EFFECTIVENESS OF COATED SEED IN THE ESTABLISHMENT OF HERBACEOUS SPECIES ON DIFFERENT MINED SUBSTRATES AS INFLUENCED BY pH AND SALINITY

By

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DECLARATION

I, Petrus Johannes Pretorius declare that the dissertation, which I hereby submit for the degree MSc (Agric) Pasture Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

P.J. Pretorius

November 2017

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ABSTRACT

Effectiveness of coated seed in the establishment of herbaceous species on different species on different mined substrates as influenced by pH and salinity

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Mining throughout the world is needed, especially coal mining to satisfy our need for electricity, and our entire economy is therefore dependant on mining. Competition exists for resources between mining and agriculture, since the rehabilitated land's agricultural potential is changed after mining and it is yet to be proven that the land will reach the same potential again. Mining produces by-product tailings materials, waste products and slurry disposed in large dams. These tailings can in addition to the physical disturbance of the soil structure due to the physical mining progress cause the soil quality to deteriorate. The decline in soil quality makes it very difficult for plants to survive and these areas usually have no or very little plant growth.

These areas that are mined, needs to be rehabilitated and made productive by law (Mining and Petroleum Resources Development Act 28 of 2002 (MPRDA)). The most commonly accepted practice to rehabilitate these mined areas is to establish pastures on them once the topsoil or subsoil is replaced. The rehabilitation of these mined areas with pastures can be

difficult and is a timely process especially in open cast mining where large areas of land have been cleared.

Deep shaft mining in gold and platinum industry result in the substrate to be brought to the surface and once processed the discarded material is placed in waste disposal sites where rehabilitation is required which includes the re-vegetation with grass.

Coating is the science that incorporates information from various agricultural companies, for example; seed, pesticide and fertilizer companies. Coating seed is done by adding polymers, nutrients, fungicides, fillers and insecticides to the outer layer of the seed. These treatments can improve the germination and emergence of species, depending on the soil it is planted in, therefore it can improve the establishment of these species. The main aim of the coating of coated seed is to overcome most of the environmental challenges, to increase the success rate of germination and emergence of these seed and to give a seed the best chance of survival.

The first aim was to determine the emergence percentage of six species, *Chloris gayana*, , *Cynodon dactylon, Cenchrus ciliaris, Digitaria eriantha Eragrostis curvula* and *Medicago sativa,* (coated and uncoated) in substrates with different salinity's (0.05M , 0.1M and 0.15M) and pH's (3, 5 , 7 and 9). The second aim was to determine the emergence percentage of the same species (coated and uncoated) in nine different mine substrates. The third aim was to determine the dry matter production, leaf area and root production in substrates with different salinity concentrations. The fourth and final aim was to determine the dry matter production, leaf area and root production in substrates with different pH's. Three species was used, namely *Chloris gayana*, *Eragrostis curvula* and *Medicago sativa.* The species were planted in silica sand placed in 400 mm tall and 150 mm wide pots. Two irrigation water treatments were tested, with different salinity's (0.05 M and 0.09 M NaCl) and water with different pH (3, 5, 7 and 9).

It was concluded for study 1 that there was no significant difference in emergence between *D. eriantha*, *E. curvula* and *M. sativa*. *Cenchrus ciliaris* and *C. dactylon* had higher emergence percentage in acidic soils while *C. gayana* being sensitive to acidic and alkaline soils. In study 1 the highest emergence for most of the species were when the soil moisture was at field capacity. Coated seed had a higher emergence for *C. cilliaris*, *C. dactylon* and *E. curvula*, while uncoated seed had higher emergence for *C. gayana* and *D. eriantha*. There was no notable difference for *E. curvula*. In the salinity trials all the emergence decreased as the salinity concentration increased. *Cenchrus ciliaris*, *C. dactylon* and *E. curvula* are very sensitive to saline soils and it is therefore not recommended to use these species in these soils. Coated *C. gayana* and *M. sativa* had a higher emergence percentage compared to the uncoated treatments of the species. Uncoated *D. eriantha* had a higher emergence percentage compared to the coated treatment.

In the second study it was concluded that the low pH and high aluminium levels prevented any species from growing in the coal discard substrate. Coated seed had a higher emergence for *C. ciliaris* in gypsym- and andalusite substrates, *C. gayana* in gold > 2% pyrite-, platinum-, fluorspar- and andalusite substrates and for *D. eriantha* in platinum substrate. Coated seed had a definitely advantage in *M. sativa* with higher emergence percentage in red sandy loam-, gold < 2% pyrite-, platinum-, gypsum- and fluorspar substrates, compared to uncoated seed. Coated seed also had a higher emergence for *E. curvula* in all the different substrates. Uncoated seed in all the different species had a higher emergence percentage in kimberlite due to kimberlites coarse texture. The texture prevented any seed to substrate contact.

