An evaluation of the germination and establishment of three selected coated grass species in different soil types for rehabilitation

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Abstract

The primary impacts of mining on the environment include the deterioration of soil properties and the loss of vegetation cover and density, often leading to increased erosion. In order to encumber further degeneration of such ecosystems and all subsequent other negative environmental impacts, active rehabilitation practices are often implemented. Active rehabilitation involves the introduction of species by different re-seeding (re-vegetation) methodologies. A higher vegetation cover and density is needed to increase soil quality, combat erosion and contribute to species richness, diversity and ground cover. Several Acts regarding environmental legislation and the conservation of the natural resource in South Africa are used to ensure that sustainable development, rehabilitation and effective environmental management of disturbed areas are enforced. Legislation therefore provides a measure to prevent pollution and ecological degradation, promotes conservation, secure ecologically sustainable development and the use of natural resources, while promoting justifiable economic and social development. Legislation also enforces and regulates the remediation of disturbed ecosystems, such as the rehabilitation of mine tailing areas. Some of this legislation mentioned above is described in the thesis.

Species selected for the compilation of seed mixtures for re-seeding and re-vegetation purposes should comply with the standards determined by the regional biodiversity framework where the disturbed area is situated. Only seed of species with non-invasive potential, that are adapted to the specific environmental conditions and have specific genetic traits, should be included in the seed mixture for rehabilitation. Since seed from local ecotype species are often not available, seed companies use seed from especially grass species that might be adapted to the environmental conditions and type of disturbance or degradation to help remediate the poor soil conditions and improve the vegetation cover. The problem is that if the morphological and physiological aspects of the seed type have not been researched properly, it may lead to poor germination and establishment results when used for the rehabilitation of certain degraded and disturbed areas, such as rangelands or mine tailings.

Advance Seed Company tries to enhance seed by adding a coating around the caryopsis (grass seed) for better germination and establishment rates. Such seeds are then referred to as “enhanced” or “coated” seed. The term “seed” will be used throughout the dissertation to describe the whole, intact caryopsis (e.g. *Anthephora pubescens*). The coatings normally refer to the physical enhancements of the seeds by the application of a water-soluble lime-based coating, which may contain nutrients, fungicides, pesticides and other polymers. This study focused on the evaluation of the germination- and establishment rates in four

soil types (growth mediums), as well as the activity of three growth enzymes on coated seed of three grass species, namely *Anthephora pubescens*, *Cynodon dactylon* and *Panicum maximum.* Advanced Seed Company provided the seeds for the three selected grass species that were coated with their newly developed certified formulae. Experimental trails were carried out in the laboratory and greenhouses (controlled conditions) at the North West University and in the field (uncontrolled conditions) at the four locations representing the different soil types, namely the clayey and sandy soils at Taaibosbult near Potchefstroom and the platinum (alkaline growth medium) and gold mine tailings (acidic growth medium) near Rustenburg and Stilfontein respectively. Detailed soil analysis was carried out by certified soil laboratories in Potchefstroom and seed purity, viability and quality determined by the Plant Protection Institute in Pretoria.

The results from the greenhouse and the field trials differed significantly for all seed types (coated and uncoated) of the three grass species in the four soil types. The germination and establishment rates in both the greenhouse (controlled conditions) and field (uncontrolled conditions) trials were overall very low. The latter can also be ascribed to the competition with other weed species that were present in the soil seed bank before re-seeding, as well as the predation by ants and guinea fowls in especially the field trials of the sandy and clayey soils. Due to the absence of competition in the field trials on the mine tailings, the germination and establishment rates were higher for most grass species. The quality of the seed batches as supplied by Advance Seed Company was not very good. Although the purity was high, many dead seeds were found, especially for *Panicum maximum.* The germination and establishment rates of *Antephora pubescens* of the uncoated seed was higher in the sandy, platinum and gold mine tailings soil types in both the uncontrolled field and controlled greenhouse trials and low for both seed types (coated and uncoated) in the clayey soils. *Cynodon dactylon* had higher germination and establishment rates for especially the gold mine tailings soil in the field trials for both seed types, as well as the sandy soils under controlled conditions in the greenhouse. Both rates were lower in the sand- and clayey soils field trials.

The germination rates for *Panicum maximum* for both seed types were similar for the clay and sandy soil types, but very low in the soils from the mine tailings, especially under controlled conditions in the greenhouse trials. The germination and establishment rates for both seed types of this species were however much higher in the field trials at both the gold and platinum mine tailings, mainly due to the absence of competition. No results for *Panicum maximum* were obtained from the field trials on the clay soils due to management and maintenance problems. The peroxidise enzyme activity was higher in the coated seed of *Antephora pubescens,* but lower in both seed types of *Cynodon dactylon* and *Panicum*

maximum. The alpha amylase enzyme activity was high in the coated seed of *Antephora pubescens* and both seed types of *Panicum maximum*, but low in both seed types of *Cynodon dactylon*. The activity of the lipoxygenase enzyme was higher in all the coated seed of all three grass species that were used in this study.

It also appears as if the storage period played a significant role in the germination of the species, especially after and during the seed coating process, as it had a negative effect on the physiology of the seed. In all species, a higher rate of gaseous exchange was observed in the uncoated seed types. However, the water content of the seed types differed between the seed types. Depending on the size and the genetic characteristics of the species, the longevity of the enzyme proteins differed. This is especially observed in the enzyme activity of three enzymes tested, i.e. lipoxygenase, peroxidase and alpha-amylase. The germination rate only improved shortly after being coated and then declined steadily. The germination capacity therefore depends on the length of the storage period. The genetic adaptation of the different species coincided with the four soil types. It is therefore recommended that only species that are adapted to a certain soil type is used in rehabilitation and if the seed is coated, it should be sown shortly after the coating process and not be stored for long periods. It is also recommended to first treat the area with herbicide before any re-seeding takes place, especially if low concentrations of seeds are used.

Keywords: germination and establishment; rehabilitation; seed coating; seed physiology; soil types.

Uittreksel

Die primêre impak van mynbou op die omgewing is die agteruitgang van grondtoestande en die verlies in plantegroei bedekking en digtheid. Ten einde verdere degenerasie van sulke ekosisteme en alle daaropvolgende negatiewe uitwerking op die omgewing te verhoed, word daar dikwels van aktiewe rehabilitasiepraktyke gebruik gemaak. Aktiewe rehabilitasie behels die inbring van spesies deur verskillende metodes van hersaai (herplant). 'n Hoër plantbedekking en -digtheid is nodig om die grond kwaliteit te bevorder, erosie te bekamp en bydraes tot spesierykheid, diversiteit en grondbedekking te maak. Verskeie wette ten opsigte van die omgewings en die bewaring van die natuurlike hulpbronne in Suid-Afrika word gebruik om te verseker dat volhoubare ontwikkeling, rehabilitasie en doeltreffende omgewingsbestuur in versteurde gebiede toegepas word. Wetgewing bied dus 'n maatreël om besoedeling en ekologiese agteruitgang te voorkom, bewaring te bevorder, ekologiese volhoubare ontwikkeling te ondersteun tydens gebruik van natuurlike hulpbronne, terwyl regverdigbare ekonomiese en maatskaplike ontwikkeling bevorder word. Wetgewing dwing ook die regulering en remediasie van versteurde ekosisteme af, soos die rehabilitasie van mynslykgebiede. Enkele van die wetgewings genoem, word in die verhandeling beskryf.

Spesies wat gekies word vir die samestelling van saadmengsels vir hersaai of hervestiging van plantegroei, moet voldoen aan die standaarde bepaal deur die plaaslike biodiversiteitsraamwerk van die streek waar die versteurde gebied geleë is. Slegs saad van spesies met 'n nie-indringende potensiaal, wat aangepas is vir die spesifieke omgewingstoestande en spesifieke genetiese eienskappe besit, moet in die saadmengsel vir rehabilitasie ingesluit word. Omdat saad van lokale ekotipes dikwels nie beskikbaar is nie, gebruik saadmaatskappye saad van veral grasspesies wat moontlik by die omgewingstoestande en tipe versteuring of degradasie aangepas is om die remediasie van die swak grondtoestande aan te spreek en plantbedekking te verbeter. Die probleem is dat, as daar nie behoorlike navorsing op die morfologiese en fisiologiese aspekte van die saad tipe uitgevoer is nie, dit tot swak ontkieming en vestigings resultate kan lei, indien dit vir die rehabilitasie van sekere gedegradeerde of versteurde areas gebruik word, soos weiveld en mynhope, wat verskille grond tipes en groeimediums verteenwoordig.

Advance Saad Maatskappy probeer om die ontkieming en vestiging van die saad te verbeter (versterk) deur die byvoeging van 'n deklaag om die kariopsis van die grassaad. Sulke sade staan dan as “omhulde” of “versterkde” saad bekend. Die term “saad” sal deur die verhandeling gebruik word as verwysing na die heel kariopsis (graanvrug), soos by *Anthephora pubescens*. Die omhulsel verwys na die verbetering van die saad deur die fisiese toevoeging van 'n water-oplosbare deklaag met ‘n kalkbasis. Dit kan

voedingstowwe, swamdoders, insekdoders en ander polimere bevat.Hierdie studie het op die evaluering van die ontkieming- en vestigingvermoë in vier grondtipes (groeimediums), asook die ensiemaktiwiteit van drie groeiensieme deur fisiologiese eksperimente op omhulde saad van drie grasspesies, naamlik *Anthephora pubescens*, *Cynodon dactylon* en *Panicum maximum* gefokus. Advance Saad Maatskappy het die saad van die drie geselekteerde grasspesies voorsien, elk omhul met die nuut ontwikkelde gesertifiseerde formule.Eksperimentele proewe is in die laboratorium en glashuise (beheerde toestande) by die Noordwes Universiteit en in die veld (onbeheerde toestande) by die vier gebiede wat die verkillende grondtipes verteenwoordig uitgevoer, naamlik in die klei- en sandgronde van Taaiboschbult naby Potchefstroom en die platinum- (alkalise groeimedium) en goudmyn slikdamme (suur groeimedium) naby Rustenburg en Stilfontein onderskeidelik. In diepte grond analises is by ‘n gesertifiseerde grondlaboratorium in Potchefstroom uitgevoer en die suiwerheid, lewensvatbaarheid en kwaliteit van die saad by die Plantbeskermingsinstituut in Pretoria bepaal.

Die resultate van die glashuis- en veldproewe het beduidend verskil vir al die saadtipes (omhul en nie-omhul) vir die drie grasspesies in die vier grond tipes. Die ontkieming en vestiging in beide die glashuis- (beheerde toestande) en veldproewe (onbeheerde toestande) was oor die algemeen baie laag. Laasgenoemde kan ook toegeskryf word aan die kompetisie met onkruid wat reeds in die saadbank teenwoordig was voor hersaai en die predasie deur miere sowel as tarentale in veral die sand- en kleigrond veldproewe. Die ontkieming en vestiging was hoër op die mynslikhope weens die afwesigheid van kompetisie by hierdie veldproewe. Die kwaliteit van die saad soos deur Advance Saad maatskappy voorsien was nie goed nie. Alhoewel die suiwerheid van die saad hoog was, het baie dooie saad voorgekom, veral vir *Panicum maximum.* Die ontkieming en vestigingsvermoë van *Antephora pubescens* van die nie-omhulde saad was hoër in die sand, platinum- en goudslik tipes gronde in beide die glashuis- en veldproewe en laag vir altwee die saad tipes (omhul en nie-omhul) in die klei grondtipe. *Cynodon dactylon* het hoë ontkieming- en vestigingsvermoëns in veral die goudslikmynhoop gronde vir beide saadtipes in die veldproewe gehad, sowel as die sandgronde onder gekontroleerde toestande in die glashuis. Beide vermoëns was laer in die sand- en kleigrondtipes in die veldproewe.

Die ontkiemingsvermoë van *Panicum maximum* vir beide saadtipes was baie dieselfde vir die klei- en sand grondtipes, maar baie laag in die gronde van die mynhope, veral onder gekontroleerde toestande in die glashuisproewe. Die ontkieming- en vestigingsvermoëns van beide saadtipes van hierdie spesie was baie hoër in die veldproewe op die mynhope, seker weens die afwesigheid van kompetisie. Geen resultate is verkry van die veldproewe in die kleigronde weens bestuur- en beheerprobleme. Die peroksidase

ensiemaktiwiteit was hoër in die nie omhulde saad van *Antephora pubescens*, maar laer in beide die saadtipes van *Cynodon dactylon* en *Panicum maximum.* Die alfa amilase ensiemaktiwiteit was hoog in die omhulde saad van *Antephora pubescens* en beide saadtipes van *Panicum maximum*, maar laer in beide saadtipes van *Cynodon dactylon.* Die aktiwiteit van die lipoksigenase ensiem was hoër in al die omhulde saad van al drie die grasspesies wat in hierdie studie gebruik is.

Dit wil ook voorkom asof die stoortydperk 'n belangrike rol gespeel het by die ontkieming van die spesies, veral gedurende en na die omhullingsproses, omdat dit ‘n negatiewe effek op die fisiologie van die saad gehad het. Die nie-omhulde saad van al drie die geselekteerde spesies het hoër vlakke van respirasie getoon as die omhulde saad, alhoewel die vlakke van voginhoud verskil het tussen die saadtipes van elke spesie. Afhangende van die grootte en die genetiese eienskappe van die spesies, het die lewensduur van die ensiemproteïene verskil na toevoeging van die omhulsel. Dit is veral waargeneem deur die aktiwiteit van drie ensieme wat getoets is, naamlik lipoksigenase, peroksidase en alfa amilase. Die ontkiemingsyfer van die drie geselekteerde spesies het slegs verhoog kort nadat dit omhul is, waarna dit weer geleidelik afgeneem het. Die tydperk van stoor beïnvloed dus die omhulde sade. Die genetiese aanpassing van die verskillende spesies kon met die vier grond tipes vergelyk word. Dit word dus aanbeveel dat slegs spesies wat by sekere grondtipes aangepas is in die rehabilitasie van gedegradeerde en versteurde gebiede gebruik word en indien die saad omhul word, dit kort na die omhullingsproses gesaai word en nie vir lang tydperke gestoor word nie. Daar word ook aanbeveel dat die gebiede wat hersaai word eers met onkruiddoder behandel word, veral as lae konsentrasies van saad gebruik word.

Sleutelwoorde: grondtipes; ontkieming en vestiging; rehabilitasie; saadfisiologie; saadomhulsel.

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What we have done for ourselves alone dies with us; what we have done for others and in the world remains, is immortal. - Albert Pike

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GLOSSARY

Aggregate. A unit of soil structure, usually <10mm in diameter (Winegardner, 1995).

Aggregation. The act of soil particles cohering so as to behave mechanically as a unit (Winegardner, 1995).

Anthropogenic soils. Newly created disposal sites cannot be included into existing soil classification systems, due to a series of unique and specific properties, created by human activity; with different characteristics and soil qualities than conventional soil (Van Deventer & Hattingh, 2004; Resulović & Čustović, 2007).

Buffering capacity. The ability of ions associated with the solid phase to buffer changes in concentration in the solution phase, and thereby exhibit resistance to pH change (Foth, 1990). The amount of base needed to produce a certain pH increase, or the amount of acid needed to lower the pH with a certain amount. The larger the amount of reactive colloids present in the soil, the larger the buffering capacity would be (Van Rensburg, 2006).

Catalyse. An enzyme, specific in its function, accelerating the rate of a biological reaction in metabolism (Garrett & Grisham, 2005).

Cation exchange capacity. The measure of the total number of equivalents of cations displaced per unit mass of solids by an extracting solution containing a high concentration of an extracting cation (Winegardner, 1995).

Degradation. Pertains to subtle or gradual changes that reduce ecological integrity and health (SER, 2004).

Dormancy. The absence of germination growth of a viable seed under otherwise optimal conditions (Vanangamudi & Natarajan, 2006).

Ecotypes. Species that have adapted to a certain environment over time to bear specific genetic characteristics, which enables it to grow in environments with a specific combination of environmental factors (Van den Berg & Kellner, 2010).

Enhanced (coated) seed. Application of enhancing substances directly to the seed, without obscuring the shape thereof (Bhaskaran *et al.*, 2006).

Hilum. Funicular scar on seed coat that marks the point at which the seed was attached via the funiculus to the ovary tissue (Hopkins & Hüner, 2004; MacAdam, 2009).

Hygroscopic. The tendency to absorb water from an atmosphere of high relative humidity (Winegardner, 1995).

Hyphae. The basic, thread-like tubes, forming the mycelium (composed of cells attached end to end) of fungus species (Webster & Weber, 2009).

Macro-aggregates. 250µm-2000µm; formed by the preceding (see 'micro-aggregates') and coarse sand bound by bacterial polysaccharides. The latter formations are consolidated by roots and mycelium to form assemblages (Gobat *et al.*, 2004).

Micro-aggregates. 2µm- 250µm; very stable, formed by organic substances bound to clay and fine silt, or by bacterial polysaccharides (Gobat *et al.*, 2004).

Micropyle. A canal or hole in the seed coat of the nucellus through which the pollen tube usually passes during fertilization. When the seed matures and starts to germinate, the micropyle serves as a minute pore through which water enters. The micropylar seed end has been demonstrated to be the major entry point for water during seed imbibition and germination. During germination the testa ruptures at the micropylar end and the radicle protrudes through the micropylar endosperm (Hopkins & Hüner, 2004; MacAdam, 2009).

Mucilage. A layer of polysaccharide slime, produced by some seeds during imbibition. Important in water uptake during imbibition and germination (Hopkins & Hüner, 2004; MacAdam, 2009).

pH. The value of expression of the concentrations of OH^- - and H^+-ions of a substance. Mathematically expressed as $pH = -\log_{10}[H^+]$, values of less than 7 indicates that H^+-ions dominate and the solution are refered to as 'acidic'. Values greater than 7 indicates that OH^- ions dominate and the solution are considered as 'basic' or 'alkaline' (Winegardner, 1995).

Polysaccharides. Large, high molecular-weight polymers of monosaccharides; consist of one or more sugars, i.e. starch or cellulose (Hopkins & Hüner, 2004).

Primary minerals. Minerals that have not been chemically altered since deposition or crystallisation in the parent rock (Foth, 1990).

Reclamation of a degraded area implies a new state in an ecosystem, where either structure or function is different from the original; this is quite likely where the soil mineralogy has been totally replaced (Bradshaw, 1998).

Rehabilitation emphasizes the reparation of ecosystem processes, productivity and services of degraded areas (SER, 2004).

Reseeding involves the introduction of seed to a disturbed area as an indirect outcome of rehabilitation. It also represents a stock of regeneration potential in plant assemblages, which is an important component of ecosystem resilience in future times of perturbation (González-Alday *et al*., 2009)

Restoration of degraded areas implies the replication of prior-existing conditions and involves the use of indigenous species of the surrounding area; the original functions of the soil and other ecosystem parameters are reinstated to a full measure (Barbour, 1992; Bradshaw, 1998).

Revegetation. Replanting selected, indigenous or exotic vegetation on degraded landscapes in order to effect restoration, rehabilitation or reallocation.

Salinity. The condition in soils which contain large amounts of soluble salts; measured by the electrical conductivity of the soil (Van Rensburg, 2006).

Sodicity. The condition in soil containing Na as a significant proportion of the total exchangeable cations; expressed as the exchangeable sodium percentage (ESP)-value and the sodium absorption ratio (SAR)-value.

Secondary minerals. Result from the decomposition of the primary minerals or the reprecipitation of the primary minerals (Foth, 1990).

Soil quality. A soil's "fitness for use"; the measurement thereof involves placing a value upon the soil in relation to fitness to fulfil a specific purpose, and becomes inseparable from the idea of ecosystem sustainability (Mills & Fey, 2003).

Soil structure. The mode of organisation of solid constituents of soil - mineral and/or organic. They may be aggregated (pedal structure) or not (apedal structure) (Gobat *et al*., 2004).

Soil texture. Particle size distribution that determines the soil's coarseness or fineness (it refers to the relative proportions of sand, silt and clay in a soil) (Foth, 1990).

Chapter 1
Introduction

1.1. Background

Exploitation of natural resources, such as overgrazing of rangelands or the development of mine tailing dams by the mining industry, often involves the degradation or complete eradication of vegetation cover and depletion of topsoil. The consequence contributes to the deterioration of ecosystems, including soil function, stability and processes and the loss of biodiversity within the area (Bradshaw, 1998; Maboeta *et al*., 2006; Mendez & Maier, 2008). A brief overview of some causes of disturbance, the importance of introduction of species by the use of seed to enhance ecosystem re-establishment, the significance of South African legislation in rehabilitation and the quality thereof is discussed. The use of enhanced (coated) seed in rehabilitation, rather than the use of seed of eco-types is discussed, followed by the motivation for the study. An overview of the project, its aims, and a hypothesis is given, where after the structure and content of the total dissertation follows.

According to Bradshaw (1998), the primary effects of mining on the environment are soil damage and inevitable destruction of vegetation cover. A great deal of pollution also often occurs at industrial sites, such as the drastic acidifying of soil, resulting in systems difficult to restore and to re-establish vegetation cover. Apart from altered growth conditions at these sites, species occurring naturally near disturbed sites also have difficulty to establish there, due to physical change of the environment, which decreases the ability of species' immigration to the degraded systems (Bradshaw, 1998). The natural succession processes that normally take place in the recovery of these systems are therefore slow or even non-existing, depending on the state of the environment (Van den Berg, 2008; Maboeta *et al*., 2006). Therefore, in order to encumber further degeneration of such ecosystems and all subsequent negative environmental impacts, active rehabilitation practices, which involves the introduction of species by re-seeding (re-vegetation) methodologies (Van den Berg & Kellner, 2005), has become an absolute necessity to increase species richness, diversity and ground cover. Several terms describe the effort of re-establishment of vegetation in an area, such as 'rehabilitation', 'reclamation', 'restoration', 'revegetation', 'reseeding' etc., which in essence all focus on the same ideology, but has different applications and end results. For the purpose of this chapter, the term 'rehabilitation' will be used as a compromise in this regard. A detailed discussion of these definitions will follow in Chapter 2.

Several Acts regarding environmental legislation of South Africa ensures enforcement of sustainable development, rehabilitation and effective environmental management of disturbed areas (Maboeta *et al*., 2006). The National Environmental Act (NEMA) (Act 107 of 1998) states that legislation provides a reasonable

measure to prevent pollution and ecological degradation, promotes conservation, secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development (South Africa, 1998). Based on the Environmental Conservation Act (ECA) (Act 73 of 1989) the terms of Section 1, Article 28 of the National Environmental Act (NEMA) (Act 107 of 1998) states that reasonable measures must be taken to minimise and rectify polluted and/or degraded environments of which the cause could not have been otherwise prevented (South Africa, 1998). In terms of the Mineral and Petroleum Resources Act (Act 28 of 2002), rehabilitation of environments affected by mining or prospecting operations must be, as far as reasonably practical, conducted towards its natural or a predetermined state, or a land-use which conforms to the generally accepted principle of sustainable development (South Africa, 2002). According to this Act, closure of mines is only granted if the mine complies with this requirement (South Africa, 2002). Furthermore, this Act also refers to the principles of Chapter 1of NEMA (Act 107 of 1998), which entail remediation of disturbed ecosystems, and consequential biodiversity loss as well as minimisation of pollution and degradation (South Africa, 1998). Seed is an important tool for active remediation practices (Brits 2007), which is used to comply with these policies and legislation in order to restore the condition of disturbed vegetation.

Not only does legislation enforce remediation of disturbed ecosystems, but the quality thereof is also regulated, by the species and the quality of the seed used. The Biodiversity Act (Act 10 of 2004) states that endangered ecosystems, that have undergone degradation of ecological structure, function or composition because of human intervention, must be protected. Contribution to the biodiversity of the disturbed area by the addition of seed during restoration actions, should comply with the standards set by the latter Act (Act 10 of 2004) as explained in Chapter 3, Part 1 of the Act. Species selected for the compilation of seed mixtures for re-vegetation should comply with the standards determined by the regional biodiversity framework of the region wherein the disturbed area is situated, thus not being alien or declared invasive species of the region (South Africa, 2004). Additionally, the Conservation of Agricultural Resources Act (CARA) (Act 43 of 1983) forbids dispersal of seeds from species recognised as weeds in a region or "cause or permit the dispersal of any weed from any location in the Republic to any other location in the Republic" (South Africa, 1983). Seed of species with non-invasive potential for the specific region requiring re-vegetation, which is adapted to the specific environmental conditions of the disturbed area, may thus be included in the seed mixture for rehabilitation.

Disturbed areas physically differ from the surrounding environment regarding the physical and chemical soil parameters, topography (such as slope) and vegetation (composition, density and cover), as well as other biodiversity parameters. One of the most limiting factors in active restoration and rehabilitation activities therefore includes the germination and establishment of plant species, such as grasses, especially in harsh

environments (Van den Berg & Kellner, 2005; Brits, 2007). In addition, it is often difficult to identify which species are better adapted to certain environmental conditions caused by the particular disturbance. Seed of certain species will only germinate and establish successfully if they are well adapted to the specific condition. This implies a need of a beforehand, in-depth study of the plant species that may be suitable in restoration and rehabilitation practices.

In terms of the Plant Improvement Act (Act 53 of 1976), seed merchants are compelled to meet certain standards regarding seed that is sold (South Africa, 1976). Seed supplied by registered seed merchants yield higher germination percentages, due to higher seed purity and viability, when compared to seed harvested and sold by local farmers without any quality control. Due to the ensured, higher quality of certified seed (South Africa, 1976), higher germination percentages of these batches of seed are therefore also expected, enhancing the rate of re-establishment of vegetation.

An important aspect that needs to be considered in the compilation of seed mixtures for rehabilitation is the adaptation of plants that takes place over time in a specific area, associated with specific environmental conditions, and specific genetic traits. These plants grow and reproduce in areas with a specific combination of environmental conditions, but no variety in the genetics of the species (Van den Berg & Kellner, 2010). However, according to Van den Berg & Kellner (2010), apart from limited knowledge about specific ecotypes that would be of use in a specific disturbed area, it is very labour intensive and often not cost effective to collect large quantities of seed representative of a specific habitat. Furthermore, if seed of a local ecotype is used, the quality, viability and purity are often of low standard, compared to certified seed. This may lead to poor restoration results (Van den Berg & Kellner, 2010).

As enhancement of the germination and establishment of non-ecotypic seed under harsh conditions, seed merchants often additionally coat selected seed types, such as grass species and crops; referred to as “enhanced” or “coated” seed. This technique mainly stimulates an advantageous microclimate for the germinating seed. These coatings refer to physical enhancements of the seeds by application of a water-soluble lime-based coating, which may contain nutrients, fungicides, pesticides and other polymers (Advance Seed, 2009). This study focuses on the effect of the compilation of substances in a seed coating on the germination- and establishment performance of seed of a specific species, e.g. acting either as a barrier against the properties of disturbed soil, or contributing to detrimental effects on the seed in the specific soil type (Brits, 2007).

Very few recorded scientific experiments have been carried out in the past to test the germination- and establishment capacity of coated grass seed types under natural conditions in different disturbed areas,

characterised by different soil types (Brits, 2007). A project was therefore launched, in collaboration with Advance Seed Company (ASC), to evaluate the germination and establishment rates of selected coated grass seed types in four different soil types as growth mediums. Formulae of seed coatings involved in previous studies were not developed according to specifications, but more on an informal, random manner (Brits, 2007). For the first time, newly developed, certified formulae of coatings were used by ASC, specifically on the three selected grass species' seed that were used in this study. Developing a specific formula for coating on each of the three selected species has the advantage that the most effective formula for the enhancement in germination, establishment and growth of each selected grass species for rehabilitation purposes in a certain soil growth medium, can be developed and duplicated if successful. The effective enhancement of the seed types that are used in seed mixtures during rehabilitation will ultimately enhance the rate of rehabilitation actions in disturbed areas. The latter will contribute to the goal of cheap and effective restoration actions (Bradshaw, 1998). The ASC regards the compilation of the formulae of the specific coatings of the three seed types as classified information; therefore it will not be discussed. In this regard, only the effect of the specific formulation of the seed enhancements, which is reflected in the percentage of germination of the seed of each specific grass species in each of the four chosen soil types, are of importance.

The Global Restoration Network (SER, 2004) comments that "the root of restoration is information". This study's contribution to a better understanding of the use of certain seed types for rehabilitation activities may thus increase the effectiveness of re-seeding activities in these environments. The joint information gained from all the phases of the study for ASC will contribute to improved knowledge in selecting grass seed types for the use in the restoration of degraded rangelands and the rehabilitation of mine tailings with certain soil characteristics.

1.2. Project overview

This project forms Phase 3 of the larger research project that is carried out in collaboration with Advance Seed Company. Phases 1 and 2 were carried out by Yvette Brits from 2006 to 2007 (Brits, 2007).

Phases 1 and -2 entailed the investigation of the difference in the germination and establishment rates between coated and uncoated selected grass seed types. The Phase 1-project included pot- and field trials, above- and below ground biomass yields, predation by insects, and the difference in vascular tissue of the transitional region of the seedlings. The grasses assessed included coated- and uncoated seed types of *Chloris gayana* (Rhodes grass), *Cynodon dactylon* (Couch grass), *Digitaria eriantha* (Common finger grass) and *Eragrostis curvula* (Weeping love grass). In the case of *E. curvula,* four seed types were tested. These included uncoated seed, seed

treated with “plain coat”, enhancement with “organic insecticide on the base of the coat” (i.e. insecticide between the enhancement and the seed) and enhancement with “organic insecticide on the base of the coat and as an overspray” (i.e. insecticide between the enhancement and the seed, as well as spraying the insecticide over the coated seed). The ASC selected and supplied all seed types. As mentioned above, the exact formula for the coating of the different seed types were unfortunately not given by ASC. This implies that the specific formula used for the coatings could not be repeated in the follow-up research project carried out during Phase 3 (the current project).

The results of Phases 1 and 2 are discussed in detail in the M.Sc.-thesis by Brits (2007).

The current, third phase of the research project differs from the first two phases in that three different species were selected by ASC. Both the coated- and uncoated seed types of the grasses *Anthephora pubescens* (GB09/8626), *Cynodon dactylon* (GC01/9454) and *Panicum maximum* (GP01/9466), were provided by ASC. The seed coating was applied according to newly developed standards and included certified compilations of coating (enhancements) which differed for each selected species. Based on the previous two phases, the third phase of the study consisted of three components; green house-, laboratory- and field trials. These are discussed in more detail in Chapter 3.

1.3. Aims

1.3.1. General objective

The general objective was to determine the effect of enhancements as coatings on the emergence and establishment of three selected grass species, in four different soil types (growth mediums).

1.3.2. Specific objectives

- Assess the germination and establishment of the seed of three selected coated- and uncoated grass species in four soil types, both under controlled- and uncontrolled conditions.
- Carry out chemical and physical analysis of soil types.
- Assess the key enzymes’ activity of germination.
- Determine the gaseous exchange of both seed types (coated and uncoated) of the different grass species.
- Evaluate the relative water content of the coated- and uncoated selected grass seed types.

1.4. Dissertation structure and content

The effect of the applied coating, of which the compilation is unique to each of the three selected species, on the germination of the seed in the four selected soil types, is the main subject matter continuous through the dissertation.

Chapter 2 discusses the literature regarding aspects of enhancement of the seed, such as the history thereof, how seed coating is related to this study, advantages and disadvantages of application of a seed coat, and the relevance of the Plant Improvement Act (No. 53 Of 1976) is included. The importance of soil characteristics and the influence it has on the germination of seed is also discussed in this chapter, including the influence that coating of the seed may have in each soil type. Furthermore, the activity of three germination enzymes and their relevant importance as physiological parameters of germination in seed are discussed, along with respiration and water content as parameters of seed viability and the germination capacity. Closely associated with these parameters, dormancy is discussed as an important characteristic of seed that prevents seed from germinating under unfavourable conditions.

Chapter 3 discusses the study areas, and materials and methods used in the three components of the study. It includes illustrations of how different methods were executed to ensure statistically referable and repeatable results.

Chapter 4 illustrates the results obtained during the third phase of the study for the objectives mentioned and presents the discussion thereof.

Chapter 5 contains the conclusions obtained by the study and also some recommendations for continued- or repeated research of similar studies.

Chapter 6 provides the references of literature used in this dissertation.

Chapter 2

Literature review

2.1. Introduction

2.1.1. Background

Human economy depends strongly on the planet's natural capital, but since global human populations escalated over the past decades, it has caused an exponential increase in demands for natural resources. Natural capital, defined by Aronson *et al.,* (2002) is the reserve of biological and physical resources, often drawn beyond its natural regenerative capacity. As a result, the natural ecosystems on which the human population is dependent are destructed and the natural capital is depleted. The causes for the decrease in the natural capital is mainly due to mismanagement by the implementation of injudicious grazing strategies, or industrial impacts, such as the physical disturbance of soils by mining. Maboeta & Van Rensburg (2002) reported that the South African mining sector is the biggest contributor to the solid waste stream and estimates the area of land used as dumping areas as 25 000 ha, covering valuable top soil that could be used for livestock or crop production. In addition, the composition of the tailings is normally of such that the area it covers, as well as the area surrounding these tailings, only has a fraction of the productive ability than before disturbance took place. Schaefer (2009) refers to the loss of ecological memory in disturbed ecosystems - the consisted loss of the species in an area as well as the ecological processes that will determine the trajectory of change for the ecosystem in the long-term - which may prevent the ecological resilience of such ecosystem.

In order to counteract these losses of ecological functioning and natural capital, active restoration needs to be implemented, which involves the introduction of seed of native species to altered and degraded landscapes in order to restore these ecosystems (Vander Mijnsbrugge *et al*., 2009). Re-seeding practices increase the vegetation cover, density and biomass (Brits & Kellner, 2009), which in turn has several ecological advantages. It includes soil stabilization, reduction of dust pollution, decrease of eolian dispersion and erosion by water runoff by plant roots, provision of a rhizosphere wherein metals precipitate and stabilize, enhancement of soil microbial activity and provision of habitat for other species that may establish over time (Mendez & Maier, 2008).

According to Tainton & Hardy (1999), the more extensively an area is degraded, the more drastic the remediative action would have to be. Tomlinson (1984) defined a single, over-arching aim for remediative actions: 'A plant community that is established should develop into a stable, self-perpetuating ecological community which will fit in with the vegetation or land use of the surrounding environment'. However, according to Van Wyk (2002), by clarifying the definitions and terminology pertaining to ecological remediation, both the remediation technique and quality of the end result thereof will be determined. Haagner

(2008) also states that standardisation of terminology is required for interpretation and meaningful exchange of results between researchers and between the different fields of application. Much emphasis is placed on the difference between the various terms in studies such as done by Haagner (2008) and Van Wyk (2002). These studies present extensive discussions on the differences of implications of these definitions in practice. However, for the purpose of this study, the focus on these definitions will be condensed to the essence thereof, and only the difference between them will be shortly discussed.

'Restoration' of a degraded area bears a strong implication of perfection (Bradshaw, 1998) where the aim is to reinstate the original functions of the soil and other ecosystem parameters to a full measure. By defining reclamation actions by this term, the replication of prior-existing conditions thus involves the use of indigenous species of the area (Barbour, 1992). Van den Berg (2008), refers to the limits of resilience between ecosystem conditions as 'thresholds' – once an ecosystem degraded beyond the threshold for that particular system, improvement thereof can only be achieved through intervention by humans, such as in the case of degraded mine waste dumps (tailings). 'Rehabilitation', which by definition bears no implication of perfection, of such landscapes would rather be implemented than 'restoration'. Human intervention would assist in remediating such ecosystems, as Jackson & Hobbs (2009) defined it, to the most functional state as governed by the biogeochemical potential of the landscape. It would only partially represent pre-existing conditions, but would be self-sustaining, often with occasional human input. 'Reclamation' of a degraded area implies a new state in an ecosystem, where either structure or function is different from the original; this is quite likely where the soil mineralogy has been totally replaced (Bradshaw, 1998).

2.1.2. Seed in disturbed environments

Several challenges have to be overcome to successfully rehabilitate degraded areas in grassland environments, one of which is the general seed scarcity of target species adapted to the specific natural environmental conditions (Knut *et al.*, 2010). The so-called "target species" refer to the desired species that are used to create a stable, restored ecosystem. Marty (2000), states that the selection of suitable plants is crucial in order to accelerate rehabilitation. Apart from reinstating the function of ecosystems in degraded areas, González-Alday *et al*. (2009) also emphasizes the importance of the condition of the soil seed bank which is determined by the introduction of seed as an indirect outcome of rehabilitation. It also represents a stock of regeneration potential in plant assemblages, which is an important component of ecosystem resilience in future times of perturbation.

