite ( $\text{Fe}_2\text{O}_3$  (University of

Oxford, 2013:273)) and jarosite ( $\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$ ) (University of Oxford, 2013:316)) crusts formations. Both limonite and jarosite are chemical crusts.

There are two main types of crusts distinguished. That is biological (organic (Brady & Weil, 2008:931; Sylvia *et al.*, 2005: 176; Van Rooyen, 1995:173)) and physical (inorganic or non-biotic (Belnap, 2001:177)) soil-surface crusts (Jakab *et al.*, 2013:147). Physical crusts can be sub-categorised as structural crusts form due to the splash erosion impact, erosional crusts are formed by wind or water erosion, and depositional crust (Sylvia *et al.*, 2005: 176) develops through the sedimentation of the delivered soil particles (Jakab *et al.*, 2013:147; Van der Watt & Van Rooyen, 1995:173).

The types of physical crusts as mentioned by Belnap (2001:177) include “the impact of raindrops, compressional forces such as animal trampling or vehicular traffic, evaporative processes (forming chemical crusts), and trapped gas bubbles (forming vesicular crusts)” (Jakab *et al.*, 2013:147; Van Rooyen, 1995:173).

Both biological and chemical factors are associated with crusts (USDA, 2008:1). Biological crusts can be defined as a living community of lichen, cyanobacteria, algae, and moss growing on the soil surface that binds the soil together (Belnap *et al.*, 2001:1; USDA, 2001:1; USDA, 2008:1).

A chemical crust can develop due to high salt content and has a white or pale colour (USDA, 2001:1; USDA, 2008:1). The most common form of crusting is the surface sealant which takes place under the place of kinetic energy of raindrops (larger raindrops result in thicker crusts (Belnap *et al.*, 2001:7; Belnap, 2001:178)) on top of the soil which breaks up soil aggregates on unprotected surfaces (Belnap *et al.*, 2001:7; Jakab *et al.*, 2013:147; USDA, 2008:1).

The formation of crusts, as described by Zejun *et al.* (2002:456) involves two main processes. The first process involves the disintegration of aggregates and

compaction of soil particles by the impact of raindrops (Belnap, 2001:177; Sylvia *et al.*, 2005: 176). The second process consists of the physical chemical action of dispersion of aggregates that move and clog the conducting pores that result in a less permeable topsoil layer (Belnap, 2001:177; Jakab *et al.*, 2013:147; Sylvia *et al.*, 2005: 176; Van Rooyen, 1995:173; Zejun *et al.*, 2002:456).

The detached soil particles are an indirect consequence of selective erosion and the different soil particles are moved differently (Jakab *et al.*, 2013:147). If the soil texture is dominated by silt and clay particles the possibility of crust formation increases in comparison with sandy soils (Lal & Stewart, 1992:319; USDA, 2008:1). The silt particles are washed into the spaces between the larger particles which clog the soil pores and in turn reduce the infiltration rate by 90% (Belnap, 2001:178; Belnap *et al.*, 2001:7) and increases runoff (Jakab *et al.*, 2013:147). The clogging can take place within the first few minutes of a rainstorm (Belnap, 2001:178; Belnap *et al.*, 2001:7; Jakab *et al.*, 2013:147). The aggregate structures are destroyed in the case of compressional forces generated for instance by machinery or hooves of grazing animals resulting in the formation of a physical crust (Belnap, 2001:178). An impermeable surface layer is formed by the trampling, which compacts the soil, especially in moist conditions (Belnap, 2001:178).

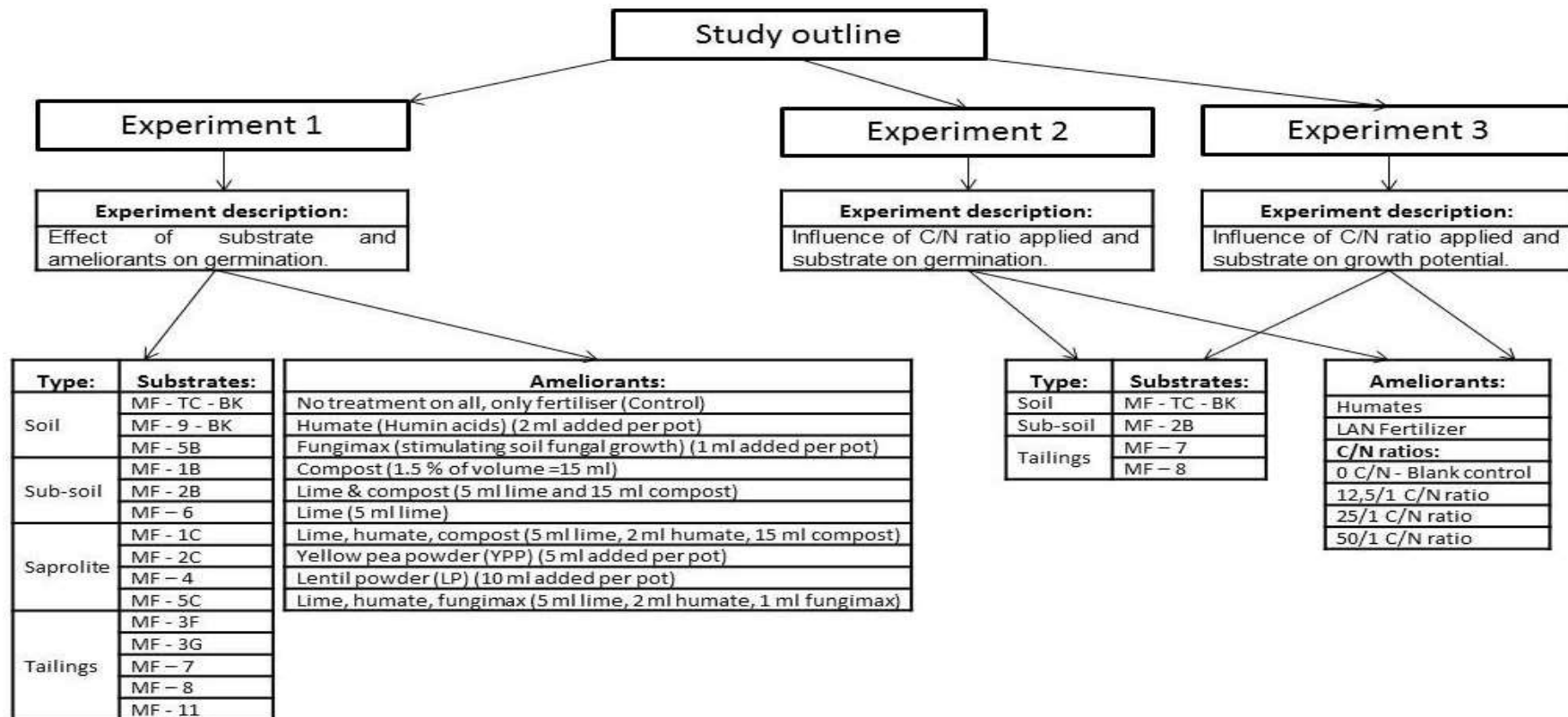
An increase in erosion will occur due to the increase in both the runoff and velocity of water (Belnap, 2001:177; Jakab *et al.*, 2013:147; USDA, 2008:1). Other consequences of crusting is that gas exchange and seedling emergence are restricted (Belnap, 2001:177; USDA, 2008:1; Zejun *et al.*, 2002:456). Crusting prohibits water infiltration which results in a higher erosion factor as well as permanent drought that the plant will experience (Jakab *et al.*, 2013:148; USDA, 2008:1). Although crusting holds a number of negative consequences there is still a positive outcome for crusting. According to USDA (2008:2) and Sylvia *et al.* (2005: 176), soils containing a small amount of sand particles (such as tailings) will protect the soil surface from wind erosion. One of the major objectives following mine closure is to have no on-going erosion and on-going pollution (such as air pollution by means of dust blowing). Crusting helps to accomplish both these objectives

(Sylvia *et al.*, 2005: 176). USDA (2008:1) stated that crusts might potentially decrease the water loss due to evaporation, for the surface area is not exposed to the air. Crusting can be counteracted by the addition of organic matter, limitation of veld fires, and reduced disturbances such as tillage, machinery and livestock (USDA, 2001:2; USDA, 2008:2).

### 3. Materials

In this chapter the geological origins as well as locations of each of the growth mediums used in the experimental pot-trials are discussed. The various ameliorants used in an attempt to alter the C/N ratios of the growth mediums are outlined in the section 3.2. The sampling methodology and experimental set-up is given in chapter 4. Diagram 1 illustrates the study outline regarding the materials utilised for all three experiments.

**Diagram 1: Study outline of experiments and materials utilised.**





### 3.1. Substrate samples

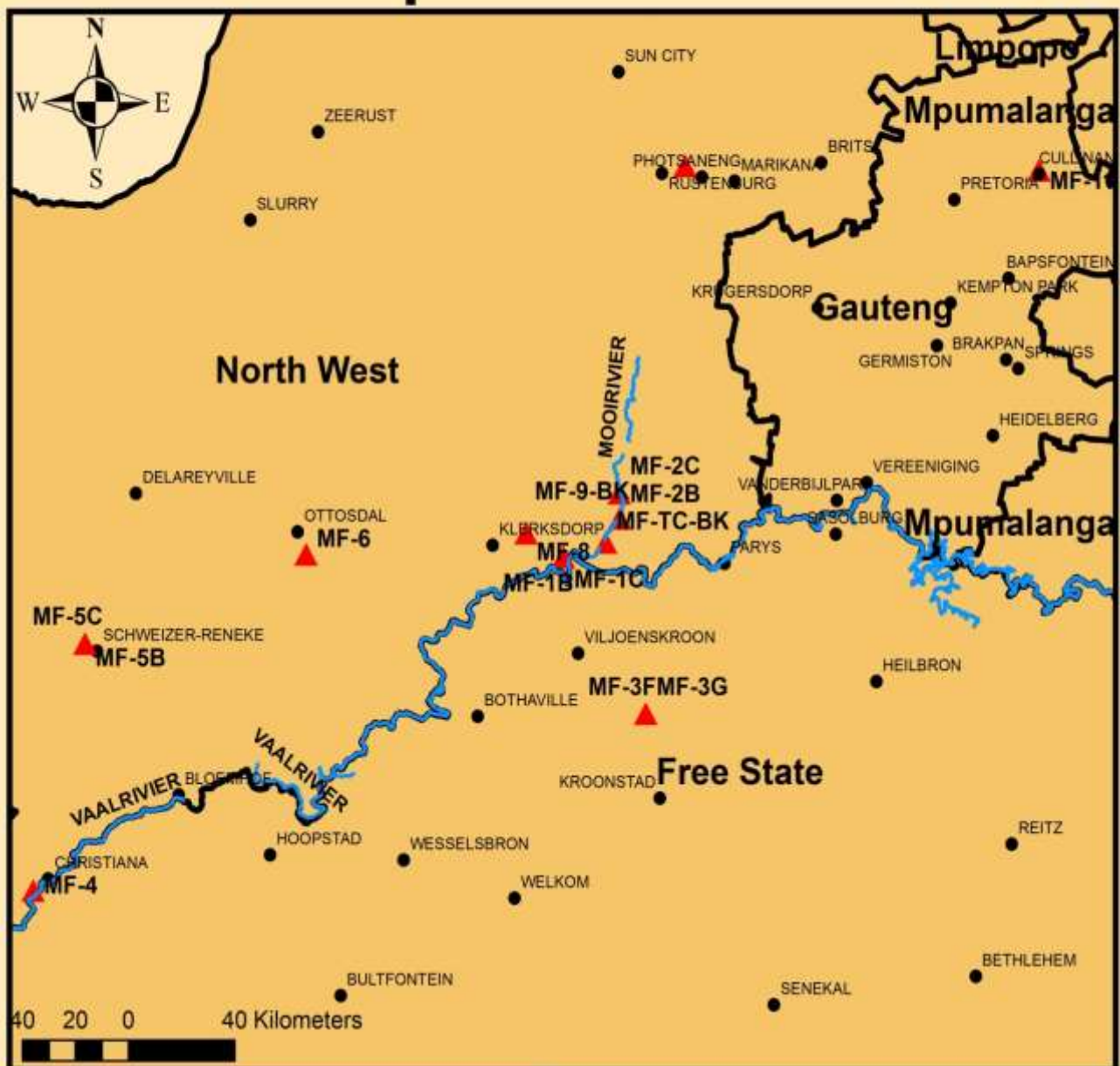
The samples (sub-soil, saprolite and tailings) listed in Table 1 were collected from the Free State, Gauteng and North West as illustrated in Figure 1 and were used as growth mediums (substrates) for the pot-trials. For a complete discussion of the materials utilised refer to section 3.2. The map was created by means of GIS (Arc Map), using data from the Geo-database of the North-West University (Potchefstroom Campus).

**Table 1: Summary of substrates collected from various localities.**

Substrate type	Sample number on map	Locality name	GPS coordinates	
			S	E
Soil	MF - TC – BK	Potchefstroom A - Horizon (Topsoil)	26.84320	27.04868
	MF - 9 – BK	Potchefstroom Vertisol	26.7658865	27.095472
	MF - 5B	Schweizer-Reneke (Borrow-pit) Kalahari Sand	27.164281	25.280987
Sub-soil	MF - 1B	Khuma (Kareerand) Red Structured B	26.88844	26.90463
	MF - 2B	Potchefstroom (North-West University) Red Structured B	26.68556	27.09458
	MF – 6	Ottosdal Kaolinite Clay	26.88091	26.03193
Saprolite	MF - 1C	Khuma (Kareerand) Acid Lava Saprolit	26.88844	26.90463
	MF - 2C	Potchefstroom (North-West University) Diabase Saprolite	26.68556	27.09458
	MF – 4	Christiana Hard Carbonate Saprolite	27.95098	25.10553
	MF - 5C	Schweizer-Reneke (Borrow-pit) Granite Saprolite	27.164281	25.280987
Tailings	MF - 3F	Viljoenskroon Kimberlite Fine Tailings	27.38811	27.18400
	MF - 3G	Viljoenskroon Kimberlite Coarse Tailings	27.38811	27.18400
	MF – 7	Rustenburg (Paardekraal) - Platinum Tailings	25.643471	27.317114
	MF – 8	Stilfontein (Chemwes Mine) - Gold Tailings	26.81279	26.77755
	MF – 11	Cullinan - Kimberlite Tailings	25.657615	28.517156

\* MF - 9 - BK and MF-TC-BK are used as control substrates.

# Sample Localities



## Legend

- Rivers
- Provinces
- Towns
- ▲ Sample Localities

Figure 1: Locality map of samples with the associated stratigraphy

## 3.2 Geological setting and pedology of materials

This section gives a comprehensive overview of the substrates utilised. The geology and pedology play a significant role in rehabilitation practices.

### 3.2.1 Geology and pedology of substrates from Khuma (Kareerand)

The Kareerand TSF is underlined by amygdaloidal and porphyritic lava (Aucamp & Van Schalkwyk, 2000:3.8), agglomerate (Rubenheimer, 2013:23), with interbedded shale, tuff, greywacke, conglomerate (fluvial and lacustrine (Groenewald & Groenewald, 2014:22)) and impure limestone with algal structures (Aucamp & Van Schalkwyk, 2000:3.8) known as stromatolites (Groenewald & Groenewald, 2014:22) (stromatolitic calcarenite (Van der Westhuizen *et al.*, 2006:196)) are reported in the Rietgat formation of the Platberg Group, Ventersdorp Supergroup (Mapukule, 2009:32) as illustrated in Table 2.

**Table 2: Geology of the Kareerand TSF (Eriksson *et al.*, 2006; Groenewald & Groenewald, 2014:22; Mapukule, 2009:32)**

Ventersdorp Supergroup	Platberg Group	Allanridge Formation (743m)
		Bothaville Formation (427m)
		Rietgat Formation (1319m)
		Makwassie Formation (1968m)
		Goedgenoeg Formation (1777m)
		Kameeldoorns Formation (1798m)

The Rietgat formation comprises predominantly alternating volcanic rocks (Groenewald & Groenewald, 2014:22) (basic to intermediate lavas, pyroclastic (Almond, 2013:3)) with minor sedimentary rocks (Mapukule, 2009:32) with varying thicknesses and ratios of volcanic to sedimentary material throughout the basin (Van der Westhuizen *et al.*, 2006:196). The composition of volcanic rocks includes basaltic andesite, dacite and feldspar porphyry (Van der Westhuizen *et al.*, 2006:196). The Khuma area also contains lavas, pyroclastic rocks and intercalated sediments in the form of greywacke and chemical sediments such as chert and carbonate, as well as lacustrine stromatolitic calcarenite (Almond, 2013:3; Van der Westhuizen *et al.*, 2006:196). Air-fallen tuffs (hard volcanic rock composed of compacted

volcanic ash) are common in the area (Mapukule, 2009:32; Van der Westhuizen *et al.*, 2006:196).

The current soil types are residual and transported red and dark-grey Vertic clays which can be classified as the Rensburg soil form (vertic A-horizon on G-horizon) (Fey, 2005:11; Fey, 2010:33; Meyer, 1984:190; Soil Classification Working Group, 1991:44). A Vertic has the ability to swell and shrink (Meyer, 1984:190; Van der Watt & Van Rooyen, 1995:55) (2:1 clays (De Wet & Jansen, 2014:16)) and contains more than 30% clay (University of Oxford, 2013:619). A vertic topsoil horizon has a particularly strongly developed structure (Fey, 2005:12; Soil Classification Working Group, 1991:16; Van der Watt & Van Rooyen, 1995:55). It might also contain either visible slickensides or a plasticity index greater than 32 (Fey, 2005:12; Soil Classification Working Group, 1991:16).

### **3.2.2 Geology and pedology of substrates from Potchefstroom (North-West University)**

The interior of the Vredefort Dome consists of basement Archaean Granites (65 Ma) averaging 18 km in diameter which are underlaid by a great deal of heavy rocks (De Villiers & Mangold, 2002; Visser, 1989:193). These outcrops occur in the Potchefstroom District (the south-eastern portion of the Province) and further west as far as the north-central portion of the Vryburg and Ganyesa Districts (De Villiers & Mangold, 2002). The Vredefort Dome, resulting from a large meteorite impact (2000 Ma), is considered to be one of the most ancient landscapes preserved anywhere in the world (De Villiers & Mangold, 2002). The dome is surrounded by a rim of Witwatersrand, Ventersdorp and Transvaal rocks that contain shatter cones indicative of violent mechanical shock (De Villiers & Mangold, 2002). The Potchefstroom syncline (20-30 km in width) comprises Transvaal rocks that are deformed by means of folding with brachy-structures on both sides of the long axis of the Dome structure (Visser, 1989:193). The rocks within the rim are over-folded to the north-west due to a flexural slip around a central vertical axis coinciding upward shifting along the strike-slip faults (Visser, 1989:193). The diabase sills intruded most likely at the same time (Visser, 1989:193).

The current soil type is a Shortlands soil form (Orthic-A horizon, Red structured B-horizon) (Soil Classification Working Group, 1991:16). The soil originated from the weathering of the diabase parent rocks (Visser, 1989:193) and accumulation of residual iron (Fey, 2010:33).

A red structured soil must have a uniform red colour (Fey, 2010:33; Van der Watt & Van Rooyen, 1995:47) which is not directly inherited from the parent rock, but is the result of iron oxide accumulation resulting from mineral weathering (Fey, 2005:13; Fey, 2010:33; Meyer, 1984:190; Soil Classification Working Group, 1991:25). This soil form must directly underlie an orthic A-horizon which often has a reddish hue. It is also diagnostic that the transition from the orthic A-horizon may be gradual but not clear with respect to texture and structure (Soil Classification Working Group, 1991:25).

This horizon is recognised in uniformly red sub-soil materials that have developed strong structures rather than being moderately blocky in the dry state (Fey, 2005:13; Fey, 2010:33; Meyer, 1984:190; Soil Classification Working Group, 1991:25). This will occur in the presence of 2:1 layer clay minerals as well as sufficient amounts of clay. It is known that the parent materials will comprise large proportions of ferromagnesian and calcium aluminosilicate minerals. The parent rocks can be basic, igneous (such as basalt, dolerite, norite, diabase and gabbro), or metamorphic rocks (such as amphibolite and basic schists). This horizon usually typically develops residually from the parent rock, but it may also originate in colluvium and rarely in alluvium. The red structured horizons are distinguished from the apedal horizons by means of the CEC ( $\geq 11$  cmol (-)), for it reflects the amount and type of clay present. Although red structured B -horizons always contain reddish coloured clay skins, eluviation of clays are counteracted by the iron oxides that coat the mineral particles as well as high clay content (Soil Classification Working Group, 1991:25).

### **3.2.3 Geology and pedology of Kimberlite substrates from Viljoenskroon**

The Voorspoed Kimberlite pipe has a large distribution of an approximately six ha surface area (Howarth, 2010:29) and is situated on the farm Voorspoed 2480 (Vaz, 1988:23). The well-known Karoo basalt block known as the Stormberg floating reef (Vaz, 1988:24) is present at the surface and is 300 m in depth (Howarth, 2010:29). Several Kimberlite dykes also occur with this basalt block (Howarth, 2010:29). Analyses of the basalt suggest it can be

associated with the Eccles shales (Vaz, 1988:24) of the Karoo Lesotho Formation (Howarth, 2010:29). It was first thought that the basalt at the surface was an ancient feeder channel for the Karoo magmatism (Howarth, 2010:29). This formed a zone of weakness that was later used by the Kimberlite to intrude through the crust. It has been proven to be incorrect due to the fact that the basalt block is only 300 m deep and is therefore incorporated during the eruption process (Howarth, 2010:29). The volcanoclastic rocks can be classified as tuffisitic Kimberlites (Howarth, 2010:29). The volcanoclastic rocks can be divided into three distinct varieties: tuffisitic Kimberlite, normal tuffisitic Kimberlite breccia and basalt-rich tuffisitic Kimberlite breccia (Howarth, 2010:29). Small scales of layering can be observed through the volcanoclastic rocks (Howarth, 2010:29). The different proportions of country rock xenoliths and Kimberlite content were used to characterise the volcanoclastic rocks (Howarth, 2010:29). The tuffisitic Kimberlite contains <30 %, normal tuffisitic Kimberlite breccia contains 50-60 % and basalt-rich tuffisitic Kimberlite contains >80 % country rock xenoliths (Howarth, 2010:29).

The Hypabyssal Kimberlite can be described as a diopside phlogopite Kimberlite (Howarth, 2010:29). The primary minerals were identified as olivine (10%), phlogopite (35%), diopside (20%), melilite (3 %) and matrix (32%) (Howarth, 2010:29). Other minerals that might be present in the minority include apatite, monticellite, leucite, amphibole, opaque spinel, perovskite, serpentine and calcite (Howarth, 2010:29). The shape of a Kimberlite pipe is characteristic as illustrated in Figure 2. The uppermost portion of a Kimberlite pipe, known as the crater, is characterised by well-bedded and poorly consolidated sediments with chaotic debris-flow deposits and pyroclasts (Vaz, 1988:5). The diatreme comprises tuffisitic Kimberlite breccia which is an easily weathered breccia (angular country rock xenoliths and fragments of mantle-derived material set in a fine-grained matrix) and is of most importance for its high volume occupied (Vaz, 1988:5). The root zone (refer to Figure 2) has an irregular shape and comprises magmatic or hypabyssal material usually porphyritic in appearance (Vaz, 1988:5). The hypabyssal material includes macrocrysts of olivine and phlogopite set in a fine-grained matrix, often with xenocrystic garnet, ilmenite, spinel and chrome-diopside (Vaz, 1988:5).

The Viljoenskroon Kimberlite substrates are exactly the same material; however, distinction can be made between the finer and coarser counterparts of the substrate and thus used as separate materials.

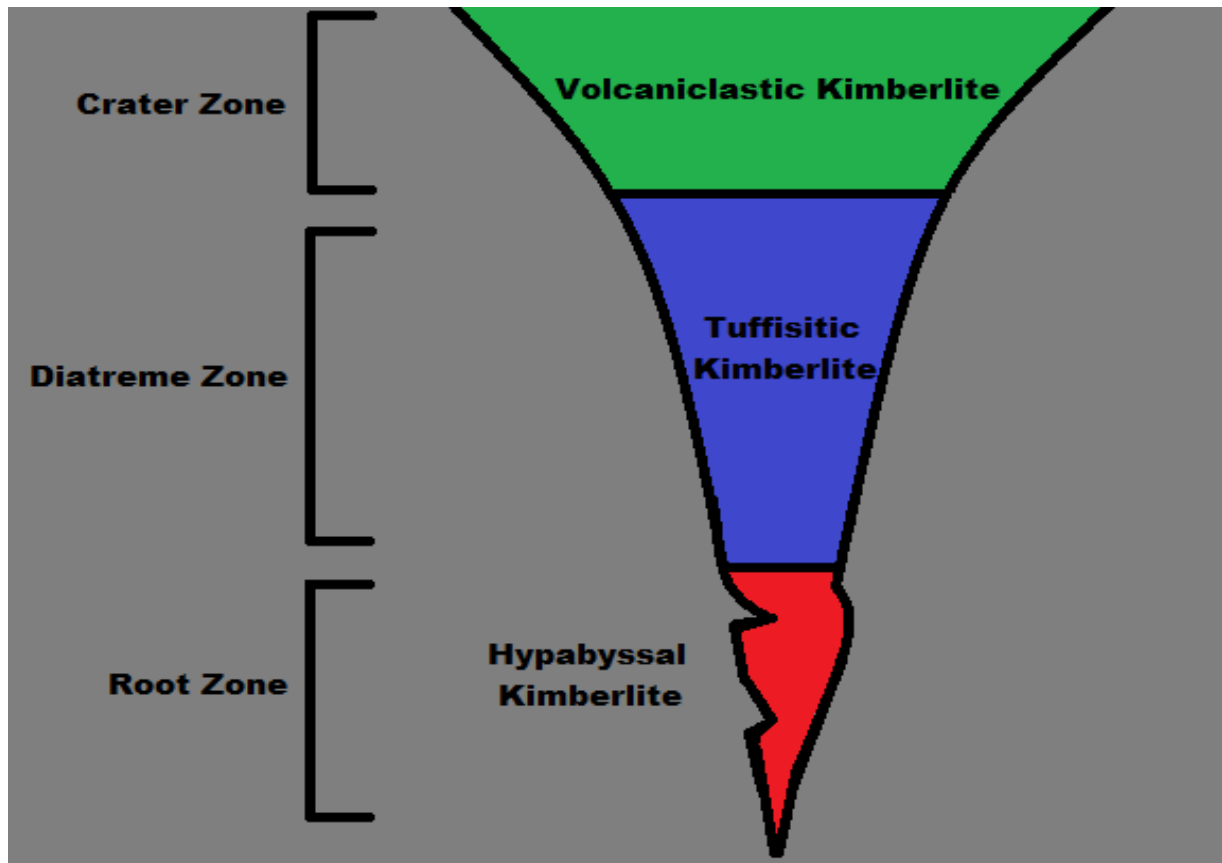


Figure 2: Model of a Kimberlite pipe (picture constructed by means of Microsoft Paint)

### 3.2.4 Geology and pedology of hard carbonate saprolite from Christiana

A hardpan carbonate horizon must be continuous throughout the pedon and is cemented by calcium and/or calcium-magnesium carbonates (secondary limestone (Fey, 2005:12)) and occurs in the lower A or in the B horizon (Brady & Weil, 2008:936; Fey, 2010:33; Soil Classification Working Group, 1991:36; University of Oxford, 2013:270; Van der Watt & Van Rooyen, 1995:48). The horizon is a barrier to roots and is slowly permeable to water (Fey, 2005:12). This horizon is also considered massive, vesicular or platy and extremely hard when dry and hard or firm when moist. This might not be applicable when the horizon is exposed to erosion, occurring beneath a red apedal B-horizon, melanic or orthic - A, yellow-

brown apedal - B, neocutanic - B or neocarbonate - B horizon (Soil Classification Working Group, 1991:36).

There are successive calcrete surface caps of remnant fluvial and pedogenic deposits of different ages preserved in the Christiana area. This calcic horizon usually occurs in arid regions (Fey, 2005:10; Fey, 2010:33). After the 20 m deposition of terraces, the youngest calcrete development occurred (Gresse, 2003:535). The Dwyka bedrock is covered by a semi-continuous calcrete layer which follows the surface contours (Gresse, 2003:535; (Meyer, 1984:188). The Rooikoppie gravels are underlaid by the calcrete caps and fluvial terrace deposits (Gresse, 2003:536). These gravels are mobile, multi-cyclic scree deposits and elevated (inverted) fluvial deposits (Gresse, 2003:536). The deposits were well-maintained and recycled constantly from one successive land surface to the next (Gresse, 2003:536). The depression recycle was only survived by the most durable silicic clasts such as banded iron formation (BIF), quartzite and chert (Gresse, 2003:536). The only areas where diamonds are present are those where the Rooikoppie gravels recycled older diamondiferous fluvial deposits (Gresse, 2003:536). According to Gresse (2003:536), barren fluvial deposits are overlaid by the Diamondiferous Rooikoppie gravels and vice versa.

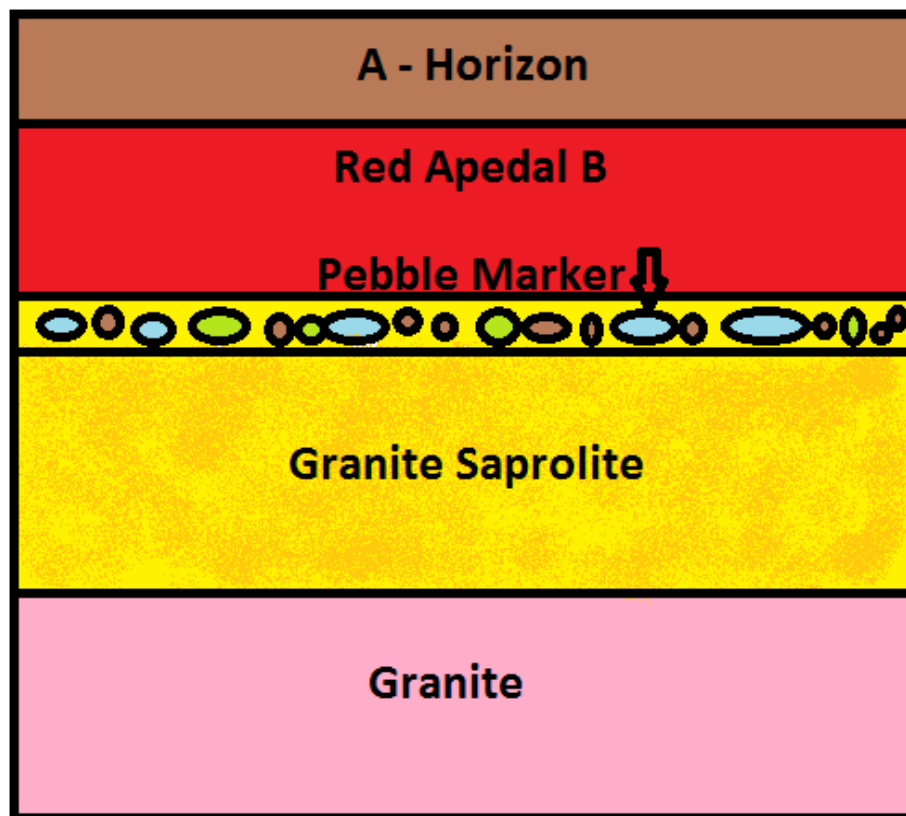
Studies have shown that regolithic gravels, diamondiferous in places, occur widely under a veneer of eolian sand in the Christiana (Sheet 2724) region (Von M. Harmse & Hattingh, 2012:35). A similar transitional layer can be observed in the Congo with better sorted eolian sand underneath (Von M. Harmse & Hattingh, 2012:35). It perhaps came from a sort of desert eluviation or a lag deposit (Von M. Harmse & Hattingh, 2012:36). Some of the pebbles were derived from the inliers of Dwyka Tillite and Eccu mudstone which are then covered by eolian sand (Von M. Harmse & Hattingh, 2012:36). There were diamonds mined from the regolithic gravels on the farms Koosfontein, Kameelkuil and Bosmansfontein as well as others in the same vicinity (Von M. Harmse & Hattingh, 2012:36). The diamonds in the vicinity could have been misidentified as older gravel (Von M. Harmse & Hattingh, 2012:36). There is in-situ diamondiferous high-level gravel of the Vaal River on the farms Vaalbank, Rustkraal, Heuningskrans, Doornbult and Baviaanskrans (Von M. Harmse & Hattingh, 2012:36).



### 3.2.5 Geology and pedology of substrates from Schweizer-Reneke (Borrow-pit)

The B-horizon originates from Kalahari sands, and is underlaid by the alluvial deposits followed by granitic saprolite originating from the Archaean granite (as illustrated in Figure 3).

Schweizer-Reneke is underlaid by Archaean granite (Van Eeden *et al.*, 1963:5). Above the granites are three distinct types of gravel (Vaz, 1988:24). These gravels include the oldest “Rooikoppie” gravel which can be described as a chemically mature unsorted lateralized colluvial gravel unit (Vaz, 1988:24). This unit is approximately a 1-2 m thick layer situated on the hillcrests and the upper sections of hill slopes (Vaz, 1988:24). The younger upward fining gravels are known as the “terrace-type” gravels that occur on the lower slopes of the present drainage valleys (Vaz, 1988:24). This layer ranges between 1 to 4 m in thickness (Vaz, 1988:24). The youngest gravels known as the “spruit type” gravels are similar in both the texture and composition to the “terrace-type” gravels and occur in the current river valley floors (Vaz, 1988:24). The transition between the younger and older land surfaces was affected by deformation and movement along the Griqualand-Transvaal axis on the Northern side of the Vaal River (Harmse & Hattingh; 2012:27). The high-level gravels occur in the Northern side of the Vaal River and are approximately 96-106 m above its present level (Harmse & Hattingh; 2012:35). The older gravels serve as evidence that the river followed the more resistant pre-Karoo surface shifting southward (Harmse & Hattingh; 2012:35). The river’s movement was stimulated by the upliftment along the Griqualand-Transvaal tectonic axis (Harmse & Hattingh; 2012:35). This statement is supported by the fact that the older gravels are confined to the northern bank, whereas the younger gravel occurs on both the northern and southern banks (Harmse & Hattingh; 2012:35).



**Figure 3: Illustration of the Schweizer-Reneke geology**

### **3.2.6 Geology and pedology of Kaolinite Clay from Ottosdal**

Ottosdal is underlaid by the Syferfontein formation of the Dominion Group (Marsh, 2006:149).

The Dominion Group comprises volcanic and minor clastic sedimentary rocks that are now metamorphosed to a green-schist-amphibolite grade (Marsh, 2006:149). The granite-greenstone basement terrain situated in the central part of the Kaapvaal Craton is overlaid by the Witwatersrand Supergroup, which is sequentially overlaid by the Dominion Group (Marsh, 2006:149). The Dominion Group is one of the earliest supracrustal sequences of the Kaapvaal Craton with an age of approximately 6 Ma (Marsh, 2006:149). Exposure of the Dominion Group on the surface is limited to the Ottosdal (Visser, 1989:29), Ventersdorp and Klerksdorp areas (Marsh, 2006:150). Minor economical gold and uranium mineralization occurs in the Dominion Group (Marsh, 2006:149). This mineral provides the maximum age of the Witwatersrand strata that represent a protobasinal phase of the greater Witwatersrand Basin and the tectonic environment prior to the deposition of the Witwatersrand strata (Marsh, 2006:149). The volcanic rocks laying conformably on the sedimentary rocks, gold-

bearing metamorphosed conglomerates, quartzites and sericite schists overlaying the Archaean granitic rocks (Visser, 1989:30) are known as the Dominion Reef Series (Marsh, 2006:149). The name originates from the Dominion Mine on the farm Rhenoster Spruit where gold was exploited from the conglomerates (Marsh, 2006:149). The Syferfontein formation comprises quartz-feldspar porphyries (phenocrysts of euhedral alkali feldspar and oligoclase, anhedral quartz, Fe-Ti oxides) intercalated (Marsh, 2006:151) with mafic to intermediate lavas (Visser, 1989:30) and volcanoclastic beds (Marsh, 2006:151). Visser (1989:30) stated that the Syferfontein formation consists of Riolite. According to Marsh (2006:151), the quartz is rounded and embayed, and the plagioclase phenocrysts present are fritted. Marsh (2006:151) stated that the “fine phenocrysts and the groundmass comprise alkali feldspar, quartz, chlorite, epidote, actinolite, stilpnomelane, sphene and calcite, the mafic minerals being from metamorphic origin”. The “wonder stone” (70m thick, 8 km along the strike) units make up a massive part of the formation which is well developed (Marsh, 2006:151) to the north of the Ottosdal area (Visser, 1989:30). The wonderstone can be described as fine-grained comprising massive fine-grained pyrophyllite (Marsh, 2006:151). Deposits of silicic ashes originated from the volcanic event and altered to bentonite via the interaction of water (Marsh, 2006:151). The bentonite was converted to pyrophyllite due to succeeding greenschist-grade metamorphism (Marsh, 2006:151). The kaolinite originated from the alkali-feldspars.

Kaolinite (dickite, nacrite, China clay, kaolin) is a 1:1 phyllosilicate (sheet silicate) with the general formula of  $\text{Al}_4(\text{Si}_4\text{O}_{10})(\text{OH})_8$  (Sylvia *et al.*, 2005:36; University of Oxford, 2013:321; Yong *et al.*, 2012:28). Kaolinite is the final product of alumino-silicates (alkali feldspars (Hamilton *et al.*, 2007:124)) that have undergone chemical weathering resulting in clay with a hardness of 2.0-2.5 (Hamilton *et al.*, 2007:124; University of Oxford, 2013:321). This secondary mineral feels like plastic and is generally used as a cheap general-purpose filler and coating material for paper, in ceramics, as well as chemicals and paints (University of Oxford, 2013:321). Kaolinitization is the process in which high temperature hydrothermal alteration and replacements of feldspars to various degrees occur, resulting in a fine-grained aggregate of mineral kaolinite (University of Oxford, 2013:321).