The study also found that there are no differences in emergence percentage between coated and uncoated seed for *C. ciliaris* in platinum tailings and for *C. dactylon* in any of the substrates, except kimberlite. There was also no differences between coated and uncoated seed for *C. gayana* in red sandy loam-, gold<2% pyrite- and gypsum substrates and for *E. curvula* in, gypsum substrate.

It was concluded as with previous studies that high levels of aluminium in combination with low pH, had a severe negative effect on germination and emergence. The low pH causes the impact of Al to be more severe. High sodium levels also had a negative impact on growth, by causing imbalances of other minerals in the seed and in turn lower the emergence percentage. The texture also had an impact on the germination rate, for example kimberlite had a coarse texture and there was a lower germination rate while in red sandy loam that had a fine texture there was a germination rate due to seed having more contact with the substrate.

In the third study it was concluded that in the *C. gayana* and *M. sativa* trials the root production was unaffected by using coated seed, the production only decreased as the salinity's increased. *Eragrostis curvula* showed an increased in root production when coated seed was used. The root diameters were slightly larger in *C. gayana* and *E. curvula* when planted to coated seed. Only in *C. gayana* was it found that using coated seed, the highest amount of roots was found deeper in the soil as compared to uncoated seed. Plants from coated seed also showed no benefit in the size of leaves, except for *E. curvula* were leaves from coated seed were larger compared to uncoated seed. The leaf area decreased as the salinity increased in all three species. The dry matter production of these species was also unaffected by using coated seed, the dry matter production only decreased when the salinity concentrations increased.

In the last study it was concluded that *C. gayan*a, *E. curvula* and *M. sativa* planted to coated seed showed no benefits in root production, root diameter, dry mater production and leaf area index compared to uncoated seed. Roots from coated *C. gayana* seed reached greater depths while roots from uncoated seed were more focused in the first 0-10cm. Coated *E. curvula* seed had an effect on the depth roots reached. Roots from coated *M. sativa* seed had roots that reached greater depths compared to uncoated seed were the majority was found in the top 0-10cm.

The benefit of using coated seed is very limited in mature plants, but it is important to note that the coated seed has other benefits, for example easing the handling and planting of seed. Coated seed has a definite place for improving the emergence percentage of seeds. It is however important to know the physical and chemical properties of the substrates that the seed are going to be planted in. The right saturation or field capacity level of a substrate should also be known before anything is planted in a specific substrate. One needs to fully know what is going on in the substrates before one can make recommendations of which seed to use, coated or uncoated. Coated seed is just one part of trying to improve the germination and emergence of pasture, but this study has made it clear that there are various other aspects that needs to be taken into consideration. Each case must be looked at individually and be treated differently, therefore findings should not be generalized, due to coated seed being area and environment specific.

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CHAPTER 1

Literature review

Effectiveness of coated seed in the establishment of herbaceous species on different mined substrates as influenced by pH and salinity

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1.1 Background

Mining throughout the world is needed, especially coal mining to satisfy our need for electricity, and our entire economy is therefore dependant on mining, however our society is dependent on food security provided by agriculture. Competition exists for resources between mining and agriculture, since the rehabilitated land's agricultural potential is changed after mining and it is yet to be proven that the land will reach the same potential again.

Mining is the second oldest form of industry in the world and is still on the increase even though there exist many negative environmental effects. Mining produces by-product tailings materials, waste products and slurry disposed in large dams. These tailings materials can often contain higher than the normal threshold of heavy metals such as copper (Cu), zinc (Zn), lead (Pb) and aluminium (Al) (Shu et al., 2002). These metals can in addition to the physical disturbance of the soil structure due to the physical mining progress cause the soil quality to decrease. The by-products of mining, for example pyrite present in coal and gold tailings can generate acids that lower the pH of the substrates which in turn affects growth of plants (Shu et al., 2002). The decline in soil quality makes it very difficult for plants to survive and these areas usually have no or very little plant growth (Shu et al., 2002).

The grass biome of South Africa is where most coal mining occurs and covers about 350 000 km² and of this total area (350 000 km²), approximately a 100 000 ha is currently under mining activity or has already been mined (Neke and Du Plessis, 2004). This information is out-dated but still shows the magnitude of mining and the challenges we face. These areas that are mined needs to be rehabilitated and made productive by law (Mining and Petroleum Resources Development Act 28 of 2002 (MPRDA) (Mentis, 2006). Under the MPRDA, owners of mines remain responsible for environmental liability, ecological degradation and pollution until the minister has issued a closure certificate (Maczkowiack et al., 2012). The certificate will only be issued when the required documents (closure plan and an environment risk report) are approved and the environment has been rehabilitated (Marais, 2013).