It is important that restoration ecologists should clarify the final use and objectives of reclamation before assembling seed compositions of different species (Bradshaw, 2000). Van Wyk (2006), states that normally the result of rehabilitation practices should resemble surrounding natural rangeland, such as the rehabilitation of mine tailings, but due to altered soil characteristics, this is not always practical or achievable. The owner of the

area also motivates the desirability and feasibility of the final use of the land after restoration or rehabilitation. The final use will therefore determine the choice of vegetation.

Before the compilation of a suitable seed mixture for a degraded area to be restored or rehabilitated, a variety of different factors, including the land-use, needs to be considered. These also include all bio-physical factors of the environment, as well as the physiological and morphological features of the plants themselves (Van Wyk, 2006). Environmental factors include aspects of the climate of the region, such as rainfall patterns (Tainton & Hardy, 1999), effects of slope and aspect (Bennie *et al.*, 2006), the properties and structure of the growth medium, and the nutrient availability and –cycling, which all determine the vigour and resilience of colonizing vegetation. Vander Mijnsbrugge *et al*. (2009), adds that it is a major challenge to also consider the genetic variation and diversity within native, or suitable, species in the selection of the seed types. Population differentiation is partly driven by local adaptation resulting in a home-site advantage for the off-spring. These species are called 'ecotypes'. Ecotype species are adapted to a certain environment over time to bear specific phenological characteristics, which enables it to grow in environments with a specific combination of environmental factors (Van den Berg & Kellner, 2010). Mendez & Maier (2008) states that not only does the already adapted traits of native species enhance the revegetation of disturbed, semi-arid areas, but the consideration of native plants for phytostabilization also avoids introduction of potentially invasive species that are conditioned to grow under harsh conditions. Therefore, according to Van den Berg & Kellner (2010), the measure of degradation of an area would determine the type of seed as well as the method to rehabilitate or restore it. Rehabilitating mine tailings and wastelands, which often remain after mineral extraction, therefore require plants that are often adapted to harsh conditions with wide ranges in moisture and temperature.

In this regard, the use of native grass species for re-seeding purposes are one of the most debated subjects in restoration ecology (Van Wyk, 2002). Bradshaw (1998) mentions that although it is assumed that processes of natural succession will reclaim a site over time, it is not always possible to make use of native seed, as derelict land is characterized by distinct flora due to habitat differences that exists in such areas. Even after soil amelioration, only a small number of species often establish, which leads to monoculture domination and ecosystems of low diversity (Roberts *et al.*, 1981). Bradshaw (2000), further states that in situations where the medium to be ameliorated is different from that of the surroundings, species may need to be brought in from elsewhere, with suited traits, adapted to survive the disturbed environment. These traits include factors such as the seasonal growth form and life cycle, perennial or annual, seed production and persistent ease of establishment and root development, as well as the resistance to drought and temperature ranges (Van Wyk, 2006). Depending on both the scale and measure of degradation, Van den Berg & Kellner (2010) states that either a single-species mixture or a multi-species mixture can be applied to re-seed a degraded area. Single-species mixtures are only employed in cases of low measures of degradation, especially a large scale. Multi-species mixtures are employed at vast-scale areas which are severely degraded, as these mixtures contain a huge

range of genetic variation. Apart from the ability to recolonise highly degraded areas at a higher rate, a mixture of adapted ecotype-species offers the re-seeded system the opportunity of higher resilience to adverse conditions. The aim is to establish a plant community that is stable, sustainable, and able to support biological diversity over the long-term.

When deciding on species for rehabilitation purposes, it is important to keep in mind that the disturbed area physically differs from the surrounding environment. The growth medium and composition thereof will also differ from the "normal" conditions. Therefore, the disturbed site cannot be rehabilitated to be completely identical to the natural surroundings, although it could be the objective. Van den Berg (2008), states that the goal of restoration should be to restore disturbed areas to an optimal, acceptable state. In order to reach this goal, non-local populations which are harvested from similar environmental habitats that demonstrate a higher fitness to current environmental conditions, would rather be included than local ecotypes. As mentioned, extensive research on the locations of already established populations of adapted vegetation should thus preferably precede any restoration attempt, in order to obtain as much information of such species for the re-vegetation of degraded areas. This practice will not only enhance frequency and density of established species, but will also enhance the rate of restoration and rehabilitation and provide faster resilience to adverse or changing conditions.

Brits & Kellner (2009) state that re-seeding activities require high input costs and are influenced by the quality and effectiveness of the seed used, especially with regard to germination and establishment under natural conditions. Better-quality commercially available grass seed, excluding the extensive amount of impurities per weight compared to locally harvested seed, is often preferred in restoration and rehabilitation practices. However, the availability of such seed types, especially with regard to ecotypes adapted to certain environmental conditions, is relatively poor (Brits & Kellner, 2009; Van den Berg & Kellner; 2010)

In the case of this study, ecotype adaptation had not been the decisive factor in choosing the grass species. The suppliers, ASC, selected the three species randomly, in order to evaluate the effect of an externally applied coating on the seed of the grass species that are used, despite the fact that not all of the selected species are known to be genetically suitable for the specific soil type. The characteristics of the species relevant to this study are as follows (Van Oudtshoorn, 2006):

- *Anthephora pubescens* (Nees.) (Wool grass)

This perennial tufted grass species has blue-green leaves, borne at the base of the tuft. Culms are unbranched and the margins of the leaves are thickened and wavy. It favours undisturbed, sandy soil in dry, subtropical areas, but also grows in loam and gravelly soil. The common occurrence in natural grazing sites indicates good veld condition, being highly palatable, the utilization thereof should be carefully managed. It is characterized as a

grass that is both palatable and drought resistant; it produces good stands of hay and performs well on soils with low nutritional values.

- *Panicum maximum* (Jacq.) (Guinea grass)

Characteristic features of this perennial tufted grass species include a large, open inflorescence with panicles which are arranged in a whorl at the lower part of the inflorescence. It is characterized by leafy tufts, and leaves sheaves possessing hair. Guinea grass prefers growing in shady areas, such as under trees and shrubs. It grows well in damp conditions in fertile soil, often along rivers. It is probably the most valuable grazing grass in areas of its distribution. Particularly palatable with characteristically high leaf- and seed production, this species occurs in abundance on well managed pastures.

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

Couch grass is a characteristically short, creeping grass, with both stolons and rhizomes. The inflorescence is exclusively digitate, with flattened spikelets without awns. This species is unique in that it grows in all types of soil, and is often found in disturbed places. It is therefore probably the most useful grass, as it is serves as good pasture that can endure heavy grazing, stays green until late into winter and its root system makes it ideal for rehabilitation purposes. However, this ability of rapid reproduction under conditions that would otherwise be unfavourable to other grass species in the seed mixture, such as *P. maximum* and *A. pubescens*, may result in strong competition by *C. dactylon*.

Theoretically, the compilation of substances in the applied coating would enhance the germination and establishment of the selected species' seed in the rehabilitation environment, by enhancing the microenvironment around the seed. This study would prove whether or not this hypothesis is true, and if so, whether it is relevant to all the newly-formulated seed coatings on each of the specific, selected species.

Understanding the need to rehabilitate disturbed areas and the possible advantage of the use of coated seed in seed mixtures, the following section discusses several aspects regarding seed coating and seed germination during rehabilitation. These aspects include the history of seed enhancement by coating, the advantages thereof to the restorationist, seed-related legislation, the influences of soil on germination, and dormancy (the reason why seed do not germinate).

2.2. Seed enhancement

2.2.1. History of seed coating as an enhancement

Bharathi *et al*., (2006), reports that many of the so-called "modern" seed enhancements have their origin thousands of years ago. Only the principles and concept has changed over time. Ancient Chinese coated rice

seed in small mud balls to anchor the seed in flooded paddy fields to prevent seed from drifting away. As improved technology lead to a better understanding of seed biology, successful results are presently obtained by coating seed with substances that enhance the establishment of seed to overcome some of the problems of plant establishment, especially in degraded and disturbed environments.

Seed coating differs from seed pelleting in that seed pelleting is the process of enclosing the seed in a small quantity of inert material just large enough to produce a globular unit of standard size to facilitate precision planting (Bharathi *et al.*, 2006). According to Bhaskaran *et al*. (2006) seed coating is the application of enhancing substances directly to the seed, without obscuring the shape thereof. Seed enhancement by means of a coating, the advantages thereof and how it is applied to this study, follows below.

2.2.2. Seed enhancement

According to Advance Seed, AgriCOTE® is a unique application technology that is used to apply various beneficial agricultural products to seed as a coating. This physical enhancement of seed by coating holds several advantages, not only in the economic sense, but also in terms of the result of growth (Swart, 2008). Firstly, the seed coat enhances seed-to-soil contact. Mayer & Poljakoff-Mayber (1989), states that it is not water potential alone that affects germination behaviour in seeds, but also the aggregation of soil particles of the particular soil type around the seed itself. If the soil aggregate is large, compared to seed size, and the water potential is low, the contact between the seed and the soil becomes a key factor in the germination process, by restricting the water availability to the germinating seed.

Under poor soil conditions the hygroscopic composition of the lime-based AgriCOTE® coating acts as an enhancement, which, theoretically, increases seedling survival. Additionally, small, light-weighted seed, which are normally sown with difficulty, are easier to handle when using a planter or even sowing by hand after coating. Apfelbaum *et al.* (1997) and Taylor *et al.* (1998) state that seed with chaff, such as *A. pubescens*, can also vary in size and shape, resulting in the difficulty of sowing the seed. According to Swart (2008), AgriCOTE® may also contain several growth stimulants and inoculants to improve the micro-climate around the seed in poor soil conditions, which could aid the breaking of seed dormancy, and seedling development. Nutrients and growth stimulants, including rhizobia inoculants, fungicide, pesticide, a binding polymer and a protective polymer can all be added to the lime-based coating. Figure 2.1 displays how the layers of this unique seed coating technology are applied to optimize the seeds' micro-environment.

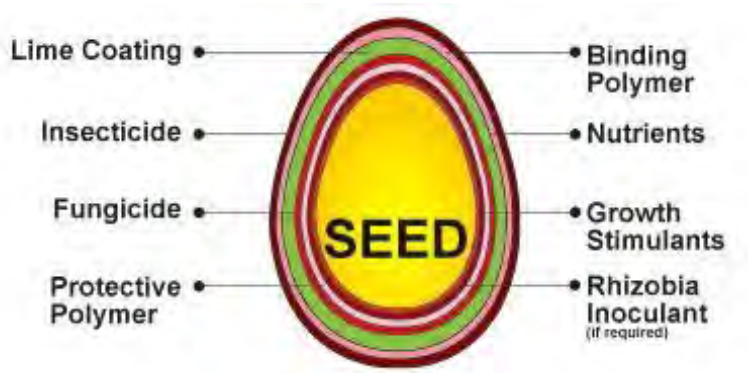

Figure 2.1. Beneficial agricultural products applied to seed in order to enhance the microenvironment around the germinating seed (Advance Seed Company, 2009).

Brits (2007), however, mentioned that there are some disadvantages in the use of enhanced (coated) seed. The increased weight of seed when coated, results in the decrease of the number of seed kernels per kilogram seed, which then requires a higher seeding rate when sown. This factor will also cause an increase in seed cost per hectare at initial stages of sowing. On the other hand, as fungicides, pesticides and other nutrients can already be applied to the seed in the coating process, additional costs for adding these materials later may cut down the overall cultivation costs.

2.3. Seed Testing and Plant Improvement Act (PIA) (No. 53 Of 1976)

To ensure the distribution and use of quality seed, the Seed Testing and Plant Improvement Act (PIA) (No. 53 Of 1976) dictates the purity and genetic maintenance of existing varieties of seed. Requirements specified by this Act prevent contamination of seed with that of any other species, other than the appropriate, or substances that may influence the integrity of quality thereof. By overarching the South African Seed Certification Scheme, it also standardises methods of seed testing, in order to compare results of seed analyses internationally, by enforcement of complying with the International Seed Testing Association's (ISTA) rules. Certified seed, such as in the case of the selected species' seed batches received from Advance Seed Company for this study, should comply with specified standards to allow declaring the batch as "certified". The South African Seed Certification Scheme, stipulates the specific requirements for each kind of plant in terms of (1) land requirements; (2) planting requirements; (3) isolation requirements; (4) requirements for plants; (5) requirements for inspections; and (6) physical requirements for seed (Handbook for the certification of seed, 2010). The use of quality seed which complies with these standards, enhance both the rate of rehabilitation and the repeatability of such a study to present comparative data.

As a supplier of quality seed to multiple facets of their industry, ASC is compelled to comply with stipulations of the latter Act. The selected species' seed is harvested at the appropriate time, referring to the stage in the life cycle of the plants and the season, to ensure the viability of the seed (Brits, 2007). Harvested seed lots are then subjected to purification (Van den Berg, 2002). Purity and germination tests have to be carried out according to

rules laid down by ISTA. These specifications ensure seed quality of high standards with high germination percentages, according to the requirements of the Plant Improvement Act (No 53 of 1976) (Mayer & Poljakoff-Mayber, 1989). More specifically, this law dictates requirements regarding germination percentages, as well as purity for each of the selected species' seed. The specifications of seed testing of the various kinds, and the standards it must attain, is recorded in the Training Manual for Seed Analysis, which is compiled by the South African National Seed Organization (SANSOR) (Anon., 2006). The manual also includes the methods and standards used by analysts of the Plant Research and Genetics Centre, Roodeplaat, Pretoria, who do seed testing for commercial trade purposes.

2.4. Important soil properties affecting seed germination

2.4.1. Conventional soils and anthropogenic soils – the properties that affects phytostabilisation

In order to study soil, knowledge of its basic definition and the properties that make it unique, must be understood (Rossouw, 2005). According to Tate (2000), soil is recognized as a natural object of properties, which are the product of the nature of its physical, biological, mineralogical and chemical composition (Press *et al.*, 2003). Furthermore, according to Gobat *et al.*, (2004), soil is one of the most essential components of the ecosystem and gives an overall reflection of the ecosystem processes, of which the health is dependant on the physical, chemical and biological processes of the growth medium it is based on. Agnelli, *et al.* (2004) and Winter (1974), describes soil as a complex mixture of inorganic and organic material, usually containing a rich variety of living and dead organisms, including bacteria, fungi, both uni- and multicellular nematodes, mollusks, insects, annelids and higher animals. Hopkins & Hüner (2004), also views soil as complex, for the reasons that it consists of a solid phase, including mineral particles derived from parent rock types in various stages of weathering, plus organic material in various stages of decomposition, a liquid phase that includes water or the soil solution, gases in equilibrium with the atmosphere, and a variety of microorganisms.

Mining activities are known to be synonymous with deprivation of these qualities of soil, thereby transforming usable land into tailings wasteland (Maboeta *et al.*, 2006). Resulović & Čustović (2007) states that these newly created disposal sites cannot be included into existing soil classification systems, due to a series of unique and specific properties, created by human activity. It is therefore regarded as anthropogenic soils (Van Deventer & Hattingh, 2004), with different characteristics and soil qualities than conventional soil. Van Deventer & Hattingh (2004) states 'soil quality' implies purpose, use and value of a soil type. In contrast to agricultural practices, this concept does not readily apply to natural ecosystems, as 'better' or 'worse' environments do not exist. In this regard, soil quality can simply be defined as a soil's "fitness for use"; the measurement thereof involves placing a value upon the soil in relation to fitness to fulfil a specific purpose, and becomes inseparable from the idea of ecosystem sustainability (Mills & Fey, 2003). According to Van Deventer & Hattingh (2004), the function of degraded substrates with regard to plant growth, are to provide a medium for plant growth, regulate and partition

water, gas and energy flow, and to serve as a buffer or filter system. Soil types providing such a supporting growth substrate, are considered to constitute a "good, physical quality".

According to Van Wyk (2002) rehabilitation of the tailing material is considered as the last phase of mining, in order to obtain mine closure. However, the importance of the effect of the 'low quality' character of the discard is often overlooked, as high rehabilitation costs are involved in remediation thereof. It is seldom kept in mind that the created environmental setting at disturbed sites, which plants are expected to endure, is crucial to the health and survival of the ecosystem (Van Deventer & Hattingh, 2004). It is therefore important to recognize the significant differences between conventional soils and anthropogenic soils, and the characteristics that influence re-establishment of vegetation in these soil types.

Essentially, all uses of any soil type are greatly affected by the physical properties thereof (Foth, 1990; Van Deventer & Hattingh (2004). The physical and chemical weathering of rocks and minerals, results in a wide range of sizes of particles in conventional soil types, such as the clayey and sandy soil used in this study (Press *et al.*, 2003). This particle size distribution determines the soil's coarseness or fineness, called the 'texture' (Foth, 1990). The texture of the soil is an important component when it comes to re-seeding activities, as it refers to the relative proportions of sand, silt and clay in a soil. Gobat *et al*., (2004) explains that soil texture is a stable property of soil, changing only with long-term soil development, except if anthropogenic activity occurs, which also makes a useful index of classifying soil. The proportion of clay to other soil particles influences the capacity of formation of clay-humus complexes, the soil's exchange capacity, fertility and root-depth of plants established in it (Gobat *et al*., 2004; Foth, 1990). Thus, soil texture directly controls soil structure, porosity and the hydric regime. With regard to the matter of soil texture, it is important to note the difference between anthropogenic- and conventional (natural) soil, in order to understand the differences in soil functionality, and therefore the extent of rehabilitation practices at the different sites.

Whilst conventional soil types have a relative wide range of particle sizes in soil texture (varying between 2 mm and 0.002 mm (Van Deventer & Hattingh, 2004), platinum- and gold mine tailings and examples of anthropogenic soil types used in this study, resulted from extensive grinding and milling of parent rock materials. According to Mendez & Maier (2008), the resulting uniform particle size found in the silt of anthropogenic soil contributes to a degraded soil texture.

Whereas soil texture is more concerned with the size of soil particles, soil structure refers to the arrangement of these particles into aggregates (Foth, 1990). It therefore modifies the influence of soil texture with regard to water-air relationships in the soil, as well as root penetration. Soil structure affects the ability of vegetation to establish by means of controlling factors such as soil-seed contact, aeration, root penetration and nutrient uptake (Winter, 1974; Ashman & Puri, 2002). Vegetation establishment can thus be significantly affected by the

variability of the soil texture and -structure to retain heat and moisture. Factors directly dependent on these soil characteristics that will have an influence on the germination and establishment of the grass, mainly involves the growth mediums' available water capacity, drainage, probability of compaction, soil strength, nutrients, salinity and soil water potentials (matric potential and osmotic potential) (Winter, 1974), collectively referring to the soil quality and performance (Van Deventer & Hattingh, 2004).

The clay fraction of anthropogenic soil does not occur as 1:1- or 2:1 clay minerals (<0.002 mm), and a more or less uniform particle size distribution occur in the tailings, although the particle shape differ especially in the clay fraction (Van Deventer & Hattingh, 2004). The function of the clay fraction in conventional soil is partially due to its origin. According to Gobat *et al*. (2004), clay minerals originally results from weathering of rocks by hydrolysis of silicate minerals, and have the particular property of being electronegative. It involves unsatisfied negative valences at the edges of the layers of the colloid, resulting in sites that retain cations. This property therefore plays a central role in the chemical activity of soil, influencing its structure, porosity, fertility, and cation- and anion exchange capacity. Thus, significant differences in soil function and -fertility between conventional clayey and sandy soil exist, based on the difference in clay particle content. Apart from significantly smaller particles in clayey soil, compared to sandy soil, it will also have higher water holding capacity, have a general small pore size, have a high porosity, have high cation exchange capacity, be high in plant nutritional value, have poor water leaching capacity, and be well-buffered against pH change (Van Deventer & Hattingh, 2004).

Conversely, anthropogenic soils originate from freshly ground rock (Maboeta *et al.,* 2006) and it has far-reaching effects on the clay fraction thereof. According to Van Deventer & Hattingh (2004), the clay fraction of the tailings does not necessarily contain a charge, such as in the case of natural soil, and consequently, anthropogenic soil has low CEC-values. Owing to the lack of ion exchange, a very low or complete lack of buffering capacity to pH change also occurs in these soil types (Van Deventer & Hattingh, 2004). Furthermore, Van Deventer & Hattingh (2004) adds that anthropogenic soil contains minerals mostly in primary form and lack secondary minerals, which also contributes to the low buffering capacity of these soil types.

Apart from the colloidal property of soil that plays a significant role in the uptake of nutrients by plants, other soil properties such as acidity and salinity have an influence on the release of the elements from the colloidal particles (Van Rensburg, 2006). Natural uptake of minerals by plant roots involves the release of H^+ by the plant roots in order to displace the nutritional elements from the colloids. The pH of soil is therefore indirectly responsible for the availability or excess of nutrients to plants in soil, due to the difference in exchangeability of ions that takes place with change in pH (Van Rensburg, 2006), which is thus also based on the availability of water.

This phenomenon transform into fatal conditions involving severe pH changes, such as in the case with anthropogenic soil. Acidification of soil, by means of added inorganic acids or cyanide during the extraction process (Van Deventer & Hattingh, 2004), involves the increase of free H^+. This results in an increased release of nutrients from the clay fraction, which may result in salinity, which may in turn lead to toxic levels of elements present in the soil (Mendez & Maier, 2008). This result in an atypical soil environment, as acidity and salinity will not occur simultaneously under natural conditions. Acidity in conventional soil arises from biological activity coupled with the vigorous leaching of salt, whereas salinity and alkalinity arises from accumulation of salts and bases with inadequate leaching and the chemical weathering of primary minerals in the tailings (Van Rensburg, 2006).

The judicial management of the availability of nutrients in a disturbed, anthropogenic soil is a crucial step in revegetation by amelioration and management of other soil properties, such as the pH thereof. Barker and Pilbeam (2007) described a plant nutrient as a chemical element that is essential for plant growth and reproduction. Plant nutrition is directly linked to the condition of the reservoir – the soil a plant is growing in (Hopkins and Hüner, 2004). Acidification of soil, often involved at anthropogenic soil such as mine tailings, involves the increase of free H+, which influences the release of the elements from the colloidal particles (Van Rensburg, 2006). The lyotrophic series of soil ($Al^{3+} > H^+ > Ca^{2+} > Mg^{2+} > K^+ = NH_4^+ > Na^+$) describes the process of exchange between adsorbed ions and ions in solution (Hopkins and Hüner, 2004). The pH of soil is therefore indirectly responsible for the availability or excess of nutrients to plants in soil, due to the difference in exchangeability of ions that takes place with change in pH (Van Rensburg, 2006). This results in an increased release of nutrients which may result in the infertility of soil, caused by leaching of the nutrients from the soil. Furthermore, very low pH levels may cause an increase in Al^{3+} concentrations in the soil, and although the vegetation might be tolerant to the extreme pH level, most plants will not be able to tolerate such high Al^{3+} -availabilities (Göransson *et al*., 2008).

Just as acidic conditions in the soil releases elements from the clay fraction, basic reactions bind all free H^+, resulting in the unavailability thereof as plant nutrients (Van Rensburg, 2006). Altering the pH thus influences reactions that involve H^+ and OH^-, generally in such a way to counteract the current pH condition. Such reactions are said to provide pH buffering (Van Rensburg, 2006). The dissolution of oxide, silicate and carbonate minerals in parent rocks are barely fast enough under natural conditions to keep up with the buffering action over years and decades, let alone to buffer disturbed soils. The way to speed up this action in order to optimise the soil for re-vegetation, is to ameliorate disturbed soils with agricultural limestone ($CaCO_3$ or $CaMgCO_3$, considering the levels of Ca and Mg in the soil type) calcium silicate, gypsum and organic matter (Van Rensburg, 2006).

Soil quality and performance is typically equated with soil organic carbon (SOC), which is directly equivalent to the soil organic matter (SOM). The lack of organic matter in anthropogenic soil (Mendez & Maier, 2008), causes several direct effects on chemical-, physical- and microbial level. These include the lack of adsorption of heavy metals and organic substances, the lack of humic and fulvic acids in the soil, a lack of anion adsorption and lessened water holding capacity (Van Deventer & Hattingh, 2004). The improvement of soil structure, by stimulating the process of aggregation by nucleation, also offers indirect advantages to structureless anthropogenic soil types, and will be discussed shortly.

According to Six *et al*. (2004) and Omweremadu *et al.* (2010), SOM and aggregate formation is directly related, as micro-aggregates are the structural units of the soil where SOM is predominantly stabilized on the long term. Particulate organic matter act as nucleation sites for soil colloids, most probably by the interaction of minerals on clay particles with organic colloidal surfaces, stimulating macro-aggregate formation (Huang *et al*., 2004; Omweremadu *et al.*, 2010), which is a measurement of soil structure (Tate, 1987). Six *et al*. (2004) states that the balance between formation and breakdown of macro-aggregates determines the macro-aggregate turn-over, which have an indirect effect on micro-aggregate formation. Several processes that take place in conventional soil are related with aggregate formation and -turn-over, which are all associated with the presence of SOM. These phenomena provide different means of amelioration of the lack of these characteristics in structureless, anthropogenic soil types.

Tate (1987), states that structureless soil, such as mine tailings, are characterised as loose sandy soils or as massive, irregular, featureless soil units with a lack of cohesiveness among the soil particles. According to Tate (1987), three distinct biological structural levels are generally accepted as contributors to aggregate formation, namely:

(1) Microscopic fungal mycelia and bacterial cells,
(2) macromolecules, as well as
(3) plant roots.

Although readily decomposed SOM contributes to soil structure as nucleation sites (Omweremadu *et al.*, 2010), Tate (1987) states that the mere presence of SOM is not as important to soil structure development as are the biological and chemical modifications of this matter by soil microorganisms. According to Six *et al.* (2004), the varying degrees of correlation between microbial activity and aggregation are related to; (1) the different scales (micro- versus macro scale) of influence of fungi versus bacteria; (2) soil texture; and (3) soil mineralogy.

Fungal mycelium forms a ‘sticky string bag’-network in which soil particles are entangled by the hyphae to form a granular structure (Tate, 1987; Guggenberger *et al*., 1999; Six *et al.*, 2004). Besides the physical effect of enmeshment of macro-aggregates by hyphae, it ‘cements’ soil particles together by extracellular produced

polysaccharides. The micro-aggregates attach to the hyphae by means of these polysaccharides and bound into stable macro-aggregates by the network of hyphae. Bacterial cells' surfaces also secrete polysaccharides, lending it also to be nucleation sites for soil aggregates. The function of microbial organisms, regarding soil structure, depends strongly on the soil texture. Six *et al.*, (2004) states that in coarse textured, sandy soils aggregation is weakly related to microbial biomass and products, as only the fungal hyphae network is involved by cross-linking sand particles to form macro-aggregates. On the other hand, both bacteria and fungi, as well as their products, play a role in aggregation in clay-containing soil types. According to Huang *et al*., (2004), in soils under neutral pH conditions, bacterial cells have a net negative surface charge. By bridging through cations, the smaller sized clay particles adhere to the bacterial cells, which cause micro-aggregation.

The presence of soil organic matter also contributes to soil structure development on biochemical level, by providing a persistent substrate for soil microbes, which leads to slow, durable formation of soil aggregates (Tate, 1987). Easily metabolised substrates leads to rapid conversion to glucose, after which the high concentrations of substrate is exploited, resulting in a rapid a shortage of substrate. Depolymerisation of cellulose, a complex polysaccharide found in SOM, requires a variety of enzymes as an energy substrate to soil microbes, which slows the process of derivation of glucose from the substrate. This 'slow release'-substrate prolongs the presence of microbial activity in soil by prolonging its presence (Tate, 1987). Other soil organic substituents that contribute to aggregate formation are soil humic acids, by reacting with clays 'in a manner conducive to the formation of micro-aggregates' (Tate, 1987). These micro-aggregates result from the neutral clay minerals that electrically bind to organic particles by polyvalent cations on exchange sites.

The presence of vegetation contributes to soil characteristics in several ways (Mills & Fey, 2003), which render it to be a successful means to remediate soil. Tate (1987) states that plant roots interact with soil particles associations at two levels, either to increase soil structure formation or to disrupt this granulation. Furthermore, Six *et al*., (2004) mentions three additional influences of plant roots on soil structure, namely a change in the soil water regime, root exudation, and dead root decomposition. The compressing action of growing roots decreases soil porosity in the zone between roots and re-orientates clay particles along the root surface, which induces formation of micro-aggregates (Six *et al*., 2004). In addition, by root penetration into macro-pores, it increases the proportion of stable micro-aggregates relative to the proportion of unstable macro-aggregates (Tate, 1987; Six *et al.*, 2004).

The direct effects of an absence of vegetation are the reduction of input of litter and root biomass (Mills & Fey, 2003; Six *et al*., 2004), as found in anthropogenic soil. Six *et al*. (2004) states that the decomposition of dead roots promotes soil structure. The magnitude thereof would depend on the decomposability and amount of material. The direct positive interactions between soil minerals and plant roots result from the adhesion of soil particles to the roots or through trapping of soil particles by fibrous root systems (Tate, 1987). The effect of

particle entanglement by root biomass that contributes to soil structure development is extrapolated by exudation of roots and arbuscular mycorrhizal (AM) fungi associated with the roots. Exudation of roots refers to organic carbon substrates; formed as a product of photosynthesis, that acts as a favourable contribution of the presence of plant roots (Tate, 1987). These mucilages produced by roots also 'stick' soil particles directly together, which leads to aggregate formation without interference of microbial activity (Six *et al.*, 2004). In addition, polysaccharide root exudates acts as a substrate to the rhizosphere microflora, which in turn is advantageous to aggregation development and soil quality (Tate, 1987). Six *et al.* (2004) also reported a positive effect of roots on soil aggregation due to the excretion of a soil protein glomalin by AM fungi. However, apart from the function of mineralogy and chemistry of soil, the mechanism of aggregate stabilisation in soil also depends on the size of the aggregates (Bearden & Petersen, 2000; Six *et al*., 2004). The presence of bigger sized aggregates in a soil type is associated with hyphal length and not root growth, whereas both hyphal length and root growth are involved in smaller aggregates.

Indirectly, the absence of vegetation causes an excessive removal of Nitrogen (N) relative to Carbon (C), possible inhibitory effects on microbial activity and the effect of temperature, and wetting and drying cycles are detrimental to the remaining SOM (Mills & Fey, 2003; Six *et al*., 2004). The removal of vegetation from conventional soils causes a rapid oxidation of the soil, during which N are freely formed and lost through leaching and volatilisation, which are even more enhanced by tillage of the soil (Mills & Fey, 2003; Omweremadu *et al*., 2010). The loss of N will inevitably cause loss of C as a result of natural reestablishment of the C: N ratio (Mills & Fey, 2003).

According to Van Rensburg (2006), anthropogenic soil often remain as scars on the natural landscape and threaten neighbouring land and -streams with their run-off water and eroded sediment. In order to stabilize it with vegetation, involving fertilizer and liming treatments, evaluation of existing condition have to be done to understand the extent of rehabilitating such a disturbance. Re-vegetation can be carried out successfully by complying with standards of soil required by the target vegetation. In the scope of this project, the supplied seed coat may act as an enhancement of the microhabitat around the seeds in the soil types of concern, joined with mentioned advantages it provides. This attempt may enhance the rate of restoration and save restoration costs in re-vegetation, e.g. fertilizing and liming treatments, regardless of the conditions the growth medium might present.

2.5. Physiological parameters in seed germination

The term germination refers to a fairly large number of processes that result in the growth of a plant from a seed. Interrelated to the role of seed dormancy, the reasons why seed do not germinate, and the characteristics of soil, the rest of the chapter is dedicated to the key function of water in the germination of seed and parameters of the activity of these processes. These include the importance of the activity of enzymes such as alpha amylase, lipoxygenase and peroxidase, as well as levels of respiration of seed.

2.5.1. Germination enzymes

2.5.1.1. Peroxidase (POD)

Peroxidases are hemoproteins that may participate in many processes of plant growth and defense (Gijzen *et al*., 1999). Environmental induced stresses in plants have detrimental effects on the physiology. In response, plants have various mechanisms to overcome these stresses to avoid fatality (Hopkins and Hüner, 2004). When exposed to stressful conditions, an increase in reactive oxygen species (ROS) are observed (Navari-Izzo & Rascio, 2005). Reactive oxygen species cause severe damage to membranes, proteins, DNA and RNA and needs to be detoxified. Increased generation of anti-oxidant or oxygen producing enzymes, such as POD, increases the ability of the plant to detoxify reactive oxygen species and thus enabling life sustaining metabolism in the plant to continue (Navari-Izzo & Rascio, 2005).

The oxidative activity of POD is also found essential in other divisions of metabolism, closely associated with germination. Schopfer *et al.* (2001) reported that reactive oxygen intermediates (ROI) comprise of incompletely reduced oxygen species, such as superoxide radicles (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-). These are byproducts of various oxygen-consuming processes and causes symptoms of oxidative damage if the ROI production exceeds the capacity of ROI-scavenging reactions. According to Schopfer *et al.* (2001), ROI has functional significance during seed germination. Seed germination presents a developmental period most sensitive to pathogen infection, during which the active release of ROI from the aleurone layer in the seed coating protects the emerging embryo from infection of microorganisms. Secreted by the aleurone layer in response to elevated levels of ROI, POD serves as a counter-acting regulator, which transforms O_2 and H_2O_2 to OH^- , which are eventually converted to H_2O and O_2. Although the production of ROI and the accompanying activated levels of POD would indicate stress levels in plants, it also demonstrates an active, beneficial biological reaction that is connected with high germination capacity and vigorous seedling development. The plant hormone indole-3-acetic acid (IAA), more commonly known as auxin, which has the function of stimulating cell elongation, is primarily degraded by oxygen and peroxide in the presence of a suitable redox system. In this case, peroxidase serves to catalyze the oxidasion of IAA while at the same time releasing CO_2 (Hopkins and Hüner, 2004). The end products of oxidized IAA are physiologically inactive, which is therefore an effective way of removing the hormone molecule once it has accomplished its purpose.

Measured activity of peroxidase in a seed sample in this study may therefore indicate highly activated levels of germination metabolism. It is expected that the effect of the applied coating, observed in assessments of the activity of α-amylase and LOX in the selected seeds' samples, should also be observed in assessments of POD.

2.5.1.2 Alpha amylase

Starch, the major energy reserve in cereal seed, is present in the form of discrete, water-insoluble granules, deposited in the endosperm of seed (Kumar & Ramesh, 2004). According to Hopkins & Hüner (2004), starch normally consists of a mixture of two polysaccharides: amylose and amylopectin. Amylose consists of strait, long-chained (1→4)-linked α-D-glucose units, whereas amylopectin is a highly branched molecule in which relatively short (1→4)-linked α-D-glucose chains are connected by (1→6)-links (Hopkins & Hüner, 2004). During the initial stages of germination, starch reserves are broken down to the component glucose residues, either by a phosporolytic pathway or by the employment of four hydrolytic enzymes, one of which the enzyme alpha amylase (α-amylase). Kumar & Ramesh (2004) states that the latter enzyme's *de novo* synthesis in granules (Van Jacobsen *et al.*, 1970) is regulated by the hormones gibberellic- and abscisic acid, which are activated by imbibition. According to Hopkins & Hüner (2004), this enzyme randomly cleaves α(1→4)-glucosyl bonds in both amylose and amylopectin, of which the product-molecules have an alpha configuration. This enzyme therefore is of prime importance during initial stages of starch degradation, and the heterogenic character thereof enhances the conversion of insoluble starch to glucose throughout the germination process to meet the metabolic demands of the growing embryo (Hopkins and Hüner, 2004). Activated levels of α-amylase in grass seed therefore indicate an increase of germination activity.

2.5.1.3. Lipoxygenase (LOX)

Lipoxygenases (linoleate:oxygen oxidoreductase), form a family of non-heme-iron-containing fatty acid dioxygenases that are widely distributed in plants (Brash, 1999). Energy reserves for seed germination are stored in the seed in the forms of complex sugars and lipids (Mayer & Poljakoff-Mayber, 1989; Feussner *et al.*, 2001). The process of germination is characterized by the mobilization of storage lipids, which serve as another major carbon source for growth of seedlings. Lipoxygenase is a product of gene expression (Feussner *et al.* (2001), which catalyses the regio- and stereospecific dioxygenation of polyenoic fatty acids, such as linoleic-, and linolenic acid (Hamberg & Samuelson, 1967). These substrates are oxygenated at either carbon atom 9 by 9-LOX or carbon atom 13 by 13-LOX of the hydrocarbon backbone of the fatty acid.