### 3.2.7 Geology of platinum tailings from Rustenburg (Paardekraal)

The Bushveld complex is approximately 2.058 Ma of age and covers an enormous area of 65 000 km<sup>2</sup> (Letts, 2007:55). The Rustenburg Layered Suite (mafic component of the complex (Harris *et al.*, 2005:579; Letts, 2007:55) is divided into five compartments of which the western limb extends for 200 km along an arc from near Thabazimbi to near Pretoria (Cawthorn *et al.*, 2006:264; Letts, 2007:55), which is mined over approximately 80 km of this length (Cawthorn *et al.*, 2006:266). The Merensky unit is included in the Critical Zone (see Figure 4) of the Rustenburg Layered Suite within the Bushveld Complex (Cawthorn *et al.*, 2006:268; Letts, 2007:57) as illustrated in Table 3.

**Table 3: Geology of platinum tailings from Rustenburg (Cawthorn *et al.*, 2006:266)**

Bushveld Complex	Rustenburg Layered Suite	Critical Zone	Upper Sub-zone (2054.4 Ma)	Western limb	Schilpadnest Sub-suite	Mathlagame Norite-Anorthosite	Merensky Reef
							Upper Group 2

The Critical Zone is characterised by its remarkable layering (Kinnaird *et al.*, 2006:12) of dunites, harzburgites, pyroxenites, chromitites, norites and anorthosites (Letts, 2007:57) This zone hosts world-class chromite and platinum deposits in several different reefs (Kinnaird *et al.*, 2006:12). The Critical Zone (1300 m (Letts, 2007:57) to 1500 m thick) is divided into a lower sub-zone and upper sub-zone (Kinnaird *et al.*, 2006:12). The lower sub-zone comprises an ultramafic lithology which is characterised by a thick succession of orthopyroxenitic cumulates (rocks that were formed as a result of magmatic segregation are called magmatic cumulates) (Letts, 2007:57; Kinnaird *et al.*, 2006:12). The upper sub-zone comprises packages of chromitite, harzburgite, pyroxenite, through norite to anorthosite (Kinnaird *et al.*, 2006:12). The first appearance of cumulus plagioclase defines the base of the upper Critical Zone which is drawn at the base of the lowermost anorthositic layer of the Rustenburg Layered Suite between two chromitite layers (Kinnaird *et al.*, 2006:12). The Merensky Reef comprises heterogeneous pegmatitic feldspathic pyroxenite bounded by narrow stringers of chromitite in its highest or normal stratigraphic position (Smith *et al.*, 2003:244). The various pegmatites might comprise very coarse-grained feldspathic

harzburgite or even medium-grained melanorite (Smith *et al.*, 2003:244). The grades increase of the platinum group elements (PGE) with coarsening of the inter-chromitite lithology (Smith *et al.*, 2003:244). The PGE are concentrated in the upper chromitite which is known as “top loading” (Smith *et al.*, 2003:244). Although the footwall to the reef comprises rare feldspathic pyroxenite or harz-burgite, it generally is noritic or anorthositic (Smith *et al.*, 2003:244). The hanging wall is uniform in lithology and comprises poikilitic pyroxenite to feldspathic pyroxenite that grades upwards into norite and anorthosite (Smith *et al.*, 2003:244). The dips occurring range from 9° to 22° in the western and north-western limbs of the Bushveld Complex (Smith *et al.*, 2003:244).

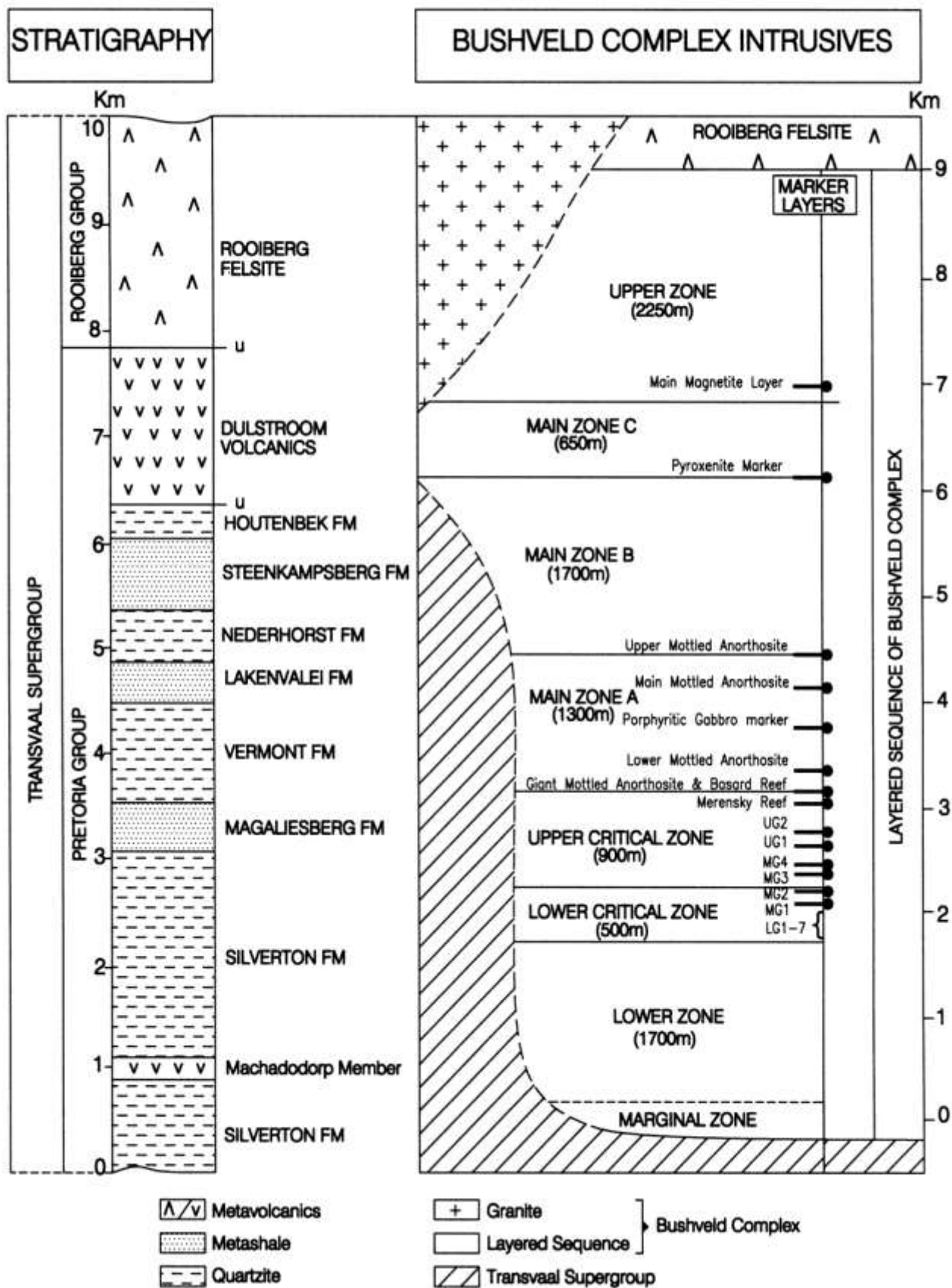


Figure 4: Stratigraphy of the Transvaal Supergroup and the Bushveld Complex Intrusives (Scoon, 2002:1038)

### 3.2.8 Geology and pedology of gold tailings from Stilfontein (Chemwes Mine)

The Vaal Reef in the Klerksdorp area belongs to the Krugersdorp Formation (Van Deventer, 2008:4) within the Johannesburg Group of the Central Rand Group which is part of the Witwatersrand Supergroup (see Table 4) (Catuneanu & Biddulph, 2001:117). There are numerous erosion surfaces within the Central Rand Group that divide it into fluvial and marine repositories of sedimentation (Manzi *et al.*, 2013:96). The Johannesburg Sub-group consists predominantly of quartzites, shales and conglomerates that were deposited in a fluvial braid-plain environment (Manzi *et al.*, 2013:96; McCarthy, 2006:168; Norman & Whitfield, 2006:324). There were periodic erosion episodes that occurred as a result of lowered base levels (Manzi *et al.*, 2013:96). During this period peneplanation of the braid-plane took place which resulted in the disconformities of a very wide extent. The placer development was associated with these so-called degradation surfaces (McCarthy, 2006:168).

**Table 4: Geology of Stilfontein tailings (Vaal Reef) (McCarthy, 2006:168)**

Witwatersrand Supergroup	Central Rand Group	Johannesburg Sub-group	Krugersdorp Formation	Klerksdorp area	Vaal Reef
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The depositional conditions were clearly different around the basin. Placer development tended to be confined to specific regions as a result of this. The base of the Krugersdorp Formation was marked by the erosive episode and is particularly important in the Welkom and Klerksdorp areas, for it gave rise to the Basal and Vaal Reefs (McCarthy, 2006:169). The Witwatersrand conglomerate is associated with the Vaal Reefs that represent a fluvial paleoplacer deposit (Catuneanu & Biddulph, 2001:120). The gold-bearing quartz pebble conglomerates of the Witwatersrand have obvious economic significance and contain pyrite in some areas (Catuneanu & Biddulph, 2001:120).

The original soils of the footprint can be described as the Myhill soil family of the Mispah soil form and the Dumisa soil family of the Glenrosa soil form (Van Deventer & Ferreira, 2013:11). After the deposition and re-mining of the tailings took place, the soil became a

Witbank soil form (chemically and physically disturbed) (Soil Classification Working Group, 1991:192).

There are significant amounts of sulphate salts that precipitate on the surface during winter or periods of drought. The main types of salts that precipitated on the surface of the TSFs are epsomite, gypsum and bldite (Van Deventer, 2011 a:6). According to Van Deventer (2011b:23), there is a correlation between the mineralogical composition of the accumulated salts and Mine Waste Solutions (MWS) No. 5 TSF. The gypsum and sulphate content are associated with acid mine drainage (AMD) that originates from the gold TSF.

### **3.2.9 Pedology of vertic soil from Potchefstroom**

The current soil type is a dark-grey vertic horizon which can be classified as the Rensburg soil form (Vertic A on an Unspecified B) (Fey, 2005:11; Soil Classification Working Group, 1991:44). This vertic soil most likely originated as a result of alluvial deposits, and is located adjacent to the Mooi River as illustrated in Figure 1.

Vertic soil comprises predominantly 2:1 swelling clays (Fey, 2005:11; Fey, 2010:33; Meyer, 1984:190; Soil Classification Working Group, 1991:44) and this sample comprises 38% clay (De Wet & Jansen, 2014:16). A vertic topsoil horizon has a particularly strongly developed structure (Soil Classification Working Group, 1991:16). It might also contain either visible slickensides (Fey, 2005:11; Meyer, 1984:190; Oxford, 2013:619) or a plasticity index greater than 32 (Soil Classification Working Group, 1991:16). A vertisol contains more than 30% clay and will expand when wet and contract when dry to produce a self-inverting soil and an undulating micro-relief (Oxford, 2013:619). They are associated with seasonally wet and dry environments (semi-arid to sun humid climates according to Fey, 2010:33, and Fey, 2005:11; Oxford, 2013:619).

According to the Soil Classification Working Group (1991:16), A-horizons that have both high clay content and a predominance of smectite clay, have the ability to swell and shrink in response to the moisture content (Fey, 2005:11; University of Oxford, 2013:619; Van der Watt & Van Rooyen, 1995:55). Strongly developed structures (Fey, 2005:11; Meyer,



1984:190; University of Oxford, 2013:619; Van der Watt & Van Rooyen, 1995:55), shiny ped faces, highly plastic consistency when moist and sticky when wet are all characteristics of this soil type. Vertic horizons in South Africa are very dark or black in colour that might be due to the high organic matter, similar to that of Melanic A-horizons (Fey, 2005:11; Soil Classification Working Group, 1991:16). Vertic soils will generally overlay a G-horizon (in wet situations), basic igneous rocks or soil material comprising a very strong structure. This soil type poses most certainly a hazard to buildings (Soil Classification Working Group, 1991:16). In order to ensure that no water accumulates next to structures one must pay special attention to adequate surface drainage. Problems associated with vegetation establishment in soils with high clay content may include drowning of plants and root penetration hindering (Rubenheimer, 2013:23).

### **3.2.10 Geology of Kimberlite tailings from Cullinan**

Kimberlite can be defined as a hybrid, volatile-rich, potassic and ultramafic igneous rock (Skinner & Truswell, 2006:651). Kimberlite originates at depths more than 100 km below the surface of the earth, which suggests that the vent will cut through all the formations above (Frick, 1970:13; Skinner & Truswell, 2006:651). The Cullinan mine was once known as the Premier Mine (Frick, 1970:13). The Premier Kimberlite is situated in Cullinan 30 km north of Pretoria (Bester, 2005:149; Frick, 1970:2; Jakubec, 2004:10; Jorgensen, 1978:36; Scarratt & Shor, 2006:120) on the farm Elandsfontein 480JR (Vaz, 1988:18). This Kimberlite pipe (1180 Ma (Frick, 1970:13)) is one of 12 Group I Kimberlites (Skinner & Truswell, 2006:652) that include the National, Schuller, Montrose and Franspoort pipes as well. Alluvial deposits occur downstream of the Kimberlites (Vaz, 1988:18). The pipe is the largest diamondiferous Kimberlite in South Africa (Frick, 1970:13; Jorgensen, 1978:36; Skinner & Truswell, 2006:651) occupying 32 hectares at surface. It comprises diatreme facies Kimberlite to a depth of 550 metres (Vaz, 1988:18) in the form of an elongated oval shape (Frick, 1970:13). This pipe comprises economical viable ore to a depth of 1500 m (Chadwick, 2012:8). Fenitised (Vaz, 1988:18) quartzites of the Transvaal Sequence are intruded by this pipe (Frick, 1970:13). A gabbro sill (1115 Ma) of 75 m thick at a depth of 350 metres below surface cuts through the Kimberlite pipe (Jorgensen, 1978:58; Vaz, 1988:18).

According to Frick (1970:17) the pipe comprises feldspathized (it is the introduction of feldspar into a rock) quartzite, felsite, granophyre and syenite. The contact is usually striated irregularly and varied in dips over short distances (Frick, 1970:17). The Premier pipe is a complex body with three distinct Kimberlite phases corresponding to three main phases of activity (Frick, 1970:18). A diatreme of “brown” tuffisitic Kimberlite breccia is produced in the south-east during the first phase (Vaz, 1988:18). This breccia (Frick, 1970:14) is characterised by the abundant shale and norite wall-rock inclusions (Vaz, 1988:18). The second phase involves the main part of the pipe and comprises “grey” tuffisitic Kimberlite breccia in the south-east (Vaz, 1988:18). The abundance of Waterberg quartzite, basement granite and gneiss inclusions are characteristic of the “grey” tuffisitic Kimberlite breccia (Skinner & Truswell, 2006:653; Vaz, 1988:18). The third phase consists of the circular plug-like (intrusive) body situated in the western part of the pipe (Skinner & Truswell, 2006:653; Vaz, 1988:18). This phase comprises “black” hypabyssal facies Kimberlite which is characterised by the dark green pseudomorphs after olivine (Vaz, 1988:18). More recent carbonatite dykes, at least six (Frick, 1970:13), radially intrude the black hypabyssal Kimberlite pipe, and massive blocks of “floating reef” comprising quartzite are found in the grey Kimberlite, all situated in the middle of the pipe (Skinner & Truswell, 2006:653; Vaz, 1988:18). The contact between the Kimberlite and the carbonate are sharp and without any indications of metasomatism. The Kimberlite is bleached near the contact with the carbonatite dykes. These carbonatite dykes vary in thickness from 0.5 m to several tens of meters with steep dips ranging from 60° -90° (Frick, 1970:17). One of the main problems associated with Kimberlite is that it will decompose when wet (Jorgensen, 1978:56).

### **3.2.11 Geology and pedology of A - Horizon from Potchefstroom**

Potchefstroom-South is underlined by amygdaloidal lava, agglomerate and tuff of the Rietgat formation, part of the Platberg Group in the Ventersdorp Supergroup as illustrated in Table 5 (Rubenheimer, 2013:23). The Geology is similar to that of Kareerand in section 3.2.1.

**Table 5: Geology of Potchefstroom A - Horizon (Eriksson *et al.*, 2006)**

Ventersdorp Supergroup	Platberg Group	Allanridge Formation (743m)
		Bothaville Formation (427m)
		Rietgat Formation (1319m)
		Makwassie Formation (1968m)
		Goedgenoeg Formation (1777m)
		Kameeldoorns Formation (1798m)

The Rietgat formation comprises alternating volcanic and sedimentary rocks with varying thicknesses and ratios of volcanic to sedimentary material throughout the basin (Burke *et al.*, 1985; Schneiderhan, 2007:67; Van der Westhuizen *et al.*, 2006:196). The Rietgat mainly comprises greenish-grey amygdaloidal and porphyritic lava (Aucamp, 2000:66). The composition of volcanic rocks includes basaltic andesite, andesite, dacite, feldspar porphyry. The area also comprises lavas, pyroclastic rocks and intercalated sediments in the form of greywacke and chemical sediments such as chert and carbonate, as well as stromatolitic calcarenite. Tuffs (air-fallen) are common in the area (Aucamp, 2000:66; Van der Westhuizen *et al.*, 2006:196).

The topsoil can be classified as eolian sand with a grain size smaller than 0.0625 mm, which is transported in suspension by wind (Harmse & Hattingh; 2012:22). The eolian sand deposits took place during the middle Pleistocene Age (Harmse & Hattingh; 2012:30). Fine sand was laid down on the developing escarpment which distinguishes the older Tertiary surface from the lower African Surface (Harmse & Hattingh; 2012:27).

The eolian topsoil (A-Horizon) can be considered apedal. In order to classify a horizon as apedal, the soils must have a uniform colour and should lack well formed pedes (structureless) (Meyer, 1984:190; Soil Classification Working Group, 1991:22; Van der Watt & Van Rooyen, 1995:47). These soils usually develop in weathering conditions of well-drained oxidizing environmental conditions, creating the coatings of iron oxides on individual soil particles (Fey, 2005:13; Fey, 2010:33; Soil Classification Working Group, 1991:22). 1:1 non-swelling types (Soil Classification Working Group, 1991:22) dominate the clay minerals occurring in this horizon.

Comprehensive chemical analyses of the substrates are given in Appendix 1.

### 3.3 Ameliorants used

Amelioration of substrates is deemed necessary because the substrate (sub-soil and mine waste) lacks organic carbon (Strohmayer, 1999:3). This is why phytoremediation poses challenges in vegetation establishment on mine wastes (Tordoff *et al.*, 2000:221). This section will give a discussion of the ameliorants utilised. Table 6 provides a summary of the different ameliorants as applied to the substrates.

**Table 6: Amelioration combinations as applied to the substrates**

Amelioration no.	Description
1	No treatment (Control)
2	Humate (Humin acids) (2 ml added per pot)
3	Fungimax (stimulating soil fungal growth) (1 ml added per pot)
4	Compost (1.5 % of volume =15 ml) added per pot
5	Lime & compost (5 ml lime and 15 ml compost) added per pot
6	Lime (5 ml lime) added per pot
7	Lime, humate, compost (5 ml lime, 2 ml humate, 15 ml compost) added per pot
8	Yellow pea powder (YPP) (5 ml added per pot)
9	Lentil powder (LP) (10 ml added per pot)
10	Lime, humate, fungimax (5 ml lime, 2 ml humate, 1 ml fungimax) added per pot

\* Fertilizer (5 g 2:3:2) was added to each pot

#### 3.3.1 Humates

Humic substances can be defined as stable dark-coloured organic fractions that accumulate as a by-product of decomposition of plant and animal residues added to the soil (Singer & Munns, 1992:446). The decomposing matter is also considered to be aerobic for part of the year (University of Oxford, 2013:285). One can also obtain it via the extraction procedures involving brown coal with alkaline chemicals (Steffen, 2003:19).

Humic substances comprise a heterogeneous mixture of compounds such as humic acid, fluvic acid and humin being made up of molecules of different sizes (Stevenson, 1986:17). As in the case of SOM, high application levels of humic substances can improve a soil's CEC, nutrient-holding capacity, carbon content and water-holding capacity significantly (Omnia

nutriology, 2014:1). Fluvic acids (low-molecular-weight) contain high amounts of oxygen and low levels of carbon (Stevenson, 1986:17).

Humates might affect the soil's chemical, biological and physical properties (Omnia nutriology, 2014:2). Crops are directly affected in a hormone-like activity manner by the addition of this product stimulating the root growth (Van Zyl, 2014:2) resulting in a greater crop yield (Omnia nutriology, 2014:2). K-humate<sup>TM</sup> is considered to be a good complexing agent for certain elements, and in certain binding sites, this is close to chelation (Van Zyl, 2014:2). Because humic substances are not made up of a regular sequence of repeating sub-units, they are mineralized very slowly, but they can be degraded co-metabolically by a variety of soil micro-organisms (De Nobili *et al.*, 2007:724).

Micro-organisms that are able to degrade humic substances to some extent include the bacteria such as *Streptomyces* (free-living saprophytes in these soils with some species colonizing the rhizosphere of plant roots) and *Pseudomonas* (Challis & Hopwood, 2003:14556; Steffen, 2003:25).

### **3.3.2 Fungimax**

Soil contains a variety of organisms such as fungi, bacteria, nematodes insects and earthworms (NRCS, 2001:1). Soils containing a high soil biodiversity are more resistant to diseases (OmniBio, 2014:1). The ratios between fungi and bacteria might differ, because they are specific to soil types and crops (OmniBio, 2014:1).

Fungimax contains nutritious elements that feed the fungi. Fungimax also contains chlorophyll, naturally chelated minerals (readily available for plant uptake), 25 vitamins, proteins, carbohydrates, auxins, cytokinins, amino acids, humates and fluvates. Thus, Fungimax only stimulates the fungi growth and does not contain any micro-organisms itself.

Micro-organisms make soil nutrients more readily available for plants by means of creating a network that transports the nutrients to the plant roots (Omnia, 2014:1). Microbes require

organic material as a food supply (Omnia, 2014:1). Poor management practices in the farming industry deplete the natural organic compounds (naturally cycled in healthy soil) in the soil (Omnia, 2014:1).

Omnia stated that crops will experience less stress if soils are healthy (Omnia, 2014:1). Fungimax was developed to stimulate fungal growth and encourage biological diversity (Omnia, 2014:1). This product spreads through the soil and restores both the water and nutrients for plants that might not have been available otherwise (Omnia, 2014:1).

### 3.3.3 Compost

Compost can be defined as organic residues or a mixture of organic residues and soil that have been piled, wetted, with or without the addition of fertilizer and lime (Sylvia *et al.*, 2005:587), and allowed to undergo thermophilic biological decomposition (Van der Watt & Van Rooyen, 1995:37) until the original organic materials are substantially altered or decomposed (Sylvia *et al.*, 2005:587). Compost can be used as a soil fertilizer or ameliorant (Van der Watt & Van Rooyen, 1995:37). For a detailed description, refer to section 2.2.1.

### 3.3.4 Lime

Lime is known as a calcium oxide (CaO (s)) derived from heating up limestone (CaCO<sub>3</sub>), coral seashells or coral (Hassibi, 1999:1). In South Africa lime is known as CaCO<sub>3</sub> (Van Deventer, 2015: Personal correspondence). The chemical reaction (calcination) is given as:

$$\text{CaCO}_3 \text{ (s)} \xrightarrow{500-600^\circ\text{C}} \text{CaO (s)} + \text{CO}_2 \text{ (g)}$$
 (Hassibi, 1999:2). According to Anderson *et al.* (2013:1), soil acidification is considered as a natural process that is accelerated by crop production practices, primarily the use of nitrogen (N) fertilizers such as urea, ammonium sulphate, or other fertilizers containing ammonium-N. Soils that experience acidic (low pH) conditions result in lower crop production due to the decrease in availability of essential nutrients and increase in toxic elements (Mamo *et al.*, 2009:1; Vossen, 2014:1). Soils can be neutralised through lime application (Mamo *et al.*, 2009:1; Vossen, 2014:1). The best pH conditions for crop production range between 6.0 and 6.8, for these conditions provide the most suitable balance of available nutrients (Mamo *et al.*, 2009:1). Phosphorus and

molybdenum become less available below this pH range (Mamo *et al.*, 2009:1). According to Anderson *et al.* (2013:1), Mamo *et al.* (2009:1) and Murphy (2014:1) some elements such as aluminium and manganese become more toxic in highly acidic soil conditions; for example at pH < 4.5.

### **3.3.5 Yellow pea powder (YPP)**

Yellow Pea Flour V- 6000 (Yellow Pea Powder) is a very fine powder (75 µ) derived from the milling of peas. It is a natural, non-GMO, gluten-free high amylose flour (>35 % amylose) with excellent cooking and pasting properties with excellent heat stability and gel formation. Some uses include the use as base flour in gluten-free baked products, pasta, Asian noodles and breakfast cereals; a thickening agent in ice cream, dairy products, dips and sauces; and as an extender in processed meat. It is also widely used as a prime natural and food safe (low micro, free from heavy metal and mycotoxin) binding agent for composite feeds which can be used in monogastric animals (poultry and pork), pet foods and aquatic feed products (Alliance Grain Traders, 2013:3).

### **3.3.6 Lentil powder**

Lentil powder is similar to the YPP in grain size and manufacturing methods. It is high in protein. It is also utilized in food formulation due to its water absorption and emulsification properties (Alliance Grain Traders, 2013:3).

## **3.4 Seeds**

Species selected during rehabilitation practices should have adaptive traits suitable to survive the disturbed environment (Anawar *et al.*, 2013:739; Bradshaw, 2000:90). Some of these traits include seasonal growth form; life cycle (perennial or annual); seed production; and root development (Muller, 2014:24), salinity (Van den Berg & Kellner, 2005:191) and pH conditions (Tanveer *et al.*, 2013:40). According to Mendez and Maier (2010:279), establishment of native vegetation is beneficial for phyto-stabilization, for it occurs naturally in the dominant climatic conditions experienced (Mendez & Maier, 2010:279).

Coated seed generally comprise a lime coating that eases the planting of seeds via a planter and tractor due to a better shape, heavier weight and smoother surface area. It is therefore recommended that more coated seed be used than natural seed (Scheepers, 2005:82)

The seed used in Experiment 1 is coated seeds comprising a lime coating, insecticide, fungicide, protective polymer, binding polymer, nutrients, growth stimulants and rhizobia inoculant. Both the seedling and soil micro-organisms thus have a reservoir of food due to the additional nutrients available. This product also enhances the seed soil contact resulting in better access to water that is necessary for the germination process and facilitates the nutrient uptake. Weed competition is alleviated due to the uniform establishment of vegetation cover. The handling of seed is improved due to the better shaped, larger seed that is easier to sow. This product allows accurate, easy and uniform placement of the seed (Advanced Seed, 2014:2-8). The same species of grass were utilised in Experiment 2, but was uncoated seed.

*Cynodon dactylon* (L.) Pers. (couch grass/*kweekgras*) is a perennial grass (Hooker, 2009:18) (“a plant whose life cycle extends over more than two growing seasons”) (Cook *et al.*, 2005; Van Oudtshoorn, 2012:225). It is characterised as a short, creeping grass, with both stolons and rhizomes. Rhizomes can penetrate up to 40-50 cm in clay soil and 70-80 cm in sand. The inflorescence (“group or cluster of flowers arranged on a stem” (Cook *et al.*, 2005)) is described as distinctively digitate, with flattened spikelets (2-3 mm) without awns (Cook *et al.*, 2005; Hooker, 2009:15; Van Oudtshoorn, 2012:225). Couch grass is often found in disturbed places (Hooker, 2009:18) due to the remarkable characteristic that it is capable of growing in all types of soil (Cook *et al.*, 2005; Van Oudtshoorn, 2012:225). It serves as good pasture that can endure heavy grazing and stays green until late into winter. Flowering occurs from September until May (Cook *et al.*, 2005; Van Oudtshoorn, 2012:225). Couch grass grows on a wide range of soils but prefers relatively fertile, well-drained soils. It can tolerate pH values from 4.5 to 8.5, yet performs best at pH levels above 5.5. It can tolerate high saline conditions up to 700 mS/m<sup>-1</sup> with a maximum yield, and 1500 mS/m<sup>-1</sup> with only 50 % growth. Although suitable for TDFs due to drought resistance, the suitability of Couch grass is limited due to not being tolerant to aluminium saturation. These characteristics as well as the root system contribute to the motivation of its being utilized in rehabilitation practices. The ideal temperatures are with mean daily temperatures above 24°C or over an optimal range of 17-



35 °C. Couch grass grows slowly at 15°C and becomes dormant when night temperatures fall below 0°C, or the average daytime temperature below 10°C. It is also not shade-resistant, but will withstand fires due to the extensive rhizome development. It is possible that some varieties can produce high levels of hydrocyanic acid (HCN), especially when high levels of nitrogen are applied, yet no severe cattle effects have been documented (Cook *et al.*, 2005; Hooker, 2009:18; Van Oudtshoorn, 2012:225).

*Chloris gayana* Kunth (Rhodes grass/*Rhodesgras*) is a summer-growing perennial grass (Cook *et al.*, 2005; Pannar, 2009:17; Van Oudtshoorn, 2012:223). It is characterised as a tufted grass, which spreads by means of stolons. The inflorescence is described as being digitate with loose fingers that typically curl when the spikelets have fallen off. The spikelets (2.5 mm long) are brown in colour and have two awns (Hooker, 2009:15). Leaf sheaths are compressed (Van Oudtshoorn, 2012:223). The leaf blade is smooth and folded open with fine tapering to a point. Rhodes grass is easy to establish and serve as worthy grazing in permanent pastures. This species is already highly nutritional in the initial stages of the season (Cook *et al.*, 2005). Flowering occurs from November until May (Van Oudtshoorn, 2012:223) and can grow in areas with a rainfall of 400 - 1000 mm / annum (Pannar, 2009:17). Roots can go down to 4.7 m deep; however, further than 2.4m is scarce. It can tolerate intense grazing conditions and high soil Na levels (conductivity >10 dS/m<sup>-1</sup>). It is fairly capable of enduring adverse pH and salinity conditions, however tolerates high pH values better than acidic conditions. pH levels ranging between 5.5 and 7.5 are favoured, yet grass will grow up to pH 10 and down to 4.5. Sodium levels as HCO<sub>3</sub><sup>-</sup> and SO<sub>4</sub><sup>-</sup> are better withstood than those as Cl<sup>-</sup> or NO<sub>3</sub><sup>-</sup>. High Li<sup>+</sup> can be tolerated, but not high soil magnesium (Mg<sup>+2</sup>) and manganese (Mn<sup>2+</sup>) (Cook *et al.*, 2005). It is consequently utilized in rehabilitation practices to stabilize exposed and disturbed soils (Pannar, 2009:17). It can also be used to restore soil structure, improve organic matter levels, and reduce nematode numbers. Rhodes grass grows on most well-drained soils if the fertility is adequate. It prefers well-structured loams and clays of volcanic origin, not including very heavy clays. It poorly tolerates shade and recovers well after fires, which makes it ideal for TSF's rehabilitation (Cook *et al.*, 2005; Pannar, 2009:17; Van Oudtshoorn, 2012:223).

Both the grass species utilised in the trials are indigenous, tolerant to most harsh pH and salinity conditions and adapted to climates experienced, thus complying with the legislation

regarding species selection to achieve rehabilitation objectives. The most important mine closure objective regarding rehabilitation is to achieve surface stability (Fourie, 2007:483; Morgan, 1986:61). Other detrimental factors such as surface runoff, dust pollution, erosion and crust formation are indirectly addressed through surface stabilization. Vegetation establishment also aids in enhancing the aesthetic value of the landscape, provides habitat to other biota as well as reducing the impact of rainfall on the soil surface (Fourie, 2007:483; Hossner & Sahandeh, 2006:154; Lange *et al.*, 2012:908; Tordoff *et al.*, 2000:220). Grasses are excellent for rehabilitation purposes due to favourable characteristics such as rapid growth, large biomass, strong resistance (Xia, 2002:345), and effective stabilization of soils (Fourie, 2007:483; Morgan, 1986:61). It is also preferred for temporary soil stabilization because of rapid and effective ground cover compared to shrubs and trees (Mendez & Maier, 2008:280; Xia, 2004:345). Improved aggregation and cohesion of soil are also a beneficial effect of dense grass stands (Koontz *et al.*, 2013:2237). Successful restoration of degraded land in semi-arid climates is significantly reliant on the plant growth (Parraga-Aguado *et al.*, 2014:135).

According to Mendez and Maier (2010:280), the most practised seeding method for rehabilitation purposes is direct seeding. This method is preferred due to the increment of the frequency, density and establishment of species attained as well as the low labour costs associated. Direct seeding using agricultural seed mixtures combined with amelioration is considered as an economical re-vegetation method (Tordoff *et al.*, 2000:219). Inorganic fertilizers and organic matter such as sewage sludge, compost or mulch are ameliorants used to alleviate nutrient deficiencies of mine waste materials (Brady & Weil, 2008:515; Cooperband, 2002:2; Chan, 2008:2; Fenton *et al.*, 2008:1; Marcel Dekker Inc., 1985:3; Singer & Munns, 1992:3; Tordoff *et al.*, 2000:216; Van Deventer & Ferreira, 2012:21).

## 4. Methods

Substrates were collected from various localities as indicated in Table 1 and are visually illustrated in Figure 1. These substrates that differ in both the chemical and physical properties were used in the experiments at the NWU nursery under controlled conditions with respect to water application. Physical crusting that might influence germination were eliminated due to the regular irrigation of samples. Amelioration was completed and planting commenced in 1 L pots. Ten seeds (5 *Cynodon dactylon* seeds (Couch grass) and 5 *Chloris gayana* seeds (Rhodes grass)) were sowed in each pot. Germination percentages and chemical analyses were subsequently determined and analysed.

Three different experimental pot-trials were repeated for each of the 15 different growth mediums. Each of the three experiments is discussed, followed by an overview of the various analytical and statistical methods employed for data acquisition and processing. The initial experiment was developed to illustrate the effect of different ameliorants on germination of coated grass as applied to the substrates. Five grams of 2:3:2 (32) fertilizer were added to each pot. The reason for making use of this fertilizer is that it was immediately available and soil corrections to 15 different substrates would not be economical. Making use of only one standard treatment ensures uniformity in the experiment. It was realised that the C/N ratio of the ameliorants was difficult to manipulate. A second experiment was then proposed in order to manipulate both the C and the N percentages to obtain the specific ratios required. During the second experiment some substrates experienced hindered growth even though high germination was obtained. The third experiment, evaluating growth potential, was incorporated to assess the suitability of grass in tailings media. Uncoated seed was utilised in both experiments 2 and 3.

### 4.1 Experiment 1: Effect of substrates and ameliorants on germination

The objective was to identify the most suitable method to ameliorate the chosen substrates to potentially be used as topsoil as mentioned previously in section 1.4.

#### 4.1.1 Sampling method

Substrate samples were collected prior to sowing as both individual and composite samples. The trial's extent is illustrated in Photo 3. Sampling of substrates is illustrated in Photo 4. The sample bags are clearly visible in this photo.

In the case of composite samples, homogeneous, uniform and representative samples were obtained and analysed. The analysed composite sample represents all four replicas created per ameliorant and substrate group. Composite sampling is also considered more economic in comparison to individual sampling. Soil samples were air-dried, split and mixed by means of a riffler and followed up with sieving to obtain particle sizes of less than 2 mm ( $< 2000 \mu\text{m}$ ).

Individual sampling was compared to composite sampling to ensure that samples were statistically equivalent as indicated in Table 7. It was found that the standard deviation was not significant except for the  $\text{SO}_4$  readings that are not applicable to this study. The composite sampling is therefore considered as an equivalent to the individual sampling.