The most commonly accepted practice to rehabilitate these mined areas is to establish pastures on them once the topsoil or subsoil is replaced. The rehabilitation of these mined areas with pastures can be difficult and is a timely process especially in open cast mining where large areas of land have been cleared (Mentis, 2006). The removal of coal (surface mining) and gold (underground mining) for example, causes large amounts of tailings and solid waste to be left behind during the mining process. This unwanted waste is left behind which pollutes the soil and changes the soils properties (Masto et al., 2010).

Coal is the most abundant fossil fuel and the economy of most countries are depended on it, and is therefore difficult to replace its use. The process of surface mining entails that large amounts of topsoil is required to be removed by draglines to get to the underlying coal resource. The soil that is removed is stockpiled into large heaps that can be seen in the most on Mpumalanga and North West province.

Deep shaft mining in gold and platinum industry result in the substrate to be brought to the surface and once processed the discarded material is placed in waste disposal sites where rehabilitation is required which includes the re-vegetation with grass. The transporting of the substrate by large vehicles contributes to the compaction of the soil and the difficulty in the establishment of grass species (Chapman and Younger, 1995). This highlights the enormous challenge to rehabilitate these areas.

The ecosystems found in mines are not always functional, but will become functional again once the rehabilitation process is completed and successful (Morgenthal and Van Rensburg, 2004). The success of rehabilitation not only depends on the species planted initially rather on the longer term species change monitored over time.

The success of rehabilitation can be assessed by using the following criteria, the amount of ground cover (plant cover) or the amount of the biomass produced (Morgenthal and Van Rensburg, 2004). The amount of ground cover by plants is the most important, the more ground that is covered the less chances there is of erosion. Erosion is influenced not only by ground cover, but also by the incline of hills and the deepness of soil. As in agriculture, the harvest of vegetation is important on rehabilitated mines, as this stimulates root growth and promotes plant regrowth, showing again that rehabilitation is a continuous process (Morgenthal and Van Rensburg, 2004). Fires can also have an effect on the success of rehabilitation. Rehabilitated mines are large open areas that can easily burn (due to large volumes of biomass especially in grasslands) and is it therefore important to monitor and manage the amount of fires. These fires can cause damage to the soil and to the grass species if it is to frequent or to hot (Morgenthal and Van Rensburg, 2004).

Mine tailings are usually high in heavy metals, for example Al and Cu. The pH is also altered in these tailings, in most of the cases being very low. Low pH can cause the effect of Al to be more severe whereas too low or high pH's can make some elements less or more available for plants, often having a toxic effect. High Al levels in the soil has the same effect on plants and in the soil as Cu, therefore high levels of Al are unwanted (Sheldon and Menzies, 2005). High levels of Cu and Al reduces the uptake of calcium (Ca), potassium (K), magnesium (Mg) and manganese (Mn) by plants, therefore contributing to reduced growth (Sheldon and Menzies, 2005).

Copper is found in areas with low pH's, as in the case with many mined areas, the phyto-availibility of Cu increases as the pH becomes lower and vice versa (Sheldon and Menzies, 2005). High levels of Cu causes the Cu molecules to bind to the root and prevent nutrients from entering the plant, therefore stunting growth (Sheldon and Menzies, 2005). The damage and reduction in root hair numbers together with restricted root development can also severely affect phosphorus uptake by the plants, The damaged roots result in poor nodulation in legumes, which cause these plants to have a lack of nitrogen (N) (Sheldon and Menzies, 2005).

The seedling phase of pastures and most plants in general are known to be the most important stage of a plants growth. This is the stage were the plant is the most vulnerable to insects, diseases and competition from other plants (Silcock, 1980, Bopp, 1995). The temperature and the radiation from the sun also plays an important role and again emphasise the importance of a quick and successful establishment (Silcock, 1980). High temperatures and too much radiation from the sun can burn the little seedling to death, therefore the seedling needs to grow and develop fast to prevent this from happening (Silcock, 1980). Apart from the above mentioned factors, the soil health also plays an enormous part in seedling health and the emergence of the seedling (Bigot et al., 2013). High levels of Al for example and low pH of the soil is detrimental to the seedling and the seedling will die (Sheldon and Menzies, 2005, Mattigod and Page, 1983).

Coating seeds is done by a treatment in which polymers, nutrients, fungicides, fillers and insecticides are added to the outer layer of the seed (Detroz and Gago, 1991). These treatments can improve the germination rate of species, depending on the soil it is planted in, therefore it can make establishment of these species more successful. The main aim of coated seeds is to overcome most of the environmental challenges and to give a seed the best chance of survival.

1.2 Problem statement

Mining activities change the profiles and properties of soil that are to be rehabilitated. Additionally, substrates of variable chemical and physical properties are introduced to the soil profile. The soil profile refers to topsoil, subsoil and tailings material, and these different layers need to be kept separate during mining and in most instances it is not always the case and then the mixing of these layers occur. Extreme variation in soil pH as a result of mining activity can result in an increased risk of poor seed germination, seedling establishment and root development or the toxicity of potentially harmful elements.