Located at the phospholipid monolayer of lipid bodies, a 13-LOX can be detected, which is capable of oxygenating esterified linoleate residues without the precedings of a lipid hydrolyzing enzyme (Feussner *et al.*, 2001). This oxygenated fatty acid fraction contains mainly 13-hydroperoxy-9,11-octadecadienoic acid [(13*S*)-H(P)OD], which is preferentially released from the lipid bodies to undergo β-oxidation. According to Feussner *et al.* (2001), the amount of linoleic acid residues in the storage lipid fraction decreases from the start of

germination, paralleled by the increase in LOX activity. Mayer & Poljakoff-Mayber (1989), states that LOX is the only enzyme active at the lowest levels of hydration. The activity of this enzyme, with specific components as a result of its metabolic pathways, therefore activates further germination processes. Therefore, the spectrophotometrical measurement of the rate at which HPOD, a product formed by the activity of this enzyme, is formed by a specific seed sample's enzyme extract, is a measurement of the activity of LOX in that specific seed sample, which indicates the levels of activated germination in seed. The effect of the applied seed coating can thus be evaluated on physiological level by assessing the activity levels of this enzyme, by comparing the activity of LOX in coated seed to seed of the same species with no applied coating.

2.5.2. Water content

Water is an essential resource for germination. According to Fenner & Thompson (2006), its uptake by seeds during germination takes place in three phases: (1) imbibition, in which the seed coat is penetrated, and the embryo and endosperm absorbs water; (2) activation, during which little further water is absorbed, but developmental processes start to occur; (3) during which the radicle elongates and breaches the seed coat. The rate of activation of germination (or the lack thereof) and the level of seed rehydration are thus closely correlated (Fenner & Thompson, 2006; Garwood & Lighton, 1990; Baskin & Baskin, 2001). Very much like osmosis, according to Hopkins and Hüner (2004), this rehydration of seed tissues involves the movement of water down a water potential gradient. It differs from osmosis, however, in that it does not require the presence of a differentially permeable membrane and is driven primarily by surface-acting-, or matric-, forces. It rather involves chemical- and electrostatic attraction of water to cell walls, proteins, and other hydrophyllic cellular materials. Mayer & Poljakoff-Mayber (1989) describes it as a hydrodynamic mass flow through the pores of a membrane, thus not diffusion.

Mayer & Poljakoff-Mayber (1989) states that imbibition is a physical process which is related to the properties of colloids within the seed, and is in no way related to the viability of the seed. It occurs equally in live seeds and seeds which have been killed by heat or by some other means. Rehydration of imbibing material causes swelling, and generates substantial pressure (Hopkins and Hüner, 2004). This "imbibition pressure" is caused by the solvation of colloid particles in the seed as well as occupation of free capillary- and intermicellar spaces in the seed. This consequential pressure may reach pressures several hundreds of times of atmosphere, and is of great importance in the process of germination. It enables the breaking of the seed coat, which permits emergence of the embryo and creates room for the seedling in the soil (Mayer & Poljakoff-Mayber, 1989). The magnitude of the imbibition pressure is also an indication of the water retaining ability, thus determining the amount of water available in the seed for rehydration of the seed tissue during germination. Each species critical water content (or water potential) required for germination activation, is genetically defined and correlates with the desiccation tolerance of the species (Fenner & Thompson, 2006).

Mayer & Poljakoff-Mayber (1989), states that the extent to which imbibition occurs is determined by certain factors, such as the composition of the seed tissue, the permeability of the seed coat, and the availability of water in the environment. Fenner & Thompson (2006) mentions other conditions of concern for the seed to take up water, namely the area of contact between the seed and the substrate, and the relative difference in water potential between the soil water and the seed.

Apart from requiring critical water content for activation of germination, seed of each species also requires a critical water content for maintenance of viability (Fenner & Thompson, 2006), which also varies among species. Based on their sensitivity to desiccation, seed are divided into two broad groups: orthodox and recalcitrant. Orthodox seed are capable of tolerating low moisture content with little effect on the seed viability. In contrast, recalcitrant seed loses viability at very low moisture content (Pritchard *et al*., 2004; Fenner & Thompson, 2006). Seed-desiccation sensitivity correlates relatively well with the ecological characteristics of an area, influenced by climate.

Pritchard *et al.* (2004), reports that desiccation-sensitive seed are shed at high moisture availability in a metabolically active state. Consequently, the period of imbibition of desiccation-sensitive seed are expected to be shorter than that of desiccation-tolerant species. This rapid germination may reduce the period of desiccation and of seed predation levels. Pritchard *et al.* (2004), also found that seed shed by desiccation-tolerant species, such as grasses, does not coincide with rainfall and their ability to withstand low moisture content enable the seed to accumulate in the soil seed bank until onset of rainfall until germination occurs. According to Fenner & Thompson (2006), a seed may become fully imbibed but remain ungerminated indefinitely if its dormancy-breaking or germination-inducing requirements are not met. Therefore, although the imbibition-requirement of a sufficient amount of water for a certain species' seed are met, low germination of a species would occur in disturbed areas if soil conditions, such as pH, nutrients and soil temperature, occur to be outside or near the edges of a species tolerance range.

Preserved and ungerminated seed loses viability with increase in age (Fenner & Thompson, 2006). The rate of ageing is determined by genetic characteristics, temperature and period of storage, and importantly, the moisture content of the seed. According to Mayer & Poljakoff-Mayber (1989) and Baskin & Baskin (2001), the moisture content of seed is determined by the relative humidity of the air and temperature of storage. Kalpana & Rao (1995) reports several changes that take place in seed with age that contributes to loss of viability: (1) increased leakage of solutes; (2) decrease in lipids and phospholipids; and (3) membrane detoriation. Fenner & Thompson (2006) also mentions chromosomal aberration as a consequence of ageing in seed. According to Mayer & Poljakoff-Mayber (1989) the loss of viability is not an abrupt failure of germination in seed. It does not imply that that all metabolic processes stop simultaneously or that all enzymes are inactivated. It is rather the total sum of processes that does not operate properly, and are based on the activity of oxidizing enzyme reactions within a

seed. It is accompanied by, and often ascribed to, various changes in lipids due to peroxidative processes. The free radicals generated by lipoxygenasion of linoleic and linolenic acids, attack intrinsic membrane proteins and cause damage to the membranes, which could be one of the causes of increased leakiness typical of deteriorating of seed. Storage of seed batches under conditions which will preserve their viability, minimizing the above mentioned changes is of great importance (Mayer & Poljakoff-Mayber, 1989). Although these conditions are genetically determined for each species' seed, the Plant Improvement Act (No 53 of 1976) specifies general conditions of storage for seed for agricultural and rehabilitation purposes in South Africa.

For optimal results, the restorationist should therefore consider the age of the seed, the conditions under which it was stored and the species-specific constraints of the selected species before using a certain batch of seed. Regarding these factors, the chemical characteristics of constituents of an applied seed coating should also be taken into account. The calcareous lime-based coating (Advance Seed Company, 2009), are known to be hygroscopic, which might increase the availability of moisture to the seed. Even at such low moisture availability, imbibition and initial germination processes such as LOX activity may be activated (Mayer & Poljakoff-Mayber, 1989).

Fenner & Thompson (2006) states that if pre-sown hydration of seed takes place, such as via the hygroscopic coating, a seed retains the physiological changes that occurred during hydration, called the "hydration memory". The final germination percentage is not affected, but the time required to germinate is reduced. This pre-sowed onset of germination may thus enhance the rate of rehabilitation of degraded areas. However, according to Fenner & Thompson (2006), a crucial aspect in this process is the length of the hydration period. Any physiological changes caused by hydration, cannot be reversed or suspended. Therefore, the dehydration of the seed may result in loss of seed viability, if the germination process has already been activated to a critical stage. Without the adequate supply of water for prolonged germination, the activation of oxidizing enzymes, such as LOX, would result in loss of viability and vigour of stored batches of coated seed. This concept is crucial for this study, for the reason that seed is wetted during the process of application of the seed coating and dehydrated by elevated temperatures to dry the coating. The effects of this coating process would be reflected in the activity levels of germination enzymes, as well as relative water content of the seed.

The entry of water into the seed is not only determined by the availability thereof in the seed's environment, but also by the permeability of the seed coat. Ahmadi *et al*. (2007) reported an increase in seedling emergence in seed with increased dry weight due to increased water uptake. Considered as the contact-area between the seed and the soil, this multi-layered membrane regulates water-uptake by the seed (Mayer & Poljakoff-Mayber, 1989). If totally impermeable under conditions otherwise considered as favorable for water uptake, it is considered as a physical state of dormancy. The possible mechanisms by such impermeability that is brought about, include the deposition of lignin or callose, cellulose and hemi-selulose. Depending on the species,

different cell layers may become impermeable (Mayer & Poljakoff-Mayber, 1989). Independent of actual dormancy of the embryo itself, the seed coat seems to have multiple functions that contribute to restriction of germination at times of unfavourable environmental conditions. Mayer & Poljakoff-Mayber (1989) mentions it exerts mechanical restraint on the embryo, it restricts water and oxygen supply, and prevents the loss of an endogenous inhibitor.

Permeability of the seed coat is generally the greatest near the micropylar end of the seed, where it is almost invariably thinner than the rest of the seed (Mayer & Poljakoff-Mayber, 1989). This fact explains the correlation of seed moisture content to relative humidity. Baskin & Baskin (1998) found that the seed moisture content is directly influenced by the relative humidity due to moisture regulation by the hilum. At low moisture content the hilum begins to act as a hygroscopic valve. It opens at low external relative humidity, through which water diffuse, and closes at high relative humidity. Therefore, seeds attain the moisture content in equilibrium with the lowest relative humidity to which they have been exposed.

In addition to maintain seed moisture content, ability of a seed to rehydrate, determine the ability to successfully imbibe and germinate. Mayer & Poljakoff-Mayber (1989) states that rehydration of seeds occurs through the imbibition of hydrophilic compounds in the seed coat, which are in the form of a gel. These particles form a micellar network, which are considered as a semi-permeable membrane due to its immobility and the bulk of it as an osmotic system. The composition of the medium in which germination takes place, determines the availability of water for imbibition. Under natural conditions, and certainly under disturbed soil conditions, the solution where seed are found is not pure water. Mayer & Poljakoff-Mayber (1989) states that imbibition decreases as the concentration of solutes increases, largely due to osmotic effects. The ability of seed to absorb water from the soil depends on both the osmotic and matric potential of soil. The water potential of the seed, compared to that of the immediate vicinity, will determine the ability of water-uptake by the seed. Under saline conditions, seed would thus be physiologically desiccated, although adequate water is present in the near vicinity of the seed.

According to Mayer & Poljakoff-Mayber (1989), a direct toxic effect of ions in solution on the seed is frequently observed in saline soil. This osmotic inhibition by salt does not persist at lowering the concentrations of the solute (Fenner & Thompson, 2006; Mayer & Poljakoff-Mayber, 1989). Thus, inhibited seed would germinate under conditions where the solute in the disturbed soil is diluted, such as rainy conditions. However, the vigour of growth may be affected if the salinity would happen to be near the edges of the selected species' tolerance range. Depending on the characteristics and ion-content of a disturbed soil, the application of a calcareous seed coating may therefore also affect the imbibition and germination of the selected seed. Depending on the pH caused by ions contained by the soil, the coating may act as a buffer, enhancing germination, or contribute even further to the adversity of conditions in the soil for the seed.

The extent to which the seed are affected by conditions in its vicinity, whether it is water availability or salinity, depends on the area of contact of the seed and the surrounding soil particles. Fenner & Thompson (2006), states that the size of a seed may affect the frequency with which its water requirements can be met. Smaller seeds, such as in the case of grasses, have the advantage that they retain their maximum water uptake more quickly than bigger seeds, and they are more likely to fall into a micro-environment that promotes water uptake in order to minimize desiccation. However, seed-to-soil-contact are reduced under conditions with soils of low clay and high silt- and sand content, which may be enhanced by the applied seed coating.

2.5.3. Respiration

Dry seed is characterized by a remarkably low level of metabolism. One of the most frequently used criteria for evaluation of the rate of metabolism is the rate of respiration in seed (Hopkins & Hüner, 2004). Woodstock & Grabe (1967) reported loss of viability which is closely related to respiration and that respiration rates are closely related with germination and seedling growth. According to Mayer & Poljakoff-Mayber (1989), the slow rate of respiration increase rapidly when seed are placed in water. Even in dry seed, a certain level of gas exchange can be detected, but it depends on the moisture level. The more the moisture level rises, the higher the rate of gas exchange.

Garwood & Lighton (1990), states that the temporal pattern of seed respiration, as seeds imbibe water and germinate, is divided into four phases. Respiration increases rapidly as seed imbibe water (Phase I), thereafter Phase II, a lag phase, follows, wherein the rate of respiration stays constant for some time. During Phase III, the respiration rapidly increases again as the seeds start to germinate. Post-germination changes in respiration (Phase IV), are variable, depending on the species and/or the moisture content of the seed. If the applied coating has enhancement effects on the imbibition and germination rate of the selected, coated seed, a general increase may thus be observed in the respiration rates of these seeds, compared to uncoated seed of the same species.

2.6. Dormancy

Few things are as important for plants to reproduce; seed germination as a means of reproduction should therefore occur at the right time, in the right place. Vanangamudi & Natarajan (2006) states that the seed phase is the most important stage in a plant's life cycle with respect to the survival of the species. Vanangamudi & Natarajan (2006), defined dormancy as the absence of germination growth of a viable seed under otherwise optimal conditions. Delayed germination is thus not accidental, and provides the species a means of escaping unfavorable conditions. Previously, the conditions under which germination will take place and the parameters thereof were discussed. However, the concept of dormancy refers to the explanations for the delay of germination in viable seed.

According to Vanangamudi & Natarajan (2006), the concept of dormancy differs from quiescence in seed. Quiescent seed will germinate under the right set of conditions, whereas dormant seed will require another set of conditions to break their dormancy. The dormancy breaking method will shift the seed from the dormant state to the quiescent state, after which germination will take place if the right set of favourable conditions are present.

According to Vanangamudi & Natarajan (2006), the classification of the types of dormancy is based on timing; i.e. primary- and secondary dormancy. Primary dormancy (exogenous) is associated with development, which involves a failure of germination when shed, and normally involve a period of "seed ripening" (Mayer & Poljakoff-Mayber, 1989). This period may be defined as any changes which occur in the seed that improves or enables germination in the seed. Secondary dormancy (endogenous) occurs essentially after dispersal and is basic to seasonal cycling in soil seed banks (Vanangamudi & Natarajan, 2006). According to Mayer & Poljakoff-Mayber (1989), this state of dormancy develops spontaneously in seed due to changes in them, and germination thereof requires the presence of favourable environmental conditions, which is determined genetically by the species.

From the discussion of water availability to the seed, it is apparent that the clearest form of dormancy is seen in seed with hard, impermeable seed coats. Vanangamudi & Natarajan (2006) stated that apart from the mechanical constraint of the hard seed coat to germination of the embryo, the coat also prevents growth of the often non-dormant embryo by prevention of uptake of water and oxygen.

The regulation of primary dormancy is furthermore regulated by the presence of abscisic acid ABA, by acting on two distinct processes: (1) the prevention of precocious germination; and (2) the imposition of primary dormancy. The suppression of precocious germination by ABA is stage specific, and is influenced by environmental factors such as water availability and temperature (Vanangamudi & Natarajan, 2006). The seed moisture content and the availability of water in the direct vicinity of the germinating seed, will therefore determine the osmotic potential of the seed. This will determine the capacity of the seed to take up water and leach away inhibitors (Vanangamudi & Natarajan, 2006) such as ABA and other phenolic compounds during imbibition (Mayer & Poljakoff-Mayber, 1989). The presence of inhibitors such as ABA, and therefore the presence of dormancy in the seed, is therefore also determined by the osmotic conditions in and around the seed. Vanangamudi & Natarajan (2006) state that degradation of ABA occurs during chilling and dry after-ripening, hence the existing method of dormancy breaking in grass seed is to prechill at 5°C (ISTA, 1985). Under natural conditions, seed is shed just before winter, when temperatures are lower, and seasons are dryer, especially in the Grassveld biome of South Africa. This contributes to ABA reduction. Due to the breakdown of the inhibitors during this seed ripening period, germination of grass seed can thus take place as soon as conditions change to the wet, warmer conditions of summer.

To ensure germination of quiescent seed, a set of conditions should be met. The absence of only one required environmental factor could result in prolonged dormancy of seed (Vanangamudi & Natarajan, 2006). Environmentally imposed secondary dormancy may be common in nature and may be considered as a response to change in the environment. It may thus be assumed that the mechanisms underlying secondary dormancy are physiological of nature, and metabolic changes occur with changes in environmental conditions (Mayer & Poljakoff-Mayber, 1989). Although the dormancy of the seed of the different grass species was not evaluated by this study, the probability existed that it could have influenced the germination of the seed types in the different soil types. Standardised dormancy breaking methods (ISTA, 1985) were applied on both the coated- and uncoated seed types of the selected species to ensure elimination of dormancy as a cause of a probable lack of germination.

2.7. Summary

To counteract losses of ecological capital and eliminate loss of topsoil and pollution, active restoration involves the introduction of seed of native species to altered landscapes in order to restore natural ecosystems (Vander Mijnsbrugge *et al*., 2009). Disturbed soil conditions offer adverse environmental conditions for seed to germinate, therefore an additional applied seed coating, offering various advantages, may enhance the rate of re-vegetation. By understanding the germination processes in the selected species as well as the influences of various environmental conditions on it, several parameters of germination may be employed to evaluate the effect of the applied coating on the germination capacity of each species.

Chapter 3

Materials and Methods

3.1. Introduction

The study was conducted during the years 2009 and 2010, and entailed three parts:

1. Greenhouse trials under controlled conditions.
2. Field trials under natural conditions.
3. Laboratory experiments.

Three grass species, namely *Anthephora pubescens* Nees (batch number GB09/8626), harvested in 2003 and coated in 2004, *Cynodon dactylon* (L.) Pers. (batch number GC01/9454), harvested and coated in 2008 and *Panicum maximum* Jacq. (batch number GP01/9466), harvested and coated in 2008. All the grass species for the study were selected by Advance Seed Company (ASC). Both coated (enhanced) and uncoated (non-enhanced) seed types of the same batches of the individual species were used. The uncoated seed types served as the control. The term "seed" will be used throughout the dissertation to describe the whole, intact caryopsis.

3.2. Study areas

3.2.1. Greenhouse trials

This part of the study was conducted in a greenhouse on the premises of the North-West University (NWU) at Potchefstroom. Four different soil types were collected at sites representing the four growth mediums (Figure 3.1) that were used in this study. These soil types included an acidic medium, from gold mine's tailings, an alkaline medium, from platinum mine's tailings, a sandy- and a clayey soil type collected from farms in the Taaiboschbult-area around Potchefstroom. The field trials were carried out at the same sites where the soil was collected for the greenhouse trials (see Section 3.2.2).

Figure 3.1. Example of soil collection at the platinum mines' tailing-site, representing an alkaline growth medium.

3.2.2. Field trials

Evaluations of the germination of the three selected grass species (both coated and uncoated) commenced under natural (uncontrolled) conditions during the growing season of the year 2010. Each seed type was sown at a site in each of the four soil types, namely sandy-, clayey- , acidic - and alkaline soil at the different locations (Figure 3.2 - 3.4 & Table 3.1).

Table 3.1. Locations of the four field trial-sites representing the different soil types.

Site (Soil type)	Location	GPS-coordinates
1. Sandy	Taaiboschbult Feedlot near Potchefstroom	lat 27°01'36.03"S; long 27°04'40.48"E
2. Clayey	Farm Taaiboschbult near Potchefstroom	lat 26º51'.11.2''S; long 26º56'.38.7''E
3. Acidic	Gold mine tailing site near Stilfontein	lat 26°48'28.87"S; long 26°48'19.33"E
4. Alkaline	Platinum tailing site near Rustenburg	lat 25°30'46"S; long 27°13'25.74"E

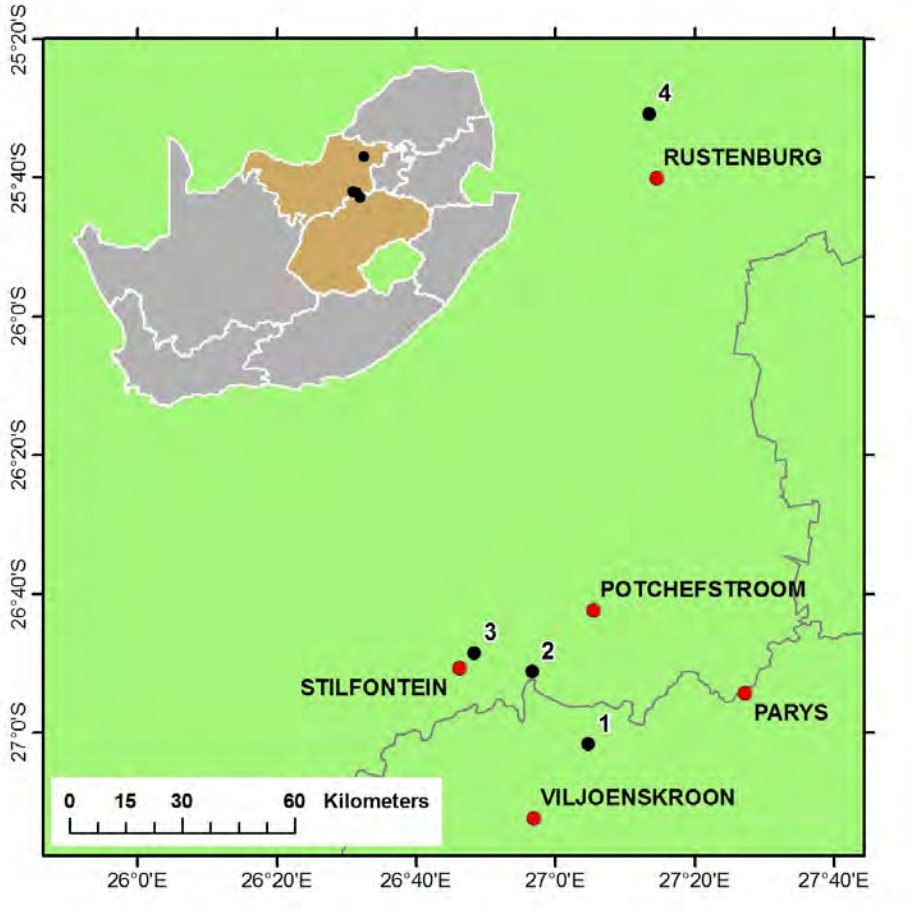

Figure 3.2. Locations of the four study sites as mentioned in Table 3.1.

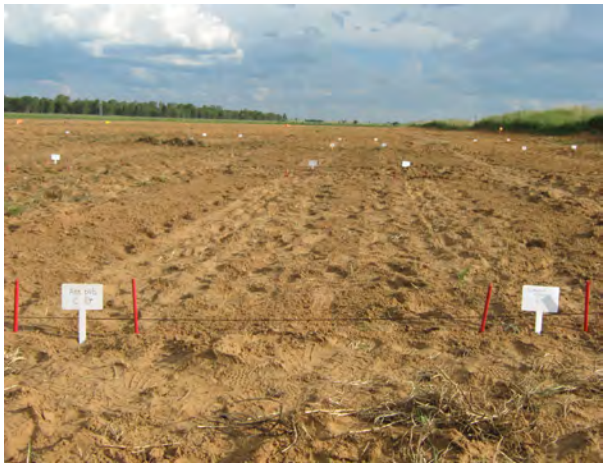

Figure 3.3. The sandy soil type site.

Figure 3.4. The acidic gold tailings-site.

For the purpose of this study, the germination and establishment data of the selected seed were only recorded and evaluated for the growth season in which the seeds were sown. Long term monitoring data will disclose the long term effect of the seed coating on long term establishment of the selected species. Data from the two nearest weather stations of the South African Weather Services located in Potchefstroom and Rustenburg was used to illustrate the climate of these regions during the 2009/2010 rainfall season (December 2009 until May 2010). The data is reflected relative to the climatic circumstances of the past thirty years. The average monthly temperature during the period of study of both the Potchefstroom and Rustenburg areas slightly exceeds that of the past thirty years in these areas (Figures 3.5.).

Both the mentioned cities and its surroundings are located in the summer rainfall zone (Mucina and Rutherford, 2006). The average monthly rainfall for these two areas is illustrated in Figure 3.6. The total precipitation for the Rustenburg area was 610.8 mm, whereas the total precipitation for the Potchefstroom area was 603.2 mm for the months December 2009 until May 2010. Compared to the long term monthly precipitation averages over the last 30 years, the precipitation in both areas were significantly higher during the months of December 2009 and January 2010. Precipitation in the Potchefstroom area was similar to the average long term values for the remainder of the growth season, whereas precipitation in the Rustenburg area was lower than expected during February and March 2010. However, significantly higher precipitation values were observed during the months of April and May 2010. The differences in temperature and precipitation regarding the long-term averages and the measurements for the study period in Rustenburg may result in the changes of the establishment of the different species and will depend on the tolerance levels of these species.

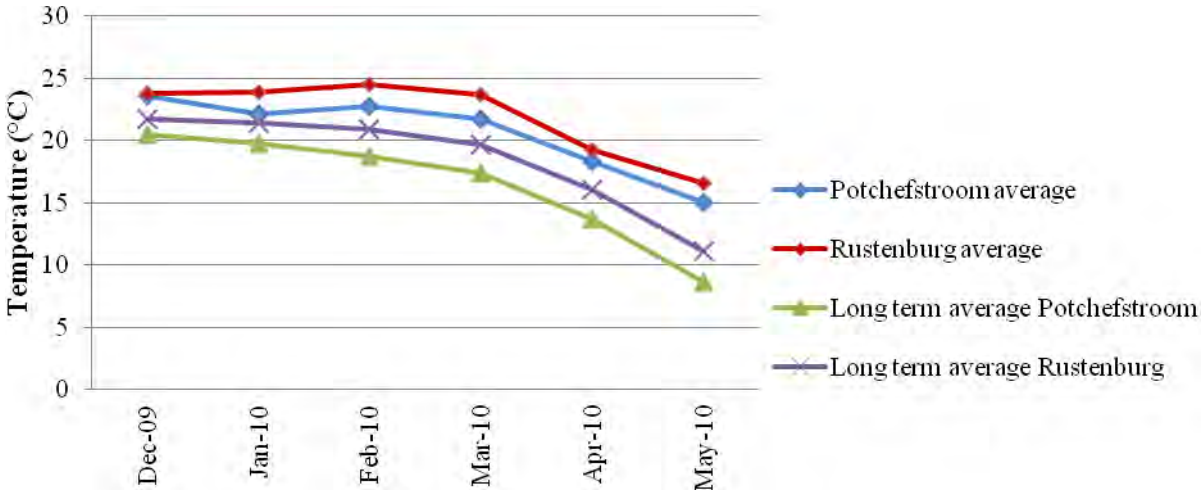

Figure 3.5. Average monthly temperatures of the growth season during the time of study (December 2009 until May 2010), as well as long term temperature averages of the same months in the Potchefstroom- and Rustenburg areas (30 years).

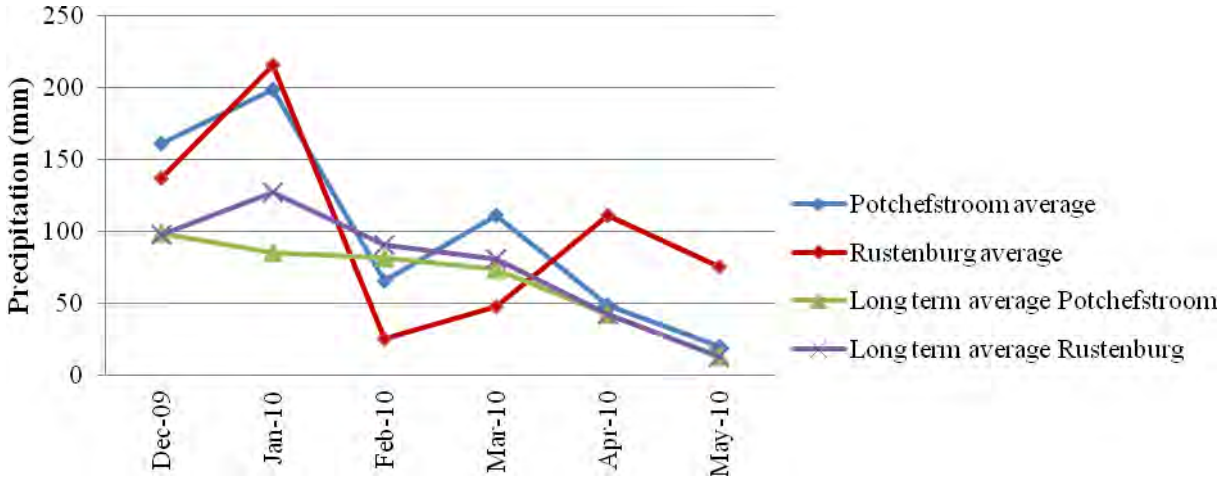

Figure 3.6. Average monthly precipitations of the growth season during the time of study (December 2009 until May 2010), as well as long term precipitation averages of the same months in the Potchefstroom- and Rustenburg areas (30 years).

3.2.3. Physiological Tests

The physiological assessment of the activities of key germination-enzymes was carried out in the certified, fully equipped plant physiology laboratories on the premises of the North-West University, Potchefstroom campus. The different physiological tests are discussed under Section 3.5.

3.3. Greenhouse trials

3.3.1. Experimental design

The study involved a 4 x 3 x 2 directional variation in statistical methodology. Rules prescribed by ISTA (Anon., 2006), as well as the methodology described by Cochran and Cox (1997), imposed a minimum amount of three replicates in the pot trials. Statistical procedures for germination included the use of an Analysis of Variance (ANOVA), as well as a split design of the plots. It involved eight incomplete blocks, which were divided into two split samples, each containing a different growth medium, which is further divided into the positions of the replicates (Figure 3.7). The position of each replicate of each seed type (coated- or non-coated) in the two split samples were determined by a chance-factor (such as by flipping a coin) in order to ensure randomization of replicates of all seed types.

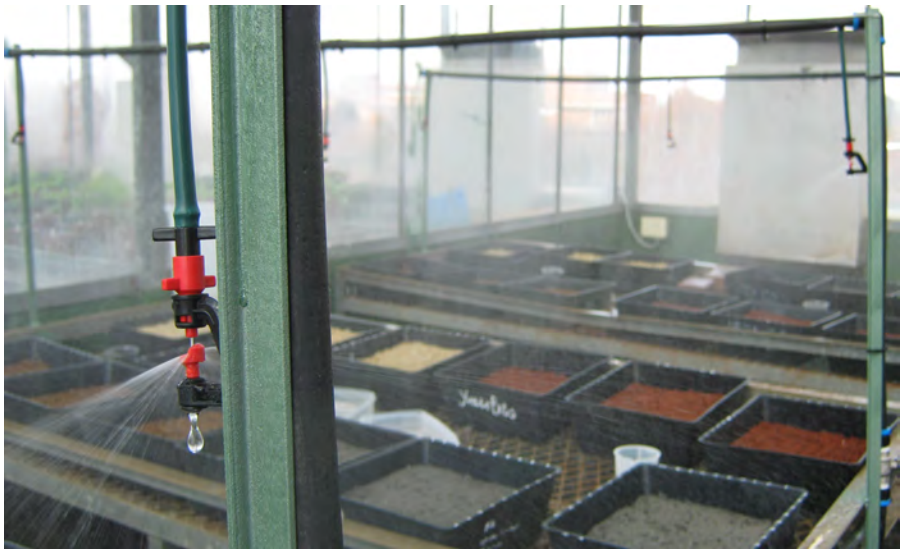

Figure 3.7. Example of trays in blocks (tables), containing the replicates of seed in the four soil types.

3.3.2 Seed analysis

The selected seed types were submitted to germination tests in four different soil types in a greenhouse as explained above. As described by Brits (2007), the submitted seed lots were tested for germination quality and purity according to the rules laid down by the International Seed Testing Association (ISTA). Van den Berg (2008) stated that the pure seed percentage and germination percentage indicates the seed value. Key factors in the determination of accurate analysis of seed quality included the method of sampling of the seed lot, the purity of the sampled seed and the germination capacity (viability) of the purified seed. This concept was developed in order to reduce the risk of planting seed of poor quality.

3.3.2.1. Sampling the seed lot

Van den Berg (2008) stated that the sample of seed tested in the laboratory is minute compared to the size of the seed lot it represents. In order to expect germination results for such large batches of seed to be statistically approved, standard procedures are prescribed by the ISTA rules. Roberts (1972) stated that the term "seed lot" denotes a nominally uniform consignment of seed, on which representative tests are done on random samples taken from it. Sampling must thus be carried out carefully, in order to obtain accurate, representative results for the total seed lot. By opening of a seed lot (in this case, a volume of seed equivalent to a 10 kg bag, depending on the physical characteristics of the seed type), the analyst identifies the type of grass seed (Anon., 2006). A submitted sample, representative of the whole seed lot, is extracted by stipulated techniques, as determined by the ISTA rules. The submitted samples are divided into a working sample, complying with the prescribed working sample mass, as stated in Column 4 of Table 2A of the ISTA rules (Anon., 2006; Brits, 2007). The estimated mass of this sample should contain at least 2500 seeds. The extraction of this sample may further involve a seed/soil divider (Riffler - (refer to section 3.3.4)) or be carried out by means of the hand division technique, depending on the seed type involved.

Techniques for sampling of coated seeds differ from the sampling techniques of the non-coated seed, especially for *A. pubescens*. Coated seed of all three the selected species, as well as the non-coated seed of *A. pubescens*, are not listed in the ISTA rules. All the seed types were sampled in the same way. In these cases, a hand sampling technique is used by extracting seed from five random places in the seed lot, from which eight replicates of hundred seeds each are counted (Anon., 2006). Seed are arranged in a funnel-shaped working sample, with the bottom of the funnel extending into a single row of seeds (Figure 3.8.). This allows impurities to be identified, noted on a purity control-sheet and excluded from the submitted sample.

Figure 3.8. Funnel-shaped working sample.

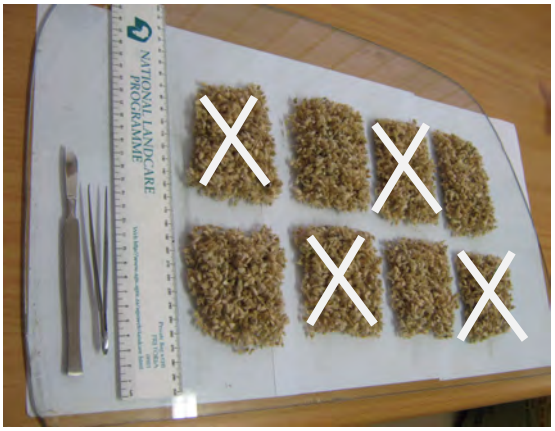

Figure 3.9. Example of the hand-halving method and the checker-patterned elimination of blocks.

The working samples of the remaining, uncoated seed types were obtained by using the hand halving method (Figure 3.9). This method entails the division of the total amount of received seed by hand into eight blocks, of which elimination is done in a checker-pattern (Figure 3.9.). The remaining blocks of seed are amassed and the process repeated until only a small amount of seed remains in the final division before sampling, enough to meet the prescribed mass for the specific species at hand. Each replicate is weighed according to the four figure rule (Brits, 2007). The variance, coefficient of variation and standard deviation are mathematically calculated. The working samples' mass should be recorded and any loss or gain of mass must be compared to the sum of components after the purity analysis is done. Noted by Brits (2007), re-testing of the sample has to be done in cases with a discrepancy of more than 5% of the initial mass (Anon, 2006).

2.3.2.2. Purity analysis

Van den Berg (2008) stated that it is impossible to remove all impurities during the cleaning process of seed lots (Figure 3.10.). Therefore, the objective of the purity analysis was to identify any seed and inert matter not being of the identified species in the submitted samples used in the experiments. Any foreign material was recorded on a purity chart and weighed in grams to the same decimal place as the working sample (Anon., 2006). The percentage of mass of foreign material in the working sample can thereby be determined. The germination tests (viability tests) of the various seed types were carried out on the pure seed component of the analyses. All seed sampling were carried out at the ecological laboratories of the North West University (NWU), while the purity tests and viability tests were carried out by the accredited laboratories of the Plant Research and Genetic Centre in Pretoria*. Dehulled seed of *A. pubescens* was used for the germination tests. Although purity and viability test were also conducted by the NWU, only data from laboratories of the Plant Research and Genetic Centre were used in the results, as the ecological laboratories of the NWU are not yet certified to conduct purity- and viability tests.