**Photo 3:** Illustration of the growth of Couch and Rhodes grass grown on various substrates with 10 different amelioration combinations, all with diverse C/N ratios





**Photo 4: Illustration of the extent and soil sampling process of Experiment 1 for standard geochemical analysis at the nursery with optimal growth conditions**



**Table 7: Comparison of soil composite samples to average of individual samples and standard deviation between the two methods**

Sample Name	pH (KCl)			EC (mS/m <sup>-1</sup> )			NO <sub>2</sub> (mg/l)			F (mg/l)		
	COMP	AVE	STDE	COMP	AVE	STDE	COMP	AVE	STDE	COMP	AVE	STDE
Red structured B with 2 % volume based LP	6,17	6,37	0,04	1,02	1,41	0,11	12,24	31,75	10,48	0,01	0,02	0,01
Diabase saprolite with 3 % volume based LP	6,01	6,02	0,11	0,53	0,53	0,02	0,02	0,06	0,01	0,10	0,08	0,01
Hard carbonate saprolite	8,14	8,17	0,01	0,96	0,89	0,10	0,33	0,24	0,02	0,15	0,09	0,02
Kalahari sand with compost	7,08	7,27	0,08	0,74	0,69	0,08	0,17	0,15	0,04	0,15	0,27	0,05
Kaolinite clay with lime, humate, fungimax	6,66	6,84	0,05	0,60	0,69	0,04	0,22	0,17	0,01	3,28	2,38	0,16
Hard carbonate saprolite with lime, humate, fungimax	8,25	8,37	0,04	0,61	0,68	0,05	3,21	2,81	0,32	0,80	0,82	0,09
Kalahari sand with 2 % volume based YPP	6,37	6,69	0,12	2,51	2,33	0,10	0,33	0,25	0,09	0,01	0,02	0,01
Granite saprolite	7,45	7,46	0,01	0,78	0,75	0,05	0,19	0,28	0,03	0,30	0,38	0,04
Platinum tailings	7,89	7,87	0,03	1,14	1,27	0,07	0,26	2,01	0,99	0,13	0,08	0,02
Platinum tailings with 4 % volume based LP	6,86	7,14	0,19	1,76	1,63	0,22	12,61	16,92	8,88	0,14	0,10	0,02

Sample name	PO <sub>4</sub> (mg/l)			SO <sub>4</sub> (mg/l)			NO <sub>3</sub> (mg/l)			NH <sub>4</sub> (mg/l)		
	COMP	AVE	STDE	COMP	AVE	STDE	COMP	AVE	STDE	COMP	AVE	STDE
Red structured B with 2 % volume based LP	0,06	0,01	0,00	106,28	123,48	8,35	330,63	517,36	72,31	0,61	0,79	0,20
Diabase saprolite with 3 % volume based LP	1,70	1,42	0,12	115,04	116,40	9,88	0,19	0,19	0,03	0,02	0,02	0,00
Hard carbonate saprolite	0,01	0,05	0,03	244,62	208,47	24,37	0,26	0,39	0,19	0,76	0,55	0,13
Kalahari sand with compost	0,61	0,41	0,19	158,21	136,52	34,49	0,42	0,22	0,04	0,07	0,13	0,10
Kaolinite clay with lime, humate, fungimax	42,12	24,30	6,81	169,52	209,05	35,74	0,71	0,32	0,04	0,45	0,28	0,03
Hard carbonate saprolite with lime, humate, fungimax	17,68	24,37	1,00	164,15	170,79	21,88	8,74	3,35	1,30	0,76	0,50	0,06
Kalahari sand with 2 % volume-based YPP	0,12	3,20	2,42	1634,75	1353,88	104,29	7,03	4,38	0,36	2,29	2,45	0,36
Granite saprolite	0,00	0,01	0,00	181,03	182,06	26,11	0,38	3,89	3,30	0,22	0,28	0,02
Platinum tailings	0,08	0,12	0,03	102,36	164,25	30,34	1,01	3,21	1,77	0,16	0,25	0,01
Platinum tailings with 4 % volume-based LP	4,94	4,88	1,86	194,60	189,08	25,56	381,92	283,05	219,91	0,83	1,13	0,53

Sample name	Cl (mg/l)			HCO <sub>3</sub> (mg/l)		
	COMP	AVE	STDE	COMP	AVE	STDE
B red structured with 2 % volume-based LP	59,95	70,53	6,24	0,50	0,93	0,15
Diabase saprolite with 3 % volume-based LP	25,29	24,43	4,22			
Hard carbonate saprolite	145,45	137,82	12,91			
Kalahari sand with compost	84,31	75,97	12,09			
Kaolinite clay with lime, humate, fungimax	60,53	68,79	4,07	1,65	1,49	0,01
Hard carbonate saprolite with lime, humate, fungimax	60,94	80,09	9,56			
Kalahari sand with 2 % volume-based YPP	44,78	57,68	4,21	2,00	2,34	0,14
Granite saprolite	93,87	86,68	11,25	2,65	2,60	0,04
Platinum tailings	87,13	91,00	3,81			
Platinum tailings with 4 % volume based LP	15,14	22,42	4,59	6,50	6,23	0,13

#### 4.1.2 Treatments

Mean calculations are estimates for the true values of the whole population of numbers. The greater the variability of the population, the more samples are required to achieve the same precision (Van Emden, 2008:40). Replicates are thus necessary to ensure that the whole population of samples are accurately represented. Four replicates were deemed acceptable for each treatment and substrate type. The International Seed Testing Association (ISTA, 2009) also requires a minimum number of three replicates for each seed type during an experiment.

- All 13 substrates (MF- 5B to MF- 11 in Table 1) x 10 amelioration combinations (Table 6) x 4 replicas = 520
- Total 520 + 4 samples for substrates 9 and TC respectively (8)
- Total 528 Samples

#### 4.1.3 Analytical methods

A description of the standard analytical methods used for characterisation of the substrates, conducted by Geolab, Eco Analytica and the Soil Science Division of the Geology – Soil Science Subject Group at the North-West University follows below:

The analyses listed below were all conducted prior to seeding. Germination percentage determinations were subsequently completed.

- Preliminary Trace elements (for background informative purposes): Total and soluble (specified in Appendix 1).
- Nutrient status on composite sample (pH (H<sub>2</sub>O & KCl), EC (saturated extract), Exchangeable cations, P<sup>4+</sup> and Nitrates) (Table 15).
- Standard geochemistry (Anions (F<sup>-</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>2-</sup>, HCO<sub>3</sub><sup>-</sup> SO<sub>4</sub>, and PO<sub>4</sub>) & Cations (NH<sub>4</sub><sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and Na<sup>2+</sup>), CEC, Texture) (Table 7, Table 8, Table 15 and Table 16).
- Fertiliser requirement determination.
- Total carbon and nitrogen status and ratio (Table 19).

- Dehydrogenase activity on selected substrates (Table 19).
- Seeding with coated grass species i.e. *Cynodon dactylon* (Couch grass) and *Chloris gayana* (Rhodes grass).
- Germination assessment and total germination % determination.

#### **4.1.3.1 Total digested metals (US EPA 3050b method {“totals”}) and soluble (ammonium nitrate) (DIN 1930 method)**

Total metallic element concentrations were extracted using the US EPA Method 3050b (acid digestion) and measured via inductively coupled plasma mass spectrometry (ICP-MS). Soluble metallic element concentrations were extracted using the DIN 1930 method (ammonium nitrate solution) and measured via ICP-MS (Non-Affiliated Soil Analysis Work Committee, 1990:15).

#### **4.1.3.2 Nutrient status**

Nutrient status includes the pH, EC, exchangeable cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ), phosphate and nitrates.

##### **pH**

$\text{H}_2\text{O}$

This method determines the pH of a soil in a saturated paste. Adsorbed  $\text{H}^+$  ions will be displaced from the charge sites in soil due to the possible presence of soluble cations that have a greater affinity for adsorption which will result in lower pH values. The presence of  $\text{CO}_2$  will decrease the pH of calcareous soils, thus care must be taken to exclude the  $\text{CO}_2$  (Non-Affiliated Soil Analysis Work Committee, 1990:3).

KCl

This method indicates the hydrogen ions activity within the soil suspension in  $1 \text{ mol dm}^{-3}$  KCl (saturated paste). The variation in salt concentration is masked by potassium chloride that originates from fertilizer residues, irrigation water and microbial decomposition of organic material. The hydrogen activity in  $1 \text{ mol dm}^{-3}$  can vary as much as 2 pH units higher or lower



than that measured in water, using the same soil solution ratio. Bulk density must be taken into account to prevent errors with regards to mass basis and not scooped quantities (Non-Affiliated Soil Analysis Work Committee, 1990:2).

### **Electrical conductivity of soil paste (EC)**

Electrical conductivity and soluble anions ( $\text{SO}_4$  - S,  $\text{NO}_3$  - N, and Cl) were determined from the extract of a saturated soil paste.

EC of the saturation extract indicates the total dissolved salts in the extract and thus the soluble salts in the soil. The EC values are used to classify any salt hazard of brackish soils, as found in the Karoo areas, and to estimate the leaching requirements of the soils for reclamation purposes. These values can be used to predict crop yield reduction as a result of high salt concentrations (Non-Affiliated Soil Analysis Work Committee, 1990:4).

Brackish soil (with regard to the reaction between the sodium and calcium ratio (SAR)) are characterised by the sodium adsorption ratio value which in return will determine whether a high sodium content is likely to be physically detrimental to a soil (Non-Affiliated Soil Analysis Work Committee, 1990:4).

The properties of a saturated paste are that the soil pores are filled with water and its characteristics are a shiny surface where the paste flows slightly when the container is tilted, no free water collects in a small drawn trench in the surface and thirdly does not cling to the spatula except for clayey soils (Non-Affiliated Soil Analysis Work Committee, 1990:4).

### **Exchangeable cations**

Exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$  and  $\text{Na}^{2+}$  were determined by atomic absorption after extraction in 1 M ammonium acetate, using a soil to solution ratio of 1 gram to 10 ml. This method is widely used in agriculture for fertilizer recommendations. The ammonium-ion replaces the cations that are situated on different exchangeable positions. The exchanged cation concentrations are subsequently measured by means of atomic absorption

spectrophotometry (AAS). Exchangeable cations represent the nutrient status of the soil. However, this method does not provide accurate results with respect to the exchangeable cation status. The amount of extractable potassium can be increased during the drying of the sample. However, the sample can be extracted while the soil is still moist. The tempo of extraction is a function of temperature. Extraction must therefore take place at 20 °C (including the extraction solution) (Non-Affiliated Soil Analysis Work Committee, 1990:12).

### **Plant available phosphorus:**

Plant available P ( $P^{+4}$ ) was extracted using the Bray 1 solution ( $0.025 \text{ mol L}^{-1} \text{ HCl} + 0.03 \text{ mol L}^{-1} \text{ NH}_4\text{F}$ ) using a soil to solution ratio of 1:7.5. A known mass of soil is shaken manually with the Bray-1-solution. The contact time between the extractant and soil should not exceed 60 seconds. This procedure extracts the more soluble phosphorus and is of most significance when analysing cultivated soils. The total inorganic phosphates which are in the extracts are determined by automated colorimetric analysis by first converting condensed phosphates present to orthophosphate by means of hydrolysis with sulphuric acid at 90°. The total phosphate concentration is subsequently determined by the reduction of phosphomolybdic acid to yield an intense blue colour, suitable for photometric determination at 660 nm. The reduction agent in this case is 1-amino-2-naphthol-4-sulfonic acid (Non-Affiliated Soil Analysis Work Committee, 1990:20).

### **Nitrogen**

Nitrogen ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$ ) are determined by means of IC (ion chromatography). Ion chromatography determines the ionic solutes, such as inorganic anions, cations, transition metals, and low molecular-weight organic acids and bases. Soil is dried and made into slurry with ten times the mass of the extracting solution. Extraction and dissolution will commence prior to IC (Jackson, 2000:2779-2796).

#### **4.1.3.3      *Standard geochemical***

##### **Cations**

Exchangeable cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$  and  $\text{Na}^{2+}$ ) are determined with 1M  $\text{NH}_4$ -Asetate solution (with a pH of 7) and measured by means of AAS. Extractable and exchangeable micro-nutrients are determined with 0.02 M  $(\text{NH}_4)_2 \text{EDTA} \cdot \text{H}_2\text{O}$  solution and measured by means of AAS (Non-Affiliated Soil Analysis Work Committee, 1990:8).

##### **Anions**

Anions ( $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{HCO}_3^-$ ,  $\text{SO}_4$ , and  $\text{PO}_4$ ) are determined by means of Ion Chromatography (IC). Bicarbonate  $\text{HCO}_3^-$  is determined by means of titration.

##### **Cation exchange capacity and exchangeable plus water soluble cations: ammonium acetate**

Colloidal particles such as clay generally have a negative charge that is neutralised by the adsorption of cations on the particle surface. CEC refer to the capacity of clay to adsorb cations (Foth, 1990:171; Non-Affiliated Soil Analysis Work Committee, 1990:12; Van Deventer & Hattingh, 2014:62; Weil & Magdoff, 2004:2). Cation exchange capacity (CEC) was determined by saturating the soil with Na and the displaced Na concentration (by ammonium acetate) was then determined by ASS (Non-Affiliated Soil Analysis Work Committee, 1990:12).

The ammonium acetate solution ( $0.2 \text{ mol dm}^{-3}$ ) is used as the extracting agent for exchangeable plus water-soluble cations. The most questionable cations extracted with this method are  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the presence of free lime and gypsum. If accurate results for exchangeable  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  or CEC are required this method should not be used in the presence of free lime and gypsum. An increase of the extractable level of  $\text{K}^+$  may also occur if the soil is dried. Nevertheless, soil samples can be extracted in a moist state. Soils that contain a significant amount of soluble salts (resistance  $<460 \text{ ohms}$ ) have the necessity of determining the soluble water cations separately. In order to obtain the exchangeable cations these soluble salts are subtracted from the extractable cations. After the exchange complex has been saturated with the index cation, the adsorbed cation and the small amount of

solution entrained by the soil after leaching can be directly displaced by another salt solution such as  $K^+$  and sulphate. Ammonia is used equal to the CEC of the soil and is separated by means of the Kjeldahl instrument (Wagner-Parnas) (Non-Affiliated Soil Analysis Work Committee, 1990:12).

### Texture (particle size distribution)

The particle size distribution (PSD) of coarse and medium-sized sand particles was determined by the standard sieving method that included the following sieve sizes: 2000, 1000, 500, 250, 100 & 53  $\mu m$  (Non-Affiliated Soil Analysis Work Committee, 1990:35). Fine sand, silt and clay particles were determined according to the standard hydrometer method as mentioned in the standard soil analysis handbook (Non-Affiliated Soil Analysis Work Committee, 1990:35). The results obtained from the analysis of the substrates used are tabulated in Table 8. For texture determination one can apply Figure 5.

**Table 8: Particle size distribution of substrates**

Particle Size Distribution										
Sample No (Figure 1):	Sample no.	> 2 mm	Very coarse sand	Coarse sand	Medium Sand	Fine sand	Very fine sand	Silt	Clay	Texture class
MF-1B	Red Structured B	1.3	2.9	4.2	9.4	17.8	14.5	28.5	22.7	Sandy Clay Loam
MF-1C	Acid Lava Saprolite	3.9	28.1	29.2	20.7	11.2	4.2	4.5	2.2	Sand
MF-2B	Potchefstroom Red Structured B	3.7	3.3	3.2	10.2	19.6	8.9	26.0	28.7	Sandy Clay Loam
MF-2C	Diabase Saprolite	4.1	33.9	28.9	16.4	10.0	3.9	4.6	2.2	Sand
MF-3F	Kimberlite Fine Tailings	1.1	5.1	29.5	29.9	21.7	7.6	4.2	2.0	Sand
MF-3G	Kimberlite Coarse Tailings	4.3	30.3	22.8	17.4	12.2	6.3	9.0	2.2	Sand
MF-4	Hard Carbonate Saprolite	13.2	17.7	12.5	13.7	21.7	14.7	12.4	7.3	Loamy Sand
MF-5B	Kalahari Sand	0.8	24.3	27.3	19.7	14.5	5.6	6.5	2.1	Sand
MF-5C	Granite Saprolite	10.4	34.3	19.3	13.9	13.4	9.5	7.3	2.3	Sand
MF-6	Ottosdal Kaolinite Clay	0.5	1.0	1.5	5.5	15.9	14.7	44.0	17.5	Loam
MF-7	Platinum Tailings	0.1	0.1	0.5	10.3	49.0	29.1	8.9	2.1	Sand
MF-8	Gold Tailings	0.2	0.3	0.9	3.7	35.1	30.4	24.7	4.8	Sandy Loam
MF-9-BK	Vertisol	1.8	3.1	4.3	9.8	11.7	13.9	21.1	36.1	Clay Loam
MF-11	Cullinan - Kimberlite Tailings	13.6	31.9	22.7	17.9	12.4	5.1	7.5	2.4	Sand
MF-TC-BK	A - Horizon	0.9	5.3	10.4	17.7	23.9	20.9	9.7	12.1	Loamy Sand

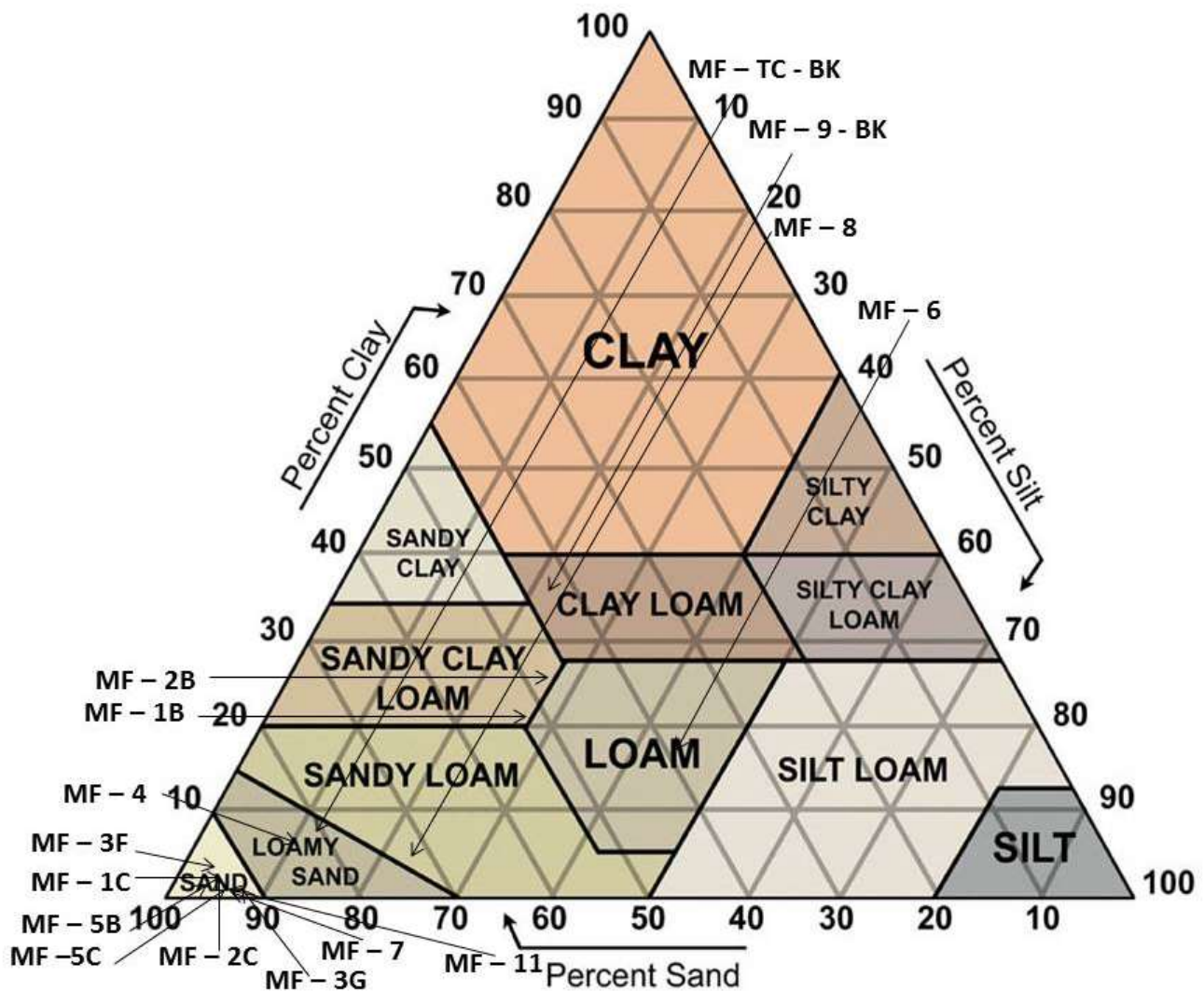


Figure 5: Texture class triangle (Soil Sensor, 2011) with the substrates plotted.

#### 4.1.3.4 Lime requirements

The net acid potential analyses done by GeoLab (Grond- en Omgewingslaboratorium) include the drying, sieving (< 2 mm) and analyses of soil and tailings according to standard methods stipulated by The Non-Affiliated Soil Analysis Work Committee (1990). Lime requirements of the tailings were done by a method developed by GeoLab and consist of two components:

- Active acidity (titrate-able acidity) gives the lime requirement to raise soil pH (KCl) to neutral and
- Latent acidity associated with acidity caused by future pyrite oxidation.

The procedure for latent acidity was followed which is described by Usher *et al.* (2003). In the case of short-term planting such as annual crops, the only lime requirement necessary is titrate-able acidity. However, the general pH of the various substrates values was acceptable when it was used for this study as it was previously amended for latent acidity (by Dries Bloem from GeoLab).

#### **4.1.3.5      *Germination***

The first germination percentage was determined after one month of sowing on 16 March 2014. This was done by counting the emerging seedlings which were then converted to a percentage. The second determination took place three weeks after the first determination. The prolonged time span prior the first determination, as well as the second determination, was due to the lowered temperatures delaying the germination (SA Explorer, 2011) as well as allowing plants to achieve the three-leaf stage. Only the total mean values of the first and second germination determination was used in the statistical analysis as mentioned in section 5. Irrigation was applied three times a week to minimise stress factors.

#### **4.1.3.6      *Dehydrogenase Activity (INT)***

The dehydrogenase activity represents the soil microbial activity which is defined as the overall metabolic activity of all the microorganisms (bacteria, fungi, actinomycetes, protozoa, algae) and micro-fauna that occupy the soil (Casida, 1977; Nannipieri *et al.*, 1990:335; Subhani *et al.*, 2001:333).

For the purpose of this dissertation, dehydrogenase activity was determined according to the INT-method developed by Von Mersi and Schinner in 1991. This method involves the incubation of soil with the substrate idonitrotetrazolium chloride (INT) at 40 °C for 2 h followed by colorimetric estimation of the reaction product idonitrotetrazolium chloride-formazan (INF) (Von Mersi & Schinner, 1991:217). The standard INF solution (stored at 4°C) consists of 0.01 g INF dissolved in 80 ml extractant which was brought up to 100 ml with the same extractant in a volumetric flask (Von Mersi & Schinner, 1991:217).

#### **4.1.3.7      *Carbon and nitrogen***

##### **Total carbon and nitrogen - Leco**

A variety of organic materials such as foods, oilseeds, fertilizers, meats and soils are analysed for the nitrogen, carbon/nitrogen, or carbon/hydrogen/nitrogen by means of a TruSpec CN instrument (refer to Figure 6) (LECO, 2008:2). This system is based on the Dumas method of combustion and can provide the information of all the elements within four minutes (LECO, 2008:2). This instrument is connected to a PC that uses a Windows-based programme that controls the system operation and data management (LECO, 2008:2).

The analysis cycle comprises the purging, combustion and analytical phases (LECO, 2008:2). The sample is placed in the loading head, sealed and any atmospheric gases that have entered whilst sample-loading are removed during the purge phase (LECO, 2008:2). The gas lines and ballast volume (zero volume at this point) are also purged (LECO, 2008:2). The samples are subsequently dropped into a hot primary furnace at 950 °C and flushed with oxygen for express and complete combustion (LECO, 2008:2). The combusted products are then placed into an afterburner (secondary furnace) at 850°C for further oxidation and particle removal (LECO, 2008:2). Moisture is removed by an additional furnace filter as well as the two-stage thermoelectric cooler. Combustion gases are collected afterwards in the ballast (collection vessel) (LECO, 2008:2). Oxygen is injected into the ballast and mixed with the combustion gases during the analysing phase (LECO, 2008:2). The combustion gases are now considered to be homogeneous in the ballast due to passive mixing, which are successively purged through the CO<sub>2</sub> infrared detector and the 3 cc aliquot loop (LECO, 2008:2). As soon as the gases are equilibrated, carbon is measured as CO<sub>2</sub> by the appropriate carbon dioxide detector (LECO, 2008:2). The gases in the aliquot loop are transferred to the helium carrier flow where it is swept through hot copper to remove the oxygen and change the NO<sub>x</sub> to NO<sub>2</sub> (LECO, 2008:2). The carbon dioxide and water are removed as they flow through the LecoSorb and Anhydrone. The nitrogen content is measured by a thermal conductivity cell. The final result is then displayed as either weight percentage (wt. %) or parts per million (ppm) (LECO, 2008:2). In order to ensure a total analysis time of less than four minutes an optimised detector is used for each element.

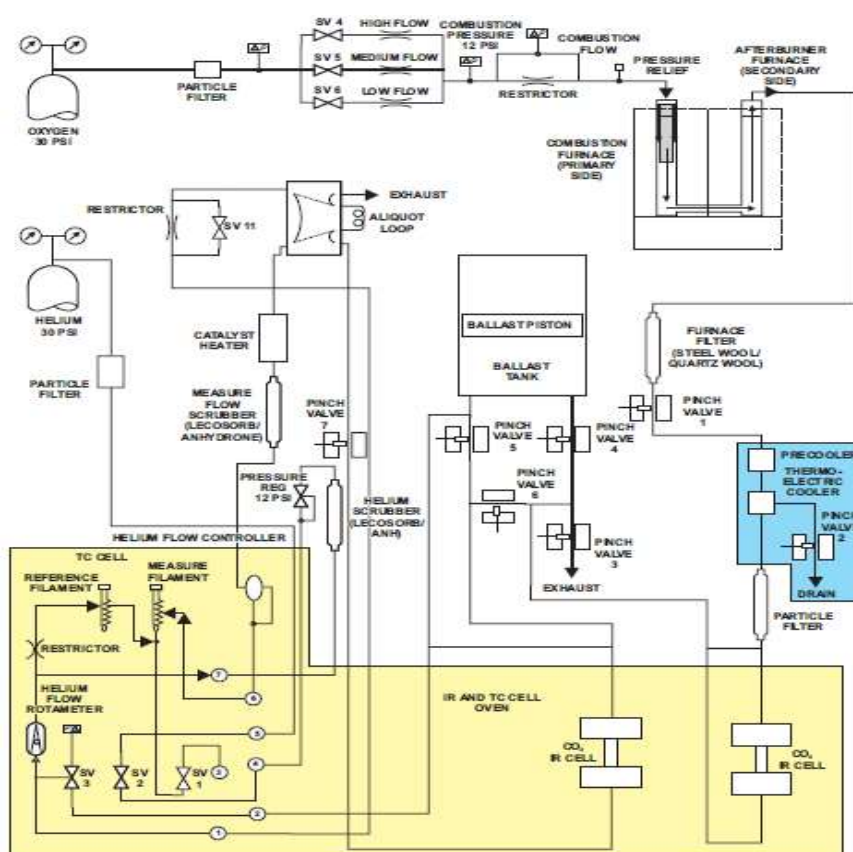


Figure 6: Illustration of the TruSpec CN instrument (LECO, 2008:2).

## C/N ratio

The nitrogen was below detectable limits (0.01 % accuracy) for the Leco analyser. The method was then adjusted by measuring the macro-elements for the total  $\text{NO}_2$ ,  $\text{NO}_3$  and  $\text{NH}_4$  (mg/L) to determine the **total C/soluble organic N**.

The total nitrogen was determined by the following equations:

<b><math>\text{NO}_2</math> (mg/L equiv. N):</b>	$\frac{\text{Value measured (mg/L)} \times 14.00672 \text{ (Atomic mass of N)}}{46.00558 \text{ (Molecular weight of } \text{NO}_2\text{)}}$
<b><math>\text{NO}_3</math> (mg/L equiv. N):</b>	$\frac{\text{Value measured (mg/L)} \times 14.00672 \text{ (Atomic mass of N)}}{62.0049 \text{ (Molecular weight of } \text{NO}_3\text{)}}$
<b><math>\text{NH}_4</math> (mg/L equiv. N):</b>	$\frac{\text{Value measured (mg/L)} \times 14.00672 \text{ (Atomic mass of N)}}{18.03846 \text{ (Molecular weight of } \text{NH}_4\text{)}}$
<b>Total N:</b>	<b><math>\text{NO}_2 + \text{NO}_3 + \text{NH}_4</math></b>



The equations can be explained by the following example (Mc Intoch, 2012:7):

$$\text{MCL} = 45 \text{ mg/L nitrate (NO}_3\text{)}$$

Calculate MCL as NO<sub>3</sub>-N:

$$45 \frac{\text{mg NO}_3}{\text{L}} \times \frac{1 \text{ mole NO}_3}{62 \text{ g}} \times \frac{1 \text{ mole N}}{1 \text{ mole NO}_3} \times \frac{14 \text{ g}}{1 \text{ mole N}} = 10 \frac{\text{mg}}{\text{L}} \text{ N}$$

$$= 10 \text{ mg/L nitrate as nitrogen (NO}_3\text{-N)}$$

**The C/N ratio was subsequently determined as follows:**

Due to the volatile properties displayed by the nitrogen during the preparation and oven drying processes, N was not accurately measured and was therefore subsequently determined as anions. The results of C (%) and N (mg/L<sup>-1</sup>) was not in the same units and required therefore conversions during Experiment 1. **This gave rise to Experiment 2. The C/N of Experiment 2 was only determined by means of the leco method. This is required to achieve uniformity in the data.**

$\text{mg.L}^{-1} = \text{mg.kg}^{-1}$	$\text{mg/L} = \text{ppm}$
----------------------------------------	----------------------------

<b>Convert C % m/m to C mg/L (ppm):</b>	$\% \text{ m/m} \times 10\,000$ $(A \% \text{ m/m} \times 10\,000 = A \text{ ppm})$
-----------------------------------------	----------------------------------------------------------------------------------------

**OR**

<b>Convert N ppm (mg/L) to % m/m:</b>	$\text{N ppm} / 10\,000$ $(A \text{ ppm} / 10\,000 = A \%)$
---------------------------------------	----------------------------------------------------------------

**C/N ratio:**

$\text{C mg.L}^{-1} / \text{N mg.L}^{-1} = \text{C/N (no units)}$
-------------------------------------------------------------------

**OR**

$\text{C \% m/m} / \text{N \% m/m} = \text{C/N (no units)}$
-------------------------------------------------------------

## 4.2 Experiment 2: Influence of C/N ratio applied and substrate on grass germination

As mentioned previously in section 1.4, the objective was to distinguish between the most suitable C/N ratio and status of various substrates. The extent of Experiment 2 is illustrated in Photo 5.



Photo 5: Illustration of Experiment 2

### 4.2.1 Treatments

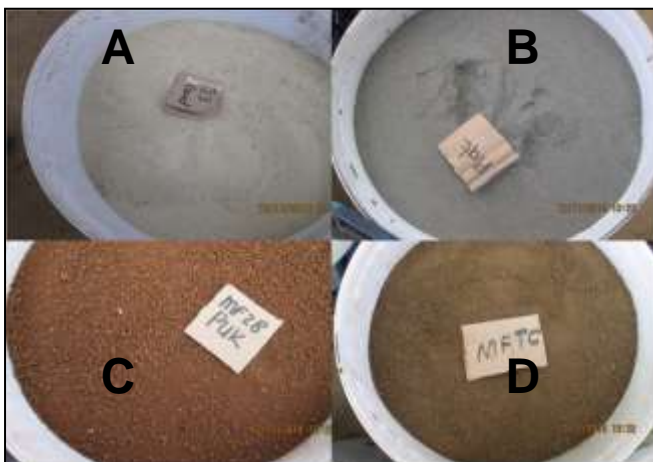
#### *Substrates*

- Only four substrates were used in the second experiment and are illustrated in Photo 6.
  - Potchefstroom (North-West University) Red Structured B (MF-2B) [MF2B in Photo 6]

- Potchefstroom A - Horizon (MF-TC-BK) [MFTC in Photo 6]
- Rustenburg (Paardekraal) - platinum tailings (MF-7) [Plat in Photo 6]
- Stilfontein (Chemwes Mine) - gold tailings (MF-8) [T23 in Photo 6]

***The C/N ratios applied was***

- Blank control
  - Humates [as illustrated in Photo 7] (High C content), 20 L/ha as highest dose recommended in combination with N as LAN to get C/N ratios of (Omnia Nutriology, 2013:6)
    - 12,5/1 C/N ratio
    - 25/1 C/N ratio
    - 50/1 C/N ratio
- Replicas were 7 of each.
- 4 Substrates x 4 C/N ratios (blank control + 3 C/N ratios ) x 7 Replicas = 112 pots



**Photo 6: Substrates used in Experiment 2.**

A = Gold tailings;

B = Platinum tailings

C = Red Structured B soil

D = A – Horizon



**Photo 7: Illustration of humates mixture used in Experiment 2**

#### **4.2.2 Analytical methods**

The following substrate and germination analysis were done:

- Total carbon and nitrogen status and ratio.
- Seeding with two grass species i.e. *Cynodon dactylon* (Couch grass) and *Chloris gayana* (Rhodes grass).
- Germination assessment and total germination % determination.

*All the analyses for the purposes of this dissertation were done by Geolab and Eco-Analytica.*

##### **4.2.2.1 Carbon and nitrogen**

- Total carbon and nitrogen status (BemLab Leco method) and C/N ratio calculated from results.

Due to the challenges encountered in Experiment 1 as explained in section 4.1.3.7, Experiment 2 was suggested. For this experiment we amended the C/N ratio of the substrates to achieve adequate germination. Only the humates could be utilized, for it comprises low quantities of N. Both the compost, LP and YYP already contain a high N value and C/N manipulation is therefore hindered. The C/N ratios used were 0, 12.5, 25 (ideal) and 50 as illustrated in Appendix 2 (Appendix Table 8). The initial carbon contents of the substrates were very low and were considered to be insignificant. One possible factor that could have affected the germination was therefore the C/N ratio of the ameliorants and substrates. The calculations to achieve adequate C/N ratios are listed in Appendix Table 8. For a comprehensive overview on the calculations refer to Appendix 2.

##### **4.2.2.2 Germination**

Preparation of the substrates with the applicable amelioration was done on 11 November 2014. Samples were kept indoors to prevent the rain from leaching the ameliorants. Sowing commenced the following day. Seeds were yet again sown on the surface and lightly pressured to make contact with the substrate, as in the case of Experiment 1. Seeding with

species i.e. *Cynodon dactylon* (Couch grass) and *Chloris gayana* (Rhodes grass) commenced.

Pots were taken outdoors in a randomised pattern to ensure randomization of replicates. The first germination percentage was determined five weeks afterwards. This was done by counting the emerging seedlings which were then converted to a percentage. A distinction was also made between the different grass species to determine which one will give the best performance. The second counting took place three weeks after the first counting. The second counting was done after the three-leaf stage. The long times allowed for germination compensated for lower temperatures. Lower temperatures delay the germination. The plants were also allowed to reach the three-leaf stage.

#### **4.3 Experiment 3: The influence of C/N ratios applied and substrates on the growth potential**

This experiment was done in order to determine the influence of the C/N ratio applied and substrate on the growth potential. It was completed during the same time as experiment 2. The same treatments discussed in section 4.2.1 are thus applicable to this experiment. The growth potential was determined by means of measuring the grass with a measuring tape. Only the total means were used in the data processing.

#### **4.4 Data processing**

A randomised pattern must be determined prior to the commencement of the experiment to ensure randomization of replicates.

The information that was directly taken from the experiments conducted is called raw data. The raw data were then processed into output data. In this dissertation the number of seedling emergence was monitored and the germination percentages subsequently determined (raw data). The data were processed using the data analysis software system, Statistica version 12 (StatSoft, Inc. 2013). First an ANOVA was done. The ANOVA is a statistical method to see the differences of the mean of several treatments on the measured

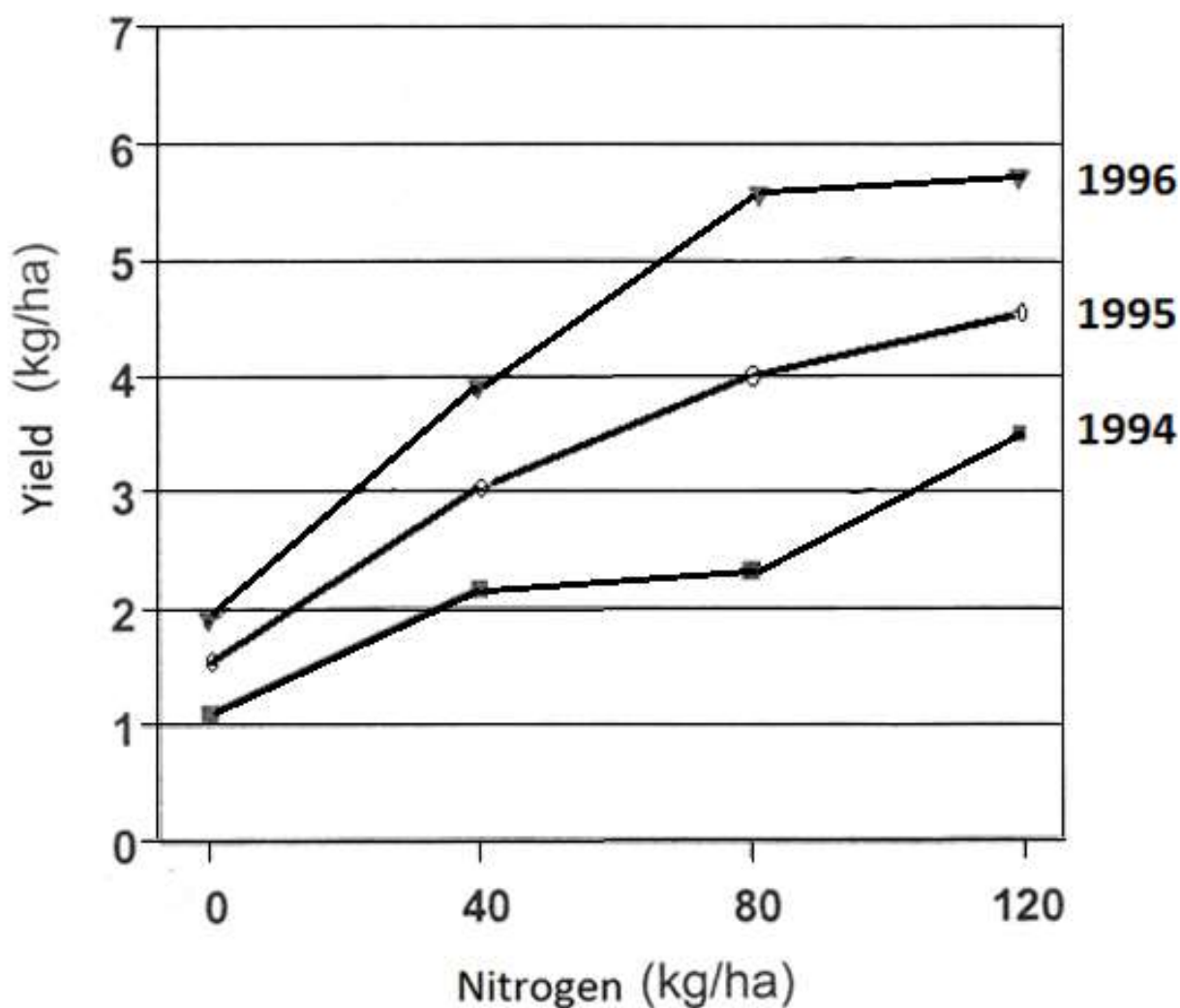
results. The ANOVA is therefore used to see the difference in the final outcome of treatments. (Asteriou & Hall, 2007:41; Buchan, 2002; Davies & Crombie, 2009:2; Gelman, 2013:70; Mc Donald, 2014:146; Senn, 2001:194; StatSoft, 2011:117; Van Emden, 2008:83-86, 140).