Coated seeds have shown much potential and can facilitate the establishment of grasses in potentially hostile environments. Seed coatings can buffer the grass seeds from extreme environmental conditions such as drought and severe heat. The seedling will have more nutrients available for growth, therefore will be able to grow roots and shoots faster. The nutrients refer to the nutrients that are added to the outer layer of the seed, this is mainly in the form of nitrogen. Once the coated seed receives water, the coating dissolves and the nutrients are released in the soil around the seed. As the seeds primary roots start to develop the root will grow directly in the soil filled with nutrients and have an advantage to grow faster.

There is currently limited information and data on the functionality of coated grass seeds planted in growth mediums with unfavourable soil pH and electrical conductivity (EC) levels. This study is therefore imperative to improve the knowledge base on such seed coatings in unfavourable growth mediums to improve re-vegetation success of rehabilitated mined areas.

1.3 Aims of research

The aim of this study is to assess whether a seed coating improves the germination potential of coated seeds planted in mined soils and substrates (mining waste).This study will also aim to determine whether the seed coating improves the primary (initial) root development which then facilitates seedling establishment.

1.4 Hypothesis

- Coated seed will have a better germination capacity than non-coated seed in different mining substrates.
- Coated seeds germinate and emerge better in extreme substrate pH's (low/high).
- Coated seeds germinate and emerge better in saline substrates.
- Coated seed germinates and emerges better in wetter soils/substrates.
- Seedlings emerged from coated seed in drier soil/substrate are more vigorous.
- Seed coatings improve the primary (initial) root system of specific grass species in mined soils/substrates.
- Plants grown from coated seeds will have a higher root and biomass in the different growth substrates.

1.5 Literature review

Recent studies focus on increasing the profits and yields of pastures, while decreasing the input costs of farmers and the costs of rehabilitation. Poor seed quality, poor establishment, environmental factors, poor management and increase in fertilizer costs are just some additional factors that contribute to the difficulty of farming (Catroux et al., 2001). These factors can be limited by using coated seed. The coated seed will not germinated if there is not enough rainfall, will lay dormant in the soil. Insects will not carry away or eat the coated seed. These are just two ways in which coatings reduces the impact of environmental conditions and to an extent poor management. The coating helps the seed to fully express its genetic potential to overcome environmental conditions (Vyn and Marua, 2001). The amount of water available and sowing method will also have an impact on the success of the seed coating. The aim is to improve the agricultural yield and production that in turn it will improve the ground cover and the sustainability in rehabilitation.

Coating is a relatively new science that incorporates information from various agricultural companies, for example; seed, pesticide and fertilizer companies. Coating seed is done by adding polymers, nutrients, fungicides, fillers and insecticides to the outer layer of the seed (Detroz and Gago, 1991). These treatments can improve the germination rate of species, depending on the soil it is planted in, therefore it can improve the establishment of these species. These coatings come in different combinations and ratios as to eliminate or reduce problems associated with specific areas (Vyn and Marua, 2001). The coating of coated seed will disintegrate when there is enough water, but will remain intact when there is not enough water, making the seed more drought resistant (Detroz and Gago, 1991). These chemicals are added to the outer layer of these coated seed, and are slow releasing so that the activity lasts longer and seed is supported for longer periods in unknown soil/substrate micro-conditions and therefore have better chance of surviving (Detroz and Gago, 1991). The main aim of coated seed is to overcome most of the environmental challenges, to increase the success rate of germination and emergence of these seed and to give a seed the best chance of survival.

The ratios of seed coating additives will differ for different industrial practioners. There is still a debate on which coated seed are economically viable or not. Coated seed is expensive, but there are many positive attributes in the long term use. The amount of nutrients and pesticides added to the outer layer of the seed in coatings is little compared to conventional fertilizers added to large fields (Ashraf and Foolad, 2005). Coatings ensure that there is a smaller impact on the environment and many of the problems associated with overuse of pesticides and large volumes of fertilizers are reduced (Ashraf and Foolad, 2005). It is also cheaper and more efficient to add all of these smaller amounts of nutrients (Harman, 1991). All the above mentioned aspects ensure fewer establishment problems and which will help overcome harsh environmental establishment conditions that can lead to a poor pasture stand.

1.6 Seed

Seed is where it all begins for a plant, the seed germinates, emerges and then grows into a mature plant. The seed is the ovule of a plant that has been fertilized by the pollen from the same or from a different plant, the ovule then develops into a seed. The seed is divided into three parts, the embryo, the endosperm and the seed coat. The seed coats main function is to provide protection to the embryo found inside. The embryo contains the cotyledons, epicotyl, plumule, hypocotyl and the radicle (Fairey et al., 1999). The attachment of the cotyledons occur at the epicotyl. The plumule is will develop into the shoot after germination and the radicle will develop into the primary root after germination (Fairey et al., 1999). Once the seed receives water the radicle and plumule develops and breaks the seed coating and germinates.