Figure 3.10. An example of inert matter as impurities isolated from a seed sample.

*Plant Research & Genetic Centre, Department of Agriculture, Forestry and Fisheries, Roodeplaat, Pretoria.
Tel: 012 808 5385; Fax: 012 808 5394

3.3.3. Germination of selected seed in four growth mediums

3.3.3.1. Procedure

The seed used in germination tests were obtained from the pure seed component (Anon., 2006) of the seed samples as described earlier. Representative samples of the selected seed types were previously submitted to pre-chilling dormancy breaking methods of 5 days at 5°C (Brits, 2007; Van den Berg, 2002; Winter, 1974). The custom method involving the use of Potassium nitrate (KNO_3) for dormancy breaking purposes was not used, because when this chemical agent is applied as a liquid, it would result in dissolving the seed coatings. Hereafter, the different seed types were germinated in the various growth mediums in 30cm x 27cm x 11cm trays, containing an approximate volume of 8910 cm^2 of the specific soil type.

Figure 3.11. Apparatus to distil water, used to wet the different soil types, was connected to the irrigation system in the greenhouse at the NWU.

Each tray was lined with Geotextile, in order to prevent the fine textured soil types, such as the mines' tailings and the sandy soil, from leaching out of the trays. The collected soil types were divided by use of a Riffler-soil divider (refer to section 3.3.4) to get a representative sample of each soil type that was put into the trays. The soil types were kept moist by watering only with distilled water [pH 7]. To ensure that contaminants contained by tap water, which could influence the germination due to change in pH, the distillation unit was connected to the irrigation system in the greenhouse at the NWU (Figure 3.11). Watering was carried out twice a day, but the exact amount of water used during the irrigation was unfortunately not determined.

Figure 3.12. Replicates of 100 coated- or uncoated seed of each species were sown by hand in rows of ten seeds each in trays lined with Geotextile.

All seed used in the pot trials were separated from the submitted samples by methods prescribed by ISTA (Anon., 2006). The seed were planted by hand with ten seeds in ten rows, thus 100 seeds per replicate tray, at a depth of 5 mm (Figure 3.12.). The raster-formation of the positions of the seed enabled the researcher to verify the progress of germination of each individual seed every time the germination counts were conducted. Each tray was replicated three times. Hereafter all replicates were placed out according to the statistical layout-plan as described earlier (see section 3.3.1.), determined by the statistical department at the NWU. Controlled temperatures were maintained at 16°C at night time and 22°C during day time respectively.

The amount of days over which the germination trail was carried out, was determined by rules laid down by ISTA (1985). The ISTA-rules stipulates a specific minimum (“initial”) and maximum (“final”) number of days at which germination should be recorded for grass species in general (ISTA, 1985) (Table 3.2). Germination was monitored daily for the prescribed number of days for each species individually. The numbers of new seedlings were recorded per day in order to provide cumulative emergence and maximum emergence over the prescribed period of observation. Only the difference in germination percentage between the coated- and uncoated seed of each species was recorded. No comparison was made between the separate species. The trial length therefore differed for the different species, although only the first 21 days of observation was recorded for logistical reasons. Following the germination trials, the establishment of seedlings was determined at the four-leaved stages.

Table 3.2. The initial- and final count days of the coated (c) and uncoated (uc) seed types of the selected species (ISTA, 1985).

Seed type	Initial count day	Final count day
Anthepora pubescens (c)	7	28
Anthepora pubescens (uc)	7	28
Cynodon dactylon (c)	7	28
Cynodon dactylon (uc)	7	28
Panicum maximum (c)	10	21
Panicum maximum (uc)	10	21

Germination data were obtained in the form of numerical counts for each grass species, coated and uncoated, in each soil type. Germination data were processed by using the computer software programs Excel of Microsoft Office and Statistica version 10.0. Line- and column graphs were drawn from tables containing germination data.

3.3.4. Soil dividing and analysis

3.3.4.1. Soil dividing

In order to obtain representative samples of all four soil types used in the trays in which germination was carried out, the total soil lot, obtained at the different sites, was partitioned by the use of a soil divider (Riffler) (Figure 3.13.). Each submitted soil sample was divided by pouring the soil into the top load tray of the Riffler, resulting into two samples of the same soil lot. After elimination of one of the bottom trays, the remaining tray's content was once more poured into the top load tray. For further division, this process was continued until the desired volume (enough to equally fill the three replicates of germination trays) was obtained. This statistical method of division of the soil ensures that random, representative samples of collected soil are used in the replicates of the experiment.

Figure 3.13. An example of the Riffler-soil divider that was used to obtain representative samples of each soil type during the pot germination trials.

3.3.4.2. Soil analysis

Soil samples were collected for analysis at the same sites where the field trials were conducted. The analyses were carried out by the laboratories of EcoAnalytica*. The amount of exchangeable cations retained on the reactive colloidal surfaces of the soil types was determined by the ammonium acetate (NH_4OAc)-method, as described by Schollenberger & Simon (1945) and Hesse (1971). Ammonium-ions replace cations on exchange positions of soil colloids (Gobat *et al*., 2004), which are then displaced into solution and measured to give the total concentration of each cation. It therefore reflects the nutritional status of the soil. Concentrations of cations were extracted at 1:10 g/ml ratio of soil: extraction reagent at 20°C. The specific base cations measured during this test included Calcium (Ca), Magnesium (Mg), Potassium (K), Sodium (Na), and Ammonium (NH_4).

The cation exchange capacity (CEC) of the four soil types was measured to indicate the total capacity of each soil type to hold exchangeable cations, and the capacity for exchange of cations between the soil and the soil solution (Gobat *et al*, 2004). The cation exchange capacity (CEC) was measured using 1 M Ammonium acetate (pH 7) (USDA, 2004). The potential exchangeability of the soils' reactive surfaces was determined by measuring the total amount of the index cation, NH_4, per base cation (Haagner, 2008).

Both the actual pH and the exchange acidity pH of the soil samples were measured. The exchange acidity (pH_{KCl}) was measured on a mass basis, using 1 N KCl as exchanging salt in a 1:2,5 soil:liquid ratio suspension. KCl masks the variations in salt concentration as a result of fertilizer applications, water quality of irrigation and microbiological composition (Haagner, 2008). A pH-meter was used to record measurements of the solution-fraction of the suspensions, four hours after mixing the soil samples with KCl.

The actual pH (pH_{water}) of each soil type was measured on mass basis by placing soil samples in distilled water in a 1:2,5 soil:water ratio suspension. In this case the electrode measures only the protons in the soil solution (Gobat *et al*., 2004), resulting in a higher pH_{water} measurements than pH_{KCl} of the same soil samples. Results were also gained from the liquid fraction of the suspension after four hours of mixing the soil with the ddH_2O, by the use of a pH-meter.

*EcoAnalitica, P.O. Box 19140, Noordbrug, Potchefstroom, 2522. Tel: 018 293 3900

The electrical conductivity (EC) of saturated extractions from the soil types in water was measured to establish the total concentration of dissolved salts of each soil type. Soil samples were saturated with de-ionised water to form a paste, left overnight or for at least seven hours, and centrifuged. Thereafter, measurements were taken with an EC-meter (Fertilizer Society of South Africa, 1974).

The more soluble Phosphate content of the soil samples was determined by using the p-Bray 1-method (Bray & Kurtz, 1945). The P-Bray 1-solution, containing Ammonium Fluoride and hydrochloric acid, was added to each soil sample in a 1:7,5 soil:extraction agent- ratio, together with a flocculent agent. An auto-analyzer was used to determine the Phosphate (P)-content of each soil sample (Fertilizer Society of South Africa,1974).

The sand, silt and clay content of the soil types were analyzed by the use of the hydro method. A 2 mm-sieve was used to sift 100g of each soil sample, after which the fraction > 2 mm of each sample was recorded. The sieved component of each sample was then saturated with distilled water, after which peroxide was added and placed on a heated sand stove for four hours. After the solution cooled down, Sodiumhexametaphosphate was added and sieved with a sedimentation cylinder. The fraction of the soil sample smaller than 53 µm was now recorded. Measurements with a hydrometer were conducted after 40 seconds, and after seven hours. The fraction left in the sieve was oven-dried and brought into suspension again. Weighing these fractions completed the sand, silt and clay analyses (Fertilizer Society of South Africa, 1974).

Particle size distribution was analyzed by sieving the soil samples through a 2000, 1000, 500, 250, 100 and 53 µm sieve separately. The fraction left on each size sieve was weighed and recorded (Fertilizer Society of South Africa, 1974).

Multivariate data analysis techniques were carried out to show the correlation of the germination of each seed type (coated- and uncoated seed) with the soil characteristics (section 4.2.5).

3.4. Field trials

3.4.1. Experimental design

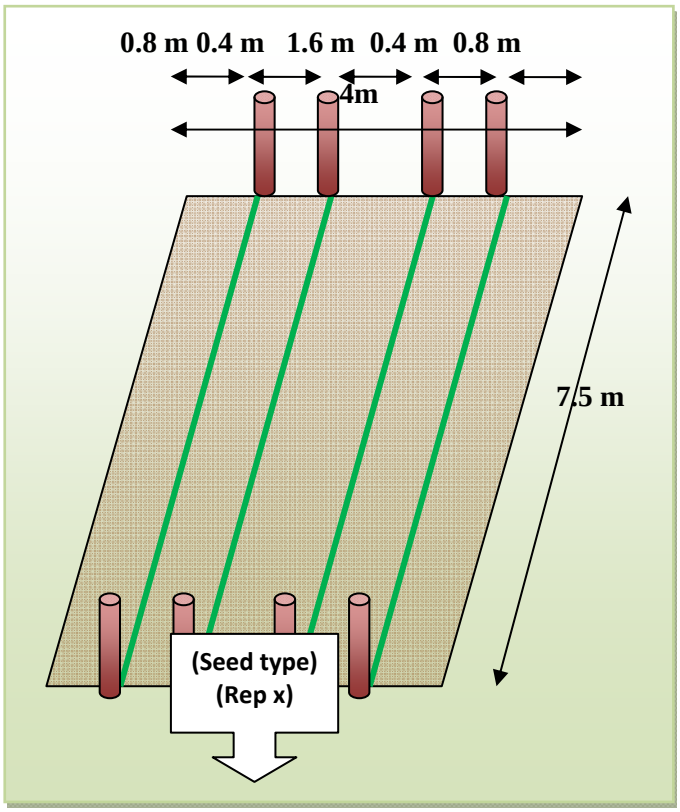

Figure 3.14. Basic design of one replicate (block) of a seed type sown in the different soil types in the field trials.

The layout of all replicates at each site was according to a split plot design. Each site was re-seeded in four rows with the prescribed seed mass as advised by ASC of each seed type. Seed masses of 15 g of uncoated- and 24 g of coated seed types were individually sown at about 1 cm deep in four rows over a length of 7.5 m. The four rows, which constitute a block (one replicate), were replicated three times at each site (Figure 3.14).

Figure 3.15. Example of one replicate (block) showing the experimental design at the gold mine tailings field trial.

The spacing of the rows in all replicates were designed to allow watering of the rows by the use of a tractor-and-water cart-combination. This is an inexpensive method in areas where no irrigation systems are installed. In each replicate, the four rows were therefore sown in two sets of two rows, which are 0.4 m apart (Figure 3.15). The two sets are separated by a distance of 1.6 m in order to allow space for the tractor wheels. The two sets of two rows were also placed 0.8 m from the side of the adjacent replicate, to eliminate competition from surrounding vegetation and thus avoiding any edge effects. In summary, the total width of each replicate was 4 m, and the length 7.5 m. All replicates were placed 1 m from each other, and replicates of the same seed types placed as far from each other as possible. The chance of any soil or micro-climate variability was therefore considered. The total site was cleared of all other existing vegetation and the surface thereof evened by machinery before the trial was established.

3.4.2. Monitoring of field trials

Figure 3.16. Determining the density of the seedlings per row in a 0.5 m x1 m quadrate placed subjectively over the two rows.

All sites were monitored on a two-weekly basis, depending on the accessibility of gravel roads due to wet conditions, and the direct surroundings of the site due to wet conditions in the rainy season. The method for surveying involved a quadrate of 0.5 m x 1 m, which was subjectively placed over two rows, at six

locations in each replicate. All seedlings of the species sown in that replicate were counted within the quadrate to determine the density (Figure 3.16).

Figure 3.17. *Cynodon dactylon* illustrating the stoloniferous growth- form.

Figure 3.18. *Anthephora pubescens* growth-, form, illustrating multiple tufts from one seed.

Figure 3.19. Separation of individuals of an *Anthephora pubescens*-tuft to ensure that the correct number of seedlings were recorded.

Figure 3.20. A matured individual *Anthephora pubescens*.

The differences in the morphological growth form between the species became significant when field surveys were carried out. In Figures 3.17 & 3.18 the differences in growth forms between species such as *C. dactylon*, which reproduce by means of stolons, and *A. pubesens*, which only grows in tufts, but may produce more than one seedling from each seed due to the multiple embryo's occurring in the spikelet, can be observed. The difference in growth form became more significant as the seedlings matured. In the case of *C. dactylon*, all daughter plants were considered the same individual as the mother-plant. For *A. pubescens*, that bear multiple embryo's, more than one seedling was counted from a single spikelet. Furthermore, when matured, the space between the individual tufts decreased, which led to added confusion of the actual number of germinated seedlings. At young stages, each plant was therefore identified and counted by either separating the combined tufts by hand, or by lifting the seedlings from the soil (Figure 3.19). As the tufts matured, it was not possible to identify separate seedlings. Every tuft was therefore counted as an individual (Figure 3.20). These differences in counts between individual seedlings in a young stage and as mature tufts could have influenced the end results.

Figure 3.21. Elimination of competition by broad-leaved species on planted grass species by manual removal.

Above-average rainfall events in the 2010 growing season (See section 3.2.2, Figure 3.6), as well as the fact that trials situated in natural sites still contained the natural seed bank of uncontrolled weed species, especially in the sandy soil trials, contributed to a rapid invasion of weedy plants in these field trials. The density of weeds, such as *Commelina benghalensis***,** *Citrullus lanatus* and *Pelargonium bifolium*, increased dramatically, which contributed to significant levels of competition for sunlight and moisture, making it difficult for the selected grass seed of the trial to germinate. The latter also caused some problems during sampling. Several measures were taken to eradicate the high densities of weedy plants at

natural field sites. At the clayey soil site, herbicide was sprayed to eliminate broad leaved-species. Spraying of herbicide was done by a contractor, Mr. Johan Bothma of the company TerraCare®*. At the sandy soil site, herbicide spraying was not applied to control the weed species. Grass species require established secondary root systems to be least affected by the toxin. Unfortunately, broad leaved species grew much faster, compared to the rate at which germination of the planted grass species took place. Therefore, the weeds were removed manually by hand at the sandy soil field trial (Figure 3.21). Weed control was not necessary at the gold- and platinum mine tailing-sites.

3.5. Physiological Tests

3.5.1. Germination enzyme activity

3.5.1.1. Plant material and treatments

The extractions of the enzyme were carried out at two stages of germination – at a stage when no germination was initiated (dry, unplanted seed) and after 96 hours of activated germination. Three grams of seed, both coated and uncoated, of each of the selected species were soaked in distilled water in order to activate germination (Figure 3.22). Three replicates of each seed type were tested for each germination enzyme. Three replicates of dry seed of each seed type served as the control.

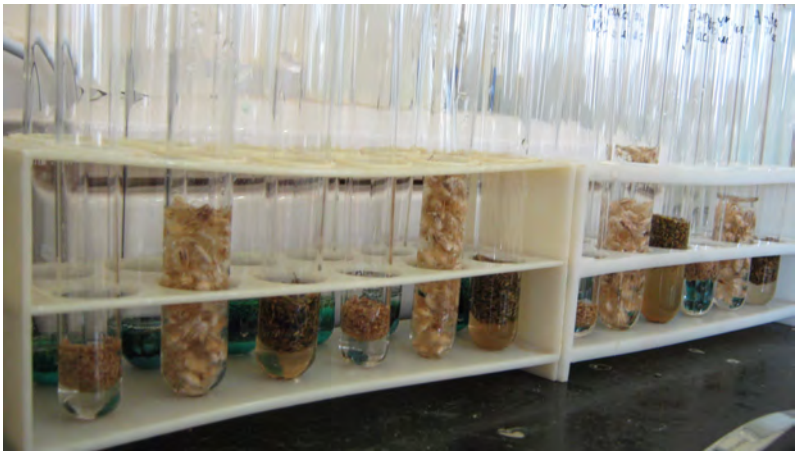

Figure 3.22. Germination metabolism of the selected coated- and uncoated grass seed, activated with distilled water (pH 7), after which the activity of germination enzymes was determined.

In addition, the activity of the enzymes was tested in seed samples extracted from three phases during the coating (enhancement) processes.

*Terracare, P.O. Box 17323, Bainvlei, 9338; Tel: 0824584558

These processes entail phases of wetting of the uncoated seed, application of the coating and drying of the coated seed at elevated temperatures (60º). Seed was sampled at the coating (enhancement)-plant of ASC, frozen with liquid nitrogen and stored at -84°C until the time of testing.

3.5.1.2. Extraction procedure

Seeds were placed in pre-cooled containers and 20 mg Dowex, which had been incubated in an extraction buffer*, pH 7.5, for 15 minutes, and 50 mg acid washed sand was added to each seed sample. The mixture was homogenised by using an Ultra Turrax in 6 ml buffer (1 g seed material: 2 ml extraction buffer). An additional 1 ml was used to rinse the container. The homogenised blend was centrifuged at 4°C, at 13 000 rpm for 20 minutes, using a JA 20 centrifuge head. The supernatant was used as the crude enzyme extract.

**** Extraction buffer (pH 7.5)***

The extraction buffer was prepared by dissolving 2.72 g KH_2PO_4 in 150 ml distilled water. The pH was adjusted to 7.5 with 2M KOH, where after the volume was filled to 200ml with distilled water. Just before use, 0.0093 g EDTA and 0.0038 g DTT was added for every 25ml stock that was used.

3.5.1.3. Protein concentration

The protein concentration was determined by the Bio-Rad Protein Assay method, which is based on the principles established by Bradford (1976). This method involved the addition of an acidic dye to protein solutions, after which a differential color change of the dye occured in response to various concentrations of protein. Subsequent measurement of each sample was carried out with a microplate reader and compared to a standard curve to determine the protein content of each sample.

3.5.1.4. Peroxidase (POD)

The activity of this enzyme was determined by blending 500 µl of 40 mM phosphate buffer (assay buffer*), 340 µl ultra pure water, 100 µl of 5 mM guiacol (the substrate for the enzyme), 50 µl of 8.2 mM H_2O_2, and 10 µl enzyme crude extract in a plastic cuvette. Spectrophotometrical methods were used to determine the activity of POD in each of the selected seed types. The change in absorbance were measured at 470 nm for 180 seconds at 30°C (Zieslin & Ben-Zaken, 1991) and the activity of POD expressed as nmol tetraguiacol mg^1 protein min^{-1}.

Assay buffer (pH 5.5)

The pH of K_2HPO_4 (80 mM) (dissolve 3.484 g in 250 ml ultra pure water) was adjusted to 5.5 by the addition of 80 mM KH_2PO_4 (dissolve 2.722 g in 250 ml ultra pure water). Just before use, 0.2 mM EDTA and 2mM phenylmethylsulfonyl fluoride (PMSF) was added.

3.5.1.5. Alpha amylase (α-amylase)

The activity of this enzyme was determined by the protocol followed by Bernfeld (1955). One ml enzyme extraction was incubated with 1 ml substrate [1 g starch dissolved in 0.02 M phosphate buffer (pH 6.9] for 3 minutes at 20°C. After 3 min, 2 ml of a mixture of 1g 3-5 dinitrosalicilic acid in 20ml 2N NaOH , 50 ml distilled water and 30g Rochelle salt (the total volume filled to 100 ml with distilled water), was added. The tube, containing the blend, was heated for 5 min in boiling water, after which it was cooled under running tap water, until the blend of reagents turned brown in color. The specific activity of alpha amylase was determined spectrophotometrically at 270 nm at 30°C and expressed as maltose mg^1 protein per min^{-1}.

3.5.1.6. Lipoxygenase (LOX)

The activity of this enzyme, involved in the germination metabolism of seeds, was determined according to the protocol followed by Grossman and Zakut (1979). The reaction mixture for determining the activity of LOX in each seed type consisted of an 50 µl enzyme extract, 150 µl substrate (2.2 mM linoleic acid in 0.15% Tween) and 1ml of 0.1M sodium citrate phosphate buffer (Ocampo *et al*., 1986). These reaction mixtures were blended in quartz cuvettes, and the rate at which the substrate is utilized was spectrophotometrically measured at a wavelength of 234 nm. The spectrophotomentrical measurements were done over a period of 600 seconds (10 minutes). By determining the protein concentration of the reaction mixture, the activity of LOX was mathematically calculated and expressed as nmol HPOD mg^{-1} protein min^{-1}.

3.5.2. Data capturing and -analysis

Spectrophotometrical data were obtained by the coupled use of spectrophotometer and Shimadzu-computer software. This data was processed by Excel-software, as well as the Sigma Plot 8.0 computer program in order to reflect the enzyme activities within the seed samples statistically.

3.5.3. Respiration of seed

Three representative samples of each of the selected seed types, sampled according to the specifications of the ISTA rules (discussed under 2.1.2. Seed analysis), were germinated for 96 hours. Respiration of

seeds was determined by measurement of the inverse of CO^2-assimilation with a photosynthesis system, including a chamber with light, humidity, and temperature control (CIRAS-1; PP Systems) (Van Heerden *et al*., 2008). Measurements were conducted at 26°C, with an irradiance of 800µmol photons $m^{-2}.s^{-1}$ at a CO_2-concentration of 360 µmol.mol^{-1}. The mass of each sample was recorded after the measurement thereof and used in a mathematical formula to determine the respiration rate.

The respiration of the sample was calculated mathematically by the following formula:

$$\textbf{Respiration} = (Pn_{value} \times 3)/(\textbf{Fresh mass of sample})$$

Pn_{value}*:* the measurement from the CIRAS-system; *3:* the standard volume of the cubicle in the CIRAS-system in which each sample was measured.
Fresh mass of sample: the mass each sample of seed that was submitted for respiration.

The influence of the additional mass of the coating (enhancement) of the coated seed samples was eliminated by subtracting a calculated average mass of the coating, which is specific for each species' seed, from the fresh mass of the seed sample.

3.5.4. Relative water content

Three samples of 10 g (at 25°C) of each selected seed type were put in petri dishes with distilled water for 48 hours (until saturated). Hereafter each sample was weighed, obtaining the "saturated mass". A complete drying of the same samples was then done, by submitting it to a temperature of 60°C in a drying oven, after which it was again weighed to obtain the "dry-mass" (DW). The relative water content (RWC) of the seed was calculated by using the following formula:

$$\textbf{RWC = Actual water content/Saturated water content = TW- DW/ } TW_{(Sat)} \textbf{ - DW}$$

RWC: Relative water content of the seed
$TW_{(Sat)}$*:* Mass of the seed when fully saturated with water (Saturated water content)
TW: Current mass of the seed with relative water content
DW: Mass of the dried seed

Chapter 4

Results and discussion

4.1. Introduction

This chapter presents the results and outcomes from the three components, namely the greenhouse trials, field trials and physiological tests, regarding the aims of the study described in Chapter 1 (Introduction). The results of the greenhouse trials showed the effect of the coating on the germination of the selected seed types in the four soil types under controlled conditions, whereas the field trials showed germination of the selected seed types under natural conditions. The results of both these trials are discussed by comparing the germination of the coated- and uncoated seed of the three selected species in the four soil types, i.e.; sandy- and clayey soil types, and soils from platinum and gold mine tailings; respectively. The physiological data gives additional insight on the effect of the seed coating on the germination potential of the seed. In order to evaluate the effect of the seed coating on the germination and physiology of the selected grass species' seed, the performance of the coated seed is compared to the uncoated seed of the same species throughout the study.

4.2. Greenhouse trials

This section of the study addresses the first objective, namely to assess the germination of the seed and the number of seedling establishment of the three selected coated- and uncoated grass species in four soil types, under controlled conditions in the greenhouse. Cumulative germination figures were obtained over the trial period of 21 days and presented as a percentage of the total of 300 hand-planted seeds of each seed type. Monitoring and recording of data was carried out on a daily basis over the 21 day period (see Chapter 3). The data obtained for each species during the greenhouse trials are discussed separately; presented by the differences in the trends in the germination rates of the seed types in each soil type (Figures 4.1, 4.3 & 4.5), as well as the comparison of the overall germination percentages of the species in each soil type after the trial period (Figures 4.2, 4.4 & 4.6). Germination % (emergence) was recorded from the first day (Day 1) when seed started to emerge, which could vary between seed types. The maximum germination refered to in the results of the greenhouse trial, only reflect the maximum emergence percentage reached during the 21 day trial period, irrespective of any further emergence after this trial.

4.2.1. *Anthephora pubescens*

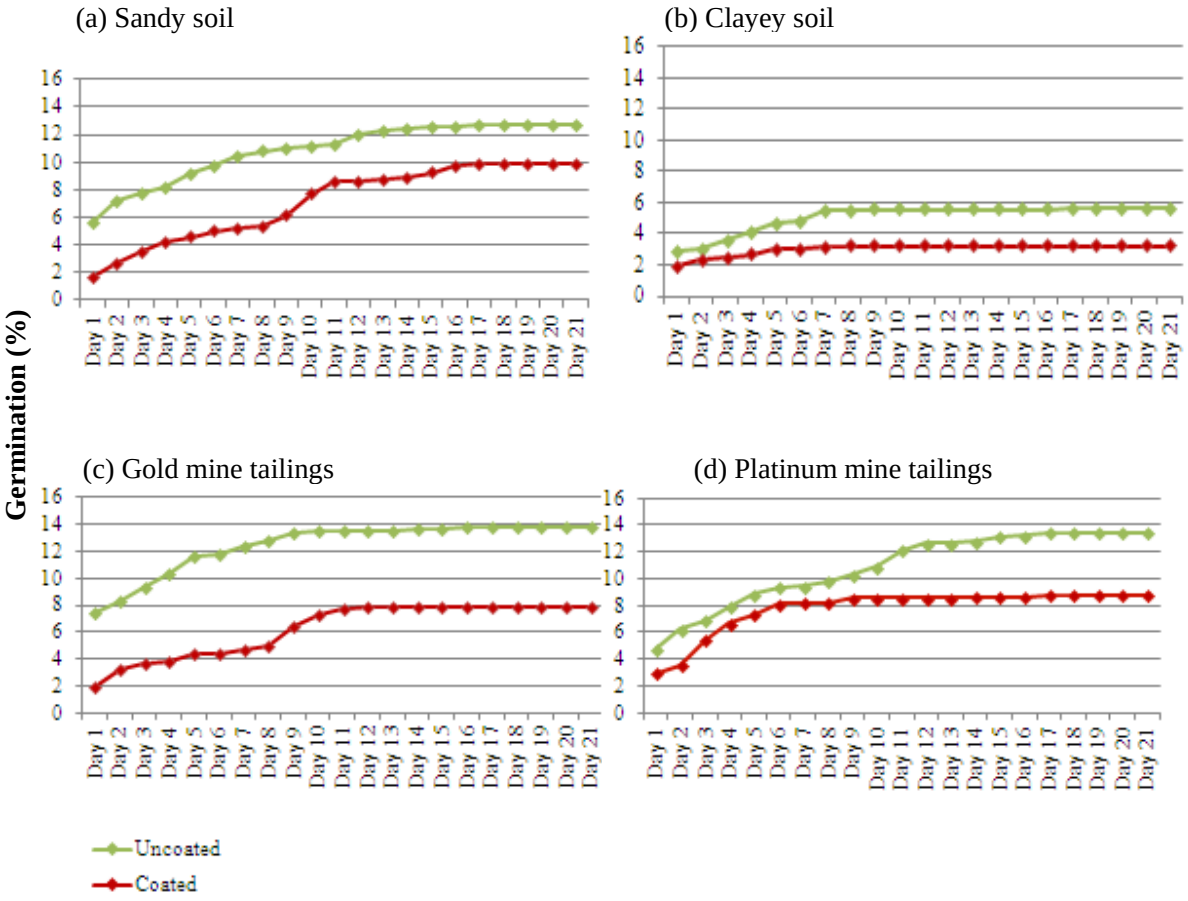

Figure 4.1. Average germination (%) of coated- and uncoated seed of *Anthephora pubescens* in the four soil types over a period of 21 days.

In general, the germination rates of both seed types of *A. pubescens* follow approximately the same trend. The maximum germination percentage for both seed types in all soil types was reached after approximately ten days, except in clayey soil, where the maximum germination percentage was reached after seven days (Figure 4.1b). In all the soil types, the uncoated seed type of this species show a higher germination rate over the germination trial period of 21 days.

The germination rate of *A. pubescens* differed significantly between the sandy- and clayey soils (Figure 4.1a & b). As mentioned above; the maximum germination rate being more than twofold in sandy soil (12.6%) than in clayey soil (5.7%). The maximum germination percentages of both seed types were also reached sooner (after 7 days) in the clayey than in the sandy soil.

Although the germination percentages of this species are initially reasonably low, higher maximum germination percentages are observed in the platinum- and gold mine tailings, 13.4% and 13.8% respectively, compared to the sandy (12.7%) and clayey (6%) soil types. The difference in the germination rate of this

species, between the coated and uncoated seeds in platinum mine tailings is especially significant, as although the germination percentages of the coated seed is overall lower, the coated seed reaches the maximum germination percentage much earlier (day six) than the uncoated seed type. Unfortunately, this result could not be explained and needs further study.

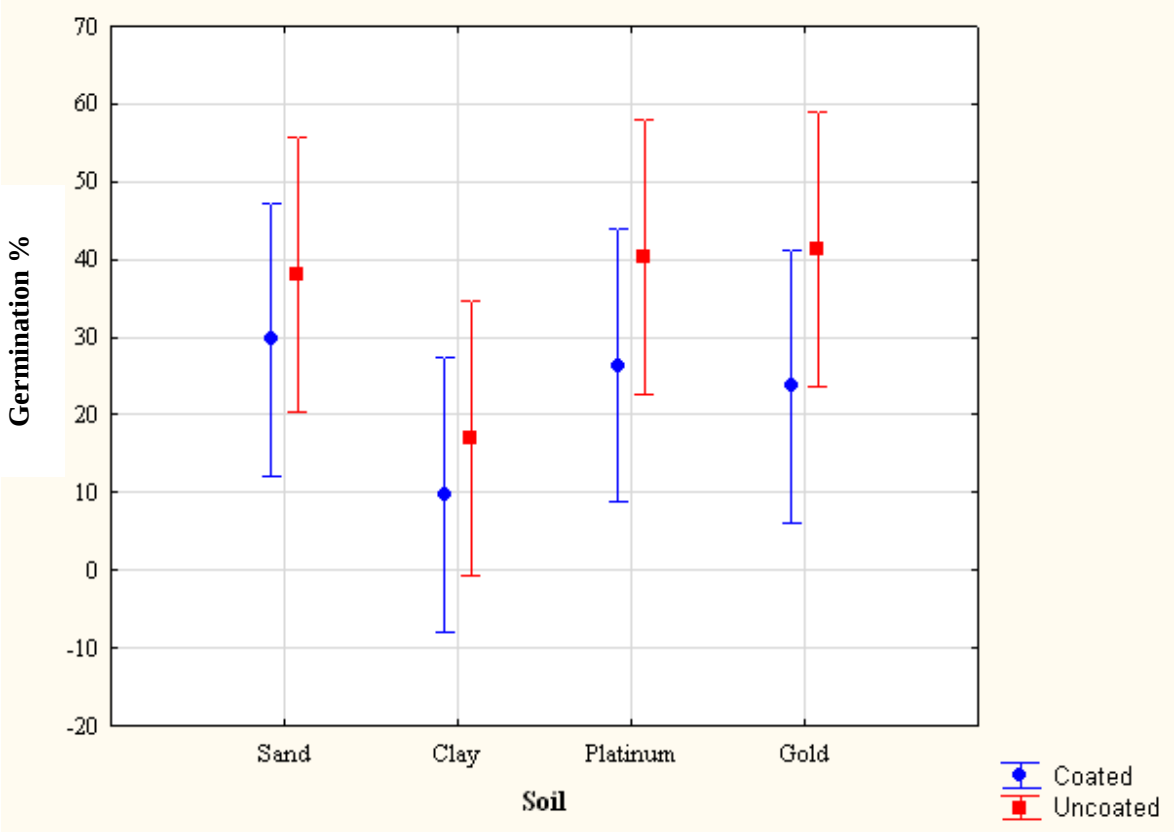

Figure 4.2. Comparison of the maximum germination (%) of the three replicates after a 21 day trial period of coated- and uncoated seed of *Anthephora pubescens* in the four soil types.

Although the maximum germination of uncoated seed exceeded that of the coated seed type, the analyses of variance (ANOVA) for the germination of *A. pubescens* during the green house trials revealed no significant difference between the coated and un-coated seed types in each of the four soil types (Figure 4.2). The germination for both seed types of *A. pubescens* is the lowest in the clayey soil. This result is expected as *A. pubescens*, has a preference for better drained, soil types (Van Oudtshoorn, 2006), such as a sandy soil.

The largest difference between the maximum germination percentages of the coated and uncoated seed is observed in the soil from the gold- and platinum mine tailings.

4.2.2 *Cynodon dactylon*

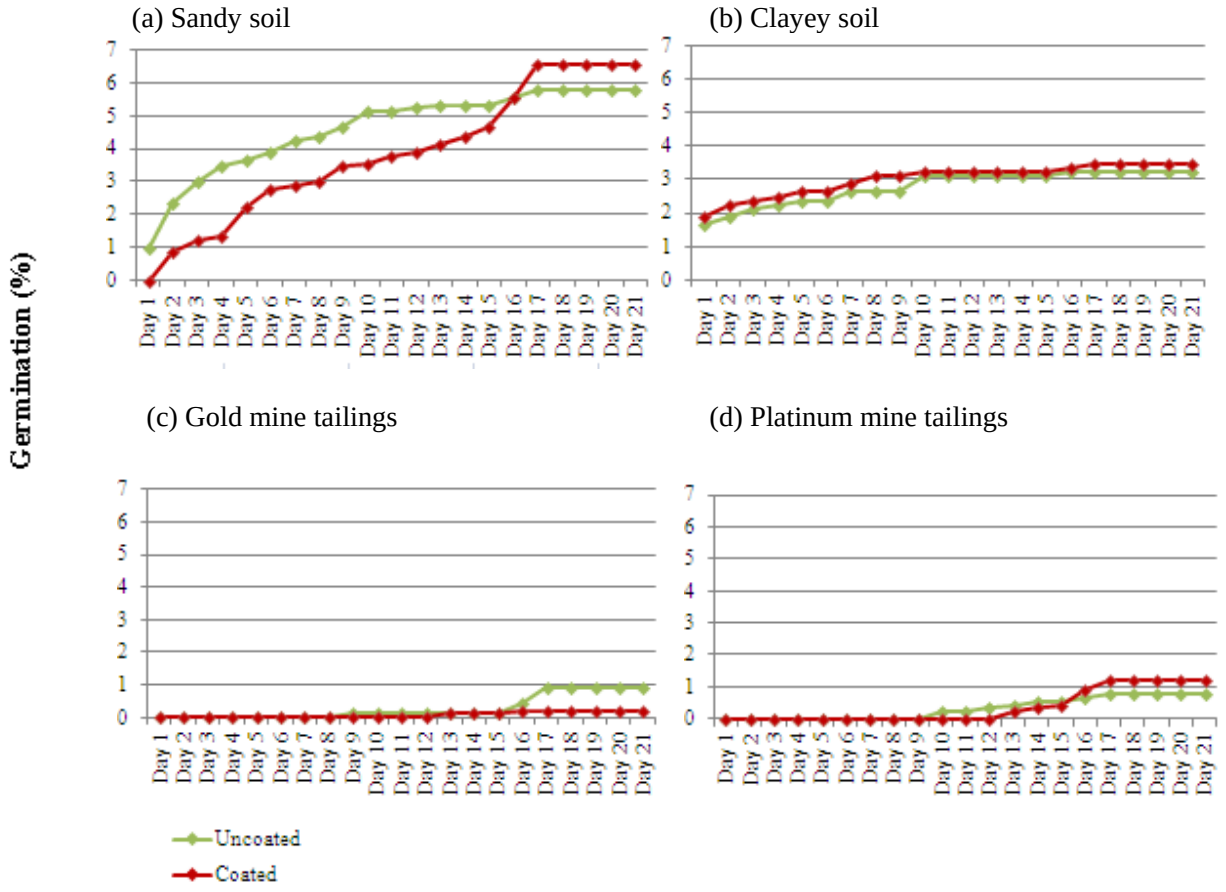

Figure 4.3. Average germination (%) of coated- and uncoated seed of *Cynodon dactylon* in the four soil types over a period of 21 days.