The ANOVA provides you with a p-value. The p-value indicates the smallest level of significance for which the observed sample statistically tells us to reject the null hypothesis ( $H_0$ ) (Asteriou & Hall, 2007:41; Gelman, 2013:70; Senn, 2001:194). In the case where the null hypothesis is rejected, the conclusion is that at least one treatment mean is different from at least one other treatment mean (Asteriou & Hall, 2007:41; Buchan, 2002; Davies & Crombie, 2009:2; Gelman, 2013:70; Mc Donald, 2014:146; Senn, 2001:194; StatSoft, 2011:117; Van Emden, 2008:83-86, 140).

Furthermore, *highly statistically significant* is referred to as  $p < 0.05$ . A p-value of  $p = 0.05$  suggests that there is a 95 % certainty that the results will be the same. A p-value of 0 indicates that there will always be differences in treatments no matter how many times you repeat it. There are no significant differences between treatments if the p-value is 1 (Asteriou & Hall, 2007:41; Buchan, 2002; Davies & Crombie, 2009:2; Gelman, 2013:70; Mc Donald, 2014:146; Rafter *et al.*, 2002:265; Senn, 2001:194; StatSoft, 2011:117; Van Emden, 2008:83-86, 140).

### **For example:**

Example to explain an ANOVA: Maize is one of South Africa's main food sources used in animal feeds and for human consumption. Take for instance maize that is planted in the field over a three-year period (1994 with a rainfall of 400 mm; 1995 with a rainfall of 550 and 1996 with a rainfall of 670 mm), with four different rates of nitrogen applied (0, 40, 80 and 120 kg N/ha). This experiment's results are represented in Figure 7 below.



**Figure 7: Maize yield at different N application rates over three seasons**

An ANOVA was done on the raw data to determine if there is a difference between treatments and show that seasons, the amount of N added and N-rate interaction with season influence the maize yield significantly (refer to Table 9).

**Table 9: ANOVA for season yield.**

Single Factors	p - Value
Season	0.0000
N – rate	0.0000
Interaction (N - rate x Season)	0.0000

From the graph in Figure 7 it seems that over the years the higher the N-application, the higher the yield obtained. For N-treatment; 0 N per ha always provides the lowest yield and 120 kg/ha N the highest yield per year.

The yield responses differ for each year. The 1994 season had the lowest rainfall and lowest yields, while 1995 experienced higher rainfall and delivered higher yields. The highest rainfall was received in 1996 and delivered the highest yield due to the higher rainfall experienced. The rainfall is directly influencing the yield attained which is dependent on seasonal changes (MVSA Bemestingshandleiding, 2007:197). This is supported by the rain measurements for 1994 which was 400 mm, 1995 with 550 mm and in 1996 with 670 mm.

There is thus clearly an interaction between season and N rate applied. Rainfall per year increased the effect of N applied. Generally if there is a significant difference with the interaction you just look at the interaction and ignore the single treatments (Van Emden, 2008:83-86, 140).

This was an example that clearly shows where the differences are. However, in a more complicated trial the statistically significant effect in the ANOVA is often followed by a post hoc test such as Tukey's range test to see where the differences in a treatment are. Tukey's Honestly Significant Difference test is a post-hoc test that is fairly easy to understand (Buchan, 2002; Hayter, 1984:61; Mc Donald, 2014:145-156; Proust, 2007:129; Rafter *et al.*, 2002:265; Skagerlund & Traff, 2014:7). The Tukey test is therefore preferable in this situation (Asteriou & Hall, 2007:41; Buchan, 2002; Davies & Crombie, 2009:2; Gelman, 2013:70; Mc Donald, 2014:146; Senn, 2001:194; StatSoft, 2011:117; Van Emden, 2008:83-86, 140).

Means within a column with the same letter(s) or no letters are not significantly different at the 95% probability level (Tukey HSD test). Means within a column with the same letter(s) are not significantly different at the 95% probability level (Tukey HSD test). Thus, treatments with different letters are significantly different from one another ("a" differs significantly from "b"). If values contain the same letter there is no significant difference ("ab" do not significantly differ from "a", "b" or "bc"; yet "ab" differ significantly from "c"). The Tukey test results are tabulated in Table 10.



For example, The ANOVA showed that the interaction between season and N application was significant. Thus, only the interaction part of Table 10 is referred to. The 1994—0 kg/ha N differ significantly from 1994 – 40 kg/ha N because the first has a Tukey HSD symbol of “a” and the second a “b” symbol. However, 1994 – 80 kg/ha N did not differ significantly from either of the above mentioned due to the “ab” Tukey HSD symbol. The 1994 - 120 kg/ha N interaction differ significantly from all three above-mentioned interactions because it contained a “c” in the Tukey test (refer to Table 10).

The highest yield was in 1996 with 120 kg/ha N with a yield of 5800 and differed significantly from all the other treatments.

**Table 10: Tukey HSD test results**

<b>Treatment</b>	<b>Value</b>	<b>Tukey symbol</b>
<b>Season</b>		
1996	4175 kg/ha	a
1995	3250 kg/ha	b
1994	2338 kg/ha	c
<b>N- applications rate</b>		
0 kg/ha N	1533 kg/ha	a
40 kg/ha N	2550 kg/ha	b
80 kg/ha N	3933 kg/ha	c
120 kg/ha N	4567 kg/ha	d
<b>Interaction</b>		
1994 - 0 kg/ha N	1100	a
1994 - 40 kg/ha N	2100	b
1994 - 80 kg/ha N	2700	bc
1994 - 120 kg/ha N	3500	c
1995 - 0 kg/ha N	1450	a
1995 - 40 kg/ha N	3000	bc
1995 - 80 kg/ha N	4000	d
1995 - 120 kg/ha N	4500	e
1996 - 0 kg/ha N	1888	a
1996 - 40 kg/ha N	3900	d
1996 - 80 kg/ha N	5100	f
1996 - 120 kg/ha N	5800	g

A statistical interaction can be described as the statistically established relationship between two or more variables that vary in some systematic way with the value of another (Asteriou & Hall, 2007:41; Buchan, 2002; Davies & Crombie, 2009:2; Gelman, 2013:70; Mc Donald, 2014:146; Senn, 2001:194; StatSoft, 2011:117; Van Emden, 2008:83-86, 140).

## 5. Results and discussions

In order to determine the germination percentages of two grass species (i.e. *Cynodon dactylon* (couch grass) and *Chloris gayana* (Rhodes grass)) based on the mean germination performance, we made use of three experiments. The first experiment was to determine the effect of ameliorants (compost, humate, fungimax, lime, yellow pea powder and lentil powder) and substrates (natural soil, topsoil proper; saprolite; subsoil and tailings) on germination.

Experiment 2 was subsequently based on various C/N ratios (0, 12.5/1, 25/1 and 50/1) applied to four substrates (Potchefstroom Red Structured B; A - Horizon; Gold Tailings and Platinum Tailings) to statistically determine the effect of the C/N ratio and substrate on the germination of the Rhodes and Couch grasses. Experiment 3 was done to determine the growth potential of germinated grasses from Experiment 2. Table 11 gives a summary of the substrates and ameliorants used in this project.

**Table 11: Amelioration and substrate legend for results**

No.	Substrates	No.	Ameliorants
<b>MF - 1B</b>	Red Structured B	<b>1</b>	No treatment, only fertiliser (Control)
<b>MF - 1C</b>	Acid Lava Saprolite	<b>2</b>	Humate
<b>MF - 2B</b>	Potchefstroom Red Structured B	<b>3</b>	Fungimax
<b>MF - 2C</b>	Diabase Saprolite	<b>4</b>	Compost (1.5 % of volume =15 ml)
<b>MF - 3F</b>	Kimberlite Fine Tailings	<b>5</b>	Lime & compost
<b>MF - 3G</b>	Kimberlite Coarse Tailings	<b>6</b>	Lime
<b>MF - 4</b>	Hard Carbonate Saprolite	<b>7</b>	Lime, humate, compost
<b>MF - 5B</b>	Kalahari Sand	<b>8</b>	Yellow pea powder (YPP)
<b>MF - 5C</b>	Granite Saprolite	<b>9</b>	Lentil powder (LP)
<b>MF - 6</b>	Ottosdal Kaolinite Clay	<b>10</b>	Lime, humate, fungimax
<b>MF - 7</b>	Platinum Tailings		
<b>MF - 8</b>	Gold Tailings		
<b>MF - 9 – BK</b>	Vertisol		
<b>MF - 11</b>	Cullinan - Kimberlite Tailings		
<b>MF - TC – BK</b>	A - Horizon		

### 5.1 Experiment 1: Effect of substrates and ameliorants on germination

Experiment 1 portrays the effect of all the substrates and ameliorants listed in Table 11 on germination as stated in the objectives (section 1.4).

According to the ANOVA done on the data, the substrates, ameliorants and the interaction between substrates and ameliorants had a highly significant effect ( $p = 0.000$ ) on germination as illustrated in Table 12.

**Table 12: The ANOVA results done on the effect of substrate, ameliorants and interaction between substrates and ameliorants on germination %**

Single factors	p - Value
Substrates	0.0000
Ameliorants	0.0000
Interaction (Substrate x Treatment)	0.0000

A Tukey HSD test was done in order to determine where the differences lie. If there is a significant difference with the interaction identified, one only looks at the interaction and ignores the single treatments (Van Emden, 2008:83-86, 140). The results of the Tukey test interactions are given in Table 13.

Tukey's Honestly Significant Difference (HSD) test at the 5 % level of significance was used to determine statistically significant differences between treatment means, where the analysis of variance (ANOVA) indicated significant effects.

**Table 13: Effects of substrate, ameliorant and interaction on germination %**

Single factors		Germination	Tukey HSD test
<b>Substrates</b>			
	Kimberlite Coarse Tailings	29.75	a
	Kimberlite Fine Tailings	39.63	b
	Cullinan - Kimberlite Tailings	39.63	b
	Granite Saprolite	40.25	b
	Platinum Tailings	43.88	bc
	Kalahari Sand	46.63	bc
	Kaolinite Clay	46.63	bc
	Acid Lava Saprolite	46.75	bc
	Potchefstroom Red Structured B	48.00	bc
	Red Structured B	49.13	bc
	Hard Carbonate Saprolite	50.13	c
	Diabase Saprolite	50.50	cd
	Gold Tailings	59.88	d
<b>Ameliorants</b>			
	Lentil powder	37.21	a
	Humate	40.96	ab
	Yellow pea powder	41.83	ab
	Fungimax	42.98	ab
	Compost	44.81	ab
	Lime	45.87	b
	Lime, humate, fungimax	47.21	b
	Lime & compost	47.40	b
	Lime, humate, compost	47.40	b
	No treatment on all, only fertiliser	58.75	c
<b>Interaction between Substrate and Ameliorants</b>			
	Kimberlite Coarse Tailings - No treatment, only fertiliser	65.00	efghij
	Kimberlite Coarse Tailings - Humate	41.25	abcdefghijkl
	Kimberlite Coarse Tailings - Fungimax	32.5	abcdefghijkl
	Kimberlite Coarse Tailings - Compost	18.75	abc
	Kimberlite Coarse Tailings - Lime & compost	43.75	abcdefghijkl
	Kimberlite Coarse Tailings - Lime	38.75	abcdefghijkl
	Kimberlite Coarse Tailings - Lime, humate, compost	28.75	abcdefg
	Kimberlite Coarse Tailings - Yellow pea powder	10.00	ab
	Kimberlite Coarse Tailings - Lentil powder	11.25	ab
	Kimberlite Coarse Tailings - Lime, humate, fungimax	7.50	ab

**Table 13 Continued: Effects of substrate, ameliorant and interaction on germination %.**

<b>Interaction between substrate and ameliorants</b>		
<b>Ameliorants</b>	<b>Germination</b>	<b>Tukey HSD test</b>
Kimberlite Fine Tailings - No treatment, only fertiliser	67.50	fghij
Kimberlite Fine Tailings - Humate	30.00	abcdefgh
Kimberlite Fine Tailings - Fungimax	18.75	abc
Kimberlite Fine Tailings - Compost	47.50	abcdefghij
Kimberlite Fine Tailings - Lime & compost	37.50	abcdefghi
Kimberlite Fine Tailings - Lime	26.25	abcde
Kimberlite Fine Tailings - Lime, humate, compost	46.25	abcdefghij
Kimberlite Fine Tailings - Yellow pea powder	45.00	abcdefghij
Kimberlite Fine Tailings - Lentil powder	31.25	abcdefgh
Kimberlite Fine Tailings - Lime, humate, fungimax	46.25	abcdefghij
Cullinan - Kimberlite Tailings - No treatment, only fertiliser	70.00	hij
Cullinan - Kimberlite Tailings - Humate	42.50	abcdefghij
Cullinan - Kimberlite Tailings - Fungimax	33.75	abcdefghi
Cullinan - Kimberlite Tailings - Compost	41.25	abcdefghij
Cullinan - Kimberlite Tailings - Lime & compost	27.50	abcdef
Cullinan - Kimberlite Tailings - Lime	35.00	abcdefghi
Cullinan - Kimberlite Tailings - Lime, humate, compost	40.00	abcdefghij
Cullinan - Kimberlite Tailings - Yellow pea powder	23.75	abcd
Cullinan - Kimberlite Tailings - Lentil powder	36.25	abcdefghi
Cullinan - Kimberlite Tailings - Lime, humate, fungimax	46.25	abcdefghij
Granite Saprolite - No treatment, only fertiliser	37.50	abcdefghi
Granite Saprolite - Humate	48.75	bcdefghij
Granite Saprolite - Fungimax	35.00	abcdefghi
Granite Saprolite - Compost	32.50	abcdefghi
Granite Saprolite - Lime & compost	42.50	abcdefghij
Granite Saprolite - Lime	52.50	cdefghij
Granite Saprolite - Lime, humate, compost	46.25	abcdefghij
Granite Saprolite - Yellow pea powder	38.75	abcdefghij
Granite Saprolite - Lentil powder	33.75	abcdefghi
Granite Saprolite - Lime, humate, fungimax	35.00	abcdefghi

**Table 13 Continued: Effects of substrate, ameliorant and interaction on germination %.**

<b>Interaction between substrate and ameliorants</b>			
<b>Ameliorants</b>		<b>Germination</b>	<b>Tukey HSD test</b>
Platinum Tailings	No treatment, only fertiliser	47.50	bcdefghij
Platinum Tailings	Humate	27.50	abcdef
Platinum Tailings	Fungimax	53.75	cdefghij
Platinum Tailings	Compost	42.50	bcdefghij
Platinum Tailings	Lime & compost	41.25	bcdefghij
Platinum Tailings	Lime	47.50	bcdefghij
Platinum Tailings	Lime, humate, compost	46.25	bcdefghij
Platinum Tailings	Yellow pea powder	41.25	bcdefghij
Platinum Tailings	Lentil powder	46.25	bcdefghij
Platinum Tailings	Lime, humate, fungimax	45.00	bcdefghij
Kalahari Sand	No treatment, only fertiliser	35.00	abcdefghi
Kalahari Sand	Humate	43.75	bcdefghij
Kalahari Sand	Fungimax	38.75	bcdefghij
Kalahari Sand	Compost	50.00	bcdefghij
Kalahari Sand	Lime & compost	46.25	bcdefghij
Kalahari Sand	Lime	62.50	defghij
Kalahari Sand	Lime, humate, compost	61.25	defghij
Kalahari Sand	Yellow pea powder	46.25	bcdefghij
Kalahari Sand	Lentil powder	43.75	bcdefghij
Kalahari Sand	Lime, humate, fungimax	38.75	bcdefghij
Kaolinite Clay	No treatment, only fertiliser	67.50	fghij
Kaolinite Clay	Humate	41.25	bcdefghij
Kaolinite Clay	Fungimax	50.00	bcdefghij
Kaolinite Clay	Compost	47.50	bcdefghij
Kaolinite Clay	Lime & compost	52.50	cdefghij
Kaolinite Clay	Lime	43.75	bcdefghij
Kaolinite Clay	Lime, humate, compost	50.00	bcdefghij
Kaolinite Clay	Yellow pea powder	43.75	bcdefghij
Kaolinite Clay	Lentil powder	27.50	abcde
Kaolinite Clay	Lime, humate, fungimax	42.50	bcdefghij

**Table 13 Continued: Effects of substrate, ameliorant and interaction on germination %.**

<b>Interaction between substrate and ameliorants</b>		
<b>Ameliorants</b>	<b>Germination</b>	<b>Tukey HSD test</b>
Acid Lava Sapolite - No treatment, only fertiliser	58.75	defghij
Acid Lava Sapolite - Humate	47.50	abcdefghijkl
Acid Lava Sapolite - Fungimax	43.75	abcdefghijkl
Acid Lava Sapolite - Compost	48.75	bcdefghij
Acid Lava Sapolite - Lime & compost	47.50	abcdefghijkl
Acid Lava Sapolite - Lime	45.00	abcdefghijkl
Acid Lava Sapolite - Lime, humate, compost	37.50	abcdeghi
Acid Lava Sapolite - Yellow pea powder	38.75	abcdefghijkl
Acid Lava Sapolite - Lentil powder	40.00	abcdefghijkl
Acid Lava Sapolite - Lime, humate, fungimax	60.00	defghij
Potchefstroom Red Structured B - No treatment, only fertiliser	61.25	defghij
Potchefstroom Red Structured B - Humate	46.25	abcdefghijkl
Potchefstroom Red Structured B - Fungimax	47.50	abcdefghijkl
Potchefstroom Red Structured B - Compost	51.25	cdefghij
Potchefstroom Red Structured B - Lime & compost	41.25	abcdefghijkl
Potchefstroom Red Structured B - Lime	52.50	cdefghij
Potchefstroom Red Structured B - Lime, humate, compost	47.50	abcdefghijkl
Potchefstroom Red Structured B - Yellow pea powder	43.75	abcdefghijkl
Potchefstroom Red Structured B - Lentil powder	37.50	abcdeghi
Potchefstroom Red Structured B - Lime, humate, fungimax	51.25	cdefghij
Red Structured B - No treatment, only fertiliser	66.25	efghij
Red Structured B - Humate	35.00	abcdeghi
Red Structured B - Fungimax	51.25	cdefghij
Red Structured B - Compost	53.75	cdefghij
Red Structured B - Lime & compost	47.50	abcdefghijkl
Red Structured B - Lime	42.50	abcdefghijkl
Red Structured B - Lime, humate, compost	57.50	cdefghij
Red Structured B - Yellow pea powder	51.25	cdefghij
Red Structured B - Lentil powder	30.00	abcdegh
Red Structured B - Lime, humate, fungimax	56.25	cdefghij

**Table 13 Continued: Effects of substrate, ameliorant and interaction on germination %.**

<b>Interaction between substrate and ameliorants</b>			
<b>Ameliorants</b>		<b>Germination</b>	<b>Tukey HSD test</b>
Hard Carbonate Saprolite	No treatment, only fertiliser	56.25	cdefghij
Hard Carbonate Saprolite	Humate	31.25	abcdefgh
Hard Carbonate Saprolite	Fungimax	55.00	cdefghij
Hard Carbonate Saprolite	Compost	30.00	abcdefgh
Hard Carbonate Saprolite	Lime & compost	72.50	lj
Hard Carbonate Saprolite	Lime	45.00	abcdefghij
Hard Carbonate Saprolite	Lime, humate, compost	47.50	abcdefghij
Hard Carbonate Saprolite	Yellow pea powder	53.75	cdefghij
Hard Carbonate Saprolite	Lentil powder	57.50	cdefghij
Hard Carbonate Saprolite	Lime, humate, fungimax	52.50	cdefghij
Diabase Saprolite	No treatment, only fertiliser	52.50	cdefghij
Diabase Saprolite	Humate	51.25	cdefghij
Diabase Saprolite	Fungimax	43.75	abcdefghij
Diabase Saprolite	Compost	48.75	bcdefghij
Diabase Saprolite	Lime & compost	51.25	cdefghij
Diabase Saprolite	Lime	43.75	abcdefghij
Diabase Saprolite	Lime, humate, compost	62.50	defghij
Diabase Saprolite	Yellow pea powder	50.00	bcdefghij
Diabase Saprolite	Lentil powder	37.50	abcdefghi
Diabase Saprolite	Lime, humate, fungimax	63.75	defghij
Gold Tailings	No treatment, only fertiliser	78.75	l
Gold Tailings	Humate	46.25	abcdefghij
Gold Tailings	Fungimax	55.00	cdefghij
Gold Tailings	Compost	70.00	hij
Gold Tailings	Lime & compost	65.00	efghij
Gold Tailings	Lime	61.25	defghij
Gold Tailings	Lime, humate, compost	45.00	abcdefghij
Gold Tailings	Yellow pea powder	57.50	cdefghij
Gold Tailings	Lentil powder	51.25	cdefghij
Gold Tailings	Lime, humate, fungimax	68.75	ghij



The interaction data are discussed simultaneously under normal circumstances. However, due to the complexity of the interaction data in Table 13, data were analysed separately.

A graph (refer to Figure 8) was subsequently constructed in order to assist in the interpretation of the complex Tukey interaction results. On the X-axis are all the ameliorants as applied to the substrates. The axis is organised from the lowest mean germination per substrate to the highest.

It seems from the graph constructed (Figure 8) that the “no treatment” amelioration performed much better in the Kimberlite coarse tailings, Kimberlite fine tailings and the gold tailings in comparison with the rest of the ameliorants.

It is evident that the seed utilised in Experiment 1 was coated when analysing the substrates and ameliorants separately. The no-treatment ameliorant was thus treated seeds; and all other ameliorants were treated seeds with extra ameliorants added. The better results of the no-treatment in Kimberlite coarse tailings, Kimberlite fine tailings, granite saprolite and acid lava saprolite was due to the coarse texture of the substrate (refer to Figure 8). The coarse texture allows the ameliorants to leach out, resulting in poor germination. There is a competition between the seed coating and the soil for readily available nutrients. If competition occurs, seeds will be deprived of essential nutrients stored in the coating because nutrients move from the coating to the soil. The coating also contains enough nutrients to sustain it until the three-leaf stage. The differences were seen statistically in these substrates due to the big difference between the highest and lowest germination averages. The texture problem was solved in coarser substrates by means of the seed coating improving the seed soil contact and water retention. Seeds seem to have enough water and nutrients stored which results in a higher “no treatment” germination.

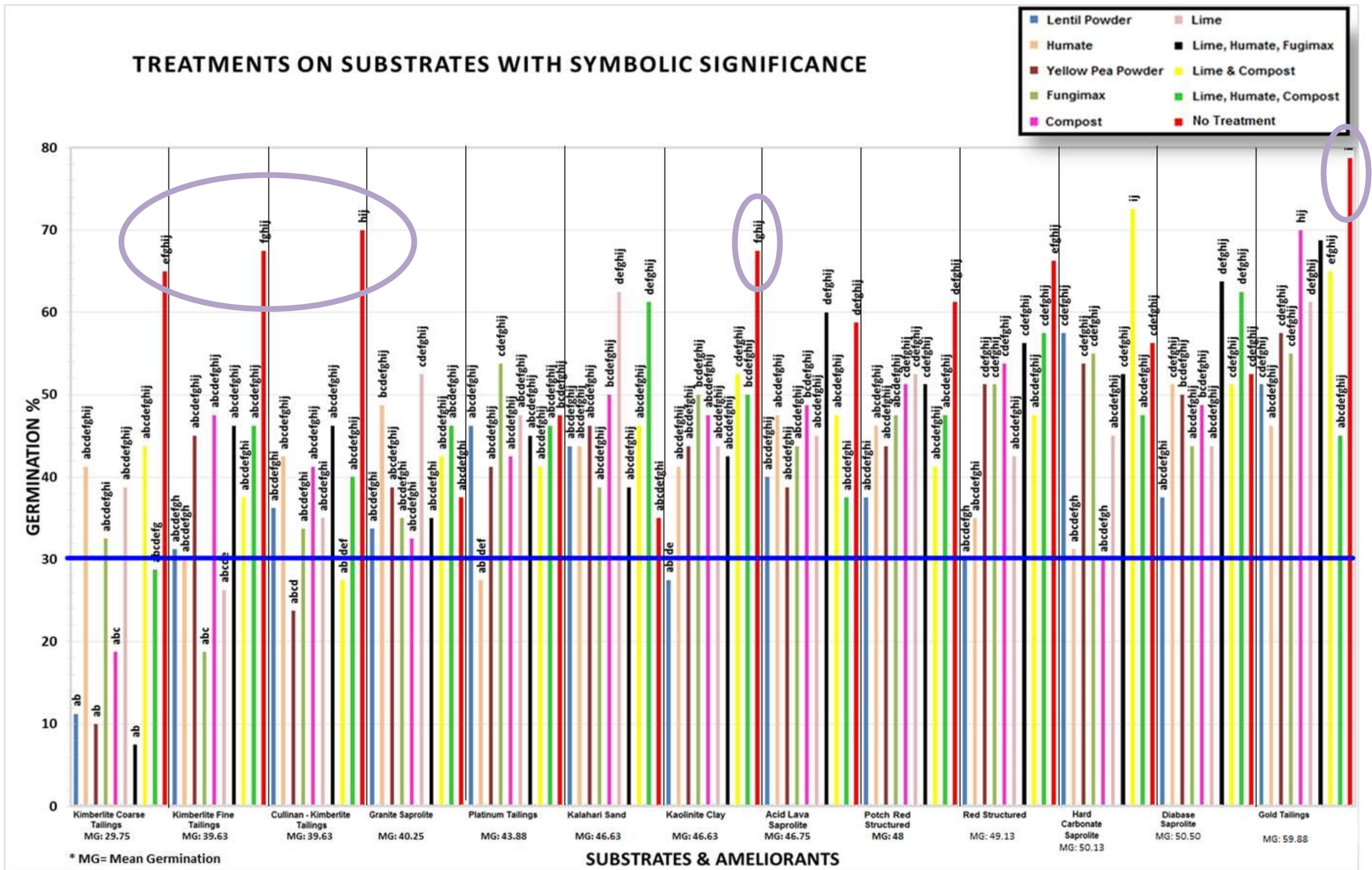


Figure 8: Effect of Substrate and Ameliorant on germination %.

The International Seed Testing Association (ISTA, 2009) requires a minimum number of three replicates for each seed type during an experiment. The cumulative data characterized in tables 13 and 14 are represented as a percentage of a total of 40 hand-sown seeds (ten seeds in each pot x 4 replicas) Data from the different species of grass used were not separated (i.e. *Cynodon dactylon* and *Chloris gayana* was counted together). The mean values reflect the emergence percentage reached during the seven weeks of the experiment, irrespective of any further emergence afterwards.

Certified seed must comply with certain standards required by the ISTA (ISTA, 2009), stating that a minimum germination of 75% must be achieved, and a refinement of 96% (Pannar, 2009:11). The ISTA rules are set for laboratory measures in a controlled environment only. The germination test thus determines a seed batch's vigour and quality under optimal conditions. The three planting methods prescribed by ISTA (1985) include on top of sand, between paper and on top of paper. The ISTA rules do not apply to field trials or experiments that utilize soil as a growth medium (ISTA, 2009). The reason is that growth mediums such as subsoil, saprolite and tailings are not included in the rules. The quality of a seed batch is tested regardless of the growth medium (tailings, saprolite or soil) that the seed will be sown in. It therefore serves solely as a guideline. The Plant Improvement Act No 53 of 1976 states that Rhodes should have at least 20% germination (South Africa Act 53, 1976). This Act does not make provision for couch grass and is only based on controlled lab environments and not applicable to field conditions.

For the purpose of this dissertation, I thus refer to above 30% germination attained as the ideal percentage for field conditions (Coetzee, 2015: Personal correspondence) and is indicated in Figure 8 with a blue horizontal line. The high overall germination percentage attained in the experiment (higher than 30% (Coetzee, 2015: Personal correspondence)) is a consequence of excessive water application (to prevent crusting (discussed in section 2.3.1)), as well as the seed coating (refer to section 3.4) that contains all the necessary nutrients to sustain the seed until the three-leaf stage. The three-leaf stage is part of the seedling emergence and occurs at three to four weeks. The four-leaf (four to six weeks after planting commenced) to five-leaf (five to eight weeks after planting commenced) stages are regarded as the tiller period (Bowden *et al.*, 2007:6; Bradley *et al.*, 2008:2; Poole, 2005:2). This period is when the plant produces additional shoots called tillers, some of which will

produce a head. Tillers form at the base (axil) of leaves from buds and do not include the main or primary shoot (Bowden *et al.*, 2007:31; Bradley *et al.*, 2008:10; Poole, 2005:5). Crown roots appear in wheat during the three to four-leaf stages (Bradley *et al.*, 2008:12). The four-leaf stage is considered to be most suitable for transplanting due to better establishment, higher biomass and better growth (Ankegowda, 2008:253).

Because of the complexity of the data, we looked at treatments separately in order to see whether there were any meaningful results.

### 5.1.1 Substrate as main effect

According to the ANOVA (refer to Table 12), the mean values of substrates differ significantly. A Tukey test was done to specify where the differences were. Table 13 signifies the Tukey HSD test results for the substrates based on their average germination percentage. The average germination attained is subsequently visually illustrated in Figure 9

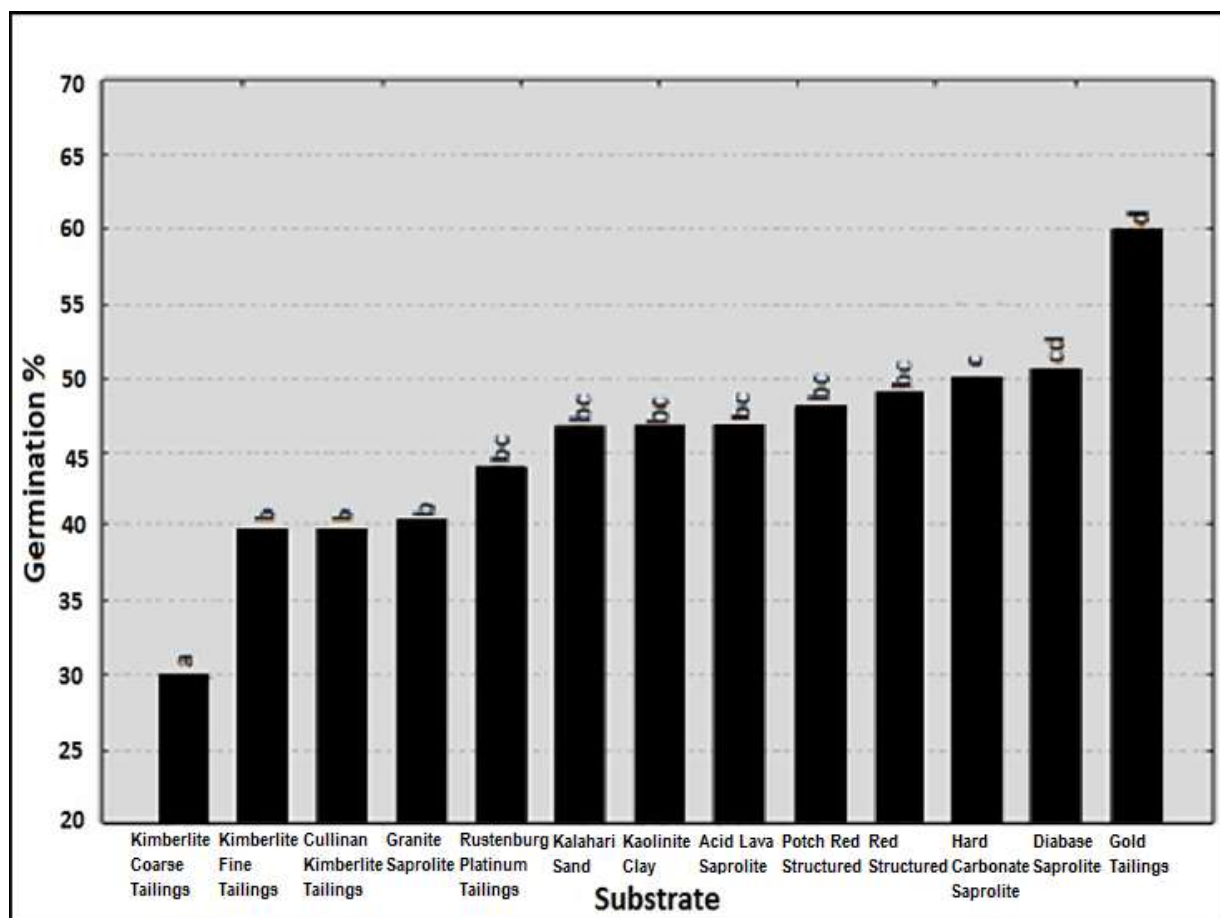


Figure 9: Effect of substrate on germination %

The Kimberlite Coarse Tailings with a Tukey HSD symbol of “a” differ statistically significantly from the Kimberlite Fine Tailings, Cullinan Kimberlite Tailings and C - Granite Saprolite with the Tukey HSD symbol of “b”. Practically half of the substrates =; i.e. Platinum Tailings, Kalahari Sand, C - Kaolinite Clay, C - Acid Lava Saprolite, Potchefstroom Red Structured B and the Red Structured B with the Tukey HSD symbol of “bc” do not statistically differ from “b” or “c”, but then again does differ from “a”. The C - Hard Carbonate with a symbol “c” differ statistically from “a”, “b” and “d”. The Diabase Saprolite (“cd”) thus differs statistically from “a” and “b”. The Gold Tailings (“cd”) differ statistically from “a”, “b”, “bc” and “c” (refer to Table 13).

The coarse texture can therefore be amended by the additional application of SOM. The SOM has the ability to not only improve the CEC (Van Deventer & Ferreira, 2012:21), but also maintain the exchangeable K, Ca and Mg. SOM provides essential humic and fluvic acids to polysaccharides; and also assists with sustaining other essential microbial activities (Duong *et al.*, 2012:197). SOM additionally aids in the promotion of water retention and porosity. It serves as a chemical buffer, maintaining soil structure (Howard & Howard, 1990:306), store and transition of air and water, improvement in nutrient retention (N, P and K), resistance of compaction. SOM provides a source of energy for soil microbes and cohesion properties of soil (Brady & Weil, 2008:515; Cooperband, 2002:2; Chan, 2008:2; Fenton *et al.*, 2008:1; Marcel Dekker Inc., 1985:3; Singer & Munns, 1992:3).

Mine tailing materials originate from excessive milling of fresh rocks (Maboeta *et al.*, 2006:150). Natural soils contain abundant amounts of secondary minerals (Van Deventer & Hattingh, 2004:62), and are also characterised by generally comprising a wide particle size distribution (PSD) due to the natural weathering processes involved (Gobat *et al.*, 2004:14). Tailings on the other hand mainly comprise primary minerals, consequently resulting in a low buffering capacity (Van Deventer & Hattingh, 2004:62). Mine waste materials have a fine homogenous texture due to the crushing and grinding processes and therefore have a small particle size distribution (Mendez & Maier, 2008:48; Rossouw, 2010:15). Some mine waste materials that have not been subjected to excessive milling result in a coarse texture as in the case of Kimberlite coarse tailings. Mine waste is therefore regarded as anthropogenic soils due to the dissimilarities in pedogenic origin and soil properties. The Witbank soil form is

the only official anthropogenic soil form included in the soil taxonomy system of South Africa (Fey, 2010:143; Van Deventer & Hattingh, 2004:62). This soil form occurs primarily in mining impacted areas resulting from coal, diamond, gypsum, and gold mining that require rehabilitation (Fey, 2010:143). Rehabilitation is intended to improve the functions of anthropogenic soil to such an extent that it is capable of functioning similarly to natural soils (Van Deventer & Hattingh, 2004:62).

The physical properties of soil determine to a great extent what it can be utilized for. Several attributes associated with the physical characteristics of the natural soil impact on the germination. These attributes were found to be the water-holding capacity, root penetration ease, aeration and retention of nutrients (Foth, 1990:27), which in turn have an effect on the mobility, mineralization, fixation, and adsorption mechanisms of nutrients (Baligar *et al.*, 2001:926). Mine waste and tailing materials lack these properties (Bradshaw, 1998:255).

Due to the growth media's hostile environment, it was deemed appropriate to investigate the physical and chemical characteristics of the mine waste materials used. This might provide insight into the germination percentages attained.

Table 14 gives the chemical characteristics such as C %, P %, N %, Mg, Ca, K, K/CEC, Mg/K, Ca/Mg and EC. The clay content, which is a physical characteristic, is also included. The exchangeable cations and CEC of the substrates are tabulated in Table 15. These tables will be used to assist in distinguishing between the substrates.