There is huge differences in the shapes and sizes of seed for example: *Cenchrus ciliaris* has a very big and woolly seed, compared so *Eragrostis curvula* that has a small and very smooth/ naked seed. *C. ciliaris* has the woolly appearance so as to catch water more easily and also to buffer more against the environment (Marshall et al., 2012). *C. ciliaris* is adapted to dry conditions and the seed has also evolved to show this drought resistant trait. The smaller the seed the more fine a seed bed needs to be to ensure that there is enough contact with the seed and the soil (Nel, 2014, Catroux et al., 2001). If there is not enough contact the seed will not germinate or it will germinate and die. Germination is the next process in the life cycle of a seed or plant.

1.7 Germination

Germination is the process where the seed moves from a dormant state and starts the formation of a new plant. It starts when the seed takes up water and ends when the radicle breaks through the surrounding tissues and becomes visible, initiating the seedling phase (Fairey et al., 1999, Koornneef et al., 2002). Many tropical species have small seed, for example *Cynodon dactylon* and these seed have small endospore reserves. Therefore with adequate temperature, heat and water, germination occurs within three days (Fairey et al., 1999). The germination process continues when the vertical coleoptile emerges together with a vertical seminal root growing downward.

The germination process or phase differs in duration between different species and cultivars, even in the same cultivar if the seed was coated. The higher the germination rate of seed that is planted, the higher the establishment of a particular plot will be, therefore seed companies strive for the highest possible germination rate. Fast emerging species influence how slow emerging species will establish, example the domination of *Chloris gayana* grass over *Digitaria eriantha* (Theunissen, 1997).

Germination rate of seed is primarily effected by two factors; the genetics of the seed and by environmental factors (Bewley and Black, 1994). The genetic part of germination is what seed breeders and seed companies strive to improve, it is the one aspect were they have control over, whereas the environment can only be managed. Genetics cannot be controlled but they can improve the success in the establishment of pasture species in certain environments (Dürr et al., 2015). The seed contains the genetic potential of the plant, but is guarded or protected by the dormancy of the seed. Different types of seed dormancy exist. Breeders select genotypes with improved affinities to specific environments, resulting in species/ cultivars/ genotypes with less need for dormancy (Dürr et al., 2015, Koornneef et al., 2002). These selections have not been applied to a large extent to subtropical grasses and seed dormancy traits have a large influence on the establishment of plants.

There are various factors that can inhibit or effect germination rate. The seed that is used needs to be of good quality, lower quality seed will result in lower germination rates and seedling vigour. The seedbed needs to be prepared correctly, the seedbed must be fine enough and all weeds and competition needs to be removed, if not, the germination rate will lower (Nel, 2014, Catroux et al., 2001). The seedbed must be fine enough to ensure that there is enough seed to soil contact and also enough water available in the soil. The depth of planting is also important, planting to deep or to shallow will lower germination (Nel, 2014). The species or cultivar selected must be suitable for that environment. The weather patterns and the climate must also be known for a specific area, therefore the timing when to plant in the particular area is important, therefore synchronising planting with rainfall events is important. High levels of salinity and high pH can also have a detrimental effect on germination.

Water, composition of air in the soil, temperature and light are the normal requirements for seed to germinate under environmental conditions (Dürr et al., 2015, Hadas, 2004). The soil or substrate that the seed is planted in should not be too compacted to prevent germination. It is not only the factors, mentioned above, on their own that impacts germination rate but the interaction between them. The quality of water is also important especially in coated seed. Water is responsible for all chemical reactions in the seed and the soil, and therefore a lower germination rate will result if the water is of lower quality (Hadas, 2004, Dürr et al., 2015). Lower germination rate is also affected by higher levels of evaporation and soil moisture contents at high temperatures. Temperature plays a very important role in the germination rates of plants. High temperature will decrease germination rate and lower temperature will increase germination rate in some plant species, this is species specific and will definitively differ between species (Zhang et al., 2010).

1.8 Environmental factors that affect seed and germination

a) Effect of pH

The term pH refers to figure indicating the acidity or alkalinity of an aqueous solution ranging from 1 up to 14 where one is very acidic and fourteen is very alkaline. A pH of 7 is referred to as neutral pH. The pH is a negative logarithm to base ten of a molar concentration of hydrogen ions in a solution that is measure in moles per liter.

The pH of the soil affects the microbial activity of microbes in the soil (Geilfus and Mühling, 2014). High levels of carbonate and bicarbonate, usually found in soils with high salinities, causes damage to plants due to salt stress but can also be caused by high pH (alkali stress) (Yang et al., 2008). These effects of high pH, alkali stress, is much worse than the effects of salinity (Yang et al., 2008). High salinity levels causes the effects of high pH to be more severe. Young leaves were found to increase in size and length with increasing salinity and increase in pH.