Very low maximum germination percentages for both seed types of *C. dactylon* were observed in all four soil types, especially during the first eight days in the two anthropogenic soil types. The highest total germination rate was observed in sandy soil (6.6%, Figure 4.3.a), whereas the lowest occurred in soil from platinum mine tailingss (0.9%, Figure 4.3d). Although initial germination percentages for both seed types of this species were relatively low in clayey soil (±1.7%, Figure 4.3b), which only reached a maximum germination percentage of 3.5% after 21 days, it was higher than germination in the soils from the gold and platinum mine tailings. In the sandy soil, the uncoated seed of *C. dactylon* exceeded the germination percentages of the coated seed during the first 14 days of germination (Figure 4.3a), where after the germination of the coated seed increased, to exceed that of the uncoated seed. The same occurrence could be observed in the soil from the gold mine tailings. The maximum germination percentage for both soil types was reached relatively late (only after 16 days) in the germination period of 21 days.

It seemed that a 'lag phase' was observed in the germination of both seed types (coated and uncoated) for *C. dactylon* in both the soils from the gold and platinum mine tailings and that germination only started after

nine to 12 days (Figure 4.3c & d). Considering the low germination percentages of this species in these soil types, it seemed that the chemical and physical characteristics of the soil types, as well as probable physiological constraints within the seed, had a negative effect on the germination rate of *C. dactylon* seeds. The average maximum germination percentages for both seed types was the lowest in the soil from platinum mine tailings.

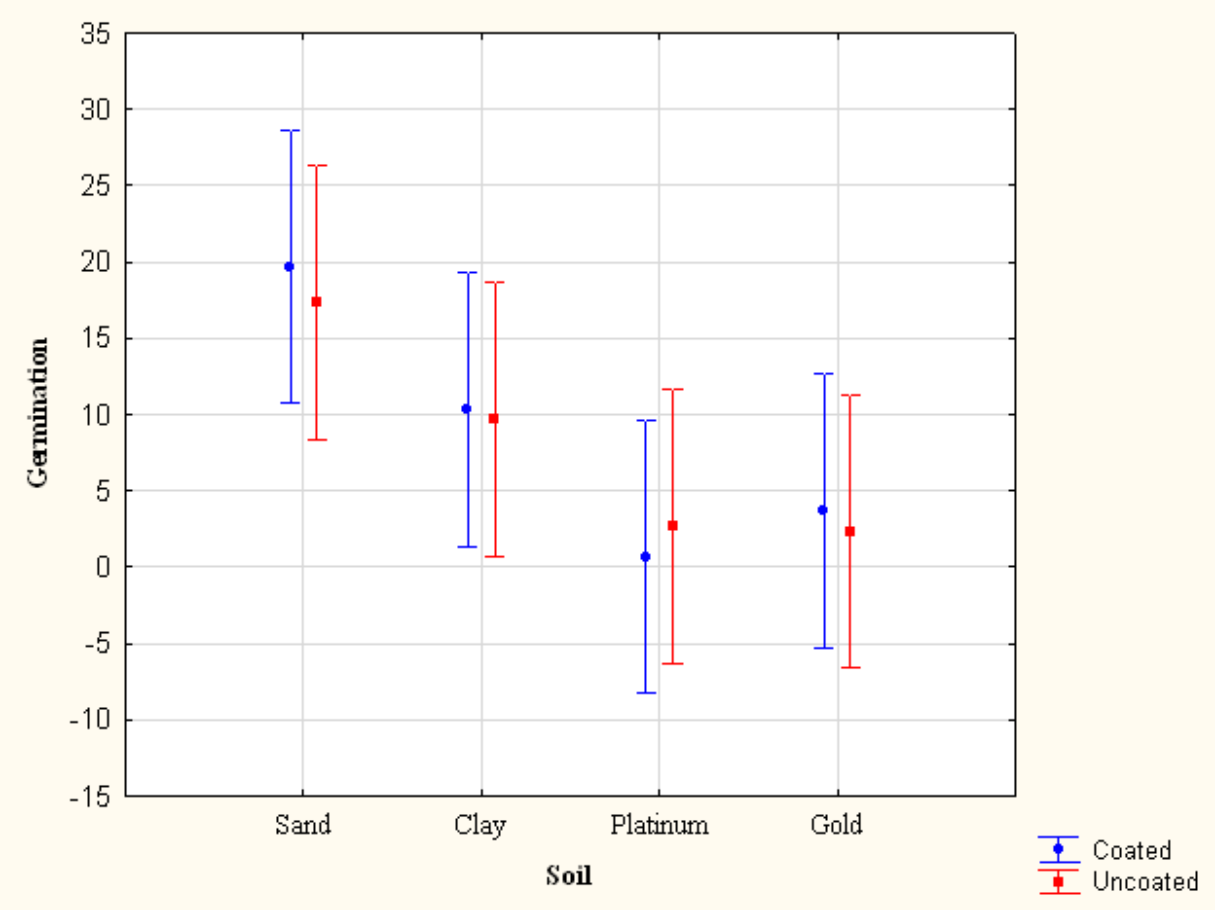

Figure 4.4. Comparison of the maximum germination (%) of the three replicates after a 21 day trial period of coated- and uncoated seed of *Cynodon dactylon* in the four soil types.

Though the difference between the seed types of this species was not significant in the separate soil types, the average maximum germination percentage of coated seed of *C. dactylon* exceeded that of the uncoated seed type in all soil types evaluated in this study, with the exception of platinum mines tailings (Figure 4.4). In the latter soil type, the average germination of the uncoated seed type exceeded that of the coated seed. According to the ANOVA statistical analysis, there was significance in the influence of the soil types as an environmental variable on germination, ($p=0.004$; $f=6.682$). The post-hoc Tukey-test however showed no significant differences between the clayey, gold mine and platinum mine tailings soil types.

4.2.3. *Panicum maximum*

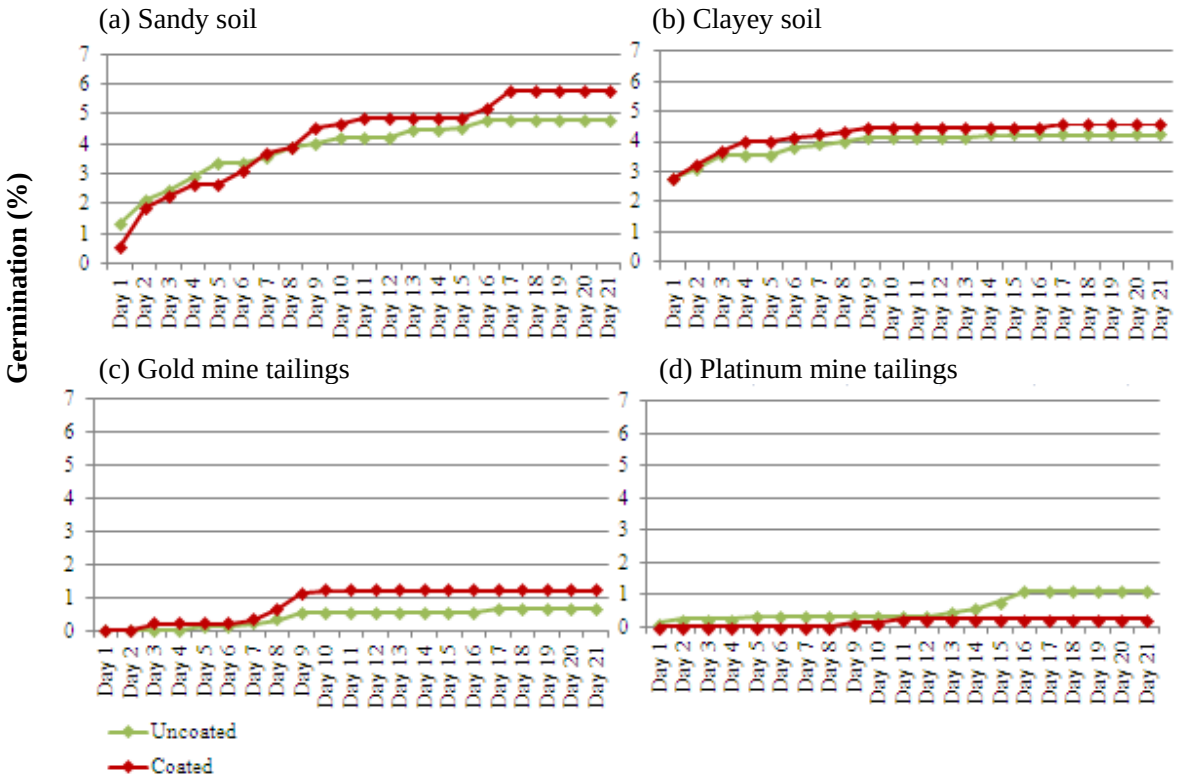

Figure 4.5. Average germination (%) of coated- and uncoated seed of *Panicum maximum* in the four soil types over a period of 21 days.

In both the sandy- and clayey soil types, the coated and uncoated seed of *P. maximum* followed very similar trends in germination rate. In the sandy soil (Figure 4.5a), low average germination percentages were observed in both seed types in the initial days of observation, where after a visible increase in germination occurred, in comparison to the remaining soil types. The germination rate of the coated seed of this species was initially lower, however, the maximum overall germination percentage was higher than that of uncoated seed in all soil types, except the platinum mine tailings soil. Although not very high, the germination percentage of coated seed for both the sandy (6%) and clayey (4.5%) soils was overall higher than for the soils of the two mine tailings. Considering the genetic adaptive characteristics of *P. maximum*; preferring less well drained soil types with a high water holding capacity and high nutrient content (Van Oudtshoorn, 2006), the low germination in clay, compared to sandy soil, was unexpected. It therefore seemed that this species can occur in a wider range of soil types than expected.

The initial germination percentages of the coated and uncoated seed of *P. maximum* in clayey soil (2.8%, Figure 4.5b) was nearly three percent higher, compared to the sandy soil (0.6% and 1.3%) respectively

(Figure 4.5a). Throughout the duration of the trial, the germination rate of the coated seed exceeded that of the uncoated seed, although with a minimal value. Both seed types reached the maximum germination percentage relatively early during the germination trial at about three to four days after seeding in the clayey soils.

The germination of both seed types was overall very low in both the soils from the mine tailings at about 1% (Figure 4.5c & d). It seemed that in soils from gold mine tailings, both the coated and uncoated seed of *P. maximum* had a "lag phase" during the first few days of the trial period (Figure 4.5c). Germination of the coated seed remained zero until the eighth day of the trial, where after only 0.2% germinated during the rest of the 21 day-period.

In the soils from the platinum mine tailings, a short interval of growth lag could also be observed in both the seed types (Figure 4.5d). The coated seed started to germinate at day two of germination, whereas germination can only be observed in the uncoated seed at day four. A slightly higher germination rate was observed in the coated seed, especially after the ninth day (Figure 4.5d).

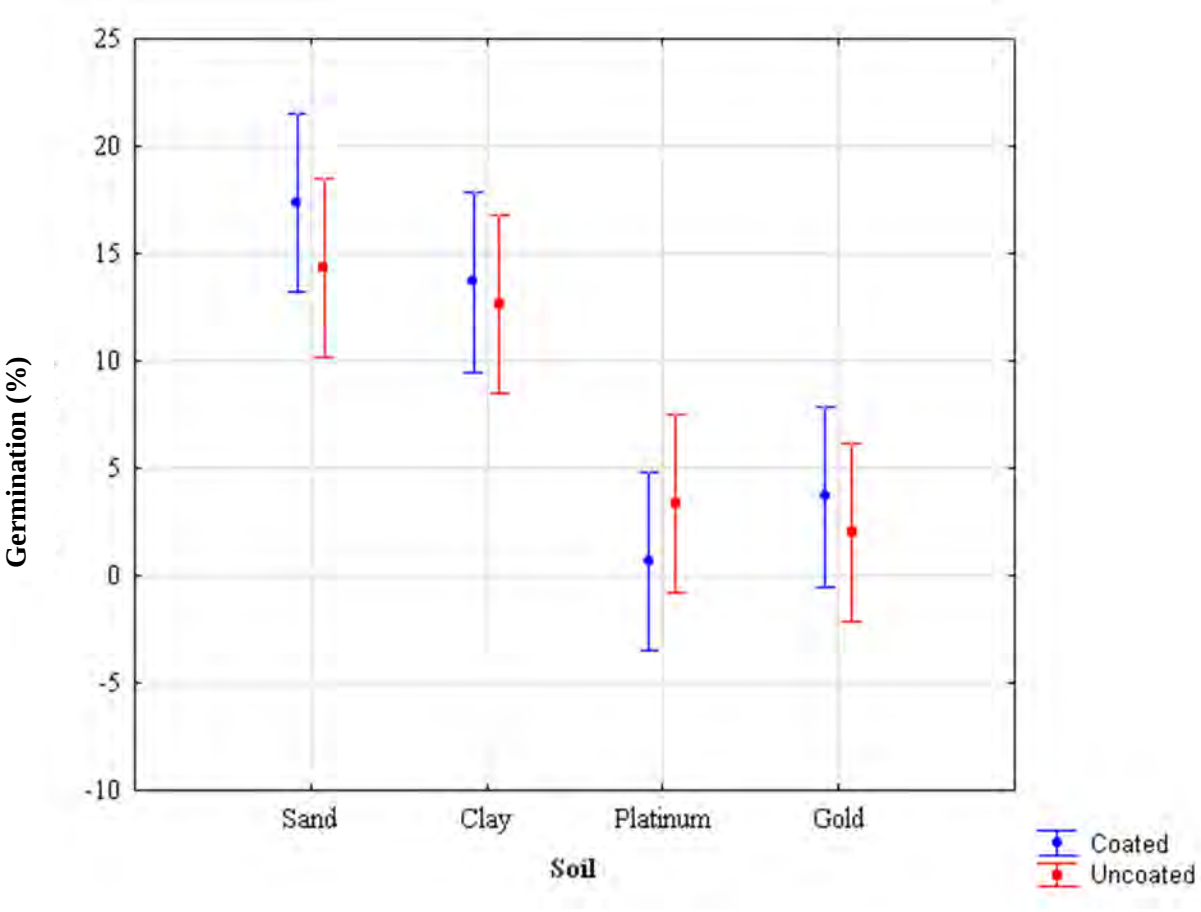

Figure 4.6. Comparison of the maximum germination (%) of the three replicates after a 21 day trial period of coated- and uncoated seed of *Panicum maximum* in the four soil types.

Differences between the coated- and uncoated seed types of *P. maximum* in each of the soil types was insignificant (Figure 4.6). The ANOVA analysis showed that there is a significant difference of germination of the seed types in the four soils (p=0.0002; f=25.9771). The post-hoc Tukey-test revealed no significant differences between gold mine tailings and platinum mine tailings and between sandy soil and clayey soil types. However, a statistical significant difference was observed when comparing the sandy soil with the two anthropogenic soil types, and when comparing the clayey soil with the two anthropogenic soil types.

4.2.4. Analysis of seed quality

The seed analyses report received by the Plant Research and Genetic Centre (see Chapter 3), showed that the pure seed percentages for all of the species is very high. These high values indicated seed batches with high purity. The other assessments conducted on these batches determined the effect of the amount of inert matter, whether the seedlings are normal, and the amount of dead seed in the batch (Table 4.1). Although the purity of the seed batch was high, the quality of the seed must also be evaluated by the number of normal seedlings, abnormal seedlings and number of dead seed in the batch. It was notable that the coated seed of all three species seed also had a higher number of dead and abnormal seedlings, except for *P. maximum*. The coating process could therefore negatively influence the seed viability over time. The possibility does exist that "dead seed" could have been in a state of secondary dormancy, but is not indicated in the results.

Table 4.1. Summary of seed quality of the selected species *(see Appendix A for detailed reports).*

Seed type	Pure pellets/seed (% by weight)	Inert material (% by weight)	Other seed (% by weight)	Weed seed (% by weight)	Normal seedlings (% by number)	Abnormal seedlings (% by number)	Dead seed (% by number)
A. pubescens **(coated)**	98.9	1	0.1	0	19	7	74
A. pubescens **(uncoated)**	98.2	1.7	0.1	0.05	72	4	24
P. maximum **(coated)**	98.3	1.5	-	-	14	0	86
P. maximum **(uncoated)**	95.5	4.3	0.2	0.2	20	0	80
C. dactylon **(coated)**	99.5	0.5	0	0	67	12	21
C. dactylon **(uncoated)**	99.8	0.2	0	0	82	12	6

In *A. pubescens,* comparison of the percentages of normal seedlings (coated seed: 19%; uncoated seed: 72%), abnormal seedlings (coated seed: 7%; uncoated seed: 4%) and dead seed (coated seed: 74%; uncoated seed: 24%) indicated a lower viability in coated seed than in uncoated seed at the time of testing. The low

germination of coated seed could not be ascribed to a low purity of the seed lot, but rather to altered seed viability, that may have been induced by the seed coating process.

The uncoated seed of *P. maximum* contained more inert matter (2.5% more than the coated seed), and 0.2% more other seed, of which 0.2% is weed seed. However, only 14% normal seedlings were found in coated seed of this species, whereas 20% normal seedlings were found in uncoated seed. Although no abnormal seedlings were found in the coated or uncoated seed type of this species, 80% of the uncoated seed, and 86% of the coated seed was dead seed. This could have contributed to the low germination percentages of P. maximum as discussed under section 4.2.3.

The seed types of *C. dactylon* contained no other seeds and very low percentages of inert matter (coated seed: 0.5%; uncoated seed: 0.2%). Both seed types of this species yielded relatively high percentages of normal seedlings, although lower values were found in coated seed (67%), than in uncoated seed (82%). Both seed types yielded 12% of abnormal seedlings. Higher percentages of dead seed were found in coated seed (21%), while only 6% dead seed were found in uncoated seed. Differences in growth results of these seed types could be expected to rather depend on the existing difference in viability between the two seed types of this species, rather than the purity thereof. Other contributing factors may have been the influence of the soil characteristics, combined with the effect of the coating after sowing of the seed.

The germination rates of all three species in the greenhouse trials must therefore be compared to the number of abnormal seedlings and dead seed per species (Table 4.1). The differences in germination results could additionally be ascribed to the combined influence of the coating and environmental conditions after the seed was sown in the specific soil types, together with the genetic adaptive traits of this species to grow in the specific soil type.

4.2.5. Soil analysis

As discussed in Chapter 3, representative samples of soil were collected from the sites where the field trials were carried out. From the soil sample analysis, it was evident that the particle size distribution of the sandy soils (88% sand) and soils from the platinum tailings (80.1%) were very similar with a higher sand fraction, while the silt (%<2mm) for both the clayey (13.2%) and gold mine tailings soil (29.8%) were more similar (Appendix A3). The silt fraction of the platinum mine tailings growth medium was also quite high at 18.1%. This explained why the germination of the species that was more adapted to sandy soils types, such as *A. pubescens*, was higher during the trails for this study.

Most of the macro elements in both the mine tailings soils was higher, especially elements such as Magnesium (22.30millimol/litre) and the Sulphate (SO_4) concentration (46.96 millimol/litre) in gold mine tailings growth medium, which proved the high acidity of these soil types (Appendix A4). The nutrient status of the gold mine and clayey soil types was also very high with extreme high Ca (2775 mg/kg), Mg (235 mg/kg) and Na (125 mg/kg) values in the gold mine tailings soil due to soil amelioration to neutralise the acidity of the soil and Mg (443.5 mg/kg) and K (171 mg/kg) in the clayey growth medium (Appendix A2). The extreme high EC value of 1475 (mS/m) of the gold mine tailings growth medium indicated that these soils were oxidised, with a very high salt content and a lower pH value (more acid – pH (H_2O of 2.53), especially in the gold mine tailings where the trial was carried out (Appendix A4).

The high micro-nutrient values of Fe (381.46 micromol/litre) explained the Iron pyride oxidation that has taken place in the gold mine tailings soil type. The high Mn value of 276 micromol/litre in these soils was due to the gold extraction process that took place during the mining operation (Appendix A2).

This soil was also used in the controlled experiments in the greenhouse trials. The detailed results of the soil analyses are presented in Appendix A. Redundancy analyses (RDA) (Figures 4.7, 4.8 & 4.9) a linear based model of a direct gradient analysis (Lepš & Šmilauer, 2003; Ter Braak, 1988), were displayed for the germination results of the coated and uncoated seed types of the three selected species in the four soil types.

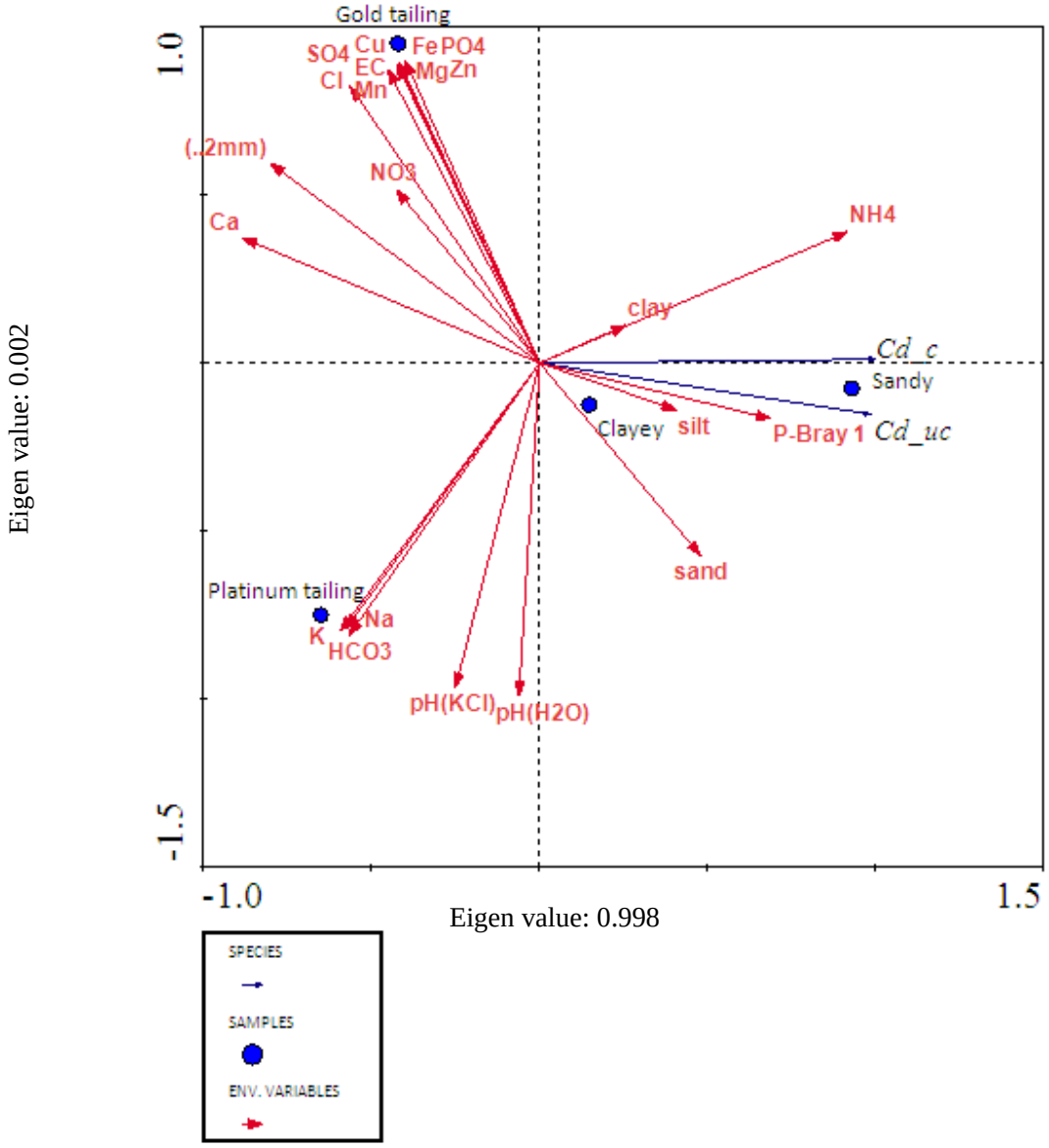

Figure 4.7. Redundancy Analysis (RDA) ordination to show the correlation for germination of coated and uncoated seed types of *Cynodon dactylon* (Cd_c & Cd_uc) and soil characteristics in the four selected soil types.

In Figure 4.7 a RDA displayed the influence of the soil characteristics on the germination of only the seed of *C. dactylon* under controlled conditions. The first ordination axis had an eigenvalue of 0.998, thus accounting for 99.8% of the variance in the dataset. According to Kent & Coker (1992), the eigenvalue indicated the highest possible degree of correlation of all variables with the principal axis, and is thus a measure of the amount of variation in the dataset accounted for by the first axis. According to the X-axis, the germination of both seed types of *C. dactylon* is therefore more positively associated with the soil properties of the sandy soil. Considering the high inflation factors of physical properties of the soil type, such as % particles >2 mm,

sand and silt, it was assumed that soil texture and related factors such as clay content and water holding capacity also played key roles in the germination performance of this seed type, since these factors were also relatively positively correlated with the X-axis. All soil elements that were positively correlated with the soils from the gold (e.g. high EC, Cu, Mn and even Ca levels) and platinum tailings (e.g. K, Na and HCO_3), were therefore less correlated with high germination rates of both *C. dactlylon* species types. Higher germination rates therefore occured in soils and elements from the sandy and clayey soil types. All elements accompanying the high EC value, including high Calcium (Ca) levels, were negatively correlated with the germination of both seed types of *C. dactylon*. These elements were thus not required as an essential stimulant for growth of this species. However, it may have provided a buffering effect in the micro-environment of the seed in acidic soil environments with an excess of available elements being low on the lyotrophic series of soil.

According to the RDA ordination, which displayed the influence of the soil characteristics on the germination of the seed of *P. maximum* under controlled conditions, the first ordination axis had an eigenvalue of 0.985, thus accounting for 98.5% of the variance in the dataset (Figure 4.8). Very similar to Figure 4.7, the germination of *P. maximum* was also positively correlated with the physical properties and elements of the sandy, clayey and silt soil types. The stronger correlation of the uncoated seed type of this species with the higher clay and silt content, might have been explained by its genetic characteristic to grow in soil types with high water holding capacity and less drainage (Van Oudthoorn, 2006). Growth in sandy soil types was assumed to be related to the hygroscopic characteristic of the lime-based coating, as growth in soil types with high availability of nutrients, such as the gold- and platinum mine tailings, was less positively correlated with germination of seed of *P. maximum*.

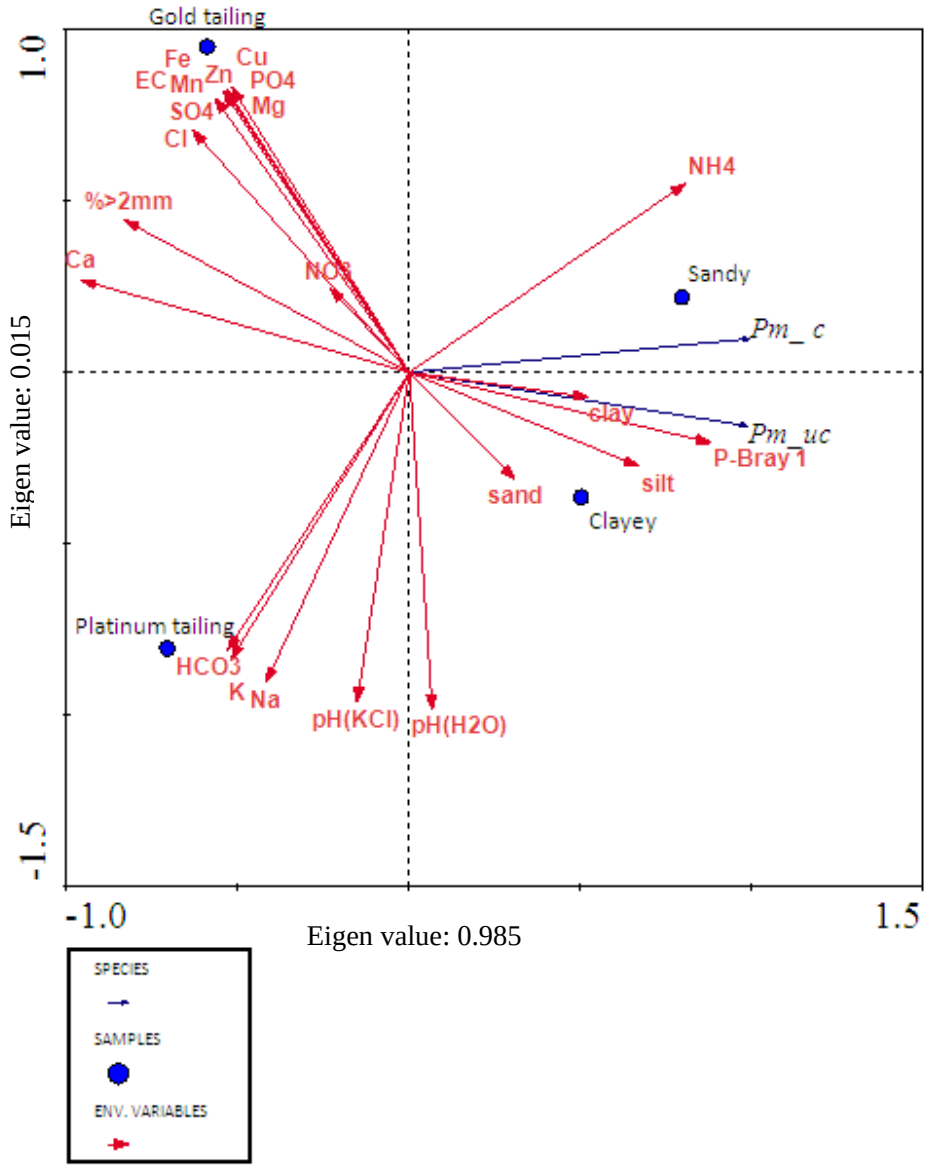

Figure 4.8. Redundancy Analysis (RDA) ordination to show the correlation for germination of coated and uncoated seed types of *Panicum maximum* (Pm_c & Pm_uc) and soil characteristics in the four selected soil types.

The RDA ordination displaying the influence of the soil characteristics on the germination of the seed of *A. pubescens* under controlled conditions, was presented in Figure 4.9. The first ordination axis had an eigenvalue of 0.963, thus accounting for 96.3% of the variance in the dataset. The germination of this species was higher in soils and their properties from platinum mine tailings, especially according to the X-axis of this ordination graph. The germination of uncoated *A. pubescens* was also more positively correlated with sandy soil, and more negatively correlated with high clay en silt content soils, as described earlier. This might have been explained by the genetic adaptive trait of this species to grow in well drained soil types with alkaline pH levels (Van Oudtshoorn, 2006). Hence also the strong positive correlation with soil from the more alkaline

platinum mine tailings, although the coating is considered to facilitate germination of seed of *A. pubescens* in the latter soil type by the closer association with the coated seed type.

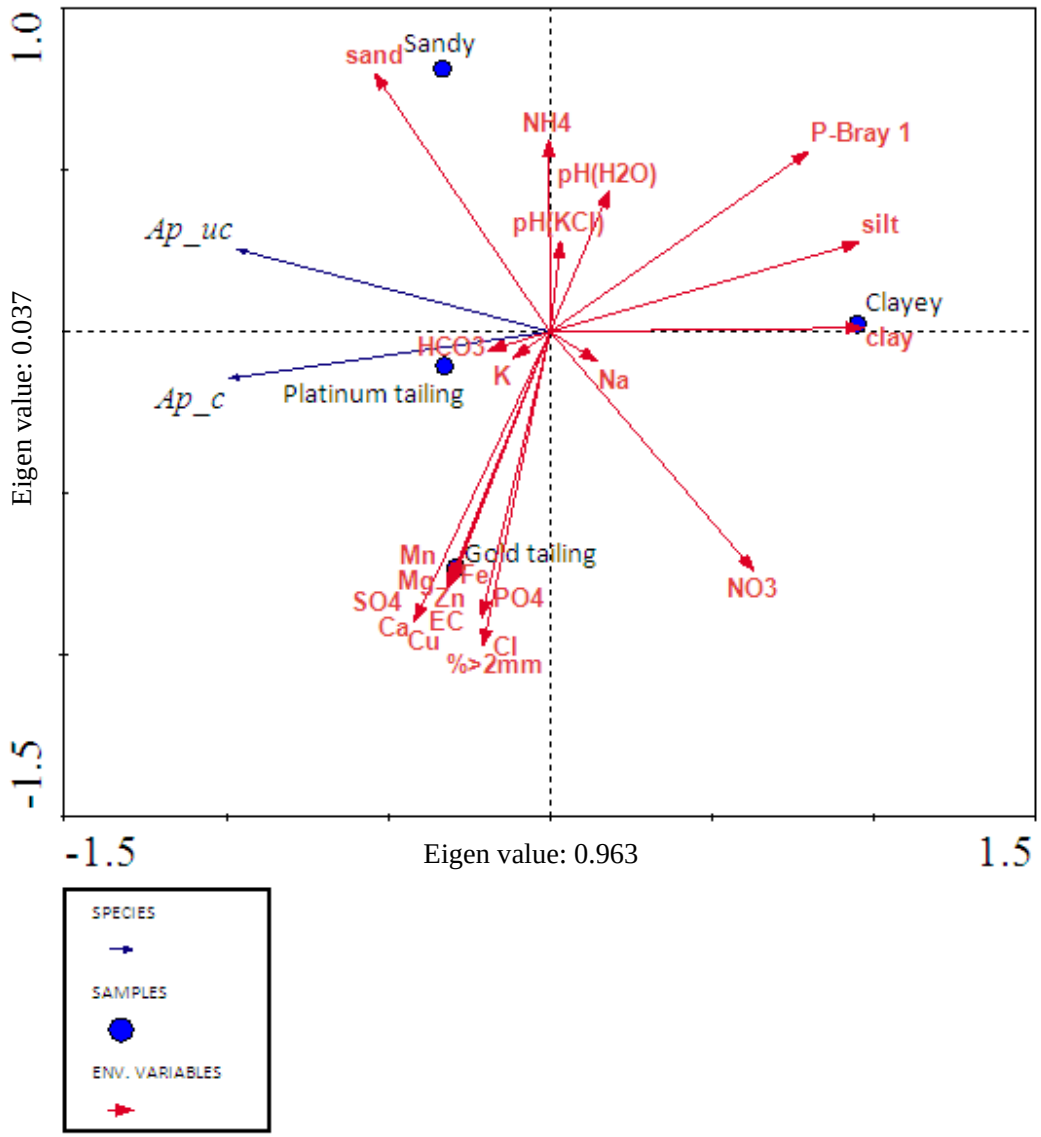

Figure 4.9. Redundancy Analysis (RDA) ordination to show the correlation for germination of coated and uncoated seed types of *Anthephora pubescens* (Ap c & Ap_uc) and soil characteristics in the four selected soil types.

4.3. Field trials

This section of the study addressed the objective to assess the establishment of seedlings of the three coated- and uncoated grass species in the four soil types under uncontrolled conditions in field trials (so called “natural conditions”). Germination and seedling establishment of the seed types were thus subjected to normal weather conditions for the specific areas. As mentioned earlier (Chapter 3) the field trials were carried out in the areas where the soils were collected for the greenhouse trials, i.e. around Potchefstroom (sand and clay soil types), Stilfontein (gold mine tailings) and Rustenburg (platinum mine tailings) (Table 3.1). Cumulative seedling growth and establishment data was collected from the four individual sites every two weeks. Comparison was only carried out between the coated- and uncoated seed of each species, and not between the species in the four soil types. Only the average number of seedling establishment per seed type (as determined in a 1 m × 0.5 m quadrat) for the four soil types at four observations over the trial period of the eight weeks was given (Figures 4.10-4.16). Although the results of the seedling establishment were given as average plants/m^2, the germination results were expressed as a percentage.