**Table 14: Chemical characteristics of different substrate types**

Substrate	%C	%N	%S	Clay %	P	K	Ca	Mg	K/CEC	Mg/K	Ca/Mg	pH	pH	EC
	(LECO)				(mg/kg <sup>-1</sup> )					%	(cmol /kg <sup>-1</sup> ) / (cmol /kg) <sup>-1</sup>		(H <sub>2</sub> O)	(KCl)
Kimberlite Coarse Tailings	n.a.	n.a.	n.a.	2.2	4.6	81.2	2923.7	15.6	1.3	0.6	112.5	9.3	7.4	74
Kimberlite Fine Tailings	n.a.	n.a.	n.a.	2.0	4.1	105.3	3540.4	17.1	1.3	0.5	126.4	9.1	7.3	94
Cullinan - Kimberlite Tailings	0.4	0	1	2.4	4.0	879.8	1695.6	226.4	11.0	0.8	4.6	9.3	7.6	108
Granite Saprolite	0.2	0.1	0	2.3	3.4	60.7	578.8	170.2	3.7	8.8	2.1	7.2	6.5	43
Platinum Tailings	0.2	0	0.09	2.1	6.0	26.6	325.4	33.1	3.2	3.9	6.0	7.6	8.0	218
Kalahari Sand	0.2	0.1	0.2	2.1	3.5	72.2	2185.1	1097.0	0.9	49.9	1.2	7.6	6.4	97
Ottosdal Kaolinite Clay	0.2	0.06	0	17.5	4.6	109.8	1238.0	228.9	3.2	6.7	3.3	7.0	6.1	59
Acid Lava Saprolit	0.3	0	0	2.2	4.0	63.7	4297.4	1213.5	0.6	62.2	2.2	8.0	6.9	27
Potchefstroom Red Structured B	0.7	0	0	28.7	3.5	148.9	1457.6	721.0	2.8	15.6	1.2	7,4	6,6	77
Red Structured B	0.9	0	0.01	22.7	3,8	90.2	1744.5	642.1	1.3	22.9	1.7	6,6	5,6	37
Hard Carbonate Saprolite	2.9	0.04	0	7.3	3.4	88.7	4248.0	67.3	2.0	2.4	38.6	7.9	7.7	56
Diabase Saprolite	0.2	0	0	2.2	3.5	79.7	2605.3	1134.2	0.9	46.5	1.4	7.4	6.1	92
Gold Tailings	0.2	0.01	0.3	4.8	4.9	19.1	1226.1	96.4	0.8	15.1	7.8	5.7	5.4	286
Vertisol	1.7	0.2	0.01	36.1	7.5	213.0	4818.9	1860.1	1.6	28.2	1.6	7.7	7.1	105
A - Horizon Topsoil	1.6	0.1	0.01	12.1	17.4	266.7	971.1	214.9	6.1	2.6	2.8	6.5	5.7	23
Ideal Values	> 1.3 % <sup>3</sup>	> 0.001 % <sub>4</sub>	0.12 % <sup>5</sup>	27 % <sup>3</sup>	>13.4 <sup>1</sup> (natural grass pastures)	<250 - >40 <sub>1</sub>	<3000 - >200 <sup>1</sup>	<300 - >50 <sub>1</sub>	7 - 12 % <sup>2</sup>	3 - 4 <sup>1</sup>	1.5 - 4.5 <sup>1</sup>	5 - 8 <sup>2</sup>	5 - 8 <sup>2</sup>	<360 <sup>2</sup>

<sup>1</sup> MVSA Bemestingshandleiding (2007:172-174, 276-286, 304) ; <sup>2</sup> Van Deventer & Ferreira (2013: 30); <sup>3</sup> Steyn & Herselman (2006:233) based on dry land use type; <sup>4</sup> Du Toit *et al.* (1994:75) based on natural C/N ratios of South African dry lands and virgin soils ; <sup>5</sup> Meyer *et al.* (1971:204) based on sugarcane; n.a. = not available;

Legend for high and low values

below ideal	ideal	above ideal
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**Table 15: Exchangeable cations and CEC of different substrate types**

Substrate	Ca		Mg		K		Na		CEC	ESP
	(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> )		
Kimberlite Coarse Tailings	14.6	2923.7	0.1	15.6	0.2	81.2	6.3	1445.5	15.6	40.2
Kimberlite Fine Tailings	17.7	3540.4	0.1	17.1	0.3	105.3	7.5	1734.0	20.3	37.1
Cullinan - Kimberlite Tailings	8.5	1695.6	1.9	226.4	2.3	879.8	8.0	1843.0	20.5	39.1
Granite Saprolite	2.9	578.8	1.4	170.2	0.2	60.7	0.2	33.5	4.3	3.5
Platinum Tailings	1.6	325.4	0.3	33.1	0.1	26.6	0.3	64.0	2.2	12.9
Kalahari Sand	10.9	2185.1	9.0	1097.0	0.2	72.2	0.5	112.5	19.5	2.5
Ottosdal Kaolinite Clay	6.2	1238.0	1.9	228.9	0.3	109.8	0.2	37.0	8.9	1.8
Acid Lava Saprolit	21.5	4297.4	10.0	1213.5	0.2	63.7	0.3	60.5	27.7	0.9
Potchefstroom Red Structured B	7.3	1457.6	5.9	721.0	0.4	148.9	0.3	62.0	13.5	2.0
Red Structured B	8.7	1744.5	5.3	642.1	0.2	90.2	0.2	44.5	18.4	1.0
Hard Carbonate Saprolite	21.2	4248.0	0.6	67.3	0.2	88.7	0.1	29.5	11.4	1.1
Diabase Saprolite	13.0	2605.3	9.3	1134.2	0.2	79.7	0.4	96.0	22.4	1.9
Gold Tailings	6.1	1226.1	0.8	96.4	0.1	19.1	0.1	32.0	6.5	2.2
Vertisol	24.1	4818.9	15.3	1860.1	0.5	213.0	0.4	88.0	33.7	1.1
A – Horizon	4.9	971.1	1.8	214.9	0.7	266.7	0.1	29.5	11.1	1.2
Ideal values for natural grass pastures	0.350 <sup>1</sup>		0.306 <sup>1</sup>		0.244 <sup>1</sup>				>5 (cmol/kg-1) <sup>2</sup>	<5 % <sup>3</sup>

<sup>1</sup> MVSA Bemestingshandleiding (2007:304); <sup>2</sup> Van Deventer & Ferreira (2012:28); <sup>3</sup> Bloem *et al.* (1992) & Du Preez *et al.* (2000)

Legend for high and low values

below ideal	ideal	above ideal
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**Particle size distribution (PSD):** Texture refers to the relative size of the particles, which in turn describes the fineness or coarseness of the soil (Foth, 1990:27). The relative proportions of clay, silt and sand-sized particles present define the soil's texture (Foth, 1990:27; Gobat *et al.*, 2004:45). Texture directly influences the porosity, structure and the hydric regime of soil. Natural soils' PSD ranges from 0.002 mm (clay size particles) to 2 mm (sand size particles) (Winegardener, 1995:19). Texture is considered as a useful index for the purpose of soil classification, for natural soils' inherent property change little over time (Gobat *et al.*, 2004:46).

PSD plays an important role in the seed soil contact properties, which in turn influences the germination attained. Coarse textures leach out essential nutrients, water and seeds. The water-holding capacity of growth media is one of the major influencing attributes in germination.

Substrate restoration via vegetation establishment is difficult in coarse-textured wastes due to the lack of moisture retained required for plant root moisture supply (Bowden *et al.*, 2007:11; Bradshaw, 1998:262; Yazdi *et al.*, 2013:1). There are significant differences in the substrates utilised based on the dissimilarity in clay particle content, in return causing differences in soil function and fertility (Van Deventer & Hattingh, 2004:62). A suitable substrate should be sufficient for proper root support and must have suitable moisture storage properties to sustain a vegetative layer (Sylvia *et al.*, 2005: 176).

**PAW:** Plant available water can be defined as the soil water content at a soil water potential of 1500 kPa. The uppermost limit of PAW is Field Capacity (FC), usually around 33 kPa (Lambooy, 1983:3). Water is essential for germination and to maintain plant growth and plant support.

**Nutrients:** Plant nutrients are the elements which are absorbed by plants and are necessary for completion of the normal life cycle. These include C, H, O, N, P, K, Ca, Mg, S, Cu, Fe, Zn, Mn, B, Cl and Mo (Van der Watt & Van Rooyen, 1995:325). Deficiencies can have a severe impact on vegetation. Phosphorus plays an important role in root development, N is necessary for vegetative growth and K for germination and nutrient

transport. For a detailed discussion refer to section 2.2.2. Nutrients can either be toxic or deficient. Both situations will influence germination and growth.

**pH:** pH ( $H_2O$ ) can be described as the logarithmic measure of the hydrogen ion concentration present in the solution and can be given as:  $pH = -\log[H^+]$  (University of Oxford, 2013:438). A pH range between 5.5 and 7 is most favourable for optimum plant growth due to the availability of plant macro and micro nutrients at this pH range (MVSA Bemestingshandleiding, 2007:96).

**EC:** Electrical conductivity (EC) is the ease of a current passing through a material ( $mS/m^{-1}$ ) (University of Oxford, 2013:191). It is associated with salts which can cause reverse osmosis, thus affecting the plant. The ideal would be below  $360 mS/m^{-1}$  (Van Deventer & Ferreira, 2013: 30).

**CEC:** CEC is defined as the total number of exchangeable cations that a particular material or soil can adsorb at a given pH. Exchangeable cations are held mainly on the surface of colloids of clay and humus and are measured in centimols per kilogram ( $cmol/kg$ ) of soil (Foth, 1990:171; Non-Affiliated Soil Analysis Work Committee, 1990; Van Deventer & Hattingh, 2014:62; Weil & Magdoff, 2004:2). According to Van Deventer and Ferreira (2013:30), a growth medium should ideally be higher than  $5 cmol/kg^{-1}$  for vegetation establishment.

Secondary clay minerals are main contributors to the soil's CEC (Sparks, 2003:64; Van Deventer & Ferreira, 2012:28). CEC is defined as the total number of exchange sites (organic and mineral colloids) of a soil (Foth, 1990:171; Non-Affiliated Soil Analysis Work Committee, 1990; Van Deventer & Hattingh, 2014:62; Weil & Magdoff, 2004:2).

Experimental work has specified that the potassium should be at least 7-12 % of the CEC, irrespective of the Ca/Mg ratio (Van Deventer & Hattingh, 2014:62). The balances between the different cations (Ca, Mg and K as well as Na) (Brady & Weil, 2008:515) are very important for optimal growth (Van Deventer & Ferreira, 2012:30). The exchangeable Ca/Mg

must be near to 6/1 (65 % Ca and 10 % Mg saturation of the CEC) (Rehm, 2015) or 1.5 - 4.5/1 (MVSA Bemestingshandleiding, 2007:174). However, research has shown that plants grow well and satisfy their Ca/Mg needs in ratios anywhere from 1/1 to 15/1 (Van Deventer & Ferreira, 2012:30). This statement is contradicted due to the Red Structured B (Ca/Mg: 1.66), the Potchefstroom Red Structured B (Ca/Mg: 1.23) and the gold tailings (Ca/Mg: 7.76) substrates that all comply with this value, yet the red structured soils still delivered lower germination percentages than the gold tailings. Van Deventer and Ferreira (2012:30) and MVSA Bemestingshandleiding (2007:174) stated that the generally accepted Ca/Mg ratio should be 1.5 to 4.5; (Ca+Mg)/K should be 10 to 20; and Mg/K should be 3 to 4.

### ***Kimberlite coarse tailings***

Kimberlite coarse tailings resulted in the lowest germination percentage achieved of only 29.75 %, which differs meaningfully (Tukey HSD test: a) from all the substrates with respect to the statistical analysis in Table 13.

- **PSD:** The poor performance regarding germination is primarily the result of the coarse texture (greater than 2 mm fraction = 4.3 %; very coarse sand = 30.3 % and 65.4 % finer material) as illustrated in Table 8 (section 4.1.3.3). The coarse texture allows nutrients and seeds to leach out (Foth, 1990:27). In the case of seeds leaching to deeper than 2 cm depths, germination is incapable (Bowden *et al.*, 2007:11; Edwards, 2008:1; Tanveer *et al.*, 2013:40; Yazdi *et al.*, 2013:1). The PSD was the main influencing factor on the germination attained. The poor seed soil contact is the overriding effect in this case affecting germination
- **PAW:** A study done by De Wet and Jansen (2014:16) specified that this medium has a very low plant available water (PAW) percentage, estimated as merely 2.86 %. A low water retention capacity is anticipated in this substrate due to the lack of finer particles (De Wet & Jansen, 2014:16). The poor PSD of the growth medium is the reason for the low PAW anticipated.
- **Nutrients:** This substrate also lacks phosphorus (P), with only 4.6 mg/kg<sup>-1</sup> (refer to Table 14) anticipated when compared with the ideal value of >13.4 mg/kg<sup>-1</sup> for natural grass pastures (MVSA Bemestingshandleiding, 2007:304). The magnesium is also deficient with only 15.6 mg/kg<sup>-1</sup> (ideal: <300 - >50) and is the lowest value anticipated

for all substrates measured. This resulted in a deficient Mg/K ratio of 0.6 and a Ca/Mg of 112.5 which exceeds the optimum value. However, both the calcium (2923.7 mg/kg<sup>-1</sup>) and potassium (81.2 mg/kg<sup>-1</sup>) are within the ideal value ranges as illustrated in Table 14. This substrate has the highest ESP (exchangeable sodium potential) value of 40.2 % which contributes to the low germination percentage attained (Table 15).

- **pH:** This substrate is alkaline with a pH (H<sub>2</sub>O) value of 9.3.
- **EC:** The EC were acceptable with 74 mS/cm<sup>-1</sup>.
- **CEC:** This growth medium has a CEC value of 15.6 cmol/kg<sup>-1</sup> (see Table 15) which is well above the minimum ideal value of >5 cmol/kg<sup>-1</sup> set by Van Deventer and Ferreira (2012:28).

Although this 29.75 % is the lowest germination attained for the experiment, it is still acceptable in practice, for a 30% germination is considered to be good in field conditions for pasture establishment in the field (Coetzee, 2015: Personal correspondence).

The next group of substrates all had a “b” value in the Tukey HSD test tabulated in Table 13 and performed significantly better than the Kimberlite coarse tailings.

Although the main influencing factor for this statistical group is the same as the Kimberlite coarse tailings, it has a better capacity for higher plant available water (PAW) percentages. For this reason, the germination attained in this group is higher than that of the statistical group “a”.

However, the Kimberlite tailings have poor physical properties (coarse texture, poor structure, and poor water holding capacity) and high pH conditions. The coarse texture allows seeds to leach out (Baligar *et al.*, 2001:926; Foth, 1990:27). The coarse tailings lack sufficient structure and an adequate water-holding capacity.

### ***Kimberlite fine tailings***

The Kimberlite fine tailings (39.63 % mean germination) did significantly better than coarse Kimberlite. This substrate performed similar to the Cullinan Kimberlite tailings.

- **PSD:** It is the same material as the Kimberlite coarse material derived from Voorspoed, but is the finer counterpart (greater than 2 mm fraction = 1.1 %; very coarse sand = 5.1 % and 93.8 % finer material; refer to Table 8).
- **PAW:** This substrate thus has a slightly higher PAW percentage attained (PAW of 8.25 %) in comparison with the coarse counterpart due to the presence of finer particles (De Wet & Jansen, 2014:15). However, it does not have great water storage and water retention abilities (De Wet & Jansen, 2014:15) due to a lack of finer material as illustrated in Table 14. The main reason for better germination attained in the Kimberlite fine tailings, when compared to Kimberlite coarse texture, is the better PAW capacity of the Kimberlite fine tailings due to higher amount of finer particles present.
- **Nutrients:** This growth medium is phosphorus-deficient with a value of  $4.1 \text{ mg/kg}^{-1}$ , slightly lower than that of the coarse counterpart. The potassium ( $105.3 \text{ mg/kg}^{-1}$ ) anticipated comply with the ideal range of  $< 250 - > 40 \text{ mg/kg}^{-1}$  (MVSA Bemestingshandleiding, 2007:172). Although the magnesium value is higher than that of the coarse counterpart, it is still deficient with only  $17.1 \text{ mg/kg}^{-1}$  as illustrated in Table 14. The calcium value of  $3540.4 \text{ mg/kg}^{-1}$  exceeds the ideal range set by the MVSA Bemestingshandleiding (2007:172). This resulted in a deficient Mg/K ratio of 0.5 and a Ca/Mg of 126.4 (highest value attained) which exceeds the optimum value. This substrate has a high ESP (exchangeable sodium percentage) value of 37.1 % which contributes to the low germination percentage attained (Table 15).
- **pH:** The pH ( $\text{H}_2\text{O}$ ) of this alkaline substrate is 9.1, somewhat more acidic than what the coarse Kimberlite counterpart is.
- **EC:** The EC were acceptable with  $94 \text{ mS/cm}^{-1}$ .
- **CEC:** This growth medium has a CEC value of  $20.3 \text{ cmol/kg}^{-1}$  (see Table 15) which is well above the minimum ideal value set by Van Deventer and Ferreira (2012:28).

### ***Cullinan Kimberlite tailings***

Cullinan Kimberlite tailings (39.63 % mean germination) delivered similar germination to the Kimberlite fine tailings. There is no significant statistical difference between the Kimberlite fine tailings derived from Voorspoed, Cullinan Kimberlite tailings and granite saprolite, which is supported by the Tukey HSD test symbolic value of b as tabulated in Table 13.

- **PSD:** This material has a PSD (particle size distribution) of greater than 2 mm fraction = 13.6 %; very coarse sand = 31.9 % and 65.4 % finer material.

- **PAW:** The PAW is 8.14 % that can be utilised by the vegetation (De Wet & Jansen, 2014:15). According to De Wet and Jansen (2014:18), there is a lack of sufficient secondary minerals supported by the PSD values anticipated, as illustrated in Table 8. This is validated by the reduced amounts of PAW present at higher suction (30 to 1500 kPa) estimating 7.37 % and only 2.12 % between 500 kPa and 1500 kPa respectively (De Wet & Jansen, 2014:15). Even though these PAW values are low, it is still higher than that measured for the Kimberlite coarse tailings. Literature also validates the findings of the PSD, for soils containing abundant secondary minerals are usually derived from natural weathering processes (Sparks, 2003:64; Van Deventer & Ferreira, 2012:28).
- **Nutrients:** However, mine waste lacks both the secondary minerals and organic matter (Van Deventer & Hattingh, 2004:62). The Ca/Mg ratio was estimated as of 4.6, signifying that a Ca deficiency will not be likely to occur as validated by Table 14. The potassium value of 879.8, highest value anticipated, exceeds the ideal range significantly. The high potassium value might be toxic to grasses resulting in lower germination percentages attained. The high potassium content suppresses the Mg and Ca uptake (MVSA Bemestingshandleiding, 2007:95). This resulted in a deficient Mg/K ratio of only 0.8. This substrate (K/CEC: 11.0) is the only one that complies with the K/CEC ratio set by Van Deventer and Hattingh (2014:62) of 7 - 12 %. The organic C% is deficient (0.4 %) as well as the %N (0 %) which does not comply with the standards set. The high potassium content and coarse texture are the main contributing factors affecting the poor germination attained.
- **pH:** The substrate derived from Cullinan has an alkaline pH (H<sub>2</sub>O) of 9.3 as tabulated in Table 14. Muller (2014:70) indicated that a net acid potential of -121 (ton/ha lime) is anticipated for this substrate. The soluble Al (0.01 mg/kg<sup>-1</sup>) is within acceptable limits. The net acid potential measurements validated the pH findings, states that the Kimberlite tailings will not be subjected to acidic conditions (Muller, 2014:70).
- **EC:** The EC (108 mS/m<sup>-1</sup>) is within acceptable limits.
- **CEC:** This growth medium has a CEC value of 20.5 cmol/kg<sup>-1</sup> (see Table 15) which is well above the minimum ideal value set by Van Deventer and Ferreira (2012:28).

Kimberlite tailing substrates seem not to be too hostile to vegetation establishment due to the high pH (H<sub>2</sub>O) conditions anticipated. With the application of additional SOM, one expects to

establish vegetation effortlessly. However, the physical characteristics (coarse texture and poor moisture retention) have enormous effects on germination. The SOM application might have been too little and insufficient, to have a noticeable effect on germination.

Mine waste commonly has poor nutrient availability due to the low CEC values anticipated (Van Deventer & Hattingh, 2004:27; Ye *et al.*, 2000:289). However, this is not the case in Kimberlite tailings, for they usually have a high CEC (Van Deventer, 2015: Personal correspondence).

The low germination percentage achieved in all the Kimberlite tailings, is rather a result of the physical properties and high pH. These properties include poor structure, poor water-holding capacity as well as the coarse texture allowing seeds to leach out (Baligar *et al.*, 2001:926; Foth, 1990:27). Depths exceeding two cm will delay germination (Bowden *et al.*, 2007:11; Edwards, 2008:1; Tanveer *et al.*, 2013:40; Yazdi *et al.*, 2013:1). The coating of seeds utilised will supply enough nutrients until the three-leaf stage. Nutrient availability and CEC will therefore not be a chief determining factor in this case.

### ***Granite saprolite***

Saprolite is defined as a “soft, earthy, thoroughly decomposed rock formed in situ by chemical weathering. It often forms a thick (as much as 100 m) layer, esp. in a humid and tropical or subtropical climate; the colour is commonly some shade of red or brown (Cf. laterite. Syn. Saprolith). Weathering rock in various stages of decomposition. It has a general organization with respect to colour, structure or consistence which still has distinct affinities with the parent rock” (Van der Watt & Van Rooyen, 1995:156). This substrate is classed as a sand in the texture triangle presented in Figure 5.

- **PSD:** The substrate has a coarse texture (greater than 2 mm fraction = 10.4 %; very coarse sand = 34.3 % and 55.3 % finer material as tabulated in Table 8) which allow seeds and nutrients to leach out (Baligar *et al.*, 2001:926). The coarse texture can therefore be amended by the additional application of SOM to improve the water holding capacity (Brady & Weil, 2008:515; Cooperband, 2002:2; Chan, 2008:2; Fenton

*et al.*, 2008:1; Marcel Dekker Inc., 1985:3; Singer & Munns, 1992:3; Van Deventer & Ferreira, 2012:21).

- **Nutrients:** Furthermore, this substrate has a very low C/N ratio of 1.8 as tabulated in Table 14. The S% is 0% and deficiencies will occur which can be observed as homogeneous yellowing of the plant after five weeks (Grobler *et al.*, 1999:205). This phenomenon was also observed in this experiment. A phosphorus deficiency is also anticipated in this growth medium with a low value of  $3.4 \text{ mg/kg}^{-1}$ . Although no deficiencies of K ( $60.7 \text{ mg/kg}^{-1}$ ), Ca ( $578.8 \text{ mg/kg}^{-1}$ ) and Mg ( $170.2 \text{ mg/kg}^{-1}$ ) occurred, the Mg/K ratio (8.8) was exceeding the ideal range as tabulated in Table 14. Yet, the Ca/Mg ratio (2.1) complied with the ideal range. Although the nitrogen of 0.1 % complies with the set standards, the carbon (0.2 %) is still deficient. This resulted in the deficient C/N ratio of 1.8 when compared to the natural C/N ratio of virgin soils set by Du Toit *et al.* (1994:75), that are based on natural C/N ratios of South African dry lands and virgin soils.
- **pH:** The granite saprolite (40.25% mean germination) has a pH ( $\text{H}_2\text{O}$ ) of 7.2, complying with the ideal pH range of 5 to 8 (Van Deventer & Ferreira, 2012:28).
- **EC:** The EC ( $43 \text{ mS/m}^{-1}$ ) is within acceptable limits.
- **CEC:** This growth medium has a CEC slightly below the ideal value of  $> 5 \text{ cmol/kg}^{-1}$  (refer to Table 15) (Van Deventer & Ferreira, 2012:28), with an anticipated value of  $4.3 \text{ cmol/kg}^{-1}$ . At low pH levels only a few type of soils hold exchangeable ions such as 2:1 type clays and allophane and some 1:1 type clays. With the raise in pH the negative charges on some of the 1:1 clays, allophane, humus, Fe and Al oxides increases, resulting in the increase of CEC (Brady & Weil, 2008:515). In order to obtain a measure of the maximum retentive capacity the CEC must be measured at a pH value of 7 or 8.2 (Hamza, 2008:32). The CEC reflects most of the pH-dependent charges as well as the permanent ones at a slightly alkaline or neutral pH (Brady & Weil, 2008:515).

The only rectifying method in current practice to increase the CEC is via additional application of substances with high CEC values (Hamza, 2008:32). Materials such as compost, specific clay materials, i.e. bentonite, smectite and zeolite have high CEC values (Baligar *et al.*, 2001:926; Foth, 1990:27). The alternative method is to create a pedogenic soil formation in the tailings to enhance soil formation processes. Pedogenic environments can be achieved by means of creating short-term favourable conditions for plant growth. Dormant



seeds slowly respire, and utilize food reserves within the seed and will initiate germination as soon as the ideal environment occurs (Jerrett & Gillen, 2015). If these conditions are set, weathering processes of the primary minerals will be accelerated in the tailings, subsequently producing secondary minerals that have a higher CEC (Van der Watt & Van Rooyen, 1995:177; Van Deventer & Ferreira, 2012:21). Weathering may be enhanced by the translocation of materials from overlying horizons (Van der Watt & Van Rooyen, 1995:177); however, this will not be promising if this substrate is utilised as a topsoil cover material.

The main factor influencing the relatively poor germination anticipated in the granitic saprolite substrate is texture, similar to all the Kimberlite tailing materials previously discussed. The substrate lack favourable physical properties such as good structure and water holding capacity. Hattingh (2012:1) & Bowden *et al.* (2007:11) stated that water is the elementary requirement for germination because it determines the speed of germination. Soil water (moisture) is considered as the most important environmental factor influencing seed germination (Yazdi *et al.*, 2013:1), for the seed requires water for the embryo to swell (Jerrett & Gillen, 2015) (enlarge) and break through the covering structures.

The next group of substrates all had a “bc” value in the Tukey HSD test tabulated in Table 13.

This statistical group has a number of main influencing factors. Firstly, the platinum tailings experience compaction, influencing root development. The relatively coarse texture of the Kalahari sand and acid lava saprolite is the reason for the low PAW anticipated, influencing germination of seeds. The kaolinite clay has a deficiency in phosphorus and the sulphur is non-existing. Both the nitrogen and sulphur are non-existent in the Potchefstroom red structured B soil and the red structured B soil. This statistical group performed better than the “b” statistical group due to an overall finer texture.

### ***Platinum tailings***

The platinum tailings (43.88% mean germination) delivered nearly a similar mean germination percentage as the Kalahari sand. This substrate does not differ statistically

significantly from the Kalahari sand, kaolinite clay, acid lava saprolite, Potchefstroom red structured B or red structured B.

- **PSD:** The platinum tailings (43.88 % mean germination) is a very fine growth medium with a well sorted PSD of greater than 2 mm fraction = 0.1 %; very coarse sand = 0.1 % and 99.8 % finer material (see Table 8). The majority of the finer material being in the fine sand fraction with a 49 % estimated.
- **PAW:** The PAW is determined as 11.0 % at 30 kPa (De Wet & Jansen, 2014:16). It is assumed that the platinum tailings will weather over time to produce a clay-rich soil due to the pyroxene minerals associated. If sufficient weathering had taken place, it might have explained the high PAW. However, the significantly lower PAW measured at high pressure (500 kPa to 1500 kPa) of 4.17 % signifies that the clay content is not high enough to dominate the water retention (De Wet & Jansen, 2014:16).
- **Nutrients:** A study done by Muller (2014:72) indicated that this substrate is associated with base metals due to the ore it is mined from, consequently resulting in a high base saturation (Muller, 2014:72). Both the nitrogen and carbon were, as expected, almost non-existent with values of 0.2 % and 0 % respectively. The Ca/Mg ratio was calculated as 6.0, exceeding that of the ideal value range, signifying no Ca deficiencies (Table 14). Although the calcium ( $325.4 \text{ mg/kg}^{-1}$ ) was ideal, deficiencies of both potassium ( $26.6 \text{ mg/kg}^{-1}$ ) and magnesium ( $33.1 \text{ mg/kg}^{-1}$ ) were identified (refer to Table 14). Aluminium toxicity will not be a contributing factor due to the high pH anticipated. This was validated by the extractable Al that was only  $0.01 \text{ mg/kg}^{-1}$  (Appendix 1). The S % was measured as 0.09 % indicating a deficiency. The phosphorus ( $6.0 \text{ mg/kg}^{-1}$ ) was also below the set standards as tabulated in Table 8. This substrate has a high ESP (exchangeable sodium potential) value of 12.9 % which contributes to the low germination percentage attained (Table 15) (Bloem *et al.*, 1992; Du Preez *et al.*, 2000).
- **pH:** This substrate is also slightly alkaline with a measured pH ( $\text{H}_2\text{O}$ ) value of 7.6. According to Muller (2014:72), the net acid potential of this medium was anticipated as -32 (ton/ha lime). The net acid potential measurements validated the pH findings, stating that the platinum tailings will not be subjected to acidic conditions and explain the high ESP.
- **EC:** This experiments' chemical analysis (Appendix 1) also revealed that the EC was  $218 \text{ mS/m}^{-1}$ .

- **CEC:** The CEC is below the ideal of  $> 5 \text{ cmol/kg}^{-1}$  (Van Deventer & Ferreira, 2012:28), with a value of only  $2.2 \text{ cmol/kg}^{-1}$ .

The main contributing factor that affected the germination in this substrate is compaction. Compaction in mine tailings is inevitable due to the lack of structure instigated by the homogenous texture of mine waste material and lack of organic material, as in the case of the platinum tailings (Bradshaw, 1998:262). Root development is stunted by compaction, which in turn ensures that vegetation establishment on mine waste materials is challenging (Bradshaw, 1998:262). Furthermore, structure influences the porosity, infiltration capacity, and root penetration (Gobat *et al.*, 2004:48; Winegardener, 1995:15).

### ***Kalahari sand***

Kalahari sand (46.63% mean germination) was derived from the same location as the granite saprolite.

- **PSD:** The PSD was measured as: greater than 2 mm fraction = 0.8 %; very coarse sand = 24.3 % and 74.9 % finer material, with the majority being in the coarse sand fraction with a value of 27.3 % determined (refer to Table 8). The clay content is very low and makes up only 2.1 % of the PSD.
- **PAW:** The relatively coarse texture is the reason for the low PAW anticipated, being 4.3 % at field capacity (30 kPa and 1500 kPa) (De Wet & Jansen, 2014:20). The PAW at permanent wilting point is extremely low with an insufficient 1.65 % anticipated. The PAW is therefore a good representation of the material's PSD.
- **Nutrients:** The total C content and N content are exceptionally low with 0.2 % and 0.1 % (LECO) respectively as tabulated in Table 14. No deficiencies of potassium ( $72.2 \text{ mg/kg}^{-1}$ ) and calcium ( $2185.1 \text{ mg/kg}^{-1}$ ) were identified. The magnesium ( $1097.0 \text{ mg/kg}^{-1}$ ), though, was available in excess, above the ideal value range. This resulted in the Mg/K ratio (49.9) exceeding the ideal range, as well as the Ca/Mg (1.2) ratio deficiency. The phosphorus (P) value ( $3.5 \text{ mg/kg}^{-1}$ ) anticipated was below the ideal limits, as is the case for all the substrates except the A - Horizon.
- **pH:** The pH ( $\text{H}_2\text{O}$ ) of 7.6 complies with the ideal range for vegetation establishment.

- **EC:** The EC was measured as 97 mS/m<sup>-1</sup>, well below the maximum threshold as tabulated in Table 14.
- **CEC:** The CEC of this substrate is significantly higher than the granite saprolite. It was measured as 19.5 cmol/kg<sup>-1</sup> (refer to Table 15) and is well above the minimum value (Van Deventer & Ferreira, 2012:28).

### ***Ottosdal kaolinite clay***

The kaolinite clay delivered a mean germination of 46.63 %.

- **PSD:** The PSD analysis conducted by Eco-Analytica revealed that the material is very fine with 44 % in the silt fraction. The remainder of the material is analysed as: greater than 2 mm fraction = 0.5 %; very coarse sand = 1.0 % and 98.5 % finer material as tabulated in Table 8. De Wet & Jansen (2014:16) revealed that the substrate derived from Ottosdal comprises 24 % clay and is dominated by 1:1 kaolinite clay.
- **PAW:** De Wet and Jansen (2014:13) stated that the silt together with the clay content in the substrate generates microscopic micro-pores as well as very small macro-pores, which release satisfactory amounts of PAW. According to De Wet & Jansen (2014:13), the PAW between 30 kPa and 1500 kPa was measured as 15.55 %.
- **Nutrients:** A deficiency of carbon (0.2 %) is anticipated in this substrate. However, nitrogen (0.06 %) complies with the set ideal values. This resulted in a C/N deficiency of 3.5. The phosphorus of 4.6 mg/kg<sup>-1</sup> is deficient, as is the case for most of the substrates utilised. However, the calcium (1238.0 mg/kg<sup>-1</sup>) potassium (109.8 mg/kg<sup>-1</sup>), magnesium (228.9 mg/kg<sup>-1</sup>) and Ca/Mg ratio (3.29) have no identified deficiencies. Still, a deficiency in the K/CEC ratio (3.16) was identified. The Mg/K ratio is also above the ideal value range (6.71) as tabulated in Table 14.
- **pH:** The pH (H<sub>2</sub>O) of 7.0 is also within the ideal standards for vegetation establishment (Van Deventer & Ferreira, 2012:28).
- **EC:** The electrical conductivity was measured as 59 mS/cm<sup>-1</sup>.
- **CEC:** Kaolinite has an adequate CEC of 8.9 cmol/kg<sup>-1</sup>.

## ***Acid lava saprolite***

Acid lava saprolite (46.75 % mean germination) achieved a slightly higher germination than the kaolinite clay (46.63 % mean germination).

- **PSD:** This material is still very coarse (greater than 2 mm fraction = 3.9 %; very coarse sand = 28.1 % and 68% finer material with the majority in the coarse sand fraction with 29.2%; refer to Table 8) for weathering is not yet finalised. The coarse texture allows seeds and nutrients to leach out (Baligar *et al.*, 2001:926).
- **PAW:** Substrates with poor water retention subsequently have a smaller amount of PAW (7.94 %) due to the lack of clay content (De Wet & Jansen, 2014:21). This can be amended by the additional application of SOM to counteract poor water retention (Brady & Weil, 2008:515; Cooperband, 2002:2; Chan, 2008:2; Fenton *et al.*, 2008:1; Marcel Dekker Inc., 1985:3; Singer & Munns, 1992:3; Van Deventer & Ferreira, 2012:21). It was also evident that much of the PAW (7.94 %) was already lost at low suctions (30 to 1500 kPa = 4.84 %) (De Wet & Jansen, 2014:21).
- **Nutrients:** Furthermore, the carbon (0.3%), sulphur (0%) and nitrogen (0%) are deficient as stated in Table 14. This substrate also has a deficiency in phosphorus (4.0 mg/kg<sup>-1</sup>) similar to most. No potassium deficiency was identified with a measured value of 63.7 mg/kg<sup>-1</sup>. Both the calcium (4297.4 mg/kg<sup>-1</sup>) and magnesium (1213.5 mg/kg<sup>-1</sup>) were exceeding the ideal values. This is a result of the Mg/K ratio above the ideal limit, and is measured as 62.2. The Ca/Mg ratio was yet within the ideal values, with an estimated 2.2.
- **pH:** The pH (H<sub>2</sub>O) value of 8.0 is marginal to the ideal pH values set.
- **EC:** The electrical conductivity was relatively low and measured at 27 mS/cm<sup>-1</sup>.
- **CEC:** This substrate has a high CEC value of 27.7 cmol/kg<sup>-1</sup>, as mentioned in Table 15. This might be due to the high Ca and Mg mineralogical (due to the pyroxene with general formula of X<sub>2</sub>Si<sub>2</sub>O<sub>6</sub>; where X is Mg, Fe, Mn, Li, Ti, Al, Ca or Na (Hamilton *et al.*, 2007:112)) content associated with the geology. The geology is associated with lavas, pyroclastic rocks and intercalated sediments (Almond, 2013:3; Van der Westhuizen *et al.*, 2006:196). This substrate will weather to a Vertic 2:1 swelling clay as mentioned in section 3.2.1. The area associated with this substrate is dominated by the red and dark-grey Vertic clays which can be classified as the Rensburg soil form (Vertic A-horizon on Unspecified B-horizon) (Fey, 2005:11; Fey, 2010:33; Meyer,

1984:190; Soil Classification Working Group, 1991:44). Higher uptake of water and improved water retention are consequences of Vertic soils (De Wet & Jansen, 2014:16). However, the saprolite material has not been subjected to sufficient weathering to develop a Vertic soil.

### ***Potchefstroom red structured B***

Potchefstroom red structured B achieved a germination of 48.00% (refer to Table 13).

- **PSD:** This fine substrate comprises mainly clay (28.7 %). This is the second highest clay% estimated for all substrates and is even above the average clay recorded for virgin soils of South Africa set by Steyn and Herselman (2006:233). The PSD analysis can be illustrated as greater than 2 mm fraction = 3.7 %; very coarse sand = 3.3 % and 93 % finer material as tabulated in Table 8. Soil structure is a consequence of the geometric positioning of the soil grains relative to each other (Winegardener, 1995:15). It is therefore explained as the aggregation of soil particles into clusters of particles that are separated from each other (Foth, 1990:38). According to Van der Watt and Van Rooyen as the “arrangement of primary soil particles into secondary units or peds” define structure. These secondary units might be arranged; however, usually are not; in the profile to give a distinctive characteristic pattern. Secondary units are classified based on the size, shape and degree of distinctness into classes, types and grades, respectively (Van der Watt & Van Rooyen, 1995:183). This substrate can be classified as “moderate structure” according to the definition provided by Van der Watt & Van Rooyen (1995:183). Moderate structure is where the peds are well formed, but not distinctly separated from one another in undisturbed soils. The structure is directly dependent on the mineralogy, texture, moisture content and organic matter of the soil. Structure influences the porosity, infiltration capacity, and root penetration (Gobat *et al.*, 2004:48; Winegardener, 1995:15).
- **PAW:** The PAW was not measured for this substrate, but due to the high clay content of this growth medium, a sufficient PAW can be predictable.
- **Nutrients:** The organic C (0.7 %; refer to Table 14) was unexpectedly within the ideal standards of South African virgin soils. However, both the nitrogen and sulphur were non-existent. No deficiencies were identified for potassium and calcium with

148.8 mg/kg<sup>-1</sup> and 1457.6 mg/kg<sup>-1</sup> respectively. Magnesium was above the set ideal range with 721.0 mg/kg<sup>-1</sup> projected. This resulted in the higher Mg/K ratio (15.6) and lower Ca/Mg ratio (1.2) anticipated.