The pH of the soil on its own is not a major problem, but is very important in the release of some minerals from the soil, making minerals more available to plants or preventing the release of some less desirable minerals from the soil (Mattigod and Page, 1983). A study was done were the uptake of Al was measured in soils with low pH, and it was found that the uptake of Al increased as the pH decreased (Dory, 1995). The uptake increased dramatically as the pH (H_2O) dropped below 4.4 (Dory, 1995). The pH also affects the amount of N that was taken up by the plants. The highest amount of ammonia (NH_4) that was taken up was at a pH (H_2O) of between 6.5 and 8.5 and the lowest was were the pH was more than 6.5 and 8.5 (Jampeetong et al., 2013).

A study where *Coix lacryna-jobi* was grown at different pH levels found that the pH effected the morphology of the plant and especially the roots of the plant. The roots had a larger number of lateral roots and had bigger roots at a pH (H_2O) of 6 compared to a pH of 3.5. Therefore plants were healthier at neutral pH compared to lower pH (Jampeetong et al., 2013).

b) Effects of Salinity

Salinity is a major problem mostly associated with land that is irrigated with saline water (So et al., 2006). Some literature estimate that 20% of dry-land and 33% of irrigated land have to high levels of salinity (Ashraf and Foolad, 2005). Saline soils are usually very high in Sodium Choride (NaCl) and this causes the exchangeable sodium percentage (ESP) to increase, this in turn will cause soil to decrease in percolation rate (So et al., 2006). Studies done in Israel show that plant growth is affected by NaCl at levels of 0.150 M (moles) and this just shows the importance of these studies to determine the effects of salts on plant growth (Radhakrishnan et al., 2006). There are limited studies that show the effect of salt water and saline soils on coated seeds, therefore studies are recommended on this topic. High levels of Na interfere with nutrient balance, osmotic regulation in plants and can change the structure of the soil (Radhakrishnan et al., 2006). The normal range of salinity in the field is between 4 and 10 dms^{-1} (EC) (Setia et al., 2011).

High levels of salinity will lower germination percentages in grasses. Germination and plant growth is lowered with increasing salinity or increases in soluble salts. The increase in salts reduce plant growth by affecting the osmotic effect of the plants (Munns and Termaat, 1986). Ion antagonism is a major problem in saline soils. Ion antagonism is when the increase in salt reduces the uptake of another mineral, for example Ca when there is too much Na in the soil (Grattan and Grieve, 1999). Too high levels of salinity will cause very low phloem mobility and this in turn will cause low levels of Ca and this can result in plant death (Grattan and Grieve, 1999). Therefore the soil requirement of Ca increases with an increase in Na. A study done on the effects of salinity on kikuyu showed that increasing the NaCl levels from 0.200 M to 0.400 M reduced the germination from 70% to as low as 5% (Muscolo et al., 2013).

Germination in plants are controlled and regulated by enzymes called amylases and glucosidases. They are responsible for the hydrolysis of starch on the reserves of seed. They are responsible for the release of energy that is needed for the metabolic responses and these enzymes are severely affected when levels of NaCl are too high (Muscolo et al., 2013). The lower effects of germination under high levels of NaCl is caused by osmotic stress due to the lack of reserve utilization caused by the lower hydrolytic enzyme activities and the lower water content within the seed and plants. The enzyme B-amylase, is most affected by these high levels of salinity (Muscolo et al., 2013). The energy that is needed for the plants to draw water from the soil increases and therefore the osmotic pressure decreases. When the salinity increases the osmotic pressure lowers even more, the concentration of salinity also increases as the soil dries.

High soil salinity is when there is high concentration of soluble salts and when the electro conductivity is more than 4 dsm^{-1} (Maas and Nieman, 1978). Soil salinity does not only effect the plant growth and production but also the microbial activity in the soil and the soil organic carbon, therefore affecting decomposition rates in soil (Setia et al., 2013). The increases in soil salinity decreases the soil organic carbon stocks. Microbes are affected because salinity effects the osmotic potential which in turn effects their activity (Rietz and Haynes, 2003). High levels of Na and Cl in very saline soils causes an imbalance of other minerals in plants, for example; K and Ca. This effects the partitioning, transport and availability of nutrients in plants and this effects the growth and germination of the whole plant because it causes imbalances (Tuteja, 2007).