4.3.1. *Anthephora pubescens*

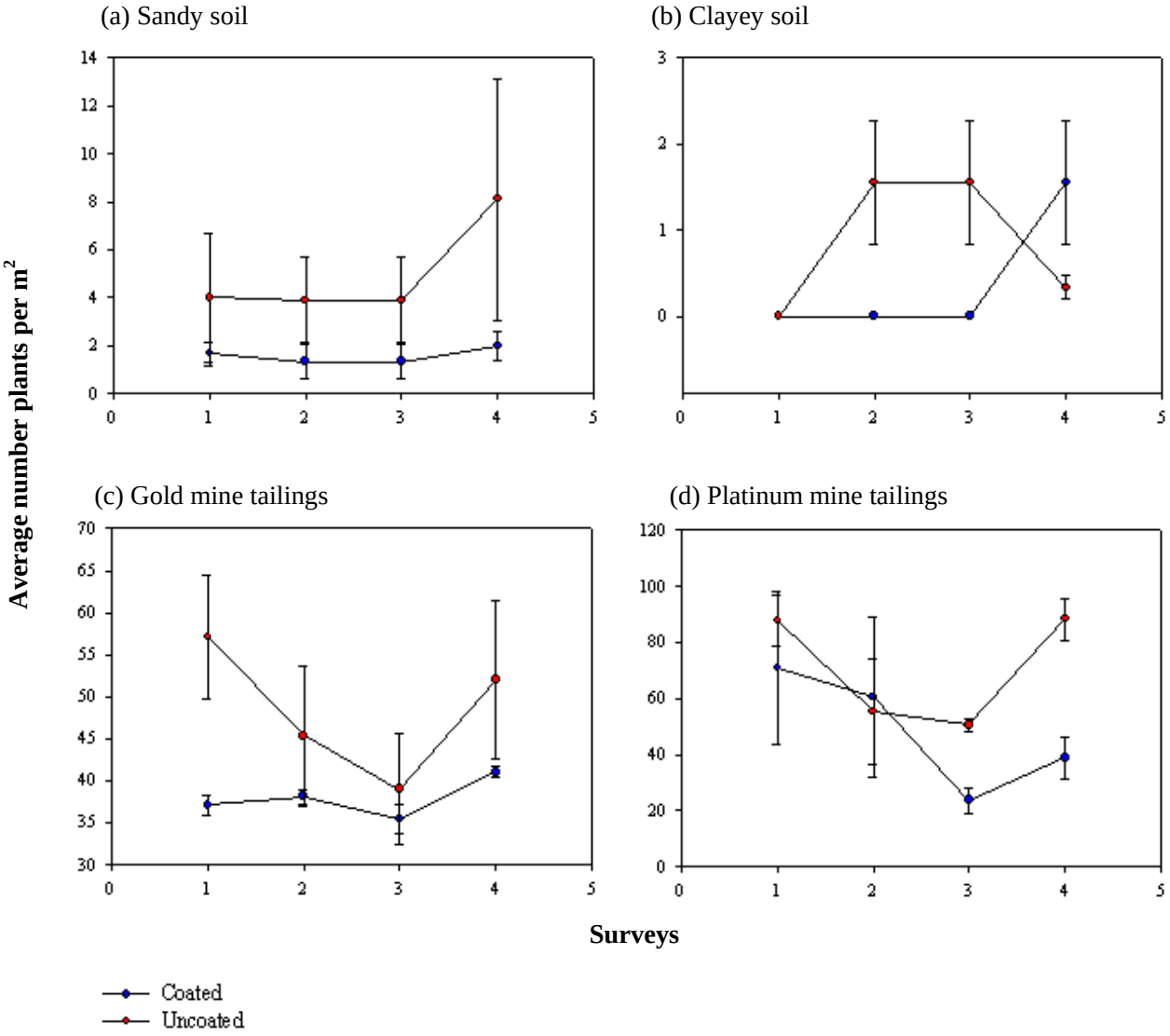

Figure 4.10. Average number of seedlings of *Anthephora pubescens* in the four soil types under natural conditions at four observations over the eight-week trial period.

The average number of seedlings from uncoated seed of *A. pubescens* were initially higher in all four the soil types (Figure 4.10). In the sandy soil, the average number of seedlings remained relatively constant during the first six weeks (±4 per m^2), where after seedling establishment increased for the uncoated (±8 per m^2) seed type (Figure 4.9a). Due to heavy rainfall over the first month after sowing, the fast growing weeds that occurred in the sandy soils, contributed to a dense overgrowth resulting in not only a strong competition for moisture, sunlight and other resources, but also completely covered the seedlings of the three grasses in the trial area (Figure 4.11). This could have caused the low germination and seedling establishment values, especially in the first few weeks of the trial. Only after removal of the competing weed cover manually by hand (as mentioned in Chapter 3), a slight increase in the average number of germinated seedlings in sandy

soil was observed. Although the hand removal of the weeds was carried out as carefully as possible, it is not excluded that some of the grass seedlings might have been removed.

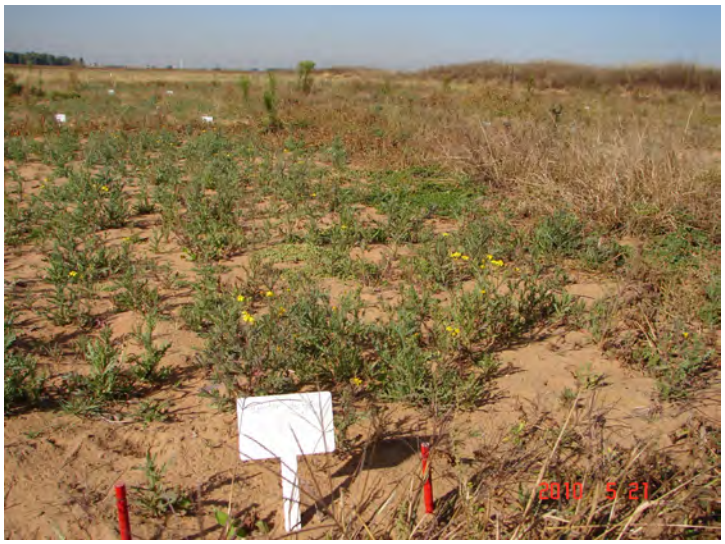

Figure 4.11. Dense overgrowth by weeds from the soil seed bank.

The seedling establishment for the uncoated seed was higher than for the coated seed over the total trial period. These results correlated with the results for the controlled (greenhouse) trials discussed above.

During the first three surveys, the average number of seedling establishment for the coated seed of this species remained zero in the clayey soil type (Figure 4.10b). Although also very low (2 per m^2), an increase was observed in the average number of seedlings in uncoated seed between the first (after two weeks) and second survey (after four weeks), after which it decreased again towards the eighth week of the trial. Only after eight weeks there were seedlings recorded for coated seed. These low seedling establishment rates corresponded with the low germination rates of the seed under controlled conditions. The reason for the low establishment rate of especially coated seed could also be ascribed to the influence of external factors, such as temperature or water stress and the poor seed quality (Table 4.1). The increase in the average number of seedlings of the coated seed in the fourth survey was either caused by benefitting from the applied coating on the seed in this specific condition, or simply the overcoming of the physiological germination-constraints induced by pre-sown conditions, such as the coating process or conditions and length of the storing period.

The number of seedlings that established from the uncoated seed type in both the gold and platinum mine tailings soil types was initially much higher at 60 and 90 plants per m^2 respectively. A significant difference was however observed in the seedling establishment rate between the coated- and uncoated seed of *A. pubescens* in the soil from gold mine tailings at the first survey, two weeks after sowing (Figure 4.10c). This could be contributed to the fact that the average number of dead seed for the coated seed was higher (Table

4.1), compared to the uncoated seed. The number of seedlings from the coated seed was fairly high (40 per m^2), even after the eight weeks of the trial. Although the number of seedlings from the uncoated seed decreased during the first six week period, an average of 50 plants per m^2 were observed after eight weeks (4^{th} survey). The low germination rates of the uncoated seed could also be ascribed to the adverse environmental condition, such as poor soil characteristics or temperature fluctuations over the eight week period.

Of all the soil types, the seedling establishment from both seed types of *A. pubescens* was the highest in platinum mine tailings under uncontrolled conditions, possibly due to a lack of competition from weeds in the seed bank of the tailings (Figure 4.10d). Similar results were shown by the RDA ordination (Figure 4.9).

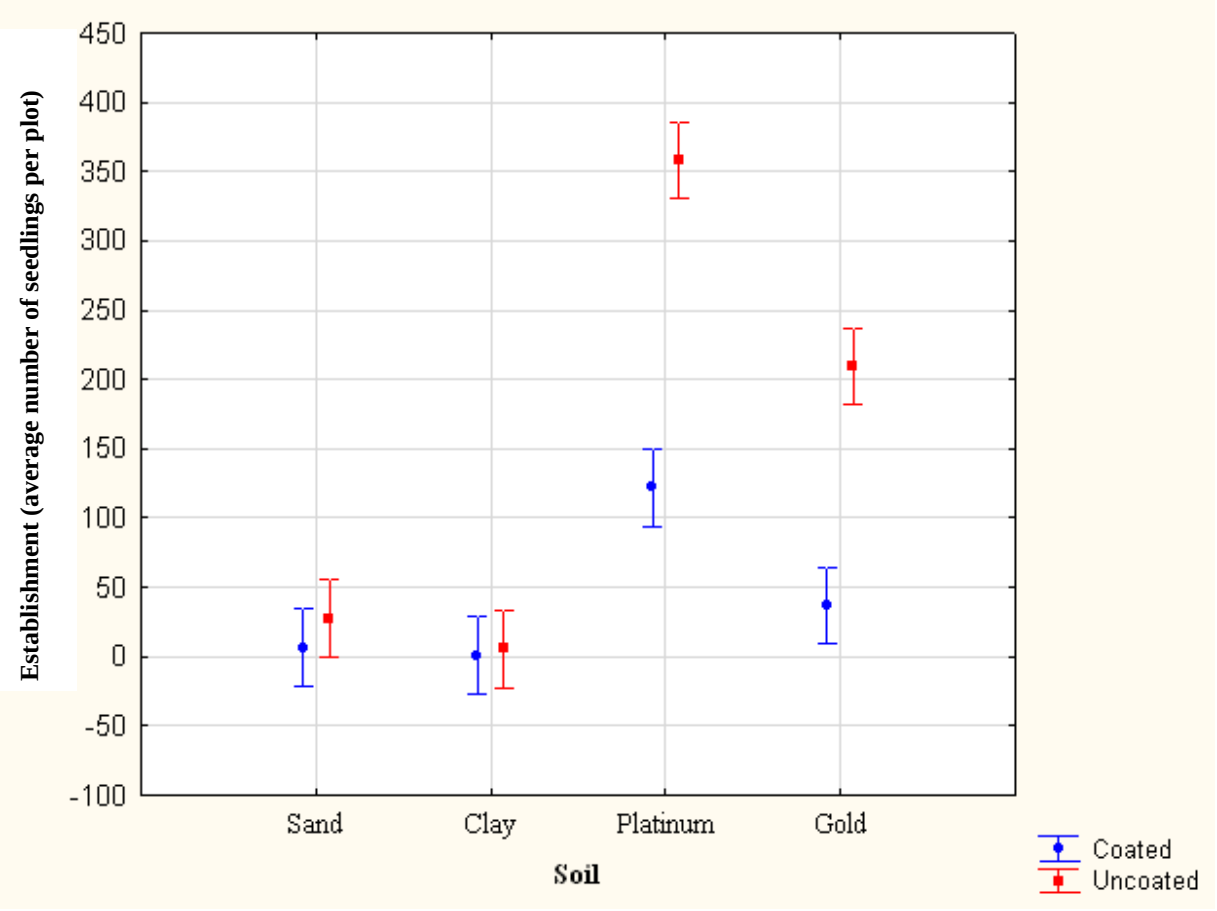

Figure 4.12. Comparison of the average seedling establishment of coated- and uncoated seed of *Anthephora pubescens* in the four soil types.

Average seedling establishment of this species was the highest in the soils from the platinum mine tailings and the lowest in the clayey soil type under uncontrolled conditions (Figure 4.12). The uncoated seed type of this species exceeded that of the coated seed type in all soil types. This corresponded with the results from the greenhouse trials (Figure 4.12). No statistical significance was found between the number of seedlings from

the coated and uncoated seed of *A. pubescens* in sandy- and clayey soils. Significant differences in seedlings from the coated and uncoated seed was however found in the soils from the gold ($p=0.0017$) and platinum ($p<0.001$) mine tailings.

The influence of the four soil types ($p< 0.001$; $f=140.412$), the coating ($p< 0.001$; $f=136.159$), as well as the combined influence of the coating and the soil types ($p< 0.001$; $f=37.480$) were significant. The post hoc Tukey-test revealed significant differences between sandy soil and the platinum and gold mine tailings. The seedling establishment between the clayey soil and both the mine tailings soils, also differed significantly.

4.3.2. *Cynodon dactylon*

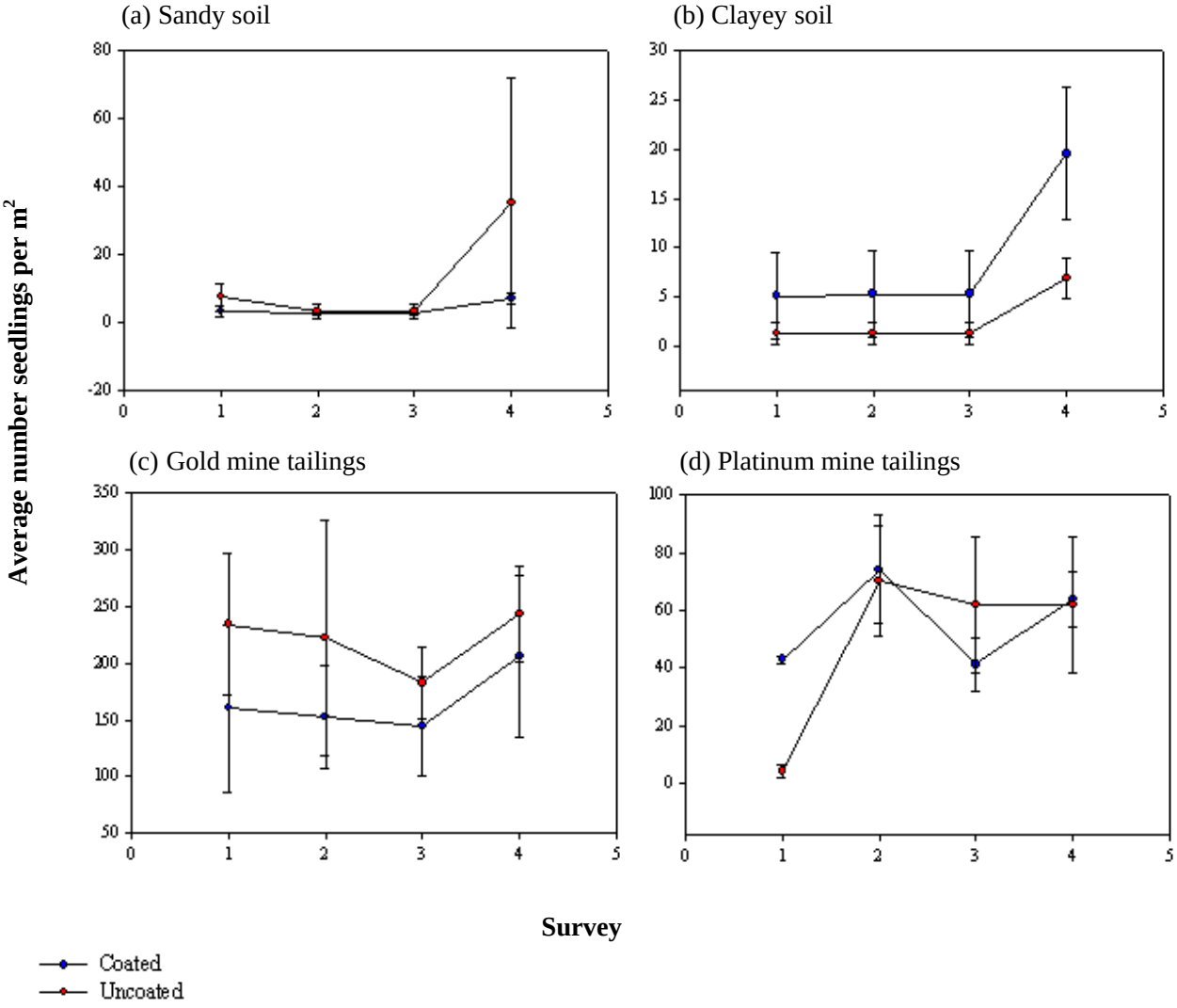

Figure 4.13. Average number of seedlings of *Cynodon dactylon* in the four soil types under uncontrolled conditions at four observations over the eight-week trial period.

Cynodon dactylon had the lowest number of dead seed (coated seed: 21%; uncoated seed: 6% - Table 4.1), and the highest number of normal seedlings (coated seed: 67%; uncoated seed: 82 % - Table 4.1) for both the coated and uncoated seed types respectively, compared to the other studied species, *P. maximum* and *A. pubescens.*

Although there were no significant difference in the average number of seedlings between the coated- and uncoated seed types of *C. dactylon* in the sandy soil at the start of the trial (1st survey after 2 weeks), the establishment of seedlings from the uncoated seed increased towards the end of the trial (30 per m^2) (Figure 4.13a).

The same pattern was observed for the clayey soil type, namely very low and constant seedling establishment until 6 weeks (Figure 4.13b). In this case however, the number of seedlings establishing from the coated seed exceeded the number of seedlings from the uncoated seed after eight weeks of observation (20 per m^2). In both the sandy- and clayey soil types, the low levels of new seedling establishment from both seed types of *C. dactylon* during the first three surveys can be ascribed to competition from rapid growing weeds in these field trials. After the weeds were controlled, either by manual removal (sandy soils) or spraying of herbicide (clayey soils), a considerable increase occurred in seedling establishment.

A considerable difference is observed in the average number of seedlings from the coated and uncoated seed of *C. dactylon* in gold mine tailings at the first survey (Figure 4.13c). The average number of seedlings from the uncoated seed type is significantly higher (almost 100 seedlings per m^2) than the coated seed. A comparison of the seedling establishment in the four soil types show that establishment was the highest in the soil from gold mine tailings for both the uncoated and coated seed after eight weeks (Figure 4.13c). High standard errors resulted from variation in the tailings at the different locations of the replicates in this soil type. Initially no uncoated seedlings germinated in the platinum soil type, whereas 40 plants per m^2 were found in coated seed after two weeks. However, after some fluctuation over the next six week-period, both seed types resulted in a rather high number of seedlings establishment (60 plants per m^2) at the end of the trial (Figure 4.13d).

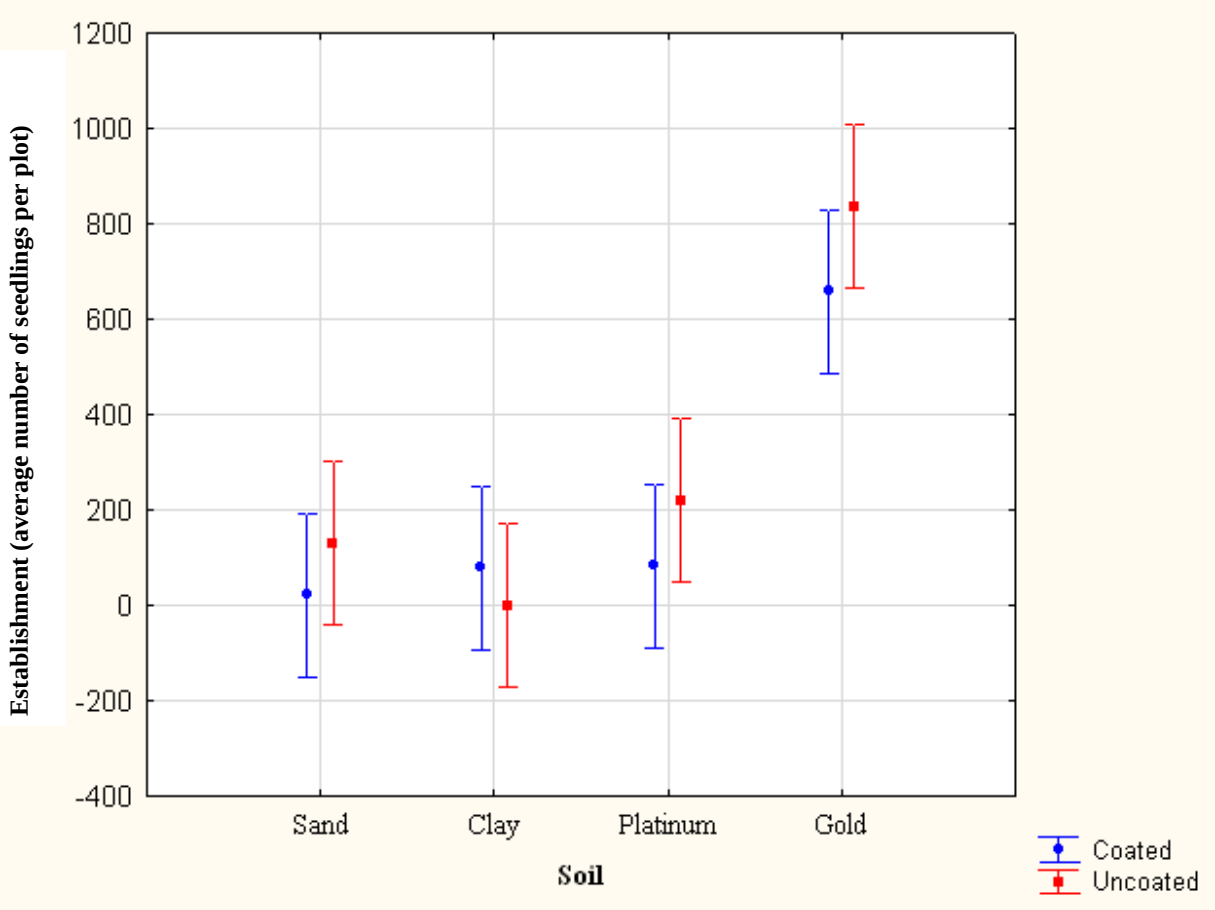

Figure 4.14. Comparison of the average seedling establishment of coated- and uncoated seed of *Cynodon dactylon* in the four soil types.

The highest mean seedling establishment rate of *C. dactylon* in the four soil types in the field trials conditions occurred in gold mine tailings (Figure 4.14). No statistical difference is observed between the coated- and uncoated seed types of this species in sandy soil, platinum and gold mine tailings. An ANOVA analysis did however show a statistical significant difference in the seedling numbers between the seed types of *C. dactylon* in the clayey soil type (p=0.008). This occurrence is explained by the zero germination of the uncoated seed type of *C. dactylon* in clayey soil during the first three observation (6 weeks) of this study.

4.3.3. *Panicum maximum*

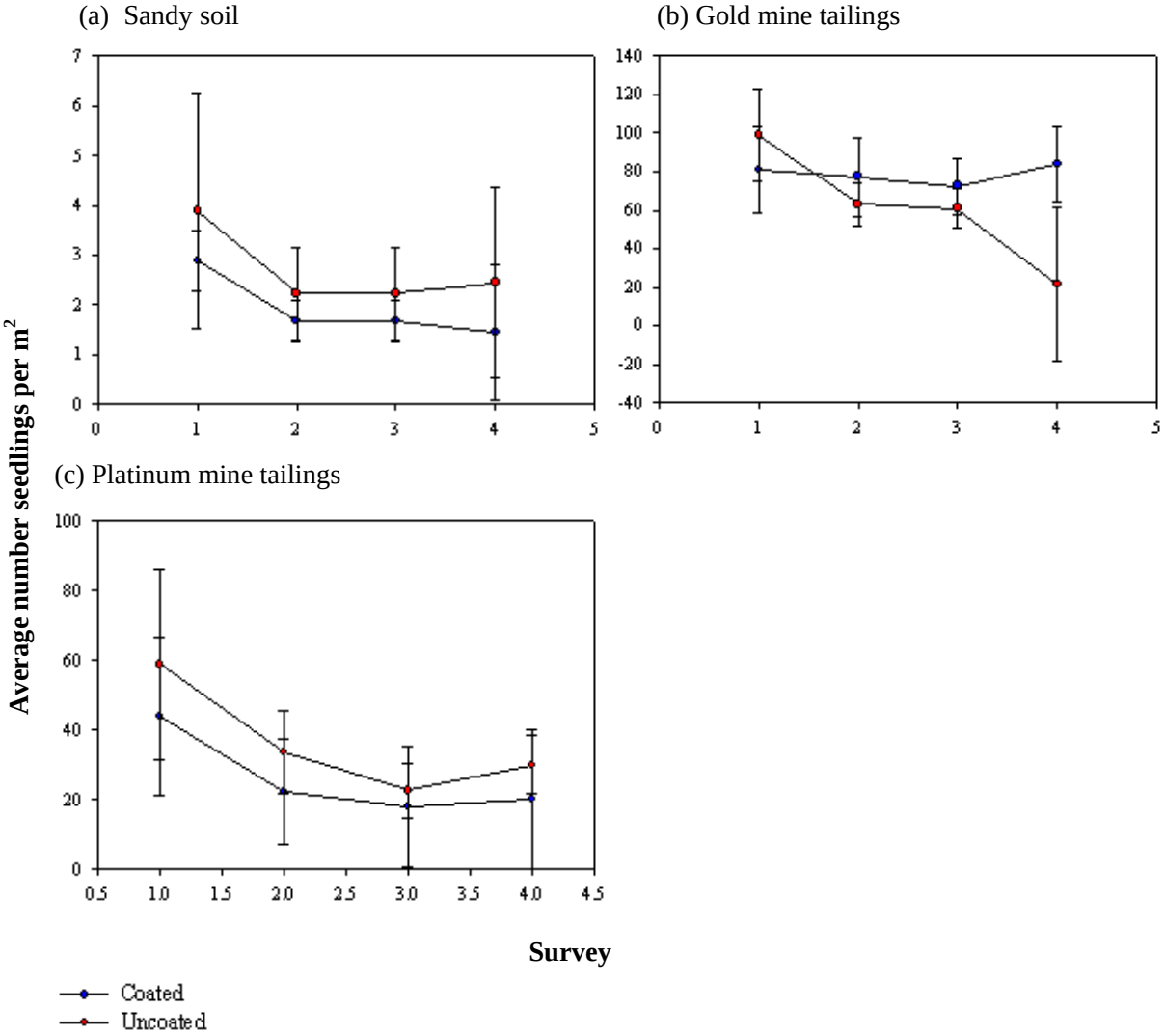

Figure 4.15. Average numbers of seedlings of *Panicum maximum* in the four soil types under uncontrolled conditions at four observations over the eight-week trial period.

No statistical significance occurred between the average number of seedlings of coated- and uncoated seed of *P. maximum* in the sandy soil. The number of seedlings was initially very low and even decreased over the eight weeks of the trial period, especially if compared to the establishment in the gold and platinum mine tailings (Figure 4.15a) where the germination and establishment rate were much higher. A high standard error is found in the results for this study in both the seed types during the last observation (survey 4), and can be ascribed to differences in the micro-habitat of the soil type. Therefore, the results of a repeated experiment under similar conditions may vary.

Although a higher average number of living seedlings of uncoated seed of *P. maximum* is initially observed (100 per m^2) in soil from gold mine tailings (first survey), if compared to the coated seed (Figure 4.15b), a

steep decline occurred towards the end of the trial after an 8 week period (20 per m^2). The number of seedlings that established from the coated seed of this species was rather constant and only varied slightly from the first (80 per m^2) to the last survey (25 per m^2). Considering the rather negative association of germination of this seed with the high salt content and low pH of gold mine tailings (Figure 4.8), this result is most likely associated with the positive influence of the applied coating. The characteristics of the applied coating on this species' seed seem to provide a buffering effect to the specific combination of environmental conditions the seed were submitted to during the field trials.

In the platinum mine tailings, a higher average number of seedlings are observed initially for both uncoated (60 per m^2) and coated (40 per m^2) seed of *P. maximum*, where after a sharp decline occurred (Figure 4.15c).

It is interesting that the seedling establishment for this species was quite high in both the gold and platinum mine tailings, whereas the germination rate was very low (Figure 4.15), also considering the high number of dead seed (Table 4.1).

No *P. maximum* seedlings germinated in the clayey soils under uncontrolled conditions. This was unexpected, as this species genetically prefers this type of environment. Considering that seedlings from neither the coated or uncoated seed established, it might have been caused by a combination of poor environmental factors and trial maintenance.

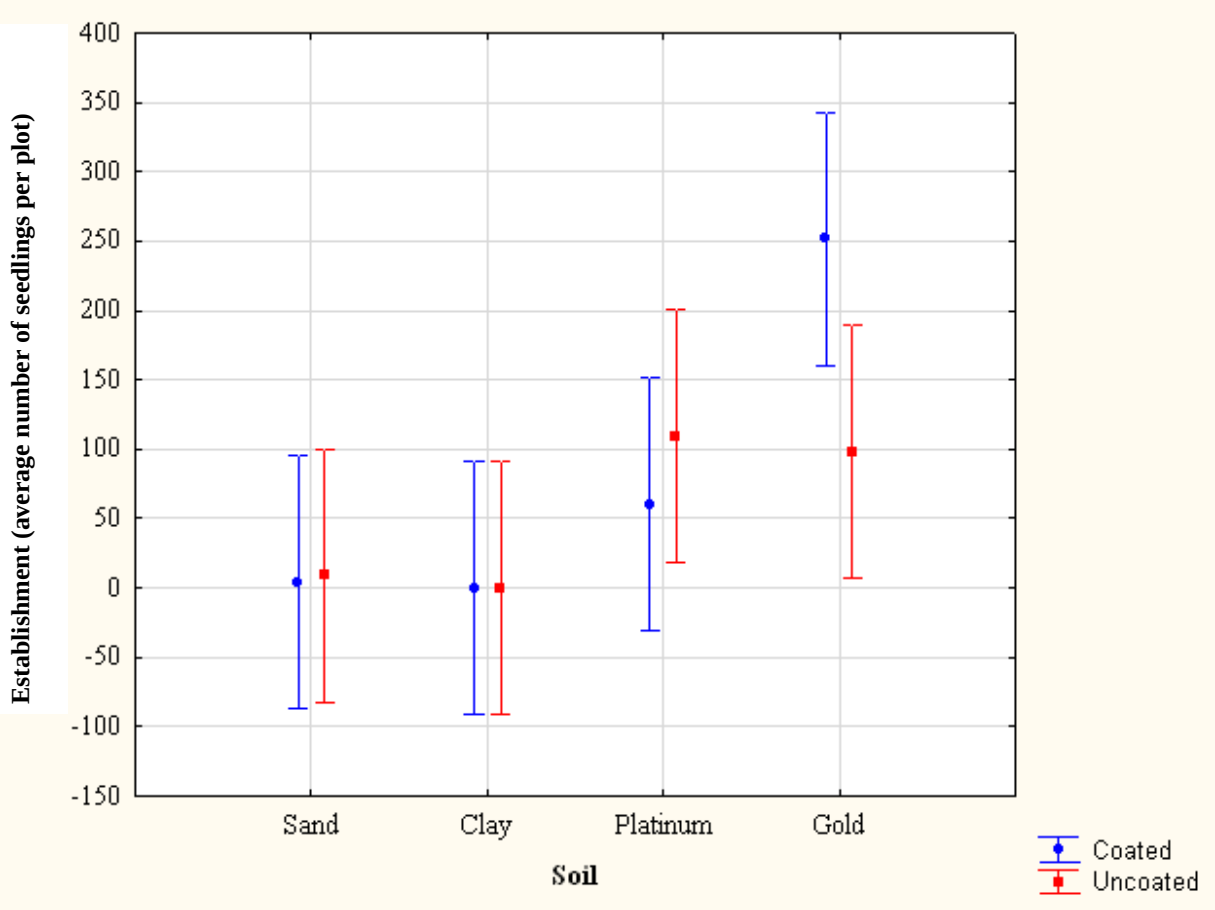

Figure 4.16. Comparison of the average seedling establishment of coated- and uncoated seed of *Panicum maximum* in the four soil types.

The highest seedling establishment of *P. maximum* is observed for the coated seed in gold mine tailings under uncontrolled conditions (Figure 4.16). The very low overall average number of living seedlings of this species in the sandy soils might be ascribed to the added effect of competition from weeds that occurred in the field trial as described earlier. In the trials on gold- and platinum mine tailings soils, no competition from weed species occurred. The seed banks of weeds in these soils are almost non-existing and no or very few weeds were observed over the eight week period of the trial.

The ANOVA analysis showed the soil component as the statistical significant factor on the seedling establishment for both the coated and uncoated seed types of *P. maximum* ($p=0.003$; $f=11.974$). The post hoc Tukey-test only indicated a significant difference in the seedling establishment of coated seed between gold mine tailings and the remaining soil types.

4.4. Physiological tests

This section addresses several of the physiological objectives of the study, namely to (1) assess the key enzymes' activity of germination; (2) determine the gaseous exchange (respiration) of both seed types (coated and uncoated) and (3) determine the osmotic potential of the coated- and uncoated grass seed types.

4.4.1. Activity of key germination enzymes

Activity of three key germination enzymes, peroxidase (POD), alpha amylase (α-amylase) and lipoxygenase (LOX), were tested in a sterile laboratory environment, excluding influences such as pH and other soil characteristics. Thus, for the purpose of this study, only the effect of the coating on the activity of the three mentioned enzymes' was evaluated. The specific enzyme activity of the three germination enzymes will be discussed for each species, followed by an evaluation of the specific enzyme's activity in seed from the separate phases of coating. Evaluation of the different phases of the coating process was only done for *P. maximum* and *C. dactylon*, as no seed batches of *A. pubescens* was coated by the supplier during the time of the study.

4.4.1.1. Peroxidase (POD) activity in the selected seed

The activity of this enzyme was determined 2, 4, 6, 8, and 10 days after germination was initiated for the seed types of the three different grass species.

After two days of germination, the activity level of POD in uncoated seed of *A. pubescens* is significantly higher than in coated seed (Figure 4.17a). An increase in POD activity was observed over the next two days, where after the activity decreased from days six to ten. Although the POD activity in uncoated seed appears to be slightly higher than in coated seed at the final observation, no statistical significance occurred at a 5% level of significance. Both were more or less the same after 10 days of observation.

The enzyme activity of POD in the seed types of *C. dactylon* is relatively low (Figure 4.17b). Although the difference between the coated and uncoated seed types of *C. dactylon* may be insignificant, the average POD activity is overall higher in the coated seed type than in the uncoated seed type. The enzyme activity of POD increased during the first eight days of germination in the uncoated seed, where after it decreased again at day 10. The POD activity for the coated seed decreased after 8 days of germination, but increased again at day 10. At the final observation of this experiment (day 10), the POD activity in coated seed therefore increased, whereas the uncoated decreased to almost no enzyme activity.

Peroxidase activity in the *P. maximum* seed types (Figure 4.17c) remained low throughout the germination period. Though the difference may be insignificant, the POD activity after two days of germination in the uncoated seed type of *P. maximum* tended to be higher than in the coated seed type. A sudden decrease to almost zero µMtetraguaiacol. $min^{-1}.mg^{-1}$protein was observed in both seed types after four days of germination. The activity of POD in both seed types of *P. maximum* varied from the sixth to the 10^{th} day with

slight increases at day eight, after which it decreased again. It appears as if the coated seed type experienced a delay in enzyme activity before increasing again over time. A decrease of POD activity occurred between the last two observations in both seed types. No statistical difference occurred between the coated and uncoated seed type of this species after 10 days of germination.