- **pH:** The pH (H<sub>2</sub>O) of 7.4 is nearly neutral with no expected adverse effects on vegetation establishment.
- **EC:** The electrical conductivity was measured as 77 mS/cm<sup>-1</sup>.
- **CEC:** The CEC is measured as 13.5 cmol/kg<sup>-1</sup> as stated in Table 15 which complies with the ideal value of >5 cmol/kg<sup>-1</sup> (Van Deventer & Ferreira, 2012:28).

### ***Red structured B***

The B-red structured delivered a mean germination of 49.13% and performed the best in this statistical group.

- **PSD:** The PSD analysis can be illustrated as greater than 2 mm fraction = 1.3%; very coarse sand = 2.9% and 95.8% finer material as tabulated in Table 8.
- **PAW:** The PAW was not measured for this substrate. But due to the high clay content of this growth medium, a sufficient PAW can be predictable.
- **Nutrients:** The carbon content (0.9 %) is somewhat higher than that of the Potchefstroom B-red structured as tabulated in Table 14. This growth medium complies with the carbon content of natural virgin soils, which is unexpected for subsoil, saprolite and fresh tailings typically have low C and N concentrations. The nitrogen (0 %) was non-existing in this growth medium and is a result of an imbalance in the C/N ratio. The sulphur content was deficient with a 0.01 % anticipated. As is the case with most substrates, this medium had a phosphorus deficiency (3.8 mg/kg<sup>-1</sup>). Although no potassium (90.2 mg/kg<sup>-1</sup>) or calcium (1744.5 mg/kg<sup>-1</sup>) deficiency was reported, magnesium (642.1 mg/kg<sup>-1</sup>) exceeded the ideal value range. This resulted in Mg/K ratio (22.9) going above the ideal range as tabulated in Table 14. The Ca/Mg ratio (1.7) however did comply with the ideal range.
- **pH:** The pH (H<sub>2</sub>O) of 6.6 is nearly neutral with no expected adverse effects on vegetation establishment.
- **EC:** The electrical conductivity was measured as 37 mS/cm<sup>-1</sup> and is significantly lower than the Potchefstroom red structured B.

- **CEC:** This growth medium has a CEC value of 18.4 cmol/kg<sup>-1</sup> (see Table 15) which is well above the minimum ideal value set by Van Deventer and Ferreira (2012:28).

The next statistical group consists of one substrate (hard carbonate saprolite) and has a “c” value in the Tukey HSD test tabulated in Table 13. It is a well-graded medium with a coarse texture. This substrate performed a little bit better than the previous statistical group “bc” due to the sufficient amounts of carbon and nitrogen present.

### ***Hard carbonate saprolite***

The C-Hard carbonate substrate (50.13 % mean germination) did significantly better than all the Kimberlite and granite saprolite. The hardpan carbonate horizon is a continuous, very hard, massive layer cemented by carbonates (secondary limestone (Fey, 2005:12)) (Van der Watt & Van Rooyen, 1995:48) occurring in the lower A or in the B horizon (Brady & Weil, 2008:936; Fey, 2010:33; Soil Classification Working Group, 1991:36; University of Oxford, 2013:270; Van der Watt & Van Rooyen, 1995:48). The horizon is a barrier to roots and is slowly permeable to water (Fey, 2005:12). For the experiment the hard horizon had to be broken up and other soil characteristics could benefit this substrate.

- **PSD:** The PSD, however, is somewhat coarser in this case due to the mining activities that the horizon was subjected to. The PSD was analysed as greater than 2 mm fraction = 13.2 %, very coarse sand = 17.7 %, coarse sand 12.5 %, medium sand 13.7 %, fine sand 21.7 %, very fine sand 14.7 %, silt 12.4 % and clay 7.3 %. This substrate is well graded and lacks clay as stated in Table 8.
- **PAW:** The PSD is the reason for the PAW (13.30 %) being mostly released at low suctions (8.49 % at 30 kPa to 1500 kPa). The PAW of this substrate is more than that of the kaolinite clay and lesser than that of the Vertic clay horizon (De Wet & Jansen, 2014:20).
- **Nutrients:** Both the carbon (2.9 %) and nitrogen (0.04 %) were sufficient in this growth medium resulting in a very high C/N ratio of 73.25. However, the sulphur content was non-existent. Yet once more a phosphorus deficiency was identified with a value of 3.4 mg/kg<sup>-1</sup>. No potassium (88.7 mg/kg<sup>-1</sup>) or magnesium (67.3 mg/kg<sup>-1</sup>) deficiency was identified. Yet an Mg/K (2.4) ratio deficiency occurred. The calcium



(4248.0 mg/kg<sup>-1</sup>) was as expected available in excess as tabulated in Table 14. The Ca/Mg (38.6) complied with the ideal range set by the MVSA Bemestingshandleiding (2007:174).

- **pH:** The pH (H<sub>2</sub>O) of 7.9 is nearly alkaline with no expected adverse effects on vegetation establishment.
- **EC:** The electrical conductivity was measured as 56 mS/cm<sup>-1</sup> and is adequate.
- **CEC:** This growth medium has a CEC value of 11.4 cmol/kg<sup>-1</sup> (see Table 15) which is well above the minimum ideal value set by Van Deventer and Ferreira (2012:28).

The next statistical group comprises only the diabase saprolite and has a “cd” value in the Tukey HSD test tabulated in Table 13. Although the main influencing factor for this statistical group is the same as the Kimberlite coarse tailings, it has a better water-holding capacity.

### ***Diabase saprolite***

The diabase saprolite (50.50 % mean germination) differ statistical significantly from the kimberlite coarse, fine and Cullinan tailings as well as the granite saprolite as mentioned in Table 13.

- **PSD:** This coarse substrate has a PSD of greater than 2 mm fraction = 4.1 %, very coarse sand = 33.9 %, coarse sand = 28.9 %, medium sand = 16.4 %, fine sand = 10 %, very fine sand = 3.9 %, silt = 4.6 % and clay 2.2 %.
- **PAW:** This medium has a PAW of 9.92 % between field capacity (30 kPa) and permanent wilting point (1500 kPa) (De Wet & Jansen, 2014). If weathering is advanced, it will result in 2:1 smectite clays that have stronger water-retention capabilities.
- **Nutrients:** Mutually the carbon (0.2%), nitrogen (0%) and sulphur (0%) are deficient in this growth medium as specified in Table 14. The phosphorus content (3.5 mg/kg<sup>-1</sup>) is deficient, as is the case for most growth media. No deficiencies were identified for both the potassium (79.7 mg/kg<sup>-1</sup>) and calcium (2605.3 mg/kg<sup>-1</sup>). Furthermore, magnesium (1134.2 mg/kg<sup>-1</sup>) was abundant and above the ideal limits which resulted in an Mg/K ratio above the ideal range with a value of 46.5. The Ca/Mg was thus below the ideal value range with an assessment of 1.4.

- **pH:** The pH (H<sub>2</sub>O) of 7.42 is nearly neutral with no expected adverse effects on vegetation establishment.
- **Ec:** The electrical conductivity was measured as 92 mS/cm<sup>-1</sup> and is adequate.
- **CEC:** The CEC of this substrate (22.39 cmol/kg<sup>-1</sup>) specified in Table 15 is well above the minimum value of >5 cmol/kg<sup>-1</sup>.

The next statistical group comprises the gold tailings and has a “d” value in the Tukey HSD test tabulated in Table 13. This substrate performed the best based on germination percentages attained. It contains plenty of gypsum which draws hygroscopic water and consequently increases the water retention and as a result has greater germination.

### ***Gold tailings***

The substrate with the highest germination percentage (59.88% mean germination) attained is the gold tailings as stated in Table 13.

- **PSD:** The PSD of the gold tailings is very fine material with the majority in the fine sand fraction with 35.1 % as quantified in Table 8 (greater than 2 mm fraction = 0.2%; very coarse sand = 0.3% and 99.5% finer material).
- **PAW:** The plant available water (PAW) is measured as 21.7 % at field capacity (30 kPa) (De Wet & Jansen, 2014:16) which is the upper limit of PAW (Lambooy, 1983:3). The PAW estimated is higher than that of Kaolinite Clay with simply 15.5 % PAW. This seems unlikely; however gold tailings contains plenty of gypsum which draws hygroscopic water and consequently increases the water retention and as a result have greater germination (De Wet & Jansen, 2014:16; Van Deventer, 2011 a:6).
- **Nutrients:** The carbon content was deficient with only 0.2%. However, the nitrogen (0.01 %) content was sufficient and resulted in a relatively high C/N ratio of 22. The sulphur content of 0.3% is, however, satisfactory due to the pyrite minerals associated with the gold ore. The phosphorus with a value of 4.9 mg/kg<sup>-1</sup> and potassium 19.1 mg/kg<sup>-1</sup> is deficient. Calcium (1226.1 mg/kg<sup>-1</sup>) and magnesium (96.4 mg/kg<sup>-1</sup>) both complied with the ideal range. The potassium (19.1 mg/kg<sup>-1</sup>) was, however, deficient which resulted in the high Mg/K ratio of 15.1. The Ca/Mg ratio of 7.8 exceeded the ideal value similar to the Mg/K ratio. However due to deficient potassium content an

inadequate K/CEC of 0.78 is projected in Table 14. The potassium deficiency is most likely caused by the formation of jarosite ( $\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$  (University of Oxford, 2013:316)).

- **pH:** The substrate is slightly acidic with a pH ( $\text{H}_2\text{O}$ ) value of 5.7.
- **EC:** This substrate is also somewhat saline with an EC value of  $286 \text{ mS/m}^{-1}$  as specified in Table 14. According to Morgenthal (2003:13), EC values are higher than  $400 \text{ mS/m}^{-1}$  regarded as saline. However, plant growth can be affected at much lower concentrations ( $200 \text{ mS/m}^{-1}$ ) (Morgenthal, 2003:13). The highest EC determined for this growth medium was however still complying with the maximum value set by Van Deventer and Ferreira (2013:30). The latent liming amendments made to the gold tailings as discussed in section 3.3.4, counteracted heavy metal toxicity, which contributed to the high germination percentage anticipated. High EC represents a high soluble salt concentration, which has a negative effect on plant growth.
- **CEC:** The CEC is  $6.5 \text{ cmol/kg}^{-1}$  (refer to Table 15) and is good enough to satisfy vegetation requirements.

Although the gold tailings had the greatest germination, it is visually noticeable that the plants are much smaller, properly due to the stress factors such as crusting not being totally eradicated and the excessive salts present as illustrated in Photo 8 (Van Deventer, 2011 a:6). Furthermore, the optimum pH is 6.5 for phosphorus availability (Verheye, 2007:125).



**Photo 8:Smaller plants anticipated in the gold tailings when compared to A – Horizon.**

The phosphorus content in South African soils is naturally low and is therefore amended to an optimal state of 25 mg P kg<sup>-1</sup> (Verheye, 2007:125). Deficiencies cause an abnormal dark green (even purplish) colour in the leaves of young plants (Hopkins & Huner, 2009:69; MVSA Bemestingshandleiding, 2007:92; Uchida, 2000:32). Growth is stunted by this deficiency (Verheye, 2007:125) and can lead to short internodes and thin stems in grains (Hopkins & Huner, 2009:69), and impede node formation in maize (MVSA Bemestingshandleiding, 2007:92). The phosphorus deficiency symptoms will first occur on the older bottom leaves (Verheye, 2007:125) as a dark greenish-purple colour (anthocyanin pigments accumulation), and later as the severity increases the leaves might become malformed and exhibit necrotic spots (Hopkins & Huner, 2009:69). The purpling of leaves was observed in this experiment and as a result explains the stunted growth in the gold tailings to a certain extent. Therefore, a combination of contributing factors is responsible for this phenomenon.

## **Control A - Horizon**

Neither the vertisol nor the A - Horizon had extensive statistical analyses done on them. The reason is that these substrates were not extensively ameliorated and could therefore not be included in the ANOVA. The natural A - Horizon therefore serves solely as an ideal control reference, for one wants to achieve similar properties in subsoil, tailings and saprolite material. The virgin topsoil is uncultivated and suitable for proper vegetation establishment. The germination anticipated in this growth medium was analysed as 65 %.

- **PSD:** The PSD of the A - Horizon is very fine material with the majority in the fine sand fraction with 23.9 % as quantified in Table 8 (greater than 2 mm fraction = 0.9%; very coarse sand = 5.3% and 93.8% finer material).
- **PAW:** The PAW was not measured for this substrate. But due to the relative high clay content of this growth medium, a sufficient PAW can be predictable.
- **Nutrients:** It has satisfactory carbon (1.6%), nitrogen (0.1%), phosphorus (17.4 mg/kg<sup>-1</sup>), calcium (971.1 mg/kg<sup>-1</sup>) and magnesium (214.9 mg/kg<sup>-1</sup>) content. An adequate C/N ratio (14.5) and Ca/Mg ratio (2.8) was anticipated. Due to the high potassium content, an inadequate Mg/K ratio of 2.6 was anticipated.
- **pH:** The pH (H<sub>2</sub>O) of 6.5 is also ideal for optimal nutrient uptake, especially phosphorus (Verheye, 2007:125).
- **EC:** The electrical conductivity was measured as 23 mS/cm<sup>-1</sup> and is adequate.


Furthermore, the statistical analysis was done to identify the significant effects between substrates on the germination percentage over all ameliorants used. The significant differences at the 95 % probability level (Tukey HSD) test are indicated in Table 16. The p-value of  $p < 0.05$  differs highly statistical significantly and is indicated in red. Thus, only the significant values are displayed. The independent variables are the known, or predictor, variables. These are the X-axis values. When the independent variables are varied, they result in corresponding values for the dependent, or response, variables, most often assigned to the Y-axis.

This interaction table (Table 16) illustrates the significant differences between the substrates. The significant differences are consequences of the adverse physical and chemical

properties. It is evident from this table that gold tailings differ statistically significantly from all the substrates except the Cullinan Kimberlite tailings. The gold tailings were also identified as the best growth medium. The lowest germination attained was in the Kimberlite coarse tailings. The germination percentages mentioned in this regression table for the various substrates are visually represented in Figure 9.

**Table 16: Tukey HSD test; Significance between substrates on the germination % irrespective of ameliorant used**

<b>Tukey HSD test 95 % confidence level; variable mean germination %</b> Approximate Probabilities for Post Hoc Tests Error: Between MS = 172.37, df = 390.00													
<b>Substrate</b>	Kimberlite Coarse 30	Kimberlite Fine 40	Cullinan Kimberlite 40	Granite Saprolite 40	Rustenburg Platinum 44	Kalahari Sand 47	Kaolinite Clay 47	Acid Lava Saprolite 47	Potchefstroom Red Structured B 48	Red Structured B 49	Hard Carbonate 50	Diabase Saprolite 51	Gold 60
<b>Kimberlite Coarse Tailings</b>			0.043	0.021	0.000	0.000	0.000				0.000		0.000
<b>Kimberlite Fine Tailings</b>	0.043										0.021		0.000
<b>Cullinan - Kimberlite</b>													
<b>Granite Saprolite</b>													0.000
<b>Rustenburg Platinum</b>													0.000
<b>Kalahari Sand</b>													0.000
<b>Kaolinite Clay</b>													0.000
<b>Acid Lava Saprolit</b>	0.000												0.000
<b>Potchefstroom Red Structured B</b>	0.000												0.004
<b>Red Structured B</b>	0.000												0.016
<b>Hard Carbonate</b>			0.021	0.043									0.049
<b>Diabase Saprolite</b>	0.000	0.013	0.013	0.028									
<b>Gold Tailings</b>			0.000										

 = p = < 0.05

### 5.1.2 Ameliorant as main effect

This experiment portrays the effect of different types of ameliorants on germination as stated in the objectives (section 1.4). However, amelioration methods seem to have a significant influence on the germination in rehabilitation practises in Table 11. One must thus distinguish between the methods in order to determine which treatment delivered the highest germination percentage. The different amelioration methods are thoroughly discussed in section 3.3.

The Tukey HSD results for ameliorants are displayed in Table 17. Mean germination percentages are displayed in Figure 10. Means within a column with the same letter(s) are not significantly different at the 95% probability level (Tukey HSD test).

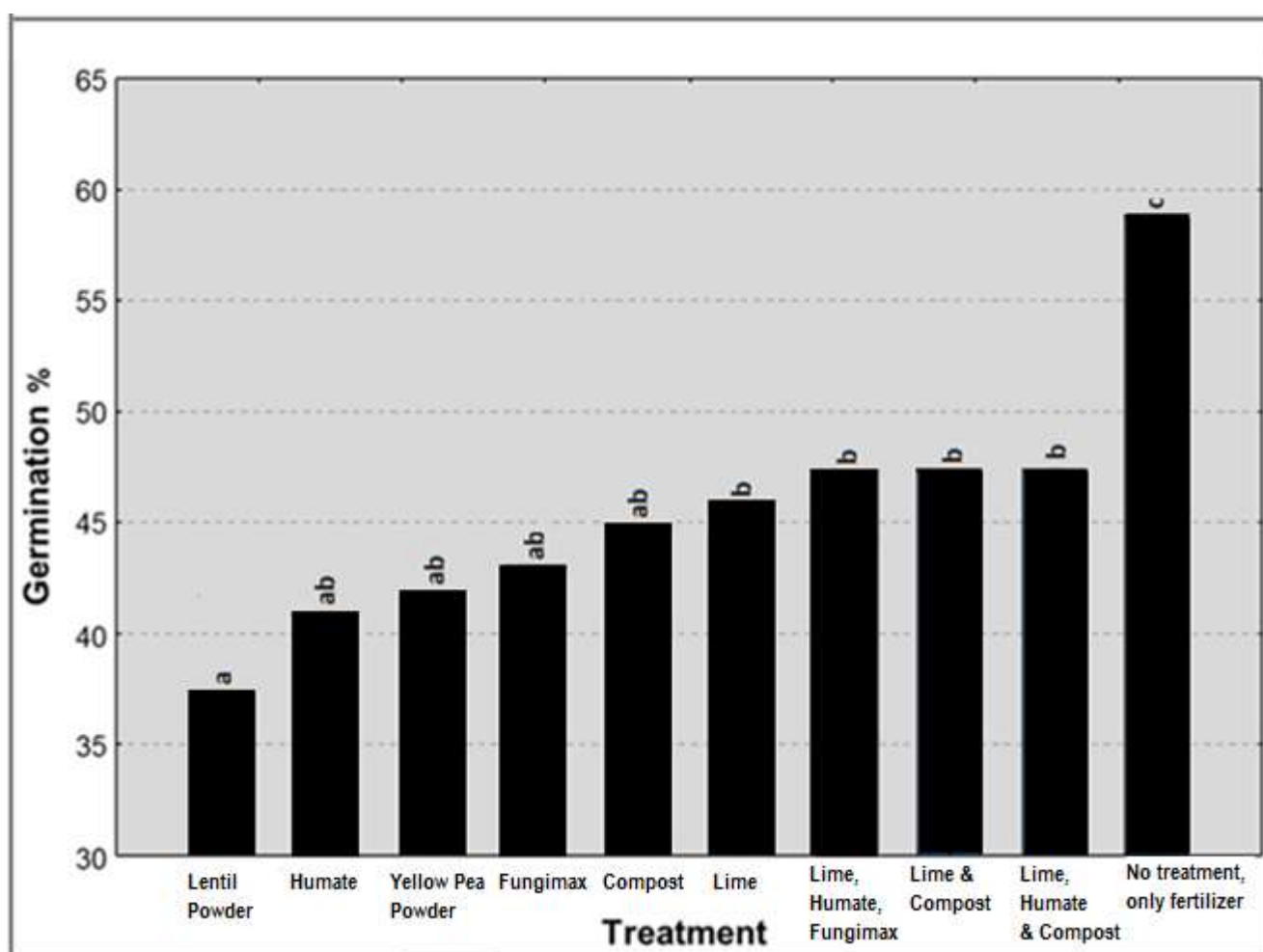


Figure 10: Effect of treatment on germination %



The highest percentage attained was with no amelioration application (58.75 %) which differs significantly from all other ameliorants (refer to Table 13). One can only state that a combination of a number of influencing factors played a role in this phenomenon.

The coated seed was a major influencing factor. The coating provides improved water access through the soil, protects against fungal attacks and insect predation, and provides sufficient nutrients to the seed. The improved seed soil contact is essential for the germination process due to better access to water (Advanced Seed, 2014:4).

The lime (45.87%); lime, humate, fungimax combination (47.21%); lime and compost combination (47.40%); and lime, humate, compost combination (47.40%) are grouped together as the “b” group. This group does not differ statistically significantly from any of the amelioration methods except lentil powder and no treatment as illustrated in Table 13. The regression table summarises the interactions in Table 18. The fungimax, compost and humates also have the same characteristics of the coating such as an increased nutrient exchange due to the chelated minerals (readily available for plant uptake). The lime combinations increase the pH of the substrates whilst counteracting heavy metal toxicity to the grass. Lime also decrease the competition between the ameliorant and the seed coating when added to humates, fungimax and compost. Yet again, water-holding capacity is increased by the application of SOM, fertilizer or lime (Haynes & Naidu, 1998:123). Lime also plays a role in water transport. Clay particles are negatively charged, whereas both Ca and Mg are positively charged. Calcium is a much larger molecule when compared to magnesium. Thus, calcium will create macro-pores and magnesium micro-pores when attached to two clay particles. Water is much easier to extract from the micro-pores due to lower adhesion forces as in the case of micro-pores. Thus, micro-pores will need stronger forces to extract the water from the soil by the plants. The PAW thus plays a significant role in germination attained (Nel, 2015: Personal correspondence).

The “ab”-group (refer to Table 13) does not differ statistically from lentil powder and amelioration combinations containing lime, but does differ statistically significantly from lentil powder and the control. Humate (40.96 %), yellow pea powder (41.83 %), fungimax (42.98 %) and compost (44.81 %) all fall in the same statistical group and thus do not differ


statistically from one another. The fungimax, compost and humates treatments compete with the coating. And due to nutrient exchange processes, it is easier to extract the readily available nutrients from the seed as the soil. The humates, fungimax and compost thus deprive the coated seed from the essential nutrients and water which might result in lower germination percentages. Generally, ameliorates are applied to soil, because it has the same characteristics as the coating. These characteristics include lime coating which counteracts acidity, heavy metal toxicity and increases the water-holding capacity; insecticide and fungicide which none of the additional treatments has; protective polymer which reduces dust on the seed; binding polymers to keep the coating together; additional nutrients that act as reservoirs of food for the seedling and the soil micro-organisms; and growth stimulants resulting in higher grass lengths attained. Lime is also known to improve the water availability for vegetation (Advanced Seed, 2014:2-8; Haynes & Naidu, 1998:123; Nel, 2015: Personal correspondence).

The “a” group consists solely of lentil powder and delivered a germination percentage of 37.21% (refer to Table 13). Lentil powder performed the worst because it did not dissolve and is therefore not readily available for plant uptake. It also forms a paste which does not want to mix with anything which has hydrophilic properties that extract all the water from the soil into the paste, resulting in water deficiencies for seeds.

Although Experiment 1 investigated both the amelioration and substrates, there is no set default solution for rehabilitation practices. Each problem is site-specific and an individual has to integrate and investigate various technical aspects prior to implementation of rehabilitation practices. This interaction table (Table 17) illustrates the significant differences between the ameliorants. The significant differences are consequences of the adverse physical and chemical properties. It is evident from this table that the “no treatments” differ statistically significantly from all the ameliorants. The “no treatments” were also identified as the best growth condition due to the properties of the coated seed. The lowest germination attained was from the lentil powder which differs statistically significantly from the ameliorants containing lime and the no treatment amelioration. The germination percentages mentioned in this regression table for the various substrates are visually represented in Figure 10.

Table 17: Tukey HSD test; significant prediction of difference between ameliorants on germination % over all substrates

<b>Tukey HSD test 95% confidence level; variable mean germination %</b> Approximate Probabilities for Post Hoc Tests Error: Between MS = 172.37, df = 390.00										
Ameliorant	Lentil powder 37	Humate 41	Yellow pea powder 42	Fungimax 43	Compost 45	Lime 46	Lime, humate, Fungimax 47	Lime, humate, compost 47	Lime & compost 47	No treatment, only fertiliser 59
Lentil powder							0.004			
Humate										
Yellow pea powder										
Fungimax										
Compost										
Lime	0.027									
Lime, humate, fungimax										
Lime and compost	0.003									
Lime, humate, compost	0.003									
No treatment at all, only fertiliser	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

 = p = < 0.05

### 5.1.3 Microbial activity

Soil microbes occur mainly in the first few centimetres (0-15 cm) of a soil profile (Hoorman & Islam, 2010:1) and contribute to the plant growth in various ways (Doi & Ranamukhaarachchi, 2009:223). Soil microbial activity is predicted by means of measuring the dehydrogenase activity (Casida, 1977:130). Dehydrogenase activity is defined as “the overall metabolic activity of all the microorganisms such as bacteria, fungi, actinomycetes, protozoa, algae and micro fauna that occupy the soil” (Subhani *et al.*, 2001:333). Factors such as the soil aeration, the soil moisture content (Kumar *et al.*, 2013:900), soil pH, nutrient availability, organic matter, addition of pesticide, heavy metals and manure (Subhani *et al.*, 2001:337) influences the microbial activity. The microbial activity also plays a significant role in the soil ecosystem’s development, functioning (Subhani *et al.*, 2001:333), stability and fertility (Kumar *et al.*, 2013:898). The microbial activity and C/N ratios of certain substrates are tabulated in Table 18 below.

**Table 18: Dehydrogenase (microbial activity) and C/N ratio of certain substrates**

Substrate	Dehydrogenase	C/N
	INF µg/g/2h	
<b>Cullinan - Kimberlite Tailings</b>	9.048	n.a.
<b>Granite Saprolite</b>	3.864	1.8
<b>Platinum Tailings</b>	2.228	n.a.
<b>Kalahari Sand</b>	4.135	1.9
<b>Ottosdal Kaolinite Clay</b>	7.033	3.5
<b>Potchefstroom Red Structured B</b>	13.572	n.a.
<b>Hard Carbonate Saprolite</b>	n.a.	73.25.
<b>Gold Tailings</b>	0.962	22
<b>Vertisol</b>	n.a.	11.07
<b>A - horizon Topsoil</b>	115.315	14.5
<b>Ideal Values</b>		<b>12.74<sup>1</sup></b>

<sup>1</sup> Du Toit *et al.* (1994:75) based on natural C/N ratios of South African dry lands and virgin soils; n.a. = not available

The substrate that has the highest microbial activity was identified as the A-Horizon with a value of 115.315 (INF $\mu$ g/g/2h) and confirms what Hoorman & Islam stated (2010:1). This is due to the gas exchange between the atmosphere and the natural topsoil. This material also contains enough carbon and nitrogen to sustain the microbes. Additionally, the A – Horizon is the only substrate that is associated with the first few centimetres of the soil profile. The rest of the growth media are either tailings or subsoil.

## **5.2 Experiment 2: Influence of C/N ratio and substrate on grass germination**

A second experiment was suggested in order to investigate whether the C/N ratio might influence the germination percentages achieved, and if so, to what extent. This will then give insight to the best possible C/N (ratio).

Experiment 2 differentiates between the most suitable C/N ratios for various substrates as stated in the objectives (section 1.4). Only four substrates were utilised in this experiment, i.e. Potchefstroom Red Structured B, Potchefstroom A - Horizon, Rustenburg - platinum tailings and the Stilfontein gold tailings. Four C/N ratios were used; a blank control (C/N 0), 12,5/1 C/N ratio, 25/1 C/N ratio and 50/1 C/N ratio. Only the humates could be utilized, for it comprises low quantities of N and the highest natural C content. Application of 20 L/ha humates as highest dose was recommended (Omnia Nutriology, 2013:6). Furthermore, LAN fertilizer (high N content) was added at different amounts to manipulate the C/N ratio. For a comprehensive overview on the calculations, refer to Appendix 2.

Table 19 provides a summary of the analysis of variance (ANOVA) done on the germination percentage for experiment 2.

It was found that substrate as main effect influences germination statistically, whilst the C/N ratios had no significant influence on germination for the both grass species. There were also no significant effects based on the interactions between the C/N ratios and substrates (Table 19).

**Table 19: The ANOVA results done on the effect of substrate, C/N ratio as well as the interaction between substrate and C/N ratio on germination percentage**

Single factors	Rhodes grass	Couch grass
	p – values	p – values
Substrates	0.000	0.0000
C/N ratio	0.6184	0.500
Interaction (substrate x C/N ratio )	0.7259	0.897

The Tukey's Honestly Significant Difference test at the 5% level of significance was used to determine statistically significant differences between means (Table 20). Only the substrates will be discussed comprehensively because it was the only significant factor identified in the ANOVA (Table 19).

**Table 20: Effect of substrate, C/N ratio and interaction on germination percentage**

Single factors	Rhodes grass		Couch grass	
	Average Final Germination %	Tukey HSD test	Average Final Germination %	Tukey HSD test
<b>Substrates</b>				
Platinum Tailings	24.28	a	1.42	a
Potchefstroom Structured B Red	49.28	b	20.72	a
A – Horizon	67.14	b	19.28	a
Gold Tailings	90.00	c	68.58	b
<b>C/N ratios</b>				
0	58.58	-	27.86	-
12.5/1	51.42	-	27.86	-
25/1	60.72	-	21.42	-
50/1	60.00	-	32.86	-
<b>Interaction</b>				
Platinum Tailings x 0	25.72	a	0	a
Platinum Tailings x 12.5	20.00	a	2.86	a
Platinum Tailings x 25	25.72	a	0	a
Platinum Tailings x 50	25.72	a	2.86	a
Potchefstroom Structured Soil x 0 Red	42.86	abc	14.28	abc
Potchefstroom Structured Soil x 12.5 Red	40.00	ab	31.42	abcd
Potchefstroom Structured Soil x 25 Red	62.86	abc	8.58	ab
Potchefstroom Structured Soil x 50 Red	51.42	abc	28.58	abcd
A - Horizon x 0	85.72	bc	20.00	abc
A - Horizon x 12.5	54.28	abc	20.00	abc
A - Horizon x 25	62.86	abc	14.28	abc
A - Horizon x 50	65.72	abc	22.86	abc
Gold Tailings x 0	85.72	bc	77.14	d
Gold Tailings x 12.5	85.72	bc	57.14	bcd
Gold Tailings x 25	91.42	bc	62.86	cd
Gold Tailings x 50	97.14	c	77.14	d

### **5.2.1 Substrate as main effect**

According to the ANOVA done on the germination data, a significant difference for substrates was identified (Table 19). This section will discuss significant interactions between substrates based on Table 20. The same characteristics of the substrates discussed in section 5.1.1 are applicable to this section.

#### ***Platinum tailings***

The platinum tailings with the Tukey HSD symbol “a” differed statistically significantly from the Potchefstroom red structured B “b”, A – Horizon “b” and Gold Tailings “c” for Rhodes grass. In the case of couch grass, a significant difference was identified between the platinum tailings “a” and the gold tailings “b”. There was no significant difference between the platinum tailings, A – Horizon and gold tailings because all three have the same Tukey HSD symbol of “a” (refer to Table 20).

The lowest overall germination for both grass species was attained by the platinum tailings. There is no statistically significant difference between the Rhodes and couch grass based on the Tukey HSD test results (refer to Table 20). Rhodes performed better, even though there is no statistically significant difference. The low germination reported (Rhodes: 24.28 % mean germination; Couch: 1.42 % mean germination) for this growth medium, is due to the associated compaction characteristics, lack of certain nutrients, and low CEC value (discussed in section 5.1.1). This also indicates that the Rhodes grass might be initially more resilient to the physical and chemical stress factors involved.

#### ***Potchefstroom red structured B***

The second lowest germination reported for the Rhodes grass was attained by this growth medium. There is a statistically significant difference between the platinum (Tukey HSD: “a”), gold tailings (Tukey HSD: “c”) and the Potchefstroom red structured B (Tukey HSD: “c”) growth medium for the Rhodes grass; verified by the Tukey HSD test’s symbolic differences in Table 19. The emergence of plants in the Potchefstroom red structured B was double that of the platinum for Rhodes grass. This substrate is has a fine texture and was classified as a



sandy clay loam (refer to Figure 5). Deficiencies of nitrogen and sulphur were identified. High amounts of magnesium probably hampered the germination of Rhodes grass (Cook *et al.*, 2005).

There is a significant difference between the grass species. This might be due to structure not fully developed in this substrate, because it was sieved prior to use.

The second best germination percentage reported for couch grass was achieved by the Potchefstroom red structured B soil. There is no statistical difference between the platinum tailings, Potchefstroom red structured B soil and gold tailings for couch grass.

### **A - Horizon**

The second best germination attained for Rhodes grass was from the A - Horizon. There is no statistical difference for Rhodes grass (both had a Tukey HSD test symbol “b”) between the A-Horizon and the Potchefstroom Red Structured B soil. However, there is a significant statistical difference between the Rhodes and couch grass for the A-Horizon. Rhodes grass delivered germination 3.5 times better than the couch grass (Rhodes: 67.14% mean germination; couch: 19.28% mean germination). There is no statistical difference between the platinum tailings, Potchefstroom red structured B and the A-Horizon for the Couch grass. The adequate carbon, nitrogen calcium, magnesium and phosphorus content is the reason for the A-horizon’s good performance (refer to Table 14; section 5.1.1). This substrate was identified as loamy sand (Table 8) and is suitable to sustain plant growth.

### **Gold tailings**

The highest overall average germination attained for Rhodes (90.00 % mean germination) and Couch grass (68.58 % mean germination) was by the Gold Tailings. There is a significant statistical difference between the grass species for this substrate, validated by a Tukey HSD test symbol difference. The Rhodes grass sown in the gold tailings differ statistically significant from all the results in the experiment. It suggests that the Rhodes grass in Gold Tailings combination will achieve the best possible germination. This better

germination achieved is the result of the gypsum content; which in turn makes the substrate hygroscopic and improves water availability to the seed. Even though this substrate delivers good germination, it is visually noticeable that the plants are much smaller than the rest of the substrates'. The reason being, this growth medium lacks some essential nutrients such as phosphorus and carbon when compared to the A-Horizon (refer to Table 14; section 5.1.1). Water is compulsory for germination to occur. Nutrients become increasingly more important for growth of the plant after germination.

### **5.2.2 General observations**

It can be observed from Table 20 that Rhodes grass germinated much better than couch grass. This is possibly due to the adaptability of grass species. When calculating the mean germination percentage of the species, Rhodes grass performed significantly better with a mean germination of 57.68 % when compared to the couch grass with a mean germination of 27.50 %.

If only one couch seed germinated on that specific tailings medium, its seed produced by the established plant was adapted to those specific growth conditions. The vegetative clones created will then cover the area and its seeds will deliver higher germination afterwards with a smaller gene pool. Thus, although the lab conditions produce much higher germination (up to 80% mean germination) compared to  $\pm 23$  % mean germination in field conditions, vegetative clones in field conditions are much more resilient once established (Nel, 2015: Personal correspondence).

Both the couch and Rhodes grasses produced more stolons and fewer seeds under stress conditions. Couch grass is more selective with ideal germination conditions when compared with Rhodes grass. When using seeds in rehabilitation you are creating a seed bank, and in the event of “disasters” such as veld fires and heavy rains, couch grass will act as a pioneer and cover the area that prevents erosion. This is not likely with vegetative growth such as using stolons or rhizomes with only a selected gene pool. The gene pool can thus not be adapted to only one condition, because if natural conditions should change, like rainfall or fires, it might result in seed dormancy (Nel, 2015: Personal correspondence).

Couch grass is both a pioneer and climax species. Couch grass will grow as a pioneer in shallow and rocky soils. However, in rehabilitation practices the engineered soils (tailings) are already stable prepared, and couch grass will thus act as climax species whereas Rhodes grass is a sub-climax species and will dominate initially. Rhodes grass begins to die off after three years when its life-cycle is completed and the couch grass will then take over by acting as climax species. This also explains the higher germination percentages attained in Table 20 (Nel, 2015: Personal correspondence).

Thus in rehabilitation practices, Rhodes grass will germinate quicker ( $\pm 10$  days) dominating in the short term; whereas couch grass takes longer to germinate ( $\pm 31$  days) and will dominate in the long term (Nel, 2015: Personal correspondence).

All the Rhodes grass in the platinum tailings germinated significantly less than that of the gold tailings (refer to Table 20). The gold tailings had the highest germination percentage. Germination for couch grass, performed significantly worse compared to the gold tailings, which is consistent with the ANOVA results.

Thus, for Rhodes grass established in substrates based on the germination attained can be statistically summarised as: Gold Tailings is smaller than A – Horizon, which is equal to Potchefstroom Red Structured B, which is smaller than Platinum Tailings. Couch grass established in substrates based on the germination attained can be statistically summarised as: Gold Tailings is smaller than Potchefstroom Red Structured B, which is equal to A – Horizon, which is equal to Platinum Tailings.

### **5.3 Experiment 3: The influence of C/N ratios as applied to substrates on the growth potential**

Due to the stunted plant growth that was experienced in the gold tailings and Potchefstroom red structured B of Experiments 1 and 2, it was deemed necessary to investigate the growth potential of the grass. The stunted grass is illustrated in Photo 9. This experiment measured

the grass length and was done at the same time as experiment 2. Therefore, the grass length was determined from the grasses germinated in experiment 2.



**Photo 9:** Visual difference between the Gold Tailings and A-Horizon's growth.

The growth potential was determined from the grasses that emerged during Experiment 2. The ANOVA consisted of three factors: substrate, C/N Ratio and the interaction effect between substrate and C/N Ratio based on the grass length (refer to Table 21). The only statistical significant difference was identified as substrate.

**Table 21:** The ANOVA results done on the effect of substrate and C/N ratio on mean grass length as well as the interaction between substrate and C/N ratio on grass length

Single Factors	p – Value
Substrates	0.0000
C/N ratio	0.6230
Interaction (Substrate x C/N ratio )	0.9321

A *post hoc* test was conducted to establish where the differences lie. The results for the Tukey test based on mean grass length per substrate are given in Table 22. The interactions from Table 22 are visually illustrated in Figure 11. Significant effects for substrate were identified (Table 21) based on grass lengths.