Osmotic potential is a more accurate way to measure the impact of salt on plant growth, because two different soils can have same the EC values but have different osmotic potentials (Setia et al., 2013). Roots are more effected than any other part of the plants. The high levels of salt causes a low osmotic potential of the soil solution that in turn results a lower amount of water that is taken up by the plants. Ion imbalance and ion toxicity is also a problem caused by high levels of salinity (Setia et al., 2013)

Salinity and pH usually have an additive effect on each other, on their own they are not harmless but together they can be harmful. The increase in pH and salinity increased the ratio of Na to K. The levels of Na increased while the levels of K decreased when the levels of salinity and pH increased (Geilfus and Mühling, 2014). In a study conducted by Geilfus and Mühling (2014) it was found that the effects of pH and salinity on their own were not as detrimental as the combined effects of high salinity and pH. High levels of carbonate and bicarbonate, usually found in soils with high salinities, causes damage to plants due to salt stress but is also caused by high pH (alkali stress) (Yang et al., 2008).

c) Water availability and field capacity

Water availability and field capacity is also important in germination. Field capacity is also referred as the saturation level of the soil. Water potential is a measurement of the energy status of the water in a system. It indicates how tightly water is bound, structurally or chemically, within a substance. Factors of a soil profile that will influence this is clay, compaction, and Mg induced hydrophobic properties and organic material (Cassel and Nielsen, 1986).

The easiest way to determine the field capacity of soil in a controlled environment is by using the WP4-T machine or also known as the Decagon's WP4 Dewpoint Potential Meter. The WP4-T machine is used to measure water potential, it is measured in MegaPascals. It measures water potential from 0 to -300 MPa with an accuracy of ±0.1 MPa from 0 to -10 MPa and ±1% from -10 to -300 MPa (Devices, 2007). The machine has mirrors on the inside and water condensates on these mirrors. At certain point there exists an equilibrium between the moisture in the sample and the moisture that condensate on these mirrors. This equilibrium gives the water potential reading (Devices, 2007).

d) Soil (influence on germination)

The soil or media in which a seed is planted in is very important in the germination process. There is a chemical and physical aspect to soils. The chemical aspect refers to the minerals and metals that are found in the soil, for example aluminium and sodium. High levels of Al and Na can have a negative effect on the germination of seed, the high levels are toxic to the seed and the seed will not germinate (Rout et al., 2001). The pH also refers to the chemical aspect, the high and low pH will also have a negative effect on the germination of plants. The amount of organic matter in the soil is also important. The organic matter is the part of the soil that is 'alive', higher organic matter will ensure higher growth and germination in plants. Organic matter refers to the decaying leaves and decaying matter found in the top layer of soil, it provides nutrients to plants and can also with hold water

The physical texture is referred to the soil texture, is there a high amount of clay or do the soil contain a very coarse structure. Soils with a higher silt and clay content will have a higher germination rate due to a better seed to soil contact (Nel, 2014). The smaller particles will ensure that there is enough contact made with the soil. In soils that are very coarse there will not be enough contact made by the soil and seed, therefore the seed will not get enough water and there will be no germination. These are very important factors to note in the soil so as to ensure maximum germination.

e) Mining substrates

The most common mining process by-products/tailings found in South Africa and the world

i. Red sandy loam

Red sandy loam is not a mining process by-product but will be used as a reference growth substrate in research studies, because it is used as the topsoil in mined land rehabilitation. Red sandy loam soil is a good growth substrate for plants to grow in, but is prone to erosion (Davenport et al., 1998). Red sandy loam has a low percentage of silt and clay but a higher amount of sand (Annexure B). The pH (KCl) of red sandy loam is under 5 and it will be suitable for adequate germination. Germination will be lower if the pH (H_2O) is lower than 3 and higher than 8 (Stubbendieck, 1974). In studies on *Medicago sativa* it was found that there was no germination at pH 2 and only 2 % germination at pH 8 (Stubbendieck, 1974).

Water holding capacity will be lower due to low levels of silt and clay because these two have large surface areas and therefore bind more water molecules. These substrates will be high in oxygen due to their low levels of clay, whereas clay and silt bind tightly and therefore exclude oxygen. The germination is expected to be high in this substrate.

ii. Gypsum

Gypsum is usually found in areas near Potchefstroom in South Africa. Gypsum have high levels of phosphorus and calcium (Annexure A) and due to these high levels Gypsum is commonly used for fertilizers and a source of lime in agriculture (Soule et al., 1952). Companies for example SA Lime and Gypsum, market gypsum specifically to lower the level of sodium in soil and to raise the level of calcium. Gypsum can also have high levels of sulphur (Annexure A). High levels of phosphorus will not have a negative effect on growth, however in some studies high phosphorus levels contributed to 51% higher root respiration rate and in turn higher yield (Peng et al., 1993). Gypsum has a very fine texture (Annexure B) and can be prone to erosion but it is a perfect growth medium for seeds due to the seed having a bigger surface area covered by the substrate (Davenport et al., 1998).

iii. Gold <2% pyrite tailings

Pyrite is known as fool's gold due having a very similar appearance to that of gold. Pyrite is also called iron sulphide. Gold < 2% pyrite will contain a smaller amount of pyrite. The pH