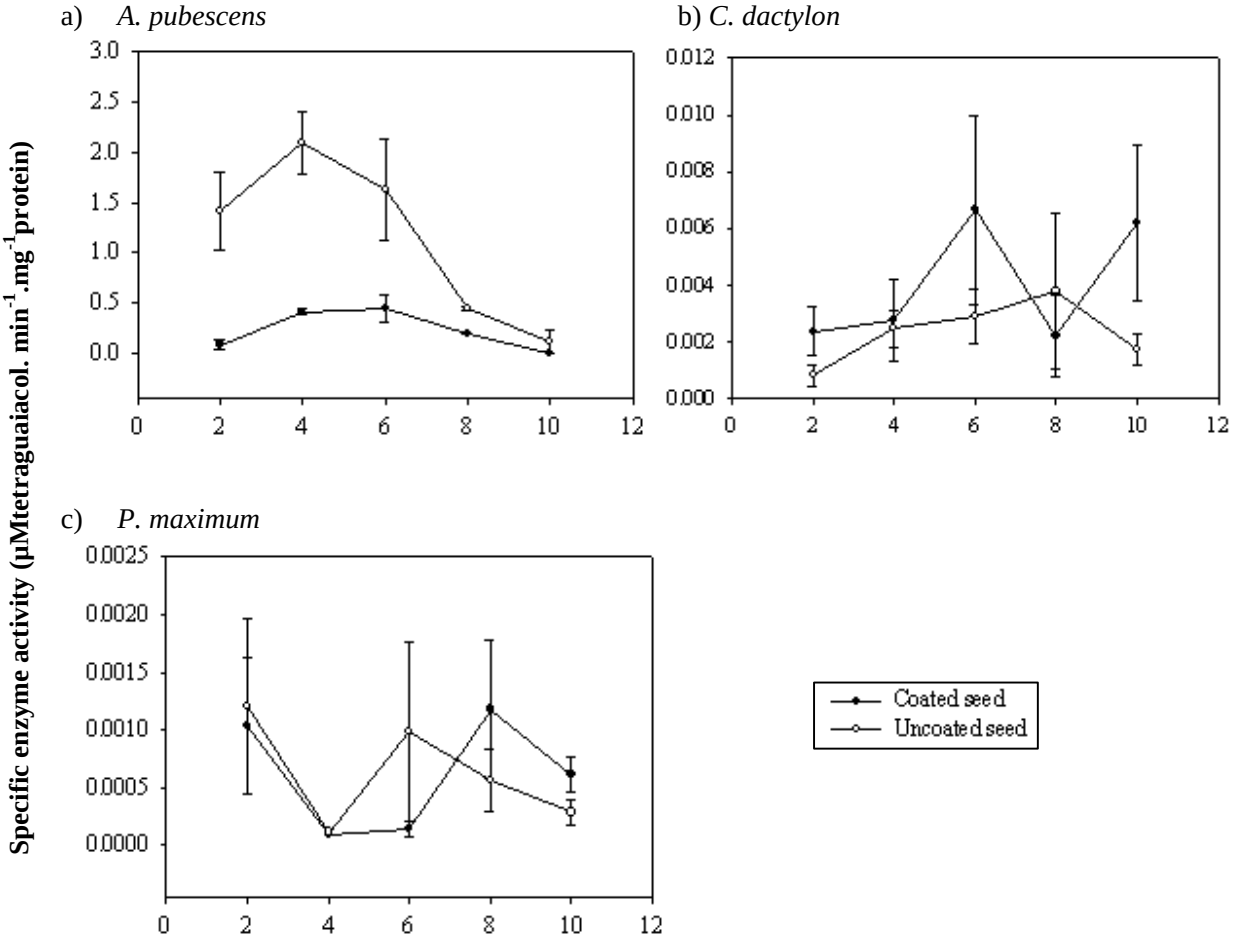

Figure 4.17. Enzyme activity of POD in the coated- and uncoated seed of the selected species after 2, 4, 6, 8 and 10 days of germination.

4.4.1.2. Peroxidase activity in seed from the phases of the coating process

The enzyme activity of POD in seed of *C. dactylon* before coating (Phase 0) or germination (uncoated) is notably higher than just after the first phase (Phase 1) of the coating process (Figure 4.18). This could be ascribed to the rapid wetting process of the seed during the application of the coating components. The second phase of the coating process (Phase 2), which involves the application of heated air to dry the coating on the seed, reveals an increase in the activity of POD in the seeds of *C. dactylon.* At the end of the coating process (Phase 3) a further increase in enzyme activity of POD is observed.

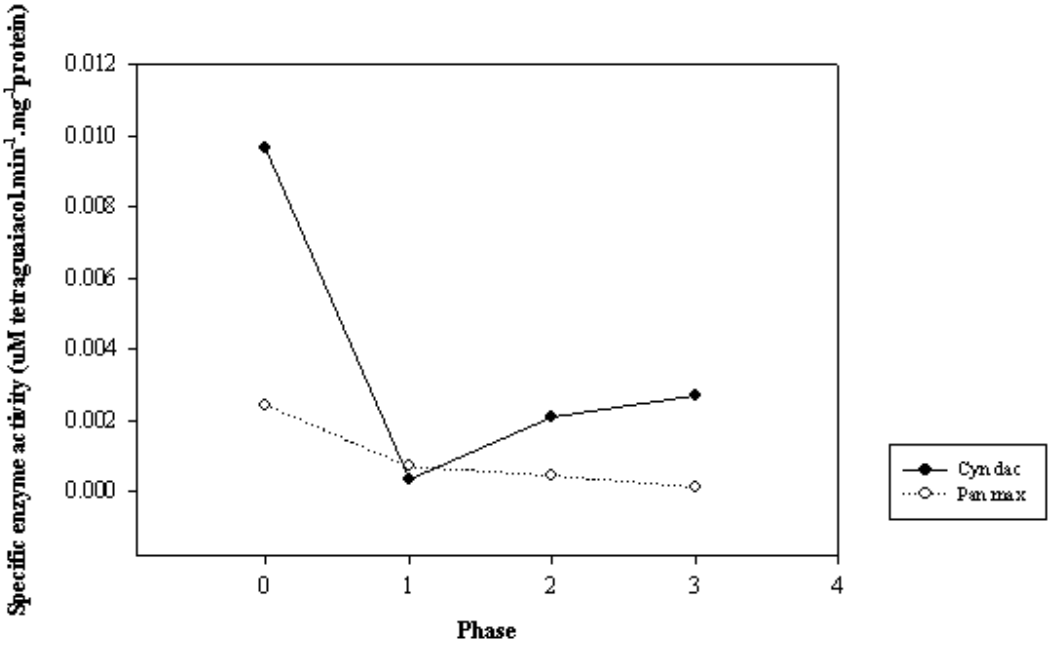

Figure 4.18. Enzyme activity of peroxidase in the seed of *Cynodon dactylon* (Cyn dac) and *Panicum maximum* (Pan max) during the different of phases of coating.

POD activity levels are considerably lower in *P. maximum* before application of the seed coat (Phase 0), after the wetting process of the seed, and decreases till the end of the drying with warm air during Phase 3 (Figure 4.18). No statistical significance was observed in the POD activity levels between the coated seed (Phase 3) and the uncoated seed (Phase 0) for both these species.

4.4.1.3 Alpha amylase (α-amylase) activity

The activity of this enzyme was also evaluated at five observations during a 10-day germination trial for the selected seed types. Assessments were made after 2, 4, 6, 8, and 10 days after germination was initiated.

The enzyme activity of α-amylase in the coated seed of *A. pubescens* was more than 500 times higher than that of uncoated seed at the first observation (Day 2) (Figure 4.19a). After four- and six days of germination the increase in α-amylase activity was even higher, especially in the coated seed. However, after 8 days a rapid decrease occurred in both seed types to almost zero after ten days of germination.

The average α-amylase activity was lower in the uncoated seed of *C. dactylon* from two to four days after germination if compared to the coated seed type of this species (Figure 4.19b). The activity of this enzyme varied from an increase after six days to a drastic decrease of especially the coated seed of *C. dactylon* after 10 days of germination. Although the difference may seem insignificant, higher activity of α-amylase is observed in uncoated seed of *C. dactylon* than in the coated seed type at the last day of observation of this experiment.

After two days of germination, the average α-amylase activity is higher in uncoated seed of *P. maximum* than in the coated seed type (Figure 4.19c). However, during the germination trial, the activity of α-amylase in the uncoated seed type decreased to zero towards the tenth day of germination. The enzyme activity of α-amylase in coated seed of *P. maximum* increased, where after it rapidly decreased to zero between the eighth and tenth day of germination, the same as for the uncoated seed. No significant difference occurred in enzyme activity of α-amylase between the seed types of *P. maximum* after 10 days of germination.

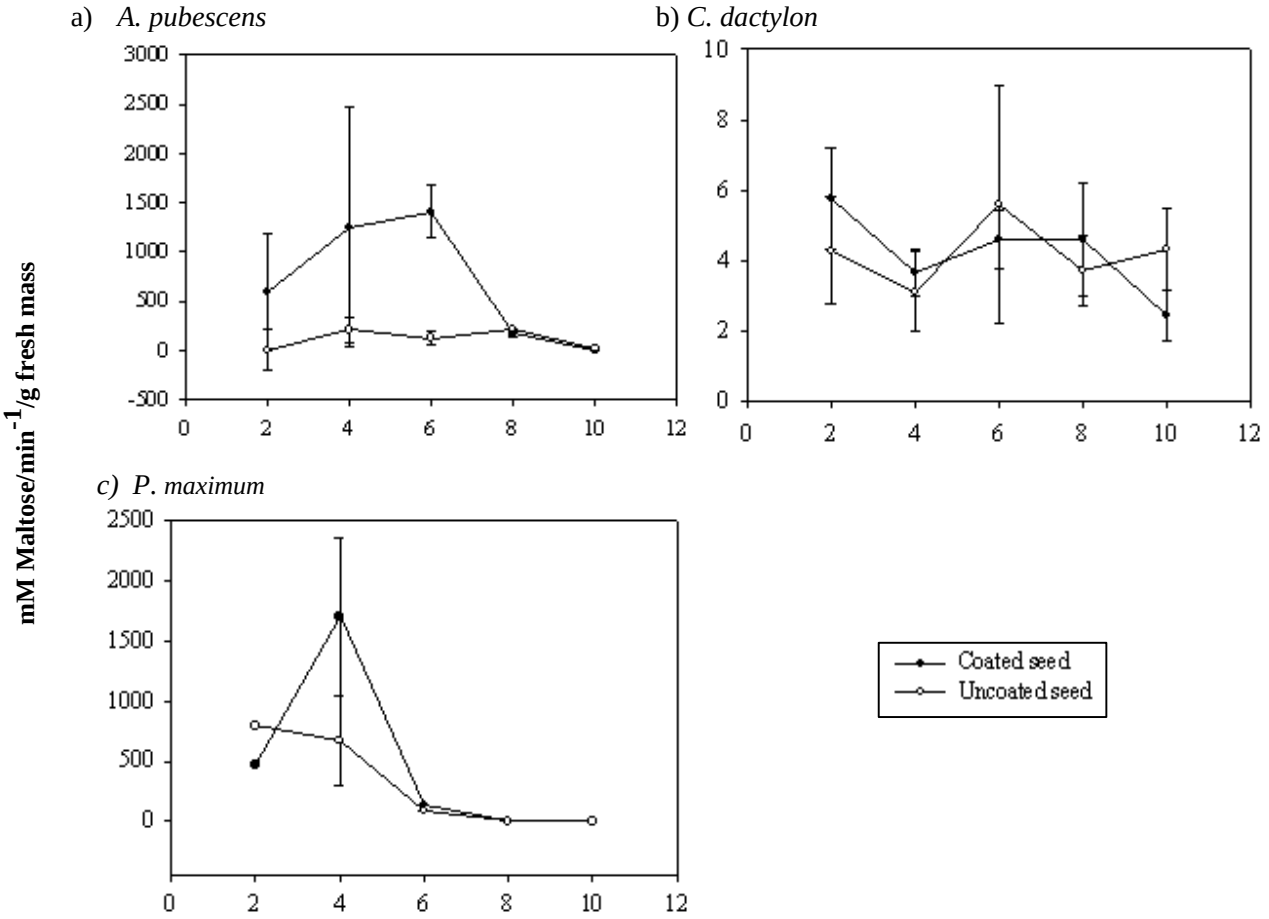

Figure 4.19. Enzyme activity of alpha amylase in the coated- and uncoated seed of the three selected species after 2, 4, 6, 8, and 10 days of germination.

4.4.1.4. Alpha amylase (α-amylase) activity in seed from the phases of the coating process

The α-amylase activity in seed of both species was very low before the coating process (Phase 0) started (Figure 4.20). During the first phase (Phase 1), which involved the wetting of the seed in order to apply the coating components, the α-amylase activity increased in both species. Although low, a constant increase was observed in the activity of this enzyme, especially in the seed of *P. maximum* at the third phase (Phase 3), which included the drying process by warm air. Although the difference was insignificant, the average activity of this enzyme was higher in *P. maximum*, compared to *C. dactylon* after the coating process.

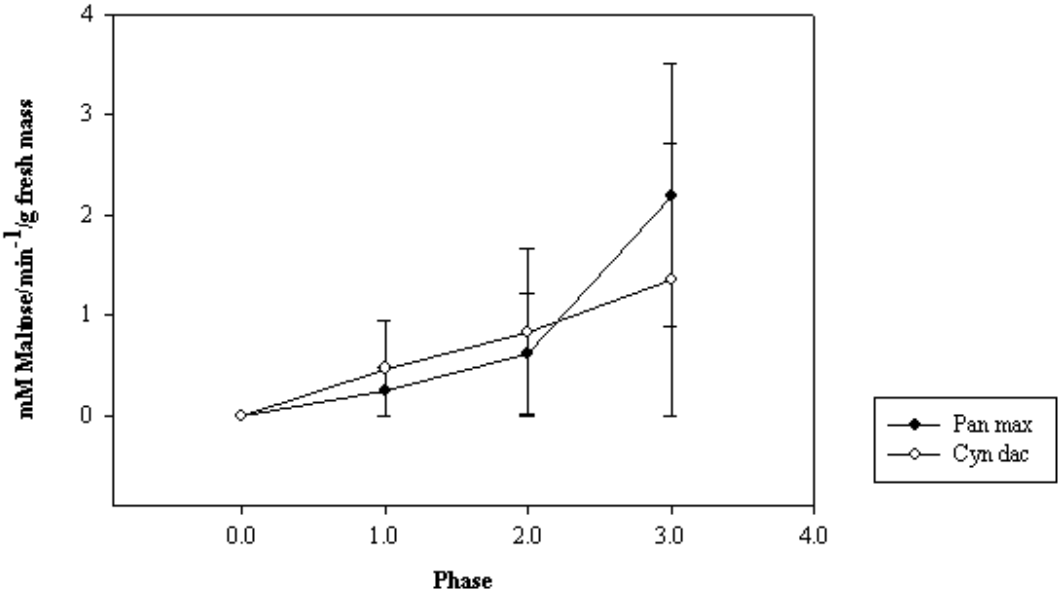

Figure 4.20. Enzyme activity of alpha amylase in the coated and uncoated seed *Cynodon dactylon* (Cyn dac) and *Panicum maximum* (Pan max) during the different phases of coating.

4.4.1.5. Lipoxygenase (LOX) activity

Evaluation of this enzyme in both seed types for each selected species was presented only for 96 hours (four days) after germination was initiated. No meaningful activity was observed in any of the seed types before germination. After four days of germination, a significant difference in the activity of LOX was observed in all the selected species at a 5% significance level. In all of the evaluated species, a higher LOX activity was observed in coated than in the uncoated seed types (Figures 4.21 - 4.23). No enzyme activity was found in lipoxygenase (LOX) in the seed of *P. maximum* and *C. dactylon* from the phases of coating at the time of testing. At Day 4, the activity of LOX was significantly higher in coated (±600 nmol HPOD.min^{-1}.mg protein) than in the uncoated seed type of *A. pubescens* (Figure 4.21). The activity of LOX in the uncoated seed type of *A. pubescens* was extremely low.

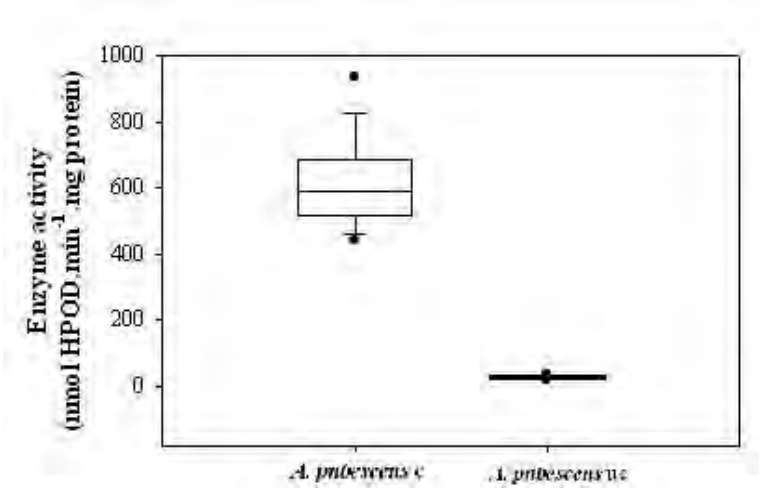

Figure 4.21. Enzyme activity of lipoxygenase in the coated- and uncoated seed of *Anthephora pubescens* after four days (96 hours) of germination.

Similar to *A. pubescence* the activity of LOX was significantly higher (18 nmolHPOD.min^{-1}.mg^{-1}protein) for coated seed after four days than uncoated seed of *P. maximum*. The activity was nearly zero for uncoated seed after four days of germination (Figure 4.22).

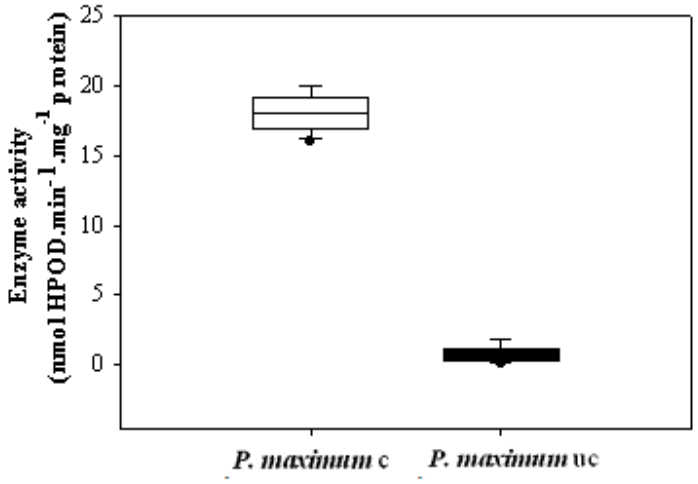

Figure 4.22. Enzyme activity of lipoxygenase in the coated- and uncoated seed of *Panicum maximum* after four days (96 hours) of germination.

After four days of germination, the activity of LOX was significantly higher in coated seed than in the uncoated seed type of *C. dactylon* (Figure 4.23). Even though the activity was relatively low, compared to the coated seed type, it was notably higher than in the uncoated seed of the other two species.

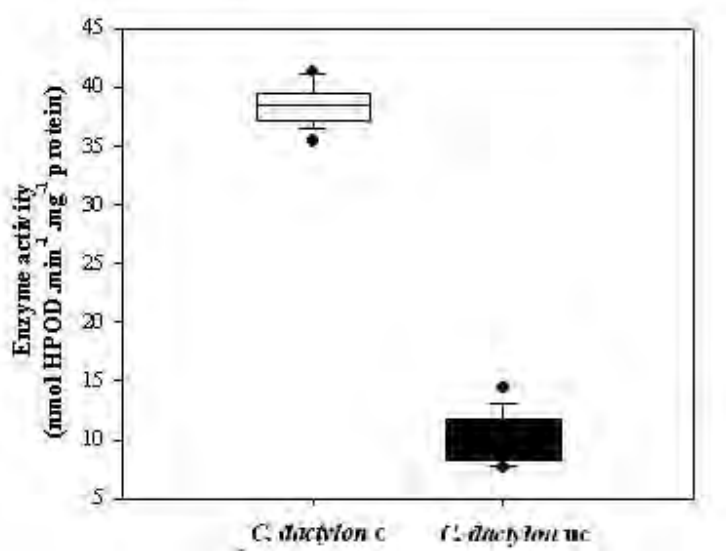

Figure 4.23. Enzyme activity of lipoxygenase in the coated and uncoated seed of *Cynodon dactylon* after four days (96 hours) of germination.

4.4.2. Respiration

The objective was to determine the gaseous exchange of both seed types (coated and uncoated) of the selected grass species to determine if the coating had any effect on the germination of the species. This data was obtained irrespective of pH or any other soil characteristics and only the difference between coated- and uncoated seed after 8 days of germination of the same species was assessed.

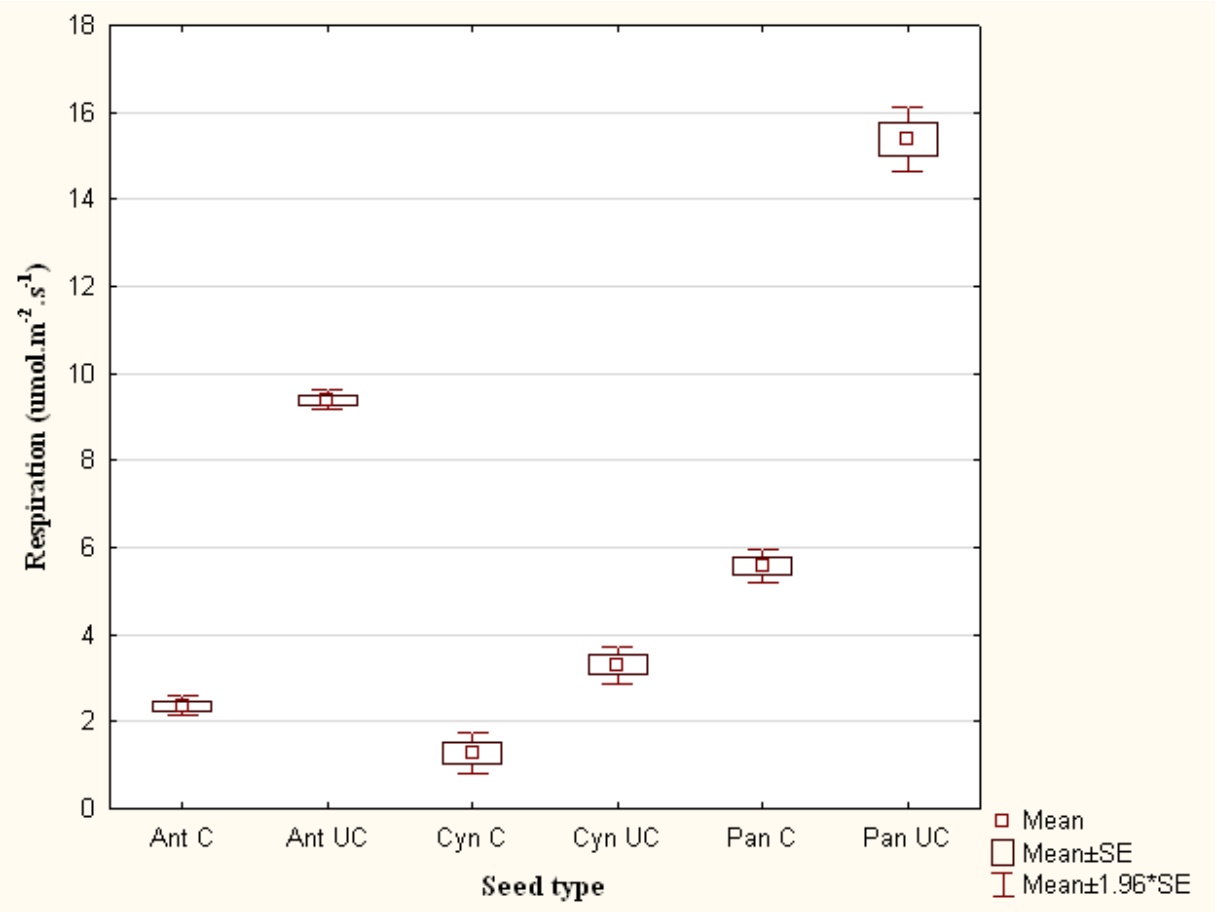

Figure 4.24. Levels of gaseous exchange in the seed types of the selected species after 8 days of germination. (Ant C: *Anthephora pubescens* (coated); Ant UC: *Anthephora pubescens* (uncoated); Cyn C: *Cynodon dactylon* (coated); Cyn UC: *Cynodon dactylon* (uncoated); Pan C: *Panicum maximum* (coated); Pan UC: *Panicum maximum* (uncoated)).

Higher gaseous exchange levels were observed in all of the uncoated seed types of the three selected species when compared to the coated seed types (Figure 4.24). A significant difference occurred in the seed types of *A. pubescens* ($p<0.001$), C. *dactylon* ($p=0.003$) and *P. maximum* ($p<0.001$). Considering the relative higher water content in coated seed of *A. pubescens* (Figure 4.25.), one would have expected higher respiration levels in coated seed, than in uncoated seed of this species, as water uptake by seed are associated with the germination process. These results could also be compared to the poor viability of coated seed of *A. pubescens* as observed in the germination tests previously discussed. The higher α-amylase activity during the first few hours of germination may have indicated elevation of germination by the seed coating process. However, the rapid decrease in α-amylase after 6 days in coated seed (Figure 4.19a) may have been caused by depleted energy in the endosperm of the seed. The loss of germination capacity in this seed type therefore might have been ascribed to a loss in the viability of the germination enzymes over time if continuation of the

germination process does not take place after imbibition. These activated proteins may have been permanently inhibited by reactive oxygen species, such as peroxide, which are metabolic products of the initial activated germination enzymes.

As predicted from the low activity of α-amylase in this seed type, the respiration in both seed types of *C. dactylon* was relatively low. The respiration in uncoated seed exceeded that of the coated seed types. These results correlated with the significantly higher relative water content (RWC) of uncoated seed of *C. dactylon* (Figure 4.27) than observed in the coated seed type. According to the seed analysis report, the coated seed of this batch yielded higher amounts of normal seedlings and less amounts of dead seeds. Considering the α-amylase activity, indicating the levels of germination within the seed, the effect of the coating process seemed to have been insignificant (Figure 4.20). The germination of the coated seed was lower than in the uncoated after a period of 10 days (Figure 4.19b). The elevated levels of germination, following the coating process, therefore also depleted the stored energy before growth could start to take place. The period of dry storage after the coating process was either relatively long, and/or because of the small caryopsis size of this species, this depletion took place relatively fast and the effect was greater than in the bigger-sized *A. pubescens* seed.

High levels of respiration was observed in both seed types of *P. maximum* (Figure 4.24). Although the levels of respiration in the coated seed were significantly higher, the relative water content of both seed types of this species was lower (Figure 4.26). The effective water uptake caused high levels of respiration after 8 days of germination. This affinity for water was probably also reflected in the effect of the seed coating process, as a relatively significant difference was observed in the α-amylase activity between the uncoated seed and the coated seed (Figure 4.19c). However, by the time physical growth started to take place, the levels of α-amylase in uncoated seed already exceeded that of coated seed, indicating a lower level of germination in coated than in uncoated seed. As for *C. dactylon* seed, the elevated levels of germination, following the coating process of this seed type, therefore also depleted the stored energy of this species before physical growth started to take place. As for *C. dactylon* seed, the period of dry storage after the coating process was either long, relative to the amount of available energy in the endosperm, or because of the small caryopsis size of this species, this depletion took place relatively fast and the effect was greater than in the bigger-sized *A. pubescens* seed.

4.4.3. Relative Water Content

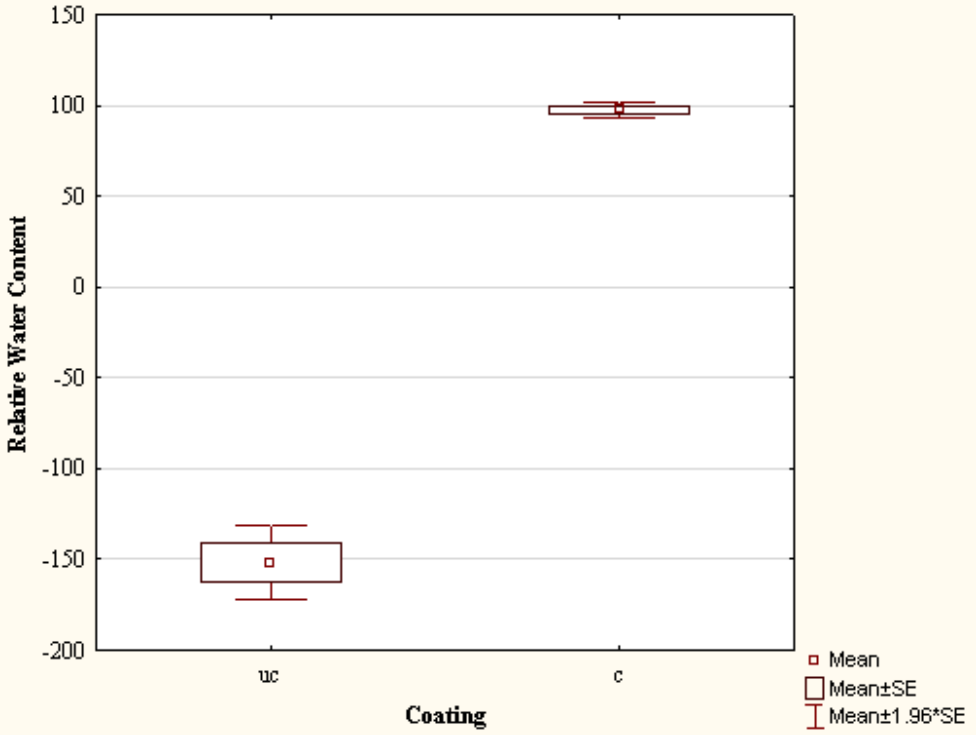

Figure 4.25. Relative water content (RWC) of the coated- (c) and uncoated (uc) seed of *Anthephora pubescens* after four days (96 hours) of germination.

The relative water content in coated seed of *A. pubescens* was higher compared to the uncoated seed type of this species after four days (96 hours) of germination (Figure 4.25). In fact, a desiccated condition was observed in uncoated seed at Time 96 (T_{96}). Seed coatings offered the possibility of including ingredients in the coating mixture that can also regulate moisture uptake (Anonymous, 2004). In this study, the uncoated seed of *A. pubescens* seemed to lose water, while the coating tended to improve water uptake under the same conditions. This higher relative water content observed in the coated seed type, correlated with elevated levels of alpha amylase, which indicated elevated levels of germination in this seed type. This might have favored the inclusion of coated seed of this species, rather than uncoated seed, in seed mixtures for rehabilitation purposes.

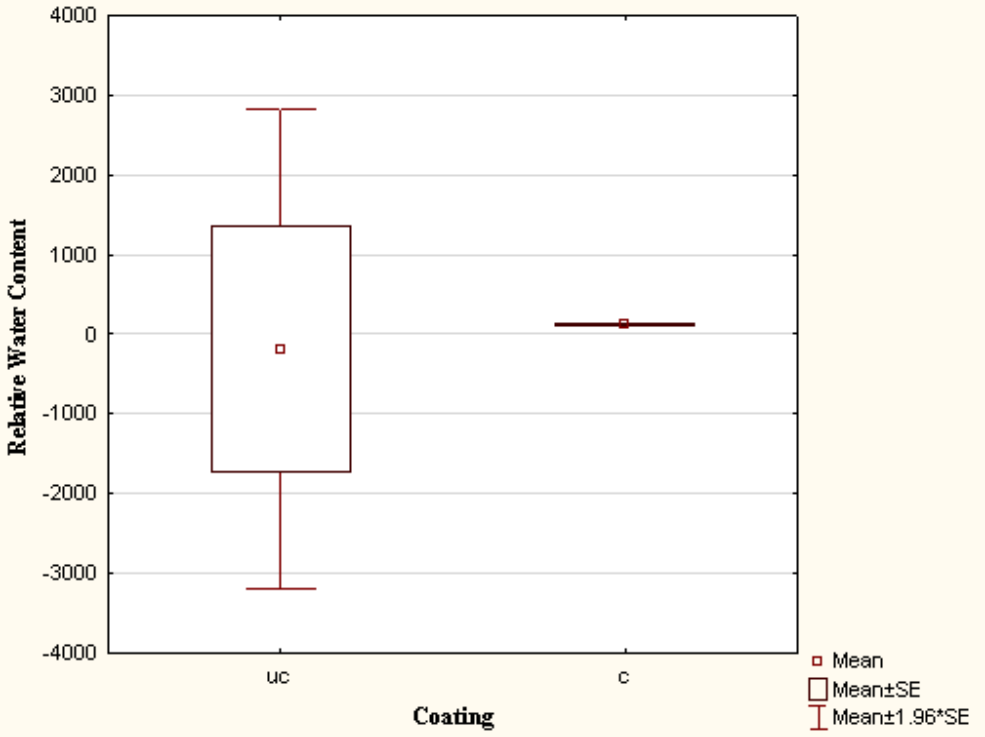

Figure 4.26. Relative water content (RWC) of the coated- (c) and uncoated (uc) seed of *Panicum maximum* after four days (96 hours) of germination.

Both seed types of this species **(***P. maximum*) contained very low levels of moisture and appeared almost desiccated (Figure 4.26). Very low levels of POD, α-amylase and LOX were observed in both seed types of this species, which indicated that general metabolism was very low. This was also observed in the seed analysis reports, as the coated and the uncoated seed types of this species yielded 93% and 92% dead seeds respectively (Table 4.1). Dormancy was therefore eliminated as a cause of low metabolism. However, the remainder of the germinating seeds were reported as normal seedlings after the duration of the test, which was reflected by the high respiration rates.

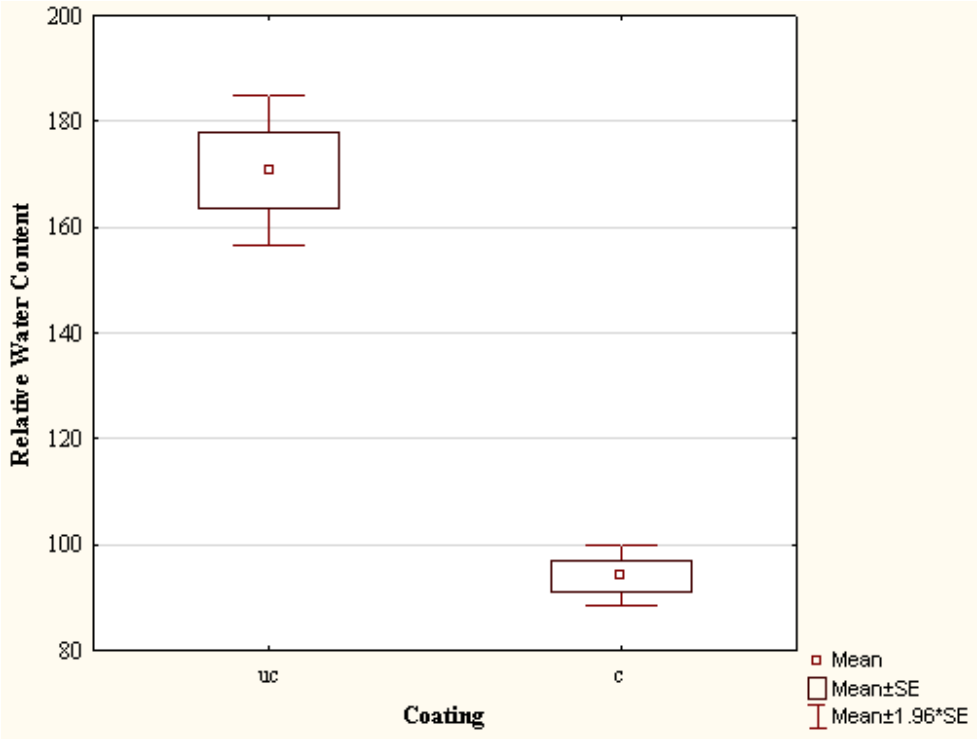

Figure 4.27. Relative water content (RWC) of the coated- (c) and uncoated (uc) seed of *Cynodon dactylon* after four days (96 hours) of germination.

Relatively high water content was observed in both seed types of *C. dactylon* with higher values for uncoated seed than for coated seed (Figure 4.27). In this case, the included ingredients in the coating mixture could have also regulated moisture uptake by causing a negative water gradient (Anon., 2004). A probability for this occurrence is discussed under section 4.4.2.

Chapter 5

Conclusions and Recommendations

5.1. Introduction

Rangeland degradation is manifested by decreases in plant species diversity, low grass height and -vegetation cover, as well as an increase in unpalatable grass species and sharp reductions in plant production yields (Van den Berg & Zeng, 2005). Recovery of such degraded arid, or semi-arid, landscapes without human intervention is a slow process and often unattainable. It also depends on how strong the positive feedback processes are, the costs and benefits from any interventions over the short and long term, and the bio-physical and socio-economic constraints for each degraded system (Leck *et al.,* 2008; Pywell *et al.,* 2002; Snyman, 2003). The soil conditions in degraded areas is normally very poor with a decrease of viable soil seed banks of especially climax, perennial tufted grasses and other plant species that contribute to the recovery of the goods and services of the ecosystem (Snyman, 2003). Since most of the plant species' occurrence is decreased, the number of seeds to improve the population structure is also depleted, meaning that seed cannot be dispersed to restore any degraded ecosystems. The greatest challenge is therefore to actively re-vegetate these degraded areas by sowing seed into the soil to enhance the ecosystem function and processes and thereby create the capacity to produce abundant biomass and vegetation cover in the shortest time possible and to slow down the erosion process (Snyman, 2003; Van den Berg & Zeng, 2005).