**Table 22: Effect of substrate, C/N Ratios and interaction on mean grass length**

Single factors	Average grass length	Tukey HSD test
<b>Substrates</b>		
Platinum Tailings	282.21	b
A - Horizon	275.37	b
Gold Tailings	107.36	a
Potchefstroom Red Structured B Soil	84.83	a
<b>C/N ratio</b>		
0	177.52	-
12.5	215.49	-
25	168.71	-
50	188.04	-
<b>Interactions</b>		
Potchefstroom Red Structured Soil x 0	64.19	a
Potchefstroom Red Structured Soil x 12.5	76.88	a
Potchefstroom Red Structured Soil x 25	98.54	ab
Potchefstroom Red Structured Soil x 50	99.70	ab
Gold Tailings x 0	118.49	ab
Gold Tailings x 12.5	106.20	ab
Gold Tailings x 25	104.91	ab
Gold Tailings x 50	99.83	ab
A - Horizon x 0	232.62	ab
A - Horizon x 12.5	351.96	b
A - Horizon x 25	230.90	ab
A - Horizon x 50	286.00	ab
Platinum Tailings x 0	294.79	ab
Platinum Tailings x 12.5	326.93	ab
Platinum Tailings x 25	240.50	ab
Platinum Tailings x 50	266.64	ab

Water is the main factor influencing germination and water combined with nutrients is essential for plant growth.

Macro-nutrients for plants include nitrogen (N), phosphorus (P), potassium (K), hydrogen (H), carbon (C), oxygen (O), magnesium (Mg), calcium (Ca) and sulphur (S) (MVSA Bemestingshandleiding, 2007:82).

Phosphorus is important for plant growth, root development and photosynthesis (Hopkins & Hüner, 2009:69). Deficiencies include a dark green to purple colour in leaves, stunted growth, short internodes and thin stems (Hopkins & Hüner, 2009:69; MVSA Bemestingshandleiding, 2007:92; Verheye, 2007:125; Uchida, 2000:32). The phosphorus content should ideally be higher than  $13.4 \text{ mg/kg}^{-1}$ , based on the natural grass pastures (MVSA Bemestingshandleiding, 2007:92).

Nitrogen is used in plants and is essential for several proteins, hormones and chlorophyll (Hopkins & Hüner, 2009:68). Nitrogen should be at least 0.001 % based on the natural content in South African dry lands and virgin soils (Du Toit *et al.*, 1994:75). Symptoms of nitrogen deficiency in plants are slow growth and chlorosis of the leaves (Hopkins & Hüner, 2009:69).

Potassium is vital for protein synthesis. This micro-nutrient is an enzyme activator in photosynthesis, thickening of cell walls and stalk strength, and serves as an osmoregulation (closing and opening of the stomatal cells) (Hopkins & Hüner, 2009:69; MVSA Bemestingshandleiding, 2007:90; Uchida, 2000:31). Potassium (osmoregulation) is essential for rehabilitation due to the water limitations (low water retention) associated with mine waste materials (Van Deventer & Hattingh, 2004:62). The potassium content should ideally range between  $40 - 250 \text{ mg/kg}^{-1}$  (MVSA Bemestingshandleiding, 2007:92). Deficiencies include mottling, chlorosis, necrotic lesions, death of leaf margins, shortened and weakened stems (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:93; Uchida, 2000:31; Verheye, 2007:125). However,  $\text{K}^+$  will have a bigger inhibition effect on germination percentage than  $\text{Na}^+$  independently of the accompanying anion due to the osmotic effects involved (Sosa *et al.*, 2005:261).

Carbon, hydrogen and oxygen are necessary for the structural backbone of all organic molecules. Carbon supply the plant with energy and nitrogen builds the tissue for the plant (Miller, 2000:20). The carbon content must be ideally greater than 1.3 % based on the dry land use type of South Africa (Du Toit *et al.*, 1994:75). Carbon deficiencies result in plant starvation, whilst water deficiencies are the consequence in the desiccation of the plants (Hopkins & Hüner, 2009:69).

Sulphur is imperative in the structure of proteins. Sulphur should be ideally 0.12 % based on sugarcane (Meyer *et al.*, 1971:204). Deficiencies in sulphur initiate chlorosis of the plants due to reduced protein synthesis. Calcium is essential for cell division and deficiencies occur in the meristematic regions (Hopkins & Hüner, 2009:70). Calcium should ideally range between 200 – 3000 mg/kg<sup>-1</sup> (MVSA Bemestingshandleiding, 2007:172-304). Magnesium is essential for chlorophyll formation; serves as an activator for enzymes; and is involved in the translocation of phosphorus (Hopkins & Hüner, 2009:70; MVSA Bemestingshandleiding, 2007:91). The ATP molecule is linked to the active site of the enzyme by magnesium (Mg<sup>2+</sup>). Magnesium should range ideally between 50 – 300 mg/kg<sup>-1</sup> (MVSA Bemestingshandleiding, 2007:172-304).

For a more detailed overview on plant nutrients, refer to section 2.2.2.

Moreover, essential trace elements are required in small concentrations for biological functions (Herselman, 2007:6). However, they can be toxic at higher concentrations. Herselman (2007:16) stated that soil pH is a dominant factor that controls the bio-availability of metal trace elements. Most metal trace elements availability reduces as the pH increase. However, arsenic (As), molybdenum (Mo), and chromium (Cr) are more mobile under alkaline conditions (Herselman, 2007:16).

As mentioned before, the phosphorus content is low in all the growth media utilised except the natural A - Horizon. These substrates require amendment to an optimal state of >13.4 mg/kg<sup>-1</sup> (natural grass pastures) (MVSA Bemestingshandleiding, 2007:304). Deficiencies cause an abnormal dark green (even purplish) colour in the leaves of young plants (Hopkins & Hüner, 2009:69; MVSA Bemestingshandleiding, 2007:92; Uchida, 2000:32). Growth is

stunted by this deficiency (Verheye, 2007:125). The phosphorus deficiency symptoms will first occur on the older bottom leaves (Verheye, 2007:125) as a dark greenish-purple colour (anthocyanin pigments accumulation), and later as the severity increases the leaves might become malformed and exhibit necrotic spots (Hopkins & Hüner, 2009:69). The purpling of leaves was observed in both experiments. Other deficiencies such as N, C and Mo were also identified and are summarised in Appendix 1.

Organic matter contributes to soil quality and is therefore a reflection of the soil to generate and sustain plant growth (Van Deventer & Hattingh, 2004:5). Anthropogenic soils undergo a reduction of SOM and related nutrients such as nitrogen (Wong, 2003:776). This deficiency can be partially rectified via the addition of organic matter and fertilizer. (Bradshaw, 2000:89; Schroeder *et al.*, 2005:318). SOM contributes to soil water-holding capacity, infiltration and porosity and adds nutrients in a slow-release organic form (Schroeder *et al.*, 2005:318).

### **Potchefstroom red structured B**

The Potchefstroom red structured B has high clay content and provided the lowest average grass length.

- **N:** The nitrogen is non-existent in this growth medium. Chlorosis and slow growth are consequential of this deficiency.
- **P:** Phosphorus is important for plant growth, and this substrate has a deficient P-content of 3.5 mg/kg<sup>-1</sup>.
- **K:** The potassium in this growth medium is sufficient with a value of 148.9 mg/kg<sup>-1</sup>.
- **Other:** The sulphur content in this medium is also non-existent. A high Mg value of 721.0 mg/kg<sup>-1</sup> was identified.

### **Gold tailings**

Even though good germination percentages are attained within this substrate, growth is still unsatisfactory.



- **N:** The nitrogen content is sufficient in this growth medium with a value of 0.1 % anticipated.
- **P:** Phosphorus is important for plant growth, and this substrate has a deficient P-content of 4.9 mg/kg<sup>-1</sup>.
- **K:** The potassium in this growth medium is deficient with a value of 19.1 mg/kg<sup>-1</sup> due to the formation of jarosite. Osmotic problems might occur.
- **Other:** The sulphur content in this medium is also non-existent. A high Mg value of 721.0 mg/kg<sup>-1</sup> was identified. The Mg content is also above the ideal with a measured value of 721 mg/kg<sup>-1</sup> (Table 14). Gold Tailings has deficiencies in C with a value 0.2 %. There is also Ni (1.9 mg/kg<sup>-1</sup>) and Zn (5.2 mg/kg<sup>-1</sup>) toxicity identified for this substrate as indicated in Appendix 1. Zinc toxicity symptoms in plants include reduced growth and development (Kabata-Pendias, 2011:284; Nagajyoti *et al.*, 2010:206). Toxic Ni concentrations in soils can stunt plant growth (Kabata-Pendias, 2011:242; Nagajyoti *et al.*, 2010:209). This explains the stunted growth experienced in the gold tailings mentioned in Experiments 1 and 2.

## A - Horizon

The A – Horizon is a suitable growth medium for vegetation establishment.

- **N:** The nitrogen content is sufficient in this growth medium with a value of 0.1 % anticipated.
- **P:** Phosphorus is important for plant growth, and this substrate has a sufficient amount of 17.4 mg/kg<sup>-1</sup>.
- **K:** The potassium in this growth medium is high with a value of 266.7 mg/kg<sup>-1</sup>.
- **Other:** The A-Horizon has sufficient C (1.6 %), N (0.1 %), Ca (971.1 mg/kg<sup>-1</sup>), and Mg (214.9 mg/kg<sup>-1</sup>) (refer to table 14). High concentrations of K will suppress the Mg and Ca uptake by plants (MVSA Bemestingshandleiding, 2007:95). There is not only a deficiency in Fe, but also in S within this substrate. All the substrates utilised had a Fe deficiency as indicated in Appendix 1.

## Platinum tailings

The platinum deficiency, however, was the lowest experienced in iron. Furthermore, this substrate delivered the highest individual length.

- **N:** The nitrogen content is non-existent in this growth medium with a value of 0 % anticipated.
- **P:** Phosphorus is important for plant growth, and this substrate has a deficient P-content of 6.0 mg/kg<sup>-1</sup>.
- **K:** The potassium in this growth medium is deficient and has a value of 26.6 mg/kg<sup>-1</sup>.
- **Other:** The platinum tailing was the only substrate utilised in this experiment with a deficient CEC value and was measured as 2.17 (Table 15).

The effect of substrate and C/N ratio on grass length is visually represented in Figure 11. The substrates can therefore be statistically summarised as: Platinum tailings, larger than A – Horizon, larger than Red structured B, larger than Gold tailings, based on the mean grass lengths attained.

The highest overall average grass length attained was by the platinum tailings (282.21; Tukey HSD test: b) as tabulated in Table 22 and visually illustrated in Figure 11. This is closely linked to the phosphorus content of the soil. The A – Horizon (P: 17.4 mg/kg<sup>-1</sup>) was the only substrate with sufficient phosphorus content. This is followed by the platinum tailings (P: 6.0 mg/kg<sup>-1</sup>), gold tailings (P: 4.9 mg/kg<sup>-1</sup>) and Potchefstroom red structured B (P: 3.5 mg/kg<sup>-1</sup>) as tabulated in Table 14. Thus, the higher the deficiency in phosphorus the lower the grass length attained. The P deficiency also affects the root development, resulting in a shorter root length.

Even though the best overall average was attained by the platinum tailings, the A - Horizon achieved the highest individual average. Although no statistically significant difference was identified, the overall average maximum grass length occurred at a C/N ratio of 12.5/1 (215.49 mm) as stated in Table 22 and visually represented in Figure 11. The findings for the A - Horizon (351.96 mm; Tukey HSD test: b). are validated by literature based on natural C/N ratios of 12.74/1 for South African dry lands and virgin soils (Du Toit *et al.*, 1994:75).

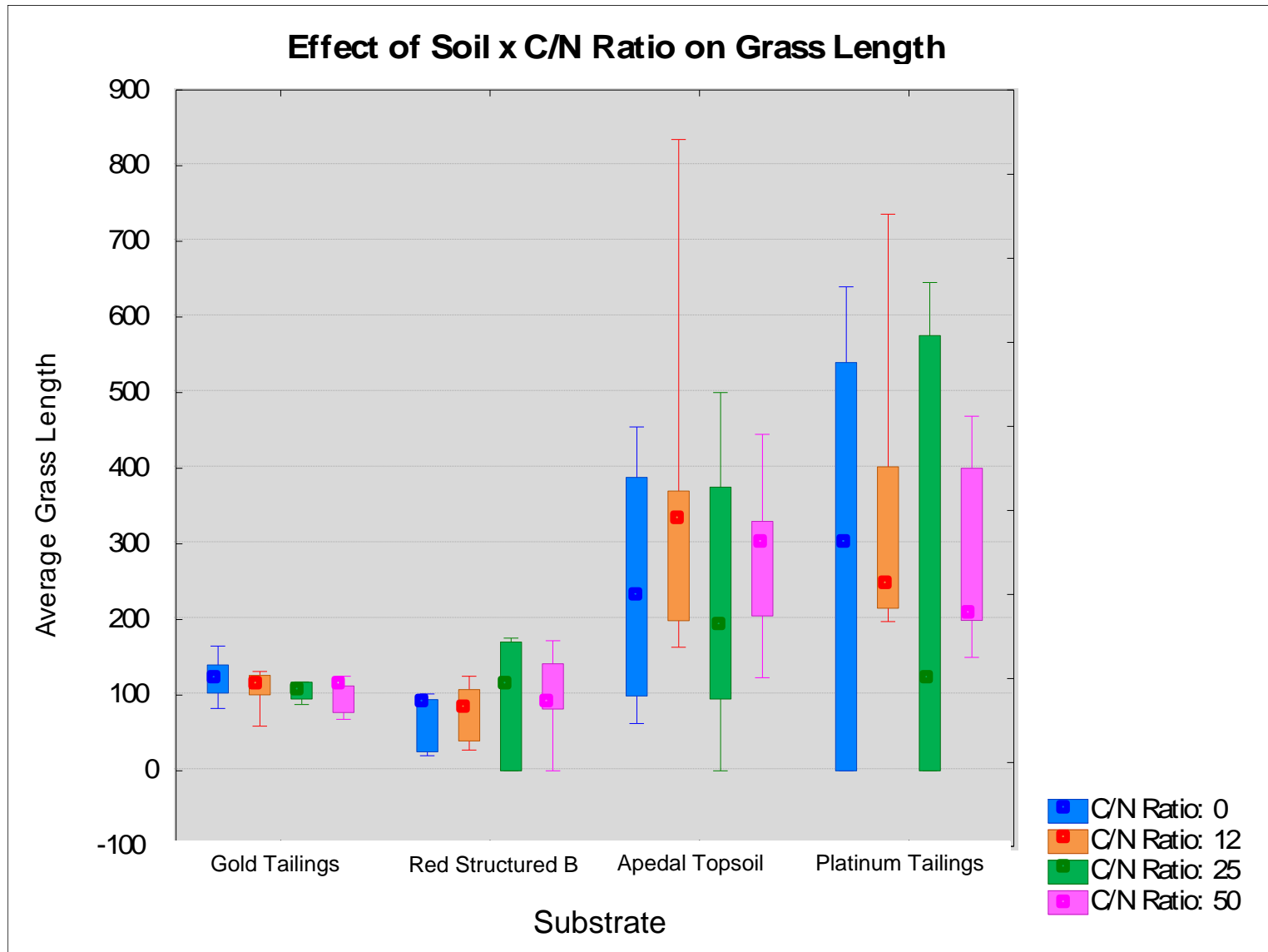


Figure 11: Effect of substrate X C/N Ratio and Grass Length

## **6. Conclusion**

Three experiments were done in order to investigate the inoculation of carbon and nitrogen in growth mediums to promote seed germination in mine rehabilitation. The first experiment illustrated the effect of different ameliorants on germination of grass on the different substrates utilised. The second experiment was completed in order to manipulate the C/N ratio by using a source with high C while applying different amounts of N to manipulate the applied C/N ratio. Experiment 3 investigates growth potential from grasses germinated in Experiment 2. This project was therefore sub-divided into three parts respectively.

### **6.1 Experiment 1: Effect of substrates and ameliorants on germination**

There was a highly significant interaction between the substrate's influences on grass germination with the amelioration treatments applied. The results were too complicated to draw a general conclusion from the interaction.

The interactions were analysed separately in order to ease the interpretation of the results.

When considering the substrates first; the coarse Kimberlite tailings delivered a significantly lower germination when compared to the rest of the substrates over all ameliorant applications. This is mainly due to the coarse texture anticipated with a lower water-holding capacity and nutrient availability. The high pH and deficiencies such as P and Mg also contributed to the germination attained. Kimberlite fine tailings, Cullinan Kimberlite tailings and granite saprolite were grouped together and did not differ statistically significantly between them. This group performed significantly better with respect to the mean germination attained in comparison with the Kimberlite coarse tailings. However the group delivered a significantly lower germination than the hard carbonate, diabase saprolite and gold tailings. Platinum tailings, Kalahari sand, kaolinite clay, acid lava saprolite, Potchefstroom red structured B and red structured B, delivered a statistically significant lower germination than the gold tailings, but did not differ statistically significantly from Kimberlite coarse tailings, hard carbonate or diabase saprolite.

According to Coetzee, 2015 (Personal correspondence, 2015) a 30% germination is considered to be good for pasture establishment in field conditions. The low germination percentages achieved in all three types of Kimberlite tailings are rather a result of the physical properties. However, the poor chemical status contributed to the state of germination. These properties include poor structure, poor water holding capacity as well as the coarse texture allowing seeds to leach out (Baligar *et al.*, 2001:926; Foth, 1990:27).

The coarse texture can therefore be amended by the additional application of SOM. The SOM has the ability to not only improve the CEC (Van Deventer & Ferreira, 2012:21), but also maintain the exchangeable K, Ca and Mg.

The main factor influencing the relatively poor germination anticipated in the granitic saprolite substrate is texture, similar to all the Kimberlite tailing materials. The substrate lacks favourable physical properties such as good structure and water-holding capacity. Hattingh (2012:1) and Bowden *et al.* (2007:11) stated that water is the elementary requirement for germination because it determines the speed of germination. Soil water (moisture) is considered as the most important environmental factor influencing seed germination (Yazdi *et al.*, 2013:1), for the seed requires water for the embryo to swell (Jerrett & Gillen, 2015) (enlarge) and break through the covering structures. Both S and P deficiencies occurred in this growth medium.

The main contributing factor that affected the germination in the platinum tailings is compaction. Compaction in mine tailings is inevitable due to the lack of structure instigated by the homogenous texture of mine waste material and lack of organic material, as in the case of the platinum tailings (Bradshaw, 1998:262). Root development is impaired by compaction, which in turn verifies that vegetation establishment on mine waste materials is challenging (Bradshaw, 1998:262). Furthermore, structure influences the porosity, infiltration capacity and root penetration (Gobat *et al.*, 2004:48; Winegardener, 1995:15).

Kalahari sand was derived from the same location as the granite saprolite. This substrate has a low carbon and nitrogen content as well as low plant available water. This is mainly due to the lack of clay that leads to nutrients leaching out. No deficiencies of potassium

(72.2 mg/kg<sup>-1</sup>) and calcium (2185.1 mg/kg<sup>-1</sup>) were identified. The magnesium (1097.0 mg/kg<sup>-1</sup>) though was available in excess (above the ideal value range) and may lead to crust formation.

The kaolinite clay is dominated by a 1:1 kaolinite clay. This substrate is deficient in carbon, C/N ratio and phosphorus.

Acid lava saprolite (46.75 %) achieved a slightly higher germination than the kaolinite clay (46.63 %). This coarse material allows seeds and nutrients to leach out (Baligar *et al.*, 2001:926). Substrates with poor water retention subsequently have a smaller amount of PAW (7.94 %) due to the low clay content (De Wet & Jansen, 2014:21). This can be amended by the additional application of SOM to counteract poor water retention (Brady & Weil, 2008:515; Cooperband, 2002:2; Chan, 2008:2; Fenton *et al.*, 2008:1; Marcel Dekker Inc., 1985:3; Singer & Munns, 1992:3; Van Deventer & Ferreira, 2012:21). This growth medium is deficient in carbon (0.34 %), sulphur (0 %), nitrogen (0 %) and phosphorus. The calcium (4297.4 mg/kg<sup>-1</sup>) and magnesium (1213.5 mg/kg<sup>-1</sup>) exceeded the ideal values.

Potchefstroom red structured B achieved a germination of 48.00%. The structure is directly dependent on the mineralogy, texture, moisture content and organic matter of the soil. Structure influences the porosity, infiltration capacity, and root penetration (Gobat *et al.*, 2004:48; Winegardener, 1995:15). The organic C (0.74 %) was unexpectedly within the ideal standards of South African virgin soils. However, both the nitrogen and sulphur were absent. Magnesium was above the set ideal range with 721.0 mg/kg<sup>-1</sup> projected. This medium can be rectified with the application of chicken manure together with compost (Van Deventer, 2015: Personal correspondence). Chicken manure contains 2.2-3.5 % N; 1.7-2.2 % P and 1.5-2.3 % K (MVSA Bemestingshandleiding, 2007:129). The B-Red structured growth medium is deficient in the nitrogen, sulphur and phosphorus. Magnesium (642.1 mg/kg<sup>-1</sup>) exceeded the ideal value range which may lead to poor physical conditions. Excess magnesium reduces the availability of potassium. High magnesium content makes soil tight which in turn causes restriction of air and water availability, water drainage as well as root development. Microbial activity and organic matter decay is also restricted by an

excess magnesium (Gobat *et al.*, 2004:48; MVSA Bemestingshandleiding, 2007:91; Verheye, 2007:126; Winegardener, 1995:15).

The hard carbonate substrate performed significantly better because of the available organic matter essential for plants. The substrate is somewhat coarse in this case due to the mining activities that the horizon is subjected to. The sulphur content was absent. P-content was deficient with a value of 3.4 mg/kg<sup>-1</sup>. The calcium (4248.0 mg/kg<sup>-1</sup>) was, as expected, available in excess.

The diabase saprolite is a coarse growth medium. Mutually the carbon (0.21 %), nitrogen (0 %), sulphur (0 %) and phosphorus content (3.5 mg/kg<sup>-1</sup>) are deficient in this growth medium. Furthermore, magnesium (1134.15 mg/kg<sup>-1</sup>) was abundant and above the ideal limits which resulted in an Mg/K ratio above the ideal range with a value of 46.5.

The gold tailings attained the highest germination percentage. The substrate is slightly acidic with a pH (H<sub>2</sub>O) value of 5.74. This substrate is fine, with PAW of 21.7 % at field capacity due to the high gypsum content that is hydrophilic. Deficiencies of carbon, phosphorus and potassium deficiencies occurred. The potassium deficiency is most likely caused by the formation of jarosite (KFe<sub>3</sub>(SO<sub>4</sub>)<sub>2</sub>(OH)<sub>6</sub> (University of Oxford, 2013:316)).

One must take not only the growth media in consideration, but also the amelioration procedure as applied to the substrates.

The lentil powder amelioration delivered the lowest germination percentage according to the ANOVA over all the substrates combined. This treatment formed a gel like substance when being exposed to water. This substance is hydrophilic and keeps the water away from the seed, inhibiting germination. Humate had the second lowest percentage possibly due to the high C/N ratio that probably results in a N negative period. N is important in the vegetative growth of plants. Humate, yellow pea powder, fungimax and compost do not differ statistically from one another.

The humates, compost and fungimax compete with the grass coating during nutrient exchange processes. This is because the nutrients from the coating are much more readily available than the ameliorants. This might cause depravation of seeds due to the nutrients pulled from the coating into the soil.

This group similarly does not differ statistically from lentil powder or amelioration combinations containing lime. The lime, humates, fungimax combination; lime and compost; and lime, humates, compost combinations are grouped together. The lime addition counteracts the competition between the ameliorant and seed, resulting in higher germination percentages.

The highest percentage attained was with the no-amelioration application, which differs significantly from all other ameliorants. One can only state that a combination of a number of influencing factors played a role in this phenomenon such as the coated seed and lower competition between the seed and ameliorant in the soil. The coating serves as a reservoir of food for the seedling and the micro-organisms due to the availability of additional nutrients. The coating also aid in the seed and soil contact enhancing water availability.

The substrate that had the highest microbial activity was the A-Horizon with 115.315 (INF $\mu$ g/g/2h). This is due to the gas exchange between the atmosphere and the natural topsoil. This material also contains enough carbon and nitrogen to sustain the microbes and correlates with literature. Most of the soil life occurs in the top 15 cm (Hoorman & Islam, 2010:1).

A second experiment was suggested to investigate whether the C/N ratio might influence the germination percentages.



## **6.2 Experiment 2: Influence of C/N ratio applied and substrate on grass germination**

Experiment 2 differentiates between the most suitable C/N ratios added to statuses of various substrates. It was found that substrate as main effect influences germination statistically, whilst the C/N ratios had no significant influence on germination for the both grass species. There were also no significant effects based on the interactions between the C/N ratios and substrates.

The experiment indicated that Rhodes grass performed significantly better than couch grass. The vegetative clones created from the initial plant will be adapted to the specific conditions anticipated. Both couch and Rhodes grasses produce more stolons and fewer seeds under stress conditions. In rehabilitation practices, Rhodes grass will germinate quicker ( $\pm 10$  days) dominating in the short term; whereas couch grass takes longer to germinate ( $\pm 31$  days) and will dominate in the long term after couch grass dies off (Nel, 2015: Personal correspondence).

## **6.3 Experiment 3: The influence of C/N ratios applied and substrates on the growth potential**

Due to the differences experienced in germination during Experiment 1, it was deemed necessary to investigate the growth potential of the grass in order to quantify the differences.

An ANOVA was done taking substrate, C/N ratio and grass length into consideration. The growth potential (measured in mm) was determined from the grasses that emerged during Experiment 2. Significant effects for substrate were identified based on grass lengths. No significant effects were identified for C/N ratios and interaction between C/N ratio and substrates.

It was observed that the best growth condition is in the Apedal Topsoil with a C/N ratio of 12.5/1 (351.96 mm). The findings for best growth condition in the apedal topsoil are validated

by literature based natural C/N ratios of 12.74/1 for South African dry lands and virgin soils (Du Toit *et al.*, 1994:75).

The phosphorus content can be summarised as: A – Horizon > platinum > gold tailings, Potchefstroom red structured B. Thus, the higher the deficiency in phosphorus the lower the grass length attained.

## 6.4 General conclusions

There is no set default solution for rehabilitation practices. Each problem is site-specific and one has to integrate and investigate various technical aspects. Scientists will only develop more effective answers when realising that *“Data is not information, information is not knowledge, knowledge is not understanding, understanding is not wisdom” (Clifford Stoll; American Author)*.

Various influencing factors have been identified making part of both chemical and physical characteristics. Factors such as macro and micro-nutrients, organic matter, water retention, particle size distribution and compaction influenced the three experiments. It was thought that C/N ratio as a single factor would contribute to the germination attained on a more superior scale. It seems on the contrary that it is rather a combination of factors that influenced the germination.

### Referring to objectives

- a. Thus, the effect of ameliorants is substrate-specific. Different substrates do not react similarly to ameliorants applied due to chemical and physical characteristics attained. Coated seeds are vital for rehabilitation practices, and need minimal additional amelioration. This is validated by the “no amelioration application” that delivered the highest germination (58.75 %) in experiment 1. Texture and PAW are very important for germination. This fact is supported by the gold tailings containing gypsum. The gypsum makes the growth medium hygroscopic (absorbing moisture (as from the air)) which result in excellent germination.

- b. Substrates had statistically significant differences, whereas the C/N ratio did not influence the germination significantly. The C/N ratio is therefore substrate and species specific. The highest overall mean germination % attained for both grass species was by the gold tailings (Rhodes: 97.14 % mean germination; Couch: 77.14% mean germination). In rehabilitation practices the engineered soils (tailings) are already stable prepared, and couch grass will thus act as climax species whereas Rhodes grass is a sub-climax species and will dominate initially. Rhodes grass begins to die off after three years when its life-cycle is completed and the couch grass will then take over by acting as climax species. Water is the main influencing factor on germination. Thus the texture is more important (due to its water-holding capacity) than nutrients for germination. The coated seed also contains enough nutrients to sustain it for germination. Nutrients become more important for plant growth.
- c. It was concluded that the best growth conditions are A - Horizon (351.96 mm) > platinum tailings (326.93 mm) > gold tailings (118.94 mm) > Potchefstroom red structured soil (99.70 mm). The A – Horizon was the only substrate with sufficient phosphorus content (P: 17.4 mg/kg). This is followed by the platinum tailings (P: 6.0 mg/kg), gold tailings (P: 4.9 mg/kg) and Potchefstroom red structured B (P: 3.5 mg/kg). Thus, the higher the deficiency in phosphorus the lower the grass length attained. There is also a general trend where the low Mg results in poor germination.

## **7. Recommendations for future research**

Rehabilitation is a necessity in complying with current legislation. A comprehensive overview of the applicable legislation regarding mine rehabilitation is summarised in Appendix 3. Vegetation establishment and soil rectification are regarded as very important aspects when rehabilitating tailings storage facilities. There is still a lot gaps to bridge in the future. It is recommended that ground cover should be further investigated due to the importance of it during rehabilitation practices. One must elaborate on this study by means of experimenting with other grass species.

It will be recommended to consider the combined effect of macro and micro-nutrients with various C/N ratios and different substrates. Dehydrogenase activity must be addressed in future studies, for it will provide insight into the interaction between plant health, microbes, macro-nutrients and rehabilitation. It is evident that substrates containing low amounts of Mg provided lower germination percentages than those containing excessive Mg. The phosphorus-deprived growth media delivered lower germination percentages than the adequate amounts.

This matrix (Table 23) is based on problems associated with the material and can be used in future studies as a reference only. Additional research is required to understand the dynamics entirely.

**Table 23: General characteristics that could cause problems for vegetation covers on different substrate types**

No.	Substrate	Chemical																		Physical				
		Macro nutrients						Micro nutrients					Organic C	C/N	K/CEC	Mg/K	Ca/Mg	ESP	Alkali -nity	Compac- tion and crusting	Infil- tration	Density Compac- -tion	Dust	Low water retent- ion
		N	P	K	Ca	Mg	S	Fe	Mn	Zn	Ni	Mo												
MF - 3G	Kimberlite Coarse Tailings	-	Low	Good	Good	Low	-	Low	Low	Low	Good	Low	-	-	Low	Low	High	High	Yes	No	High	No	No	Yes
MF - 3F	Kimberlite Fine Tailings	-	Low	Good	High	Low	-	Low	Low	Low	Good	Good	-	-	Low	Low	High	High	Yes	No	High	No	No	Yes
MF - 11	Cullinan - Kimberlite Tailings	Low	Low	High	Good	Good	Good	Low	Low	Low	Good	Low	Low	-	Good	Low	Good	High	Yes	No	High	No	No	Yes
MF - 5C	Granite Saprolite	Good	Low	Good	Good	Good	Low	Low	Low	Low	Good	Low	Low	Low	Low	High	Good	Good	No	No	High	No	No	Yes
MF - 7	Platinum	Good	Low	Low	Good	Low	Low	Low	Low	Low	Good	Low	Low	-	Low	Good	High	High	No	Yes	Medium	Yes	Yes	Yes
MF - 5B	Kalahari Sand	Good	Low	Good	Good	High	Good	Low	Low	Low	Good	Low	Low	Low	Low	High	Low	Good	No	No	Medium	No	Yes	Yes
MF - 6	Ottosdal Kaolinite Clay	Good	Low	Good	Good	Good	Low	Low	Low	Low	Good	Low	Low	Low	Low	High	Good	Good	No	Yes	Low	Yes	No	No
MF - 1C	Acid Lava Saprolit	Low	Low	Good	High	High	Low	Low	Low	Low	Good	Low	Low	-	Low	High	Good	Good	No	No	High	No	No	Yes
MF - 2B	Potchefstroom Red Structured B	Low	Low	Good	Good	High	Low	Low	Low	Low	Good	Low	Good	-	Low	High	Low	Good	No	Yes	Low	No	No	No
MF - 1B	Red Structured B	Low	Low	Good	Good	High	Low	Low	Good	Low	Good	Low	Good	-	Low	High	Good	Good	No	Yes	Low	No	No	No
MF - 4	Hard Carbonate Saprolite	Good	Low	Good	High	Good	Low	Low	Low	Low	Good	Low	Good	High	Low	Low	Good	Good	No	No	High	No	No	Yes
MF - 2C	Diabase Saprolite	Low	Low	Good	Good	High	Low	Low	Low	Low	Good	Low	Low	-	Low	High	Low	Good	No	No	Medium	No	No	Yes
MF - 8	Gold	Good	Low	Low	Good	Good	Good	Low	Good	High	High	Low	Low	Good	Low	High	High	Good	No	Yes	Medium	Yes	Yes	No
MF - 9 - BK	Vertisol	Good	Low	Good	High	High	Low	Low	Low	Low	Good	Low	Good	Low	Low	High	Good	Good	No	No	Low	No	No	No
MF - TC - BK	A - Horizon	Good	Good	High	Good	Good	Low	Low	Low	Low	Good	Low	Good	Good	Low	Low	Good	Good	No	No	Medium	No	Yes	No

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## 9. Appendices

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## Appendix 1: Physical and chemical analysis of substrates

Appendix Table 1: Legend for Appendices.