Seedlings are the most vulnerable stage in the life cycle of plants, being subjected to a plethora of biotic and abiotic factors that may affect their emergence, survival, and establishment (Kolb & Barsch, 2010). The identification of the factors affecting plant recruitment is thus fundamental for understanding plant population processes in certain environments, as well as plant distribution and abundance (Harper, 1977). The influences of environmental factors on plant establishment are reflected in both germination percentages and the speed of germination. Some studies by Turner *et al*., (2006), Venning (1988) & Anonymous, (2004), found a significant advantageous effect by coating of seed to overcome certain disadvantageous conditions, such as pH of soil or nutrient deficiency.

Numerous research studies were conducted in the past to determine how effective grass seed types can be used to re-vegetate disturbed areas. There are however very few studies in the literature describing the benefit and applicability of seed coating technologies for native seeds in the land rehabilitation and restoration industries (Turner *et al.,* 2006). The findings and recommendations of this study in which the germination and establishment, as well as physiological activities of coated and uncoated seed types normally used for active re-vegetation were addressed, will thus be a valuable contribution to restoration practices in natural- and industrially disturbed areas. In the following sections, the outcomes of the germination trials will be briefly summarized and discussed. As for the discussion of

the results, the conclusions will be presented per species. The outcomes of the field trials (uncontrolled conditions) and greenhouse trials (controlled conditions) were compared. In addition, some insight on the effect of the applied seed coatings on the physiology of the selected seed will be summarized and discussed, regardless of the influence of soil characteristics. Some recommendations will also be made for future research efforts regarding the application of the coated- and uncoated seed of the three selected grass species, *Anthephora pubescens*, *Panicum maximum* and *Cynodon dactylon*, in the restoration of different soil types. A few shortcomings of this study will also be addressed.

5.2. Germination and establishment

5.2.1. *Anthephora pubescens*

The uncoated seed of *A. pubescens* germinated at a faster rate, compared to the coated seed in all four soil types during this study under controlled conditions in the greenhouse trials. The highest number of seeds germinated after seven days (hereafter referred to as the 'maximum germinated percentage') where after the germination rate remained constant, especially in the clayey soil. This was relatively early compared to the germination of the seed of this species in the other soil types, where the maximum germinated percentages were reached only after ten days. The maximum germination percentages of both seed types of this species was overall significantly lower in the clayey soils than in the sandy soil, unexpected, almost the same in platinum and gold tailings material. If the germination levels of *A. pubescens* in the two main tailings growth material was compared, which have quite distinct difference in pH levels, i.e. platinum mine tailings medium had a higher pH value (more alkaline) compared to the gold mine tailings material with a much lower pH value (more acidic), then it seemed as if there are soil factors, other than pH, that contributed to the establishment of this species in the mine tailings material. According to Van Oudtshoorn (2006), *A. pubescens* prefer to grow in soil types with good drainage, i.e. more sandy soil types. This concurred with the results of this study, namely that the overall germination potential was much lower in the high percentage clay soil type used in this study, relative to the remaining three soil types with lower clay contents and better drainage capacity.

The results from the experiments carried out under controlled conditions and subjected to the elimination of competition by other species, indicated that this seed type may be considered for inclusion into seed mixtures for the re-vegetation of higher drainage soils, including the sandy soil, platinum- and gold mine tailings. Although the germination rate of the seed of *A. pubescens* did not differ significantly between the two mine tailings soil types, the difference between the coated (lower) and uncoated (higher) seed in these soil types was the highest. The effect of the coating was therefore more evident in these soil types.

The maximum germination rate was reached much sooner in the soils from the mine tailings than that of the sandy soil type. The coating around the seed and the microbial activities in the soil could have influenced in germination potential of this seed. Kolb and Barsch (2010) stated that the abundance of seedlings at any given point in space and time can be regarded as a function of the quality and quantity of micro-sites available for germination and establishment, as well as the abundance of seeds. This heterogeneity has to be considered when restoring large areas in the landscape.

If the differences in pH of the sandy soil, platinum – and gold mine tailings as growth mediums had any considerable influence, the maximum germination percentages would have differed more significantly between the two tailings soil types. In fact, in this case pH is regarded as inferior as an environmental influence on the germination of the seed types. It rather seems as if the water holding capacity of the soil types, which are closely related to the clay percentages thereof (see Chapter 2) may have played a key role in the germination percentages of this species. It therefore seems that as this species prefers well drained sandy soils, *A. pubescence* was subjected to too much water in the clayey soil experiments leading to lower germination rates. Katerji *et al.* (2003) reported that when seed are put in the soil, germination, observed as emergence, is affected by the water content and structure of the soil. The clay content in platinum – and gold mine tailings was probably just adequate enough to provide an above-required supply of moisture to enable maximum germination of this species. Regular irrigation in the greenhouse trials supplied sufficient moisture to germinating seeds in the well drained sandy soil, which also made maximum germination possible for the duration of the trial, despite the drainage of water from the very top layer of the soil, i.e. the growth region. These results imply that the rate of establishment is much faster in the tailings material where less competition exists than in sandy soils, especially under natural, uncontrolled conditions.

5.2.2. *Cynodon dactylon*

Low germination percentages was observed in both seed types of this species in all four soil types under controlled conditions. The initial emergence of the coated seed was lower than that of the uncoated seed in sandy soil, but in the duration of the trial, the germination rate exceeded that of the uncoated seed to reach the highest maximum germination percentage in all the soil types. The maximum germination percentages of both seed types were significantly lower in clayey soil, with no significant difference between the two seed types. Germination of both seed types of this species in the gold- and platinum mine tailings soil types remained almost zero. Apart from the particularly low germination rates in the latter soil types, both seed types also showed an initial delay in germination in these soil types.

Germination and establishment of both seed types were also the lowest in the clayey soil type under uncontrolled conditions, as zero emergence was recorded for especially the uncoated seed of this

species. This species grows in various types of soil, often found in disturbed places (Van Oudtshoorn, 2006). Although better drained soil types are preferred, this species also grows in moist, nutrient-rich soil types (Van Oudtshoorn, 2006), such as clayey soils (Appendix A). The very low germination and establishment results were therefore unexpected and could be ascribed to other factors, such as the competition with weeds, poor seeding circumstances, poor physical conditions of the soil, or predation by ants or guinea fowl and the high amount of dead seed in the batch. Through personal observation, several nests of scavenger ant species were noted, as well as guinea fowl activity. According to Baskin and Baskin (1998) seed are eaten within a few days after dispersal.

Thompson (1987), states that surface seed foragers, such as ants, select seed on the basis of size, although selectivity varies with species and abundance of seed and distance to the nest. In areas of low seed density, such as natural seed populations or degraded areas with scarce vegetation and seed, both ants and mammals prefer smaller seeds. The probability therefore also exist that predation of seed may have preceded burial, which also reduced the emergence numbers. Although a considerable amount of rainfall occurred prior to the sowing of the field trials, the ploughing resulted in the formation of huge clumps of clayey soil. Some mechanical crushing of these clumps was carried out, but due to the small size of the seed, a lot of seed was buried much deeper than anticipated and the seed also clung to the wet clay clumps, which could have lowered the germination and establishment rates. The deeper the seed are in soil, the lower and slower the germination and seedling establishment, due to the death of the seedling before the shoot reaches the surface (Baskin & Baskin, 1998; Ren *et al*., 2002; Zhu *et al*., 2007).

5.2.3 *Panicum maximum*

The relatively low germination percentages of the coated (14%) and the uncoated (20%) seed of *P. maximum*, suggested low germination and establishment rates of this species. The 0% abnormal seedlings (Table 4.1) furthermore implied that the low germination rate was mainly owed to a loss in viability and high amount of dead seed, but not to dormancy (broken at 5˚C) or the presence of an inhibitor protein of this seed. Although enzyme activities of the three germination enzymes evaluated in this study suggested higher levels of metabolic activity in the coated seed of *P. maximum*, this effect of the coating process was not reflected in either the greenhouse or the field trials.

Physical properties and elements contained by the soil types, especially observed in the sandy, clayey and tailings soil types, seemed to play a key role in the germination and establishment of *P. maximum*. The strong correlation of the uncoated seed type of this species, with the higher clay and silt content, as shown in the multivariate data analyses graphs (Figures 4.7 & 4.8) might have been explained by the genetic characteristic of this species, namely to grow in soil types with high water holding capacity and less drainage. Better growth of the coated seed in sandy soil types, was assumed to be

related to the hygroscopic characteristic of the lime-based coating around the seed. Growth in soil types with high availability of nutrients, such as the gold- and platinum mine tailings, was less positively correlated with germination of coated seed of *P. maximum*.

In both the greenhouse and the field trials, lower germination and establishment of both seed types of this species was observed in the clayey soil type, compared to the other three soil types. The number of established seedlings of this species differed notably between the field- and greenhouse trials. The effect of the coating was more pronounced in the remaining soil types in the greenhouse trial, noted in the differences between the coated and the uncoated seed type in these soil types, while different results were found in these same soil types under uncontrolled influences under natural circumstances. Apart from the improved water holding capacity in the tailings soil types, the lack of competition from a naturally occurring soil seed bank in these soil types, might also explain the higher germination and establishment rates of *P. maximum* in the mine tailings soil types.

5.2.4 General concluding remarks

Recruitment of vegetation at disturbed sites depends on the availability of seed and the appropriateness of micro-environment conditions (Baskin & Baskin, 1998). Gibson (2009) stated that forbs are better represented in natural seed banks than grasses, of which annuals are better represented than perennials. The interactions between these species can be defined as ‘those mechanisms that affect community structure allowing the community to be viewed as possessing the emergent properties greater than the sum of the individual plants’ (Gibson, 2009). One of these interactions often encountered in grassland establishment, is competition. Goldberg (1990) recognized that most plant-plant interactions occurred through intermediaries, such as the availability of resources. Competitive ability can thus be evaluated in terms of the ability of an individual to suppress other individuals by depleting resources, or the ability of an individual to avoid being suppressed by responding to shifts in resource availability (Lauenroth & Aguilera, 1998). Gibson (2009) mentions that the outcome of competition between seedlings of different species is primarily determined by the emergence time and growth rate. The species that commenced growth first and grew the fastest always had an advantage. This competition effect was evident at the uncontrolled, natural field sandy- and clayey soil sites of this study, and also explains the lower amounts of established seedlings at these sites, compared to the results of the platinum- and gold mine tailings sites.

It is therefore recommended that in order to establish grasses in disturbed natural sites, such as former fields, the sowing of any seed, whether coated or non-coated, should be accompanied by weed control mechanisms, such as the spraying of herbicides. Considering that this method may not always be cost-effective and will depend on the rate and amount of herbicide that has to be used, as well as the time

of restoration, seed may also be applied at very high sowing rates to enhance seed availability and increase the competition effectiveness of the grass species.

On the other hand, the low germination rates in clayey soils under controlled conditions could not only be ascribed to predation, burial or competition, as temperature is reported to be an important regulator of germination of seed caryopses (Alvarado & Bradford, 2002; Zhu *et al.*, 2007). In addition, Rivas-Arancibia *et al*. (2006) stated that, although the importance of rainfall, temperature and water availability are acknowledged as separate influences on plant establishment, the interaction of these three factors are barely recognized. The sole influence of the substrate is not significant in terms of influencing germination, but it does play an important role combined with the influences of rainfall, soil moisture availability and temperature. Temperatures in the greenhouse were maintained at 16º C - 22ºC and germinating seed was watered frequently, resulting in wetter substrates with lower soil temperatures compared to the natural field sites. Water uptake at lower temperatures is reported to impair germination and induces damage to seedling development, and excess water interferes with oxygen uptake (Mayer & Shain, 1974). These factors may have lead to the low germination rates in the greenhouse trials.

The composition of the soil type in which germination took place, determined the availability of water for imbibition. Mayer & Poljakoff-Mayber (1989) states that imbibition of seed decreases as the concentration of solutes increases, largely due to osmotic effects. The ability of seed to absorb water from the soil thus depends on both the osmotic and matric potential of soil. The water potential of the seed, compared to that of the immediate vicinity, will determine the ability of water-uptake by the seed. Under saline conditions, such as in platinum- or gold mine tailings soil types, seed would thus be physiologically desiccated, although adequate water is present in the near vicinity of the seed.

According to Mayer & Poljakoff-Mayber (1989), a direct toxic effect of ions in solution on the seed is frequently observed in saline soil. This osmotic inhibition by salt does not persist when lowering the concentrations of the solute (Fenner & Thompson, 2006; Mayer & Poljakoff-Mayber, 1989). Thus, inhibited seed, such as from the seed coating process, would germinate under conditions where the solute in the disturbed soil is diluted, expected after high rainy conditions. However, the vigour of growth may be affected if the salinity would happen to be near the edges of the selected species' tolerance range. Depending on the characteristics and nutrient-content of a disturbed soil types, the calcareous seed coating may have therefore also affected the imbibition and germination of the selected seed. Depending on the pH caused by ions contained by the soil, the coating may act as a buffer, enhancing germination, or contribute even further to the adversity of conditions in the soil for the seed to germinate. Under controlled conditions, without the presence of competition from other species, this effect was noted especially for *P. maximum,* which had much lower germination rates in the soils from platinum- and gold mine tailings, compared to the clayey and sandy soils. Therefore,

the hypothesis that germination and establishment of the selected species will be effected only by the coating is therefore not correct. Although the exact compilation of the applied coating is unknown, the effect thereof can be observed in the selected species in combination with the genetic traits of the seed types and the influence of the environmental circumstances, such as soil characteristics, temperature and moisture availability.

5.3 Effect of the coating on seed physiology

Seed quality is a complex trait that depends on a number of various factors. Seed quality is determined by its genetic, physical and health condition properties that are influenced by agro-ecological conditions during the development of the seed and specific ecotype, as well as seed processing, storage conditions and storage period (Rozman *et al.*, 2010, Van den Berg & Kellner, 2010). During the period of storage, seed quality is influenced by factors such as seed moisture content, temperature, and relative humidity in the warehouse. Optimum conditions for storage are thus dependant on the species of which the seed is stored. If the relative humidity (10-20%) and temperature (2^{o}C) is controlled during storage, some studies reported that the preservation of grass seed viability can be as long as twelve years (Marshall & Lewis, 2004).

As discussed under section 2.5.2, and mentioned under 5.2.4, a seed retains the physiological changes that occurred during pre-sown hydration, such as during the seed coating process. Any physiological changes, caused by hydration, cannot be reversed or suspended (Fenner & Thompson, 2006). Without the adequate supply of water needed for prolonged germination, starch supplies becomes depleted, and the activity of oxidizing enzymes such as LOX and POD, results in the loss of viability of the seed. Lower germination metabolism, expressed as lower enzyme activity in alpha amylase, LOX, POD, as well as lower respiration, are observed in the coated seed type of all the species, except in *A. pubescens*. As expected, this effect is thus also seed size-dependant. Considering that dormancy breaking methods were applied to the seed types of the selected seed, the low germination can not be ascribed to dormancy, but rather to physiological constraints within the seed of the selected species, caused by the periodic water uptake during the seed coating process. (Also see following section 5.3).

As mentioned earlier, high purity of the selected seed batches, does not necessarily imply high quality of the seed. Although no abnormal seedlings were recorded in the seed batch of *P. maximum*, 80% and 86% dead seed were recorded in the uncoated- and coated seed of this species respectively (Table 4.1). Furthermore, higher percentages of dead seed and abnormal seedlings were recorded in the coated seed of *A. pubescens* and *C. dactylon*, compared to the uncoated seed. Considering the higher values of germination metabolisms, reflected in the higher enzyme activity of the three germination enzymes in coated seed from the phases of the coating process, it is concluded that the seed coating process does stimulate germination metabolism. However, the stimulated germination metabolism

appear to only last for a specific time, before the stored energy becomes depleted and oxidising enzymes causes loss of viability. It is therefore recommended that seed should be sold or sown as soon as possible after coating of the seed took place to ensure a higher germination rate and faster establishment of the species.

5.3.1 Activity of key germination enzymes

5.3.1.1 Peroxidase

The average peroxidase (POD) activity of the uncoated seed was generally higher compared to the coated seed of the three grass species evaluated in this study. Although the POD activity of both the coated and uncoated seed types were relatively low after 2 days of germination (Figures 4.1 a, b & c), the POD activity of the uncoated seeds tended to be higher than the coated seeds. According to Wang *et al.* (2009), plants that have induced levels of antioxidant enzymes are usually associated with higher levels of tolerance towards environmental stresses. An increase in the active oxygen species (AOS) occur in plants when exposed to a specific type of environmental stress. If these increases in active oxygen species are not detoxified by antioxidant mechanisms, the plant usually succumb to the environmental induced stress. Chaitanya & Naithani (1993) reported that extensive peroxidation of large quantities of stored polyunsaturated fatty acids in seed may contribute to loss of seed viability. The products of lipid peroxidation can have a negative impact on the plants' metabolism. A consequence of exposure to salt and drought stresses is the generation of reactive oxygen species and the associated peroxidation of lipids (Wang *et al.*, 2009).

The detoxification of active oxygen species is an important defence mechanism of plants towards environmental stresses and to ensure the viability of seeds. During the antioxidant defence mechanism, superoxide dismutase converts superoxide radicles (O_2^-) into hydrogen peroxide (H_2O_2), which is further reduced by peroxidise to water, in order to prevent oxidative cell damage. Besides mediating lipid peroxidation, O_2^- and derived forms of activated oxygen are capable of oxidising protein thiol groups, causing enzyme deactivation, initiating generation of more reactive oxidizing nucleic acids (Chaitanya & Naithani, 1993). It can therefore be concluded that high levels of peroxidase does indicate active seed metabolism during seed metabolism. However, if desiccated conditions and elevated levels of peroxide are found accompanying high levels of these enzymes, it may indicate that the seed are subjected to conditions outside the species' tolerance range, which may influence the quantity and health of the seedlings and thus also the survival and establishment rate of the seedlings in a specific soil type.

In both *P. maximum* and *C. dactylon*, a decrease in POD activity was observed after the first phase of the coating process, suggesting that the seed coating process may be responsible for lowering POD activity in the coated seed.

5.3.1.2 Alpha amylase

Much higher enzyme activity of alpha amylase (α-amylase) was visible in coated seed of *A. pubescens* at day two after germination (Figure 4.19a). This is an indication that the germination process of the coated seed is most probably better initiated. As germination levels increase after 4-6 days of the trial, the levels of metabolic activity also increases. However, after 10 days of germination, α-amylase activity almost totally ceases, probably due to the depletion of starch.

In *C. dactylon*, higher levels of alpha amylase activity was also found in the coated seed, compared to the uncoated seed. After 10 days, the enzyme activity levels in uncoated seed exceeds that of the coated seed, probably ascribed to a faster depletion of starch substrate, which can again be ascribed to quicker initiation of germination in the coated seed.

Relatively high alpha amylase activity was observed in both the coated and uncoated seed of *P. maximum*, after 2 days of germination (Figure 4.19c). Alpha amylase activity decreased in both the coated and uncoated seed after 6 days of germination. This decrease in activity can be contributed to the depletion of the starch content of the seed. After the seed coating process, which involves the wetting of the seed and high temperatures, the activity of α-amylase was much higher than before the coating process. This indicates that the process of germination was already initiated during the coating process. It is uncertain for how long this enzyme can stay active after activation. The increased activity of lipoxygenase (LOX) in the coated seed of all three selected species also indicated the initiation of germination.

Considering the relative higher water content in coated seed of *A. pubescens* (Figure 4.25), one would expect higher respiration levels in the coated seed than in the uncoated seed, as water uptake by seed are associated with the germination process. The higher α-amylase activity during the first few hours of germination may have indicated an increase in the rate of germination by the seed coating process. However, the rapid decrease in α-amylase after 6 days in coated seed (Figure 4.19a) might have been caused by depleted energy in the endosperm of the seed. The loss of germination capacity in this seed type might therefore be ascribed to a loss in the viability of the germination enzymes over time if continuation of the germination process does not take place after imbibition. As predicted from the low activity of α-amylase in the coated seed type, the respiration rate in both seed types of *C. dactylon* was relatively low (Figure 4.24). The respiration in uncoated seed exceeded that of the coated seed types. These results correlated with the significantly higher relative water content (RWC) of uncoated seed of *C. dactylon* (Figure 4.27) than was observed in the coated seed type. According to the seed analysis report, the coated seed of this batch yielded higher amounts of normal seedlings and less amounts of dead seeds. Considering the α-amylase activity, which is also an indication of the germinating capability of the seed, the effect of the coating process seemed to be insignificant, as the germination of the coated seed is lower than in the uncoated after a period of 10 days. The elevated

levels of germination, following the coating process, therefore also depleted the stored energy before growth could start to take place. The period of dry storage after the coating process was either relatively long, and/or because of the small caryopsis size of this species, this depletion took place relatively fast and the effect was greater than in the bigger-sized *A. pubescens* seed.

5.3.1.3 Lipoxygenase

No meaningful activity of lipoxygenase (LOX) was found before germination was initiated in the seed types of any of the three species. Also, no meaningful activity was found in the seed from any of the phases of the seed coating process. However, four days (96 hours) after germination was initiated, a significantly higher enzyme activity of LOX was found in the coated seed type of all three the selected species. Combined with the results of other physiological tests, such as the higher respiration rates of the coated seed of the selected species, this indicates a higher germination metabolism in the coated seed of the selected species. It would be important to conduct this assessment over a longer period of time (longer than four days), in order to compare the concentration and period of activity of this enzyme in both seed types of the different species and evaluate whether the concentration and period of activity coincided with the germination rate of the seed in the different soil types.

5.3.2 Respiration and relative water content of the selected seed

High levels of respiration were observed in both seed types of *P. maximum*. Although the levels of respiration in the seedlings from coated seed were significantly higher, the relative water content of both seed types of this species was lower. The effective water uptake caused high levels of respiration after 8 days of germination. This affinity for water was probably also reflected in the effect of the seed coating process, as a relatively significant difference was observed in the α-amylase activity between the uncoated seed and the coated seed. However, the levels of α-amylase of the uncoated seed already exceeded that of the coated seed (Figure 4.19c), indicating a lower level of germination in coated than in uncoated seed. When growth did not take place, although the rate of metabolism was increased by the coating process of this seed type, it contributed to the depletion of the stored energy. The period of dry storage after the coating process was either too long, relative to the amount of available energy in the endosperm or because of the small caryopsis size of this species. This depletion therefore took place much faster and the effect was greater than in the bigger-sized *A. pubescens* seed.

In this study, the uncoated seed of *A. pubescens* seemed to lose water, while the coating tended to improve water uptake under the same conditions. Van den Berg & Zeng (2005) reported a loss in germination capacity as the osmotic potential decreased in the seed. This higher relative water content observed in the coated seed type, correlated with elevated levels of alpha amylase, which is an indication that the metabolic process associated with germination is functioning. This might have

favored the inclusion of coated seed of this species, rather than uncoated seed when used in seed mixtures for rehabilitation purposes, if the reseeding takes place very soon after the coating process.

5.4.1. Recommendations for similar studies

These include**:**

- Seasonal rains have to be considered during the monitoring process in the natural environment, as this could influence the time and frequency of monitoring. The accessibility of the trial terrain is mostly dependant by the soil type, which can be problematic if the site is unreachable.

- The choice of the area in which the field trial is carried out, greatly influences the layout of the trial, especially if the trial is on the farmer's land and not at an experimental research station. Although this is not always possible, due to practical reasons, sample sites of the field trials for each soil type cannot be exactly the same size. The layout of all sample sites will then not be identical and the statistic integrity of the data affected. If the natural field trials are not on an experimental station, but on the farmers land, as it was for this study, the management practice of the farmer or land user must also be considered. Often the aims of the researcher and the farmers are not the same which could negatively impact on the data and research trial.

- Care should be taken when counting individual seedlings of *A. pubescens.* Multiple seedlings, germinated from an individual seed, may result in oversampling of this species. Likewise, the stoloniferous growth form of *C. dactylon* can also result in oversampling of this species' seedlings. Only the plant of origin should be recorded, which is often difficult as the plant matures.

- Competition from other species in the field trial experiments should be controlled either mechanically or chemically on a regular basis and at the beginning of the trial, especially if the soil seed bank is not analysed before the experiment to monitor the amount and type of weeds that could compete with the seeds sown for the trial.

- Further experiments should be conducted to evaluate the effect of the coating on predation of seeds by insects and guinea fowl. Considering the shallow depth of sowing of grass seed, care must be taken to avoid removal of seeds by these predators.

- Any physiological tests to determine the impact of the coating process, such as the longevity/viability of the activated enzymes, periodic assessments of the seed during and directly after the coating process should be carried out, and not after long periods when the coating process is completed.

5.4.2. Shortcomings of the study

- The effect of the seed coating on root development was also attempted by using a sterile, transparent agar medium. The agar medium used in these experiments was however overgrown by fungi before the growth of the roots took place. It was suspected that the agar was inoculated by fungi from the seed, as all other apparatus in the experiment, including the tubes and the agar itself, was sterilised beforehand. The difficulty of sterilising the seed was that the applied seed coating would have been removed during the sterilizing process.

- Testing the relative water content of the seed after a storage period, as was carried out for this study, only indicated the ability of the seed to take up or loose water. Determining the osmotic potential, by using an osmometer, would give a better indication of the effect of the seed coating on the osmotic potential of the seed. The latter could not be done due to financial and logistical problems.

- As for the seed of *C. dactylon* and *P. maximum*, activity of the key germination enzymes in seed of *A. pubescens* should also be assessed following a coating process to evaluate the effect thereof on the key germination enzymes.

Chapter 6
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APPENDIX A

Reports of soil analyses, as received from laboratories of EcoAnalytica.

Appendix A1: Table of nutrient status of the four growth mediums

Sample	Ca	Mg	K	Na	P	pH(H_2O)	EC
	(mg/kg)						(mS/m)
Sandy Soil	218.5	115.5	11.5	55.5	2.9	5.70	8
Clayey soil	933.5	443.5	171.0	66.0	2.9	6.57	38
Platinum tailing	500.0	35.0	10.0	60.5	3.2	8.44	108
Gold tailing	2775.0	235.0	135.0	125.0	4.7	2.53	1475

Appendix A2: Table of micro-nutrients of the four different growth mediums

Sample	Fe	Mn	Cu	Zn	B	pH	EC	P-BRAY 1
	Micromol per litre						*(mS/cm)*	*ppm*
Sandy soil	1.43	0.09	0.06	0.01	<1	6.00	0.03	2.81
Clayey soil	6.15	0.20	0.11	0.13	<1	6.46	0.21	2.95
Platinum tailing	9.50	0.27	0.17	0.12	<1	7.59	0.53	3.13
Gold tailing	381.46	276.90	92.93	90.11	<1	2.63	9.69	4.75

Appendix A3:Table of particle size distribution of the different growth mediums

Sample	> 2mm	Sand	Silt (% < 2mm)	Clay
Sandy soil	3.5	88.0	5.8	6.2
Clayey soil	12.5	64.4	13.2	22.4
Platinum mines' tailing	0.0	80.1	18.1	1.8
Gold mines' tailing	0.1	63.1	29.8	7.1

Appendix A4:Table of macro elements contained in the four growth mediums

Sample	Ca	Mg	K	Na	PO_4	SO_4	NO_3	NH_4	Cl	HCO_3
	Millimol per litre									
Sandy Soil	0.07	0.02	0.03	0.02	0.01	0.01	0.08	0.07	0.07	0.10
Clayey soil	0.35	0.40	0.15	0.38	<0.01	0.43	0.48	0.06	0.44	0.30
Platinum mines' tailing	1.32	0.63	0.57	0.77	<0.01	1.50	0.20	0.05	0.47	1.60
Gold mines' tailing	1.75	22.30	0.01	0.01	0.15	46.96	0.45	0.06	2.02	0.0

APPENDIX B

Detailed germination reports as received from laboratories of the Plant research and Genetic Centre.

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REPUBLIC OF SOUTH AFRICA

DIVISION: SEED TESTING
DIRECTORATE: PLANT PRODUCTION
Private Bag X250
Pretoria
0001
Ref: 20/3/3

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
DIRECTORATE: PLANT PRODUCTION
2011 -03- 31
PRIVATE BAG X 250, PRETORIA, 0001
OFFICIAL SEED TESTING LABORATORY

SEED ANALYSIS REPORT

INTERNAL BOX 284
BUILDING E6 (J. S. VAN DER MERWE BUILDING)
POTCHEFSTROOM CAMPUS
NORTH WEST UNIVERSITY
POTCHEFSTROOM
2520

ATTENTION: MS M WESTCOTT

INFORMATION SUPPLIED BY APPLICANT:

Seedkind: *Anthephora pubescens* (**UNCOATED**)
Variety:
Code:
Company:
Sampler:

ANALYSIS RESULTS

Ref. No.: **INV/11-0057A**
Weight of submitted sample (gram): 158.2

Date received: 2010-11-02
Date test concluded: 2011-01-25

Botanical name: *Anthephora pubescens* (**UNCOATED**)

Purity (% by weight)	Pure seeds	Inert matter	Other seeds
	-98.2-	-1.7-	-0.1-

Inert matter: Stalks, dead insect, empty units of other seeds and chaff

Other seeds: thereof 0.05 **-TR%-** weed seed. *Cenchrus ciliaris, Chloris gayana, Cynodon dactylon, Digitaria eriantha., Eragrostis curvula* and *Verbesina* sp.

Germination method:	PP:20-30°C; remove outer structures of the seed unit before planting					
Germination (% by number)	Duration of test (days)	Normal seedlings	Hard seeds	Fresh seeds	Abnormal seedlings	Dead seeds
	-21-	-72-	-0-	-0-	-4-	-24-

Other test: NONE

Description of abnormal seedlings: Seedlings decayed and deformed.

Remark: No testing method in the current International Seed Testing Association (ISTA) rules

Date: 2011-03-31

for DIRECTOR: PLANT PRODUCTION

The analysis results relate only to the sample received for testing and not necessarily to the seedlot from which the sample was taken.

Enquiries: SO Phahladira Tel No.: 012 8085052 Fax No.: 012 8085394 E-mail: ObedP@daff.gov.za

agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

DIVISION: SEED TESTING
DIRECTORATE: PLANT PRODUCTION
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SEED ANALYSIS REPORT

INTERNAL BOX 284
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POTCHEFSTROOM CAMPUS
NORTH WEST UNIVERSITY
POTCHEFSTROOM
2520

ATTENTION: MS M WESTCOTT

INFORMATION SUPPLIED BY APPLICANT:

Seedkind: *Anthephora pubescens* (**COATED**)
Variety:
Code:
Company:
Sampler:

ANALYSIS RESULTS

Ref. No.: **INV/11-0057B**
Weight of submitted sample (gram): 231.1

Date received: 2010-11-02
Date test concluded: 2011-12-21

Botanical name: *Anthephora pubescens* (**UNCOATED**)

Purity (% by weight)	Pure pellets	Unpelleted seed	Inert matter
	-98.9-	-0.1-	-1.0-

Unpelleted seed: *Anthephora pubescens*, *Cynodon dactylon* and *Digitaria* sp

Inert matter: loose pelleting material, stalks and chaff

Germination method:	PP:20-30°C; remove outer structures of the seed unit before planting

Germination (% by number)	Duration of test (days)	Normal seedlings	Hard seeds	Fresh seeds	Abnormal seedlings	Dead seeds
	-29-	-19-	-0-	-0-	-7-	-74-

Other test: NONE

Description of abnormal seedlings: Seedlings decayed and deformed.

Remark: No testing method in the current International Seed Testing Association (ISTA) rules

Date: 2011-03-31

for DIRECTOR: PLANT PRODUCTION

The analysis results relate only to the sample received for testing and not necessarily to the seedlot from which the sample was taken.

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POTCHEFSTROOM CAMPUS
NORTH WEST UNIVERSITY
POTCHEFSTROOM
2520

ATTENTION: MS M WESTCOTT

INFORMATION SUPPLIED BY APPLICANT:

Seedkind: *Cynodon dactylon* (**UNCOATED**)
Variety:
Code:
Company:
Sampler:

ANALYSIS RESULTS

Ref. No.: **INV/11-0058A**
Weight of submitted sample (gram): 39.95
Date received: 2010-11-02
Date test concluded: 2010-12-20

Botanical name: *Cynodon dactylon* (**UNCOATED**)

Purity (% by weight)	Pure seeds	Inert matter	Other seeds
	-99.8-	-0.2-	-0.0-

Inert matter: Stalks

Other seeds: NIL

Germination method:	TP:20-30°C; KNO_3; Prechill

Germination (% by number)	Duration of test (days)	Normal seedlings	Hard seeds	Fresh seeds	Abnormal seedlings	Dead seeds
	-19-	-82-	-0-	-0-	-12-	-6-

Other test: NONE

Description of abnormal seedlings: Decayed seedlings and insufficient root system

Date: 2011-03-31

for DIRECTOR: PLANT PRODUCTION

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2520

ATTENTION: MS M WESTCOTT

INFORMATION SUPPLIED BY APPLICANT:

Seedkind: *Cynodon dactylon* (**COATED**)
Variety:
Code:
Company:
Sampler:

ANALYSIS RESULTS

Ref. No.: **INV/11-0058B**
Weight of submitted sample (gram): 49.83
Date received: 2010-11-02
Date test concluded: 2010-12-20

Botanical name: *Cynodon dactylon* (**COATED**)

Purity (% by weight)	Pure pellets	Unpelleted seeds	Inert matter
	-99.5-	-0.0-	-0.5-

Inert matter: Stalks and loose pelleting material

Unpelleted seed: NIL

Verification of species: 100 seeds examined – 100 *Cynodon dactylon*

Germination method:	TP:20-30°C

Germination (% by number)	Duration of test (days)	Normal seedlings	Hard seeds	Fresh seeds	Abnormal seedlings	Dead seeds
	-28-	-67-	-0-	-0-	-12-	-21-

Other test: NONE

Description of abnormal seedlings: Decayed seedlings and insufficient root system

Date: 2011-03-31

for DIRECTOR: PLANT PRODUCTION

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2520

ATTENTION: MS M WESTCOTT

INFORMATION SUPPLIED BY APPLICANT:

Seedkind: *Panicum maximum* (**UNCOATED**)
Variety:
Code:
Company:
Sampler:

ANALYSIS RESULTS

Ref. No.: **INV/11-0059A**
Weight of submitted sample (gram): 30.12

Date received: 2010-11-02
Date test concluded: 2010-12-20

Botanical name: *Panicum maximum* (**UNCOATED**)

Purity (% by weight)	Pure seeds	Inert matter	Other seeds*
	-95.5-	-4.3-	-0.2-

Inert matter: Plant materials, stalks, sterile units of other seeds and chaff

Other seeds: * thereof **-0.2%-** weed seed. *Amaranthus* sp, *Bothriochloa* sp., *Chenopodium* sp., *Chloris gayana* and Unknown Poa sp

Germination method:	TP:20-30°C; KNO_3; Prechill

Germination (% by number)	Duration of test (days)	Normal seedlings	Hard seeds	Fresh seeds	Abnormal seedlings	Dead seeds
	-34-	-20-	-0-	-0-	-0-	-80-

Other test: NONE

Date: 2011-03-31

for DIRECTOR: PLANT PRODUCTION

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INFORMATION SUPPLIED BY APPLICANT:

Seedkind: *Panicum maximum* (**COATED**)
Variety:
Code:
Company:
Sampler:

ANALYSIS RESULTS

Ref. No.: **INV/11-0059B**
Weight of submitted sample (gram): 49.13

Date received: 2010-11-02
Date test concluded: 2010-12-20

Botanical name: *Panicum maximum* (**COATED**)

Purity (% by weight)	Pure pellets	Unpelleted seeds	Inert matter
	-98.3-	-0.2-	-1.5-

Inert matter: Plant materials, stalks and loose pelleting material

Unpelleted seed: *Chloris gayana* and *Panicum maximum*

Verification of species: 100 seeds examined – 100 *Panicum maximum*

Germination method:	TP:20-30°C; KNO_3 ;Prechill

Germination (% by number)	Duration of test (days)	Normal seedlings	Hard seeds	Fresh seeds	Abnormal seedlings	Dead seeds
	-19-	-14-	-0-	-0-	-0-	-86-

Other tests: NONE

Date: 2011-03-31

for DIRECTOR: PLANT PRODUCTION

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