No:	Sample number on map	Locality name
1	MF - 1B	Khuma (Kareerand) Red Structured B
2	MF - 1C	Khuma (Kareerand) Acid Lava Saprolit
3	MF - 2B	Potchefstroom (North West University) Red Structured B
4	MF - 2C	Potchefstroom (North West University) Diabase Saprolite
5	MF - 3F	Viljoenskroon Kimberlite Fine Tailings
6	MF - 3G	Viljoenskroon Kimberlite Coarse Tailings
7	MF - 4	Christiana Hard Carbonate Saprolite
8	MF - 5B	Schweizer-Reneke (Borrow-pit) Kalahari Sand
9	MF - 5C	Schweizer-Reneke (Borrow-pit) Granite Saprolite
10	MF - 6	Ottosdal Kaolinite Clay
11	MF - 7	Rustenburg (Paardekraal) - Platinum Tailings
12	MF - 8	Stilfontein (Chemwes Mine) - Gold Tailings
13	MF - 9 - BK	Potchefstroom Vertisol
14	MF - 11	Cullinan - Kimberlite Tailings
15	MF - TC - BK	Potchefstroom A - Horizon (Topsoil)

Appendix Table 2: Soluble trace elements of substrates

Soluble Trace Elements (Ammonium Nitrate [NH <sub>4</sub> NO <sub>3</sub> ])																
(mg/kg 1)	Ideal values	Red Structured B	Acid Lava Saprolit	Potchefstroom Red Structured B	Diabase Saprolite	Kimberlite Fine Tailings	Kimberlite Coarse Tailings	Hard Carbonate Saprolite	Kalahari Sand	Granite Saprolite	Ottosdal Kaolinite Clay	Platinum Tailings	Gold Tailings	Vertisol	Cullinan - Kimberlite Tailings	A - Horizon
Be 9	–	0,000045	0,000044825	0,0000446	0,000044825	0,000044825	0,000045125	0,0000446	0,00004455	0,0000449	0,0000441	0,0000446	0,000040425	0,000045075	0,000044825	0,000044375
B 11	–	0,00258	0,00292	0,002287	0,002342	0,00206075	0,002291	0,0020795	0,00239575	0,00233975	0,0022465	0,001929	0,0024685	0,0024405	0,00118	0,0021365
Na 23	–	20,4625	42,725	41,375	88,55	1955	1594,75	6,6625	92,775	11,875	11,3875	46,1	9,475	69,6	2170,75	2,7
Mg 24	<300 - >50 <sup>2</sup>	581,5	1038,5	690,75	1013,25	25,275	19,1675	76,275	904	170,1	214,125	39,2	100,575	1531	217,5	194,225
Al 27	–	0,407	0,87175	0,38925	0,8645	0,78225	0,34225	1,0275	2,975	0,213575	0,03305	1,6725	0,57325	0,537	0,0849	0,97225
P 31	>30 <sup>2</sup>	0,0051925	0,005375	0,005875	0,0060575	0,00449	0,0054925	0,003475	0,005635	0,005505	0,00571	0,0040025	0,00611	0,001099	0,0052075	0,001416
K 39	<250 - >40 <sup>2</sup>	65,875	49,525	122,85	65,75	91,125	74,95	83,875	50,175	52,525	93,8	21,44	12,8775	181,7	797,5	234,725
Ca 43	<3000 - >200 <sup>2</sup>	1446,75	3480	1293,5	2448,25	1878,25	1772,75	1451,5	2019	448	978,5	356,25	2174,25	3267,5	1162,5	746
Ti 47	–	0,0197675	0,028925	0,0109975	0,0359	0,070625	0,031825	0,04985	0,08035	0,015105	0,0011325	0,0288	0,007095	0,0303	0,0020045	0,0841
V 51	<150 <sup>4</sup>	0,00038925	0,00032875	0,000398	0,0003715	0,00031	0,00035275	0,00025775	0,0003385	0,0004045	0,0004075	0,0003085	0,000406	0,0002058	0,000364	0,000367
Cr 53	<0.1 <sup>1</sup> - >0.05 <sup>6</sup>	0,000044525	0,001231	0,00005775	0,000142325	0,000019235	0,0000571	0,000040525	0,0074375	0,000069375	0,000086975	0,68425	0,000083975	0,000012722 <sup>5</sup>	0,00005595	0,00004695
Mn 55	<740 <sup>4</sup> - >5 <sup>6</sup>	27,625	0,28925	0,02133	0,0186425	1,2075	1,417	0,030025	0,12365	0,197425	1,11725	0,35225	13,3675	0,033325	0,2935	1,4915
Fe 57	>35 <sup>6</sup>	5,595	13,305	4,8675	9,235	7,845	6,8075	5,885	9,2	1,75725	3,56	3,1925	8,095	11,635	4,1075	3,2775
Co 59	<10 - >0.5 <sup>6</sup>	0,08205	0,0146875	0,000032325	0,000569	0,001735	0,00066375	0,000013415	0,0014915	0,0000287	0,00075125	0,008275	0,5645	0,0030425	0,000405	0,000031325
Ni 60	<1.2 <sup>1</sup>	0,6635	0,0491	0,013835	0,049375	0,0283	0,028725	0,0026725	0,06295	0,0429	0,062425	0,27025	1,85675	0,023475	0,09005	0,0142475
Cu 63	<60 - >1.2 <sup>1</sup>	0,00025325	0,106825	0,0002385	0,0002895	0,121925	0,140675	0,000082725	0,000046675	0,000219225	0,00025975	0,0921	0,2171	0,005345	0,0218825	0,000153675
Zn 66	<5.0 <sup>1</sup> ; 2 <sup>2</sup>	0,00153125	0,0014275	0,0016285	0,00155925	0,00143925	0,00165025	0,00171225	0,0014025	0,00154275	0,001051	0,00150625	5,2175	0,0016325	0,00069125	0,00054
As 75	<0.014 <sup>1</sup>	0,00017665	0,000170375	0,0001705	0,000176275	0,0001507	0,000158475	0,000165	0,000176025	0,00017665	0,000167625	0,00015545	0,000084375	0,000165375	0,000114225	0,000172375
Se 82	0.05-0.3 <sup>5</sup> ; <1 <sup>1</sup> - >0.05 <sup>6</sup>	0,000753	0,0007755	0,00077075	0,00077225	0,000753	0,0007595	0,000769	0,0007675	0,00081	0,0007925	0,00075775	0,00074425	0,000725	0,000774	0,00078025
Rb 85	–	0,60075	0,2775	0,9025	0,33125	0,231825	0,228225	0,244925	0,381	0,3765	0,13395	0,0358	0,04665	0,76825	1,579	0,67725
Sr 88	–	3,8675	7,3875	2,5675	5,345	60,675	53,925	2,6975	4,4325	3,6	5,8425	0,789	1,0985	8,115	37,625	3,9525
Mo 95	<2 <sup>5</sup> ; <0.5 - >0.05 <sup>6</sup>	0,004835	0,0034325	0,00341	0,0029575	0,04675	0,019005	0,00543	0,0027725	0,00274	0,0027925	0,00576	0,0043075	0,004245	0,0298	0,0028925
Pd 105	–	0,0034575	0,009455	0,0018975	0,005805	0,078775	0,0667	0,005435	0,0045625	0,0034275	0,00563	9,765E-07	0,0025675	0,0099725	0,044175	0,003555
Ag 107	–	0,000168775	0,000054875	0,000163675	0,000164475	0,000155425	0,00016565	0,000151975	0,00016915	0,0001492	0,0001702	0,000170575	-0,017085	0,000170025	0,000141875	0,0001699
Cd 111	<0.1 <sup>6</sup>	0,000057375	0,000065325	0,000064975	0,0000645	0,0000588	0,00006125	0,0000638	0,000064925	0,000064	0,0000638	0,000063925	0,0135175	0,0000642	0,000062875	0,000061825
Sb 121	–	0,0025825	0,0038225	0,0028875	0,0028825	0,0040225	0,0033075	0,0031475	0,002725	0,00307	0,002765	0,0034375	0,004895	0,0031975	0,0041275	0,002845
Ba 137	–	21,4425	25,85	9,56	5,145	8,5875	7,09	7,005	5,845	6,065	7,9175	0,522	0,38775	34,75	9,63	10,2725
Pt 195	–	0,0000358	0,00000695	0,00002965	0,0013	0,000031725	0,000026925	0,000035025	0,0000335	0,0000306	0,00002775	0,000034	0,000035825	0,000035475	1,12225E-05	2,20975E-05
Au 197	–	0,0129475	0,0131525	0,01302	0,0128325	0,01326	0,01284	0,0153575	0,0129125	0,0128175	0,0128325	0,0128825	0,013125	0,0131775	0,012755	0,012845
Hg 202	<0.007 <sup>1</sup>	0,000012595	0,000012507 <sup>5</sup>	0,00001251	0,000012572 <sup>5</sup>	0,0000124125	0,0000124175	0,0000126325	0,000012625	0,0000126175	0,0000127	0,000012712 <sup>5</sup>	0,000012697 <sup>5</sup>	0,00001287	0,00001276	0,0000127375
Tl 205	–	0,000035925	0,000089325	0,000076025	0,000093125	0,00008155	0,00008345	0,00008125	0,00008945	0,000085375	0,0000933	0,00008365	0,000060875	0,000063875	0,00000728	0,0000748
Pb 208	3.5 <sup>1</sup>	0,00042	0,00038075	0,000391	0,00039525	0,00036975	0,00036075	0,00039125	0,000399	0,00040125	0,0004015	0,00037575	0,000304	0,00041775	0,00037475	0,00039575
Bi 209	–	0,028225	0,02825	0,028225	0,028225	0,028225	0,028225	0,0283	0,028325	0,0282	0,028225	0,028225	0,02825	0,028275	0,028225	0,028275
Th 232	–	0,0166175	0,01664	0,016575	0,0165875	0,0166425	0,0166125	0,0169175	0,01665	0,016585	0,0165925	0,01668	0,0167525	0,017355	0,0166475	0,0167425
U 238	0.04 <sup>3</sup>	0,000050025	0,0000423	0,0000498	0,0000496	0,0064025	0,002645	0,0000252	0,00005015	0,0000496	0,00004955	0,000049225	0,0141	0,0000322	0,000000768 <sup>3</sup>	0,000047825
Be 9	–	0,000045	0,000044825	0,0000446	0,000044825	0,000044825	0,000045125	0,0000446	0,00004455	0,0000449	0,0000441	0,0000446	0,000040425	0,000045075	0,000044825	0,000044375

<sup>1</sup> Herselman (2007:A7-A8)

<sup>2</sup> MVSA Bemestingshandleiding, 2007:172

<sup>3</sup> Rösner *et al.* (2001:90)

<sup>4</sup> NEMWA (2014;6)

<sup>5</sup> Tainton (2013:344) [based on toxic levels for cattle]

<sup>6</sup> (Steyn & Herselman, 2006:234 - 235)

blue = below ideal; red = above ideal

Total trace elements (EPA 3050b Method )															
	Red Structured B	Acid Lava Sapolit	Potchefstroom Red Structured B	Diabase Sapolite	Kimberlite Fine Tailings	Kimberlite Coarse Tailings	Hard Carbonate Sapolite	Kalahari Sand	Granite Sapolite	Ottosdal Kaolinite Clay	Platinum Tailings	Gold Tailings	Vertisol	Cullinan - Kimberlite Tailings	A - Horizon
Be 9	0,27	0,0533	0,3865	0,048325	0,279	0,28975	0,1217	0,059925	0,169075	0,2975	1,86E-05	0,0575	0,352	0,113325	0,32
B 11	0,0103675	0,014725	0,014248	0,022133	0,001197	0,312	0,01044	0,020193	0,023118	0,0218	0,018088	0,02147	0,001093	0,2685	0,01898
Na 23	394,75	1362,75	369,5	2152,5	5517,5	6245	301	2306	309	303,5	2166,75	338,5	387,25	2415,5	288,25
Mg 24	2590	10237,5	2005,75	8110	22950	22392,5	1616,5	8337,5	578	2044,5	8897,5	1216,75	6115	56325	628
Al 27	21917,5	19062,5	22590	20272,5	13877,5	14865	4230	20505	2670	3715	12952,5	1872	15852,5	10005	7785
P 31	330	334,25	328,25	286	963	894,5	377,25	305	315,75	361,5	314	349	391,75	784,75	510,5
K 39	642,25	408,25	956,25	300,5	4515	3687,5	992	316,75	557	2115	322,25	220,625	1055,25	5400	1031
Ca 43	2687,5	12470	1702,5	10280	23280	21437,5	80250	11017,5	436,25	1095	11255	1990,75	10707,5	9540	1211,5
Ti 47	366,75	416	249,975	333	1770,75	1575,5	54	327,25	33,175	13,02	90,8	11,655	53,425	1041,75	84,1
V 51	92,05	50,4	87,25	28,2	91,775	81,975	19,105	27,1	12,0625	12,8875	10,2775	4,635	67,525	25,95	37,75
Cr 53	279,25	135,8	217,275	86,175	104,45	95,025	20,9575	85,5	27,4	6,7125	444,5	14,68	119,675	236,825	95,1
Mn 55	645,5	547	834	412,75	508	494	90,575	332,5	44,325	586	106,575	136,925	823	257,5	263,75
Fe 57	27550	23380	31500	18297,5	27400	26375	4695	17220	4392,5	10997,5	11902,5	4362,5	15007,5	19597,5	11420
Co 59	19,4	22,05	24,28	22,625	21,87	21,2825	2,6025	19,3225	1,837	4,6975	55,4	12,4925	14,14	36,925	5,655
Ni 60	57,55	58,95	66,6	62,175	92,9	90,65	7,4525	56,95	3,25	6,2425	171,95	29,825	33,95	387,75	17,2225
Cu 63	41,525	52,75	37,175	33,775	63,8	68,025	6,4225	36,625	5,44	9,3375	128,1	21,7375	22,165	41,05	17,86
Zn 66	15,8975	23,6125	13,9725	15,285	40,175	39,6	6,0575	15,085	4,775	22,6325	19,2525	47,25	24,4375	21,975	19,315
As 75	0,88325	0,23935	1,896	0,239475	1,5205	4,575	0,88375	0,2178	0,4405	1,08475	0,04765	26,3	1,02825	0,54625	1,25825
Se 82	0,0035325	0,006503	0,003475	0,006005	0,002745	0,0035	0,005788	0,006093	0,006663	0,0048	0,005475	0,005765	0,004358	0,006455	0,005628
Rb 85	9,5525	4,0725	14,5625	3,9925	24,065	19,7575	6,59	4,6525	7,0425	10,825	1,323	1,12475	12,035	43,35	14,84
Sr 88	6,295	17,0925	4,36	18,43	260	246,2	26,975	18,5775	4,2925	7,355	44,85	5,6025	16,48	146,775	6,5725
Mo 95	0,35625	0,1383	0,57575	0,134325	0,61225	0,69475	0,157975	0,131	0,1466	0,7875	0,48925	0,6205	0,29225	0,3405	0,441
Pd 105	0,233025	0,127825	0,20485	0,12895	0,57575	0,5635	0,0945	0,1225	0,052425	0,1238	0,15395	0,100375	0,20115	0,25075	0,115825
Ag 107	0,00097475	0,001449	0,001038	0,00138	0,000555	0,1113	0,116125	0,001406	0,0337	0,001305	0,000906	0,001198	0,001151	0,000805	0,001402
Cd 111	0,000100275	0,00014	0,000175	0,000267	0,049175	0,010313	0,000119	0,000311	0,000326	0,000268	2E-05	0,047275	0,000529	0,008693	0,000186
Sb 121	0,09075	0,048025	0,163725	0,0601	0,08275	0,17705	0,080925	0,054975	0,058525	0,1295	0,050275	0,257	0,141275	0,055	0,155325
Ba 137	61,225	118,875	69,175	90,675	235,425	176,45	30,05	63,975	15,175	176,5	21,405	15,4375	123,975	295	37,075
Pt 195	0,058425	8,9E-05	0,000155	0,000216	0,000288	0,02364	0,000313	0,000313	0,00035	0,0003	0,151425	0,00034	0,000326	0,00027	0,000347
Au 197	0,147525	0,14135	0,141275	0,135825	0,1478	0,15055	0,151225	0,1357	0,13325	0,144125	0,151325	0,28325	0,142875	0,1389	0,1406
Hg 202	0,0001166	0,00012	0,00012	0,000121	0,000118	0,000119	8,09E-05	0,000121	0,000121	0,000121	0,000123	0,000113	0,000121	0,000124	0,000122
Tl 205	0,00023165	0,000753	0,03145	0,000722	0,000175	0,015238	0,000432	0,000743	0,000545	0,000557	0,000468	0,000663	0,007155	0,06575	0,000131
Pb 208	3,07	0,30775	5,69	0,2048	2,885	3,69	1,60675	0,406	3,4675	88,55	24,835	11,5525	7,255	5,645	4,855
Bi 209	0,3735	0,3295	0,406	0,336	0,404	0,4615	0,3345	0,30775	0,33075	0,3465	0,347	0,57725	0,37475	0,3315	0,376
Th 232	1,994	0,54325	3,0025	0,53275	3,19	3,2575	1,259	0,5505	6,3625	4,29	0,472	4,36	3	2,79	2,372
U 238	0,236975	0,025325	0,34275	0,002126	0,74575	0,81125	0,100425	0,000151	0,248175	0,09955	0,029325	7,2425	0,1741	0,47475	0,2575
Be 9	0,27	0,0533	0,3865	0,048325	0,279	0,28975	0,1217	0,059925	0,169075	0,2975	1,86E-05	0,0575	0,352	0,113325	0,32

Appendix Table 3: Total Trace Elements of Substrates. Aluminium

## Appendix 2: Experiment 2 calculations

**Appendix Table 4: C/N ratio of ameliorants**

Product	%C	%N	C/N ratio	C-content/l or kg	N-content/ l or kg	C/N	g/20L	g/20L	
K-humate	15	0,15 % (wt./vol)	100	136 g/kg	1,36 g/kg	100	2720	27,2	
Compost	17,42	2,96 % (wt./wt.)	6	174,2 g/kg	29,6 g/kg	6			500ml K-humate
YPP	41,13	1,11 % (wt./wt.)	37	411,3 g/kg	11,1 g/kg	37			

**Appendix Table 5: C/N ratio of substrates**

Substrate	%C	%N	C/N ratio	C-content/L or kg	N-content/ L or kg	C/N	g C	g N	C/N
Platinum tailings	0,28	0,06 %(wt/wt)	5	2,8 g/kg	0,6 g/kg	5	138,8	1,96	70,82
Potchefstroom Red Structured	0,44	0,13 %(wt/wt)	3	4,4 g/kg	1,3 g/kg	3	140,4	2,66	52,78
Gold tailings	0,39	0,17 %(wt/wt)	2	3,9 g/kg	1,7 g/kg	2	139,9	3,06	45,72
Kalahari sand	0,16	0,05 %(wt/wt)	3	1,6 g/kg	0,5 g/kg	3	137,6	1,86	73,98

**Appendix Table 6: Amendment to achieve C/N ratios 0, 12.5/1, 25/1, and 50/1.**

Substrate	C	C/N = 25/1			C/N = 50/1			C/N = 12.5/1		
		N for 25	Short for 25	g LAN for 25	N for 50	short for 50	g LAN for 50	N for 12.5	short for 12.5	g LAN for 12.5
Platinum tailings	138,8	5,552	3,592	12,8285714	2,776	0,816	2,91428571	11,104	9,144	32,65714286
Potchefstroom Red Structured	140,4	5,616	2,956	10,5571429	2,808	0,148	0,52857143	11,232	8,572	30,61428571
Gold tailings	139,9	5,596	2,536	9,05714286	2,798	-0,262	-0,93571429	11,192	8,132	29,04285714
Kalahari sand	137,6	5,504	3,644	13,01428571	2,752	0,892	3,185714286	11,008	9,148	32,67142857



**Appendix Table 7: Applied amounts of humates and LAN for ratio**

C/N ratio	K-humate (L)	LAN (g)
25	20	288,6
50	20	97,5
12,5	20	680

**Appendix Table 8: Amount of humates and LAN added per pot and mixed in drum**

	60 ml per pot			9000 ml drum		
	LAN	K-humate		LAN	K-humate	
Ratio C/N	LAN	K-humate	Mixture	LAN	K-humate	Mixture
	g/pot	ml/pot	In 60 ml	g/150 pots	ml/150 pots	9000 ml
25	0,05	2,88	C	6,94	432,00	c
50	0,02	2,88	D	2,31	432,00	d
12,5	0,11	2,88	B	16,20	432,00	b
0	0,00	0,00	A	0,00	0	a

**Appendix Table 9: Amount of C and N per 20 L humates**

K-humate calculations:	20l K-humate		x	y	z
	C	N			
Total C=15 % (wt./vol)	150g/l X20L	1.5g/l x20l	0,01728	0,00864	0,03456
Total N=0.15 % (wt./vol)	3 000 g C	30 g N			

**Appendix Table 10: C and N in 20 L humates**

K-humate calculations:			
Total C=15 % (wt./vol)	Total N=0.15 % (wt./vol)	20 l K-humate = 3000 g C	20 l K-humate = 30 g N
15 % wt./vol	0.15 % wt./vol	2.88 ml K humate = 0.432 g C	2.88 ml K humate =0.00432 g N
15g/1000L	0.15g/1000L		
15 % = 100 X g/1000ml	0.15 % = 100 X g/1000ml		
150g C/l K-humate	1.50g C/l K-humate		

**Appendix Table 11: C/N ratios to achieve and amounts of C and N**

K-humate calculations:							
C/N	ideal=25	50	12.5	LAN (28 % N) = 280g/kg LAN = 280g N/ 1000 g LAN			
100	0.432:x	0.432:y	0.432:z	(28/100) x X =	0,0130	X = (0.01296*100)/28	X = 0,046
	x=0.01728	y=0.00864	z=0.03456	(28/100) x Y =	0,004	Y = (0.00432*100)/28	Y = 0,015
	0.01728-x=	0,01296 g N needed for 25 C/N		(28/100) x Z =	0,030	z = (0.03024*100)/28	Z = 0.108
	0.008648-y=	0,00432 g N needed for 50 C/N					
	0.03456-z=	0,03024 g N needed for 12.5 C/N					

**Appendix Table 12: Compost C and N content**

Compost calculation:						
Total C= 17.41 %	Total N=2.96 %	2.481 g compost = 0.42177 g C	C/N	Ideal = 25	50	12.5
174.1g/1000g X100= 17.41 %	29.6g/1000g X 100 = 2.96 %		6,126	0.432:x	0.432:y	0.432:z
1kg compost=174.1g C	1kg compost=29.6g N			x=0.01728	y=0.00864	z=0.03456
174.1/0.432= 402,7778	2.481 g x 2.96 %	2.481 g compost = 0.0734 g N	0.01728-x=	-0,09068 g N needed for 25 C/N	(28/100) x X = 80.8 g	
1000g/402.778= 2.4828			0.00865-y=	-0,06476 g N needed for 50 C/N	(28/100) x Y = 27.3 g	
C/N = 5,881757			0.03456-z=	-0,03884 g N needed for 12.5 C/N	(28/100) x Z = 190.4 g	

**Appendix Table 13: Yellow pea powder C and N content**

YPP calculation						
<b>Total C= 41.13 %</b>	411.3g/1000g X100= 41.13 %	1kg YPP=411.3g C	C/N	2720/411.3 = 6.6132	6.6 kg = 2720 g C	C/N
<b>Total N=1.11 %</b>	11.1g/1000g X 100 = 1.11 %	1kg YPP=11.1g N	37,054		6.6 kg = 73.3 g N	37,108

**Appendix Table 14: Lentil powder C and N content**

LP calculation			
<b>Total C= 41.13 %</b>	407.7g/1000g X100= 40.77 %	1kg LP=407.7 g C	C/N
<b>Total N=1.11 %</b>	19.8g/1000g X 100 = 19.8 %	1kg LP=19.8g N	20,591

## **Appendix 3: Applicable legislation**

Rehabilitation is required by the legislation of South Africa. This section will address the applicable acts.

### **Constitution of South Africa (Act 108 of 1996)**

All the legislation is underpinned by the Constitution of South Africa, 1996 (Act No. 108 of 1996). The Constitution states that: the State must respect, promote, protect, and fulfil the rights preserved in the Bill of Rights (the cornerstone of democracy in SA as, in compliance with Section 7 (2) of the Constitution.

#### **Section 24 of the Constitution – Environment**

*“Everyone has the right-*

- *to an environment that is not harmful to their health or well-being; and*
- *to have the environment protected, for the benefit of present and future generations, through reasonable legislative and other measures that-*
- *prevent pollution and ecological degradation;*
- *promote conservation; and*
- *secure ecologically sustainable development and use of natural resources while promoting a justifiable economic and social development.”*

The environment must be protected through avoidance of pollution and ecological deterioration. Conservation must be promoted and ecologically sustainable developments be secured.

### **National Environmental Management Act (NEMA) (Act 107 of 1998)**

The national environmental management policy is regulated by this act. NEMA focuses primarily on co-operative governance, sustainable development and public

participation by establishing principles for decision-making and providing for matters affecting and connected to the environmental functions.

NEMA sets out the requirements for environmental assessment for a number of activities associated with mining.

## **Section 28 of NEMA**

According to Section 28 (1) of NEMA, soil management plans promote sustainability such as:

- *“Assessing the impact on the environment;*
- *Informing and educating employees about the environmental risks of their work and ways of minimising these risks;*
- *Ceasing, modifying or controlling actions which cause pollution/degradation;*
- *Containing pollutants or preventing movement of pollutants;*
- *Eliminating the source of pollution or degradation; and*
- *Remedying the effects of the pollution or degradation.”*

## **Applicable NEMA sections**

### ***Section 28: Principles that may significantly affect the environment***

This section addresses the general duty of care on every person who causes, has caused or may cause significant pollution or degradation of the environment. They must take reasonable measures to prevent such pollution or degradation from occurring, continuing or recurring. It must be done to the extent that if such harm came to the environment and cannot be avoided or stopped, it is authorised by law that reasonable measures must be taken to avoid or minimise and rectify such pollution or degradation of the environment.

### ***Section 30: Control of emergency incidents***

Pollution incidences must be reported to the Department.

### **Section 34: EMP**

Included in a draft environmental management plan must be the following –

EIA regulations state that information on any proposed management or mitigation measures that will be taken to address the identified environmental impacts in a report contemplated by these regulations, must include objectives in respect of environmental impacts

#### **“(iv) Environmental rehabilitation”**

Must take place as far as reasonably practicable, so that measures to rehabilitate the environment affected by the undertaking of any listed activity or specified activity to its natural or predetermined state or to a land use which conforms to the generally acceptable principle of sustainable development can be taken, including where appropriate, progressive or concurrent rehabilitation measures

### **The Minerals and Petroleum Resources Developments Act (MPRDA) Act 28 of 2002**

In terms of the MPRDA, a company has to have a licence to mine and an approved EMP with regards to the MPRDA process. The applicable MPRDA sections are summarised below.

#### **Applicable MPRDA sections**

##### ***Section 37 - Environmental management***

Requires that the principles set out in section 2 of NEMA must apply to all mining and prospecting operations, and that the generally accepted principles of sustainable development must be applied by integrating economic, social and environmental factors during both planning and implementation phases of mining projects.

**Section 38 - Environmental management**

It requires the applicant to manage all environmental impacts in accordance with either the approved environmental management program, or his or her environmental management plan.

**Section 39 - Environmental management**

Deals with the requirements of either an environmental management programme or plan, whichever is applicable to the above-mentioned.

**Section 41 - Financial provision**

Financial provision needs to be provided and annually assess the environmental liability thereof.

**Section 43 - Closure certificate**

The holder of a mining right is responsible for all EMP-identified environmental liabilities and application for the closure certificate needs to be made to the regional manager.

**Section 44 - Removal of infrastructure**

The mine may not remove buildings, structures or objects when the mining operation comes to an end which may not be removed or demolished in terms of any other law

**Section 45 - Remedial measures**

The minister has the right to evaluate, investigate, assess and report on the impact of any ecological degradation or pollution that requires remedial measures

## **Regulation 527 of the MPRDA**

The published Government Notice No. R.527, as published in the Government Gazette on 23 April 2004 (GG No. 26275, Volume 466), Mineral and Petroleum Resources Development Act (Act 28 of 2002): regulations stipulate clearly that the following closure objectives must form part of both the Environmental Management Programme (EMP) and the Soil Management Plan (SMP):

- *“Identify the key objectives for closure of the operation to guide the project design;*
- *Development and management of environmental impacts;*
- *Provide future land use objectives for the site; and*
- *Provide proposed closure costs.”*

## **Applicable regulations**

### Requirements of the Government Notice 527

#### Regulation 42(1) - The need to prevent and alleviate pollution arising from mining activities

Section 42(1)a of the MPRDA stipulates that the closure process of the mine must start right at the commencement of a mining operation and continue throughout the entire life of the mine. Furthermore, future closure and land use objectives must be included in the applicable Environmental Management Programme (EMP). Section 42(1)d stipulates furthermore that any environmental damage or identified residual impacts during the Environmental Risk Assessment (ERA) phase must be acceptable to all I&APs in line with Section 24(a) of the National Constitution.

#### Regulation 43 - The need to prevent and alleviate pollution arising from mining activities

A summary of the results of progressive rehabilitation undertaken must be included in a closure plan contemplated in Section 43(3)(d) of the Act, and as the case may



be form part of the environmental management programme or environmental management plant.

Regulation 56 - Part III of R527 deals with environmental regulations for mineral development, petroleum exploration and production

In accordance with applicable legislative requirements for closure of a mine, the holder of a prospecting right, retention permit, mining right or mining permit must ensure that the land is rehabilitated to its natural state, or to a predetermined and agreed standard or land use, as far as it is practicable, which conforms with the concepts of suitable development.

### **The National Water Act (Act 108 of 2008)**

For the benefit of all water users, the National Water Act, 1998 (Act 36 of 1998) (NWA) aims to provide management of the national water resources to achieve sustainable use of water. This requires that the quality of water resources is protected and also that management of water resources must be integrated with the delegation of powers to institutions at the regional or catchment level. The purpose of the NWA is to ensure that the nation's water resources are protected, developed, used, managed, conserved, and controlled in ways which take into account:

- *“Meeting the basic human needs of present and future generations;*
- *Promoting equitable access to water;*
- *Redressing the results of past racial discrimination;*
- *Promoting the efficient, sustainable and beneficial use of water in the public interest;*
- *Facilitating social and economic development;*
- *Providing for growing demands for water use;*
- *Protecting aquatic and associated ecosystems and their biological diversity;*
- *Reducing and preventing pollution and degradation of water resources;*
- *Meeting international obligations; and*
- *Managing floods and droughts.”*

## ***Applicable regulations***

### **Section 19 - Prevention and remedying effects of pollution**

Any existing situation which may cause or is likely to cause pollution of a water resource, must take all reasonable measures to prevent any such pollution from occurring, continuing or recurring.

### **Section 20 - Control of emergency incidents**

Incidences of pollution need to be reported to the Department as well as the relevant catchment agency.

### **Section 21 - Water uses**

Any type of water uses

## **Regulation 398 of the National Water Act (March 2004)**

### **Applicable regulations**

#### **1.7. (1)(c)(vii) - Rehabilitation of river diversions –Section 21(c)**

All necessary measures are taken to stabilise the diversion structure as well as the surrounding area,

This will include:

- “(aa) rehabilitation of the riparian habitat integrity by ensuring that during rehabilitation only indigenous shrubs and grasses are used in restoring the biodiversity;
- (bb) rehabilitation of disturbed and degraded riparian areas to restore and upgrade the riparian habitat integrity to sustain a biodiversity riparian ecosystem;”

### 1.7. (1)(c)(vii) - Rehabilitation of river diversions –Section 21(c)

All reasonable and available measures must be taken to ensure that:

- “(c) rehabilitation of the watercourse, including riparian and in stream habitat, is undertaken after any impendence or diversion of flow”

### **Government Notice 704**

This notice was published in Government Gazette 20119 of 4 June 1999. The Minister of Water Affairs is responsible for the protection, use, development, conservation, management and control of the water resources of South Africa on a sustainable basis and has prescribed requirements, in terms of the regulations, that must be seen as minimum requirements to fulfil this goal.

The Department of Water Affairs (“DWA”) subscribes to the principles of co-operative governance and recognises the role of the Department of Mineral Resources to co-ordinate environmental management within the mining industry and the role of the Department of Environmental Affairs as the lead agent on matters affecting the environment.

The roles of Environmental Management Programme Reports and Environmental Management Programme Performance Assessment Reports required in terms of the Mineral and Petroleum Resources Development Act, 2002 (Act 28 of 2002), and Environmental Impact Assessment Reports required in terms of the National Environmental Management Act, 1998 (Act 107 of 1998) as well as the National Environmental Management Waste Act, 2008 (59 of 2008) are recognised and supported by the DWA, and as a result any information, obligations, programmes, permissions and commitments contained in the above reports, procedures, consultation requirements and decision-making processes will be recognised by the Department.

To promote coordination, copies of relevant exemptions from the requirements of the regulations will be forwarded to the Department of Mineral Resources as well as the Department of Environmental Affairs by the Department of Water Affairs as they are issued. National Environmental Management: Waste Act No 59 of 2008.

The rehabilitation measures must be aligned with the objections of the national Environmental Management: Waste Act, 2008 (Act 59 of 2008) (NEM:WA) which includes:

- *“(a) to protect health, well-being and the environment by providing reasonable measures for—*
- *minimising the consumption of natural resources;*
- *avoiding and minimising the generation of waste;*
- *reducing, re-using, recycling and recovering waste;*
- *treating and safely disposing of waste as a last resort;*
- *preventing pollution and ecological degradation;*
- *securing ecologically sustainable development while promoting justifiable economic and social development;*
- *promoting and ensuring the effective delivery of waste services;*
- *remediating land where contamination presents, or may present, a significant risk of harm to health or the environment; and*
- *achieving integrated waste management reporting and planning;*
- *(b) to ensure that people are aware of the impact of waste on their health, well-being and the environment;*
- *(c) to provide for compliance with the measures; and*
- *(d) generally, to give effect to section 24 of the Constitution in order to secure an environment that is not harmful to health and well-being.”*

## **Waste Classification and Management Regulations, 2013 (Government Notice NR: 634)**

Both Waste Classification and Management Regulations (WCMR) promulgated under the National Environmental Management: Waste Act, 2008 (NEM: WA) (effective 2013) provide mechanisms to:

- *“Facilitate the implementation of the waste hierarchy to move away from landfill*
- *Reuse, recovery and treatment;*
- *Separate waste classification from the management of waste;*
- *Divert waste from landfill and into utilisation where possible; and*
- *Provide measures to monitor the progress.”*

The Waste Classification and Management Regulations (**GNR: 634 – 635**) ultimately enable the improved and more efficient classification and management of waste; provide for safe and appropriate handling, recovery, storage, reuse, treatment, recycling and disposal of waste and will also enable relevant and accurate reporting on waste generation and management. All waste generators, excluding domestic generators, must ensure that the generated waste is classified within 180 days of its generation. All classified wastes in terms of “Minimum Requirements for the Handling, Classification and Disposal of Hazardous Waste in terms of the Department of Water Affairs” (2<sup>nd</sup> Edition, 1998; Department of Water Affairs and Forestry) or alternative approved classifications that were prior to the WCMR taking effect, must be assessed and re-classified within three years from the commencement of these Regulations.

### **Reference is made to the NEM: WA, part 8 of Chapter 4 regarding contaminated land**

All owners of significantly contaminated land become obliged to report that contamination is occurring. Part 8 of Chapter 4 addresses the concern with the remediation of contaminated land. The new legal regime for identifying contaminated

land, determines its status and the risk that it poses, introducing the regulating of the remediation process. This specific law imposes significant legal obligation on the owners of land as well as on those who cause contamination, with potentially serious financial consequences of the mentioned offence. Part 8 applies where the pollution only manifest sometime after the contamination occurred and also where the person's action (for example, the excavation of land pursuant to a development) and result in a noticeable change to pre-existing contamination. Along with the notice bringing Part 8 into effect, standards and norms for the remediation of contaminated land and soil quality (list certain contaminants and specify soil screening values for human health and environmental protection) must be set. This also has several important implications for the sale of land where sellers who know that their lands are contaminated, can no longer keep silent and can be classified as an offence.

### **Applicable sections**

- *“Section 16: General duty in respect of waste management;*
- *Section 17: Reduction, re-use, recycling and recovery of waste;*
- *Section 18: Extended producer responsibility; and*
- *Section 21: General requirements for storage of waste.”*

## **National Environmental Management: Biodiversity Act no. 10 of 2004**

### **Applicable sections**

- *“Section 2: Management and conservation of Biological diversity;*
- *Section 72: Duty of care relating to listed invasive species.”*

## **National Air Quality Act**

### **Section 27, 32, 34 & 35: Prevention of air pollution that also includes dust, smoke and noise variants**

Studies on dust fall-out are conducted in accordance with the National Environmental Management: Air Quality Act, 39 of 2004. The ASTM International measurement system to determine monthly average fall-out concentrations in

accordance of the South African National Standards (SANS) 1929: 2005, Edition 1.1 will be utilised during the process of dust sampling and analysis.

A four-band scale is used in the evaluation of dust fall where aspects like target, action levels and alert are measured. These environmental limits for dust levels were established to minimize effects such as the negative environmental impact on society as well as air pollution and the prevention of any polluting developments that may occur.

**Appendix Table 15: Dust fallout guideline**

<b>Band Number</b>	<b>Band Description Label</b>	<b>Dust-Fall Rate (D) (mg/m<sup>2</sup>/day, 30-day average)</b>	<b>Comment</b>
1	Residential	$D < 600$	Allowable for residential and light commercial
2	Industrial	$600 < D < 1\,200$	Acceptable for heavy commercial and industrial
3	Action	$1\,200 < D < 2\,400$	Needs investigation and remediation if two sequential months lie in this band, or more than three occur in a year
4	Alert	$2\,400 < D$	Immediate action and remediation required following the first exceedance. Submit an incident report to the relevant authority.

**Appendix Table 16: Target, alert and actions thresholds**

<b>Level</b>	<b>Dust-Fall Rate (D) (mg m<sup>-2</sup> day<sup>-1</sup>, 30-day average)</b>	<b>Averaging Period</b>	<b>Permitted Frequency of exceedance</b>
Target	300	Annual	-

Action residential	400	30 days	No two sequential months within three times any year,
Action industrial	1200	30 days	Three within any particular year, not sequential months
Alert threshold	2400	30 days	None. The first exceedance requires remediation and compulsory report to relevant authorities.

### **National Heritage Resources Act, No. 25 of 1999**

The heritage impact assessment forms an important part of the environmental impact assessment as required by the EIA regulations in terms of the National Environmental Management Act, 1998 (Act No. 107 of 1998). The heritage impact assessment was compiled in accordance with Section 38 of the National Heritage Resources Act, and has therefor been submitted to be part of the South African Heritage Resources Agency (SAHRA). A phase 1 assessment report was compiled for the EIA / EMP process during the completion of the MPRDA process.

### **The Conservation of Agricultural Resources Act, 1983 (Act 43 of 1983)**

The South African Mineral Resource Committee (SAMREC) Code is of particular importance in this regard where the determination of whether the product (mineral) has made an adequate provision for environmental rehabilitation in terms of Section 41 of the MPRDA is important, because this will be considered by analysts when making their assessment of a product's shares prior to investing.

### **Best Practice and International Guidelines**

Closure of mines is an international challenge. South Africa has produced various well-known and reputable guidelines through the years on matters directly linked to and/or associated with mine closure.



Such was the need for guidelines to manage the successful closure of mine provisions in a consistent manner provided for by the (Department of Minerals and Energy [DME] 2005). These defined guidelines are the only official mine closure guidelines as contemplated in Regulation 54(1) in the Mineral and Petroleum Resources Development Act, 2002 (Act 28 of 2002). This guideline document is of particular importance as it also governs the closure cost assessment process in South Africa, applied by the DMR through its respective regional managers in each province.

In 2012 the World Wildlife Fund (WWF) published a discussion document named the “Financial provision for the rehabilitation and closure in South African Mining: Discussion Document on Challenges and recommended improvements”. The specific document focuses on the adequacy of financial provisions and draws a very strong link between insufficient financial allocations and that of abandoned and rundown mines in South Africa.

The document further emphasizes the great importance of establishing a dependency between the both the EMP (Environmental management plans or programmes) and financial provision which is updated and adequate

The Chamber of Mines (CoM) (2007) issued a guideline for the successful rehabilitation of mined land. This specific document is a result of scientific knowledge experts. It is an on-the-ground reference document which provides important and useful written guidelines on the best rehabilitation techniques available. How the document distinguishes between the financing, planning and the licencing components of a typical mining program is of great value.

Recently a released guideline from the Government of Western Australia provides insight to the importance of mine closure. These guidelines in particular state the importance of planning for mine closure as a critical component of environmental management in the mining industry. Notably is that this industry leading practice also requires planning for mine closure that should start before mining commences and

should also continue throughout the life of the mine (LoM) until final closure and relinquishment has taken place. This approach enables better environmental outcomes at the end. It is also good business practice as it should avoid the need for costly remedial earthworks late in the project lifecycle of the mining process.

### **Other legislation which relates to soil management includes**

The Bill of Rights states that primarily existing environmental rights have to ensure good health and well-being, and secondarily has to protect the environment through reasonable legislation, ensuring the prevention of the degradation of important resources.

- The Environmental right is furthered in the National Environmental Management Act (No. 107 of 1998), which prescribes the three principles, namely firstly the precautionary principle, secondly the “polluter pays” principle and thirdly the preventive principle. It is further stated that the group/individual responsible for the degradation/pollution of natural resources is also required to rehabilitate the polluted source.
- The National Environmental Management Act 107 of 1998 requires that pollution and degradation of the environment be avoided at all costs, or, where it cannot be avoided, be minimized and remedied.
- The National Veld and Forest Fire Bill of 10 July 1998 and the Fertiliser, Farm Feeds, Agricultural Remedies and Stock Remedies Act 36 of 1947 can also be applicable in some of the cases that might occur.”

## *Declaration*

*This is to declare that I, Annette L Combrink, accredited  
language editor and translator of the South African  
Translators' Institute, have language-edited the dissertation  
by*

**M Ferreira (22128115)**

*with the title*

**Inoculation of carbon and nitrogen in growth mediums to  
promote seed germination in mine rehabilitation**



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*Date: 10 November 2015*