(KCl) of Gold < 2% pyrite is 5.3 (Annexure A) and this is an adequate pH for germination and growth (Stubbendieck, 1974). Gold <2% pyrite will have a higher water holding capacity than Red sandy loam due to higher level of silt, but only slightly and will also have high levels of oxygen (Annexure A and B). Gold <2% pyrite has the same level of metals as red sandy loam except for cobalt, copper, zinc, uranium and arsenic that are higher. Copper can have a negative effect on germination by affecting root development and also have health implications for humans and animals (Muccifora and Bellani, 2013).

iv. Gold > 2% pyrite tailings

Gold > 2% pyrite contain a larger amount of pyrite compared to Gold < 2%, therefore will have a higher iron and sulphur content. The pH (KCl) of Gold>2% pyrite is 6.4 (Annexure A) and this is very close to a neutral pH and pH will not affect germination (Stubbendieck, 1974). Gold>2% pyrite will have a low water holding capacity due to the low levels of clay and silt (Annexure B). Gold>2% pyrite is also high in calcium, magnesium and sodium this can cause the soil to have a high salinity (Annexure A).

v. Platinum Tailings

Platinum mining in South Africa is big and therefore there are a number of platinum mines and in turn huge amount of tailings. Platinum tailings has a fine texture with high amount of silt in (Annexure B). This texture would provide adequate support for germination. The pH (KCl) of Platinum tailings is high and is around 8 and therefore seen as very high for a growth medium to sustain growth (Annexure A). The pH range where plant growth is possible is between 3 and 8, however the optimal pH is 6 and therefore germination rate will be lower at this pH level (Stubbendieck, 1974). Platinum tailings have high levels of aluminium that can have a negative effect of the germination (Annexure A).

vi. Kimberlite

Kimberlite from Cullinan has a whitish/grey appearance with a very coarse texture (Annexure B). Kimberlite is formed from magma and very hot temperatures and is a source of diamonds (Swami et al., 2007). Kimberlite is crushed so that the diamonds can be removed by a screening process (Ndlovu et al., 2014, Reid and Naeth, 2005). When diamonds are removed large amount of kimberlite by-product remains (Reid and Naeth, 2005, Swami et al., 2007). Kimberlite has a pH (KCl) of above 7. Kimberlite will have a low water holding capacity due to high percentage of coarse and very coarse sand but will have high oxygen levels.

vii. Fluorspar

Fluorspar is the by-product of mining for calcium fluoride. Fluorspar is usually found in the area near Zeerust (Limpopo Province). Fluorspar has a texture with a high percentage of very fine sand component (Annexure B) that will ensure adequate seed to substrate contact that will improve the germination rate. The pH (KCl) of fluorspar is 7.9 and the CEC is 1.1, the high pH can reduce the germination rate due to high alkalinity (Annexure A) (Stubbendieck, 1974). Fluorspar will have a low water holding capacity due to the high percentage of sand, but will have high oxygen in the soil.

viii. Andulusite

Andulusite is a gem and is mined to extract these gems and therefore these andulusite tailings remains behind. Substrate is usually found in the Groot Merico area. This substrate is very high in silt and clay and will have a very good water holding capacity because water more water molecules will be able to be bound (Annexure B and C). Andulusite is very high in aluminium (Annexure A). The pH (KCl) of Andulusite is 5.5 which is optimal for germination and also has a CEC of 3.6, this will have a positive effect on binding minerals, and therefore have a low leaching risk.

ix. Coal discard tailings

Coal discard tailings are usually in areas near Witbank, Mpumalanga, but the mine fields in South Africa are increasing and it is reported that there is 1120853 million tons of this discard and slurry coal in South Africa (Wagner, 2008). Coal tailings are very high in aluminium and has a pH (KCl) of below 4 (Annexure A). Aluminium is very toxic to plants and this high level will definitely lower germination. Coal tailings is also very high in sulphur this will also definitely limit growth. Some reports show that sulphur in coal tailings material can go up to 8% (Wagner, 2008). Coal tailings have a very fine texture (Annexure B).

1.9 Influence of environment on plant roots

Roots are divided into two categories; tap roots and fibrous roots. Tap roots are thick roots that develop downwards and have a small amount of lateral roots, Lucerne is an example of tap root plant. Fibrous roots are when the roots branch and develop into many directions, most grasses fall into this category. Root meristems are found on the ends of roots and these are the growing points of the roots and are also sensitive to damage or where damage occurs.

The root cap protects the root meristems from damage when the root moves through the soil. The root caps are constantly replaced due to the damage that occurs when growing (Hartmann et al., 1981).

Roots of plants are very essential in absorbing nutrients and water from the soil and they are very sensitive to stress. Roots are in direct contact with the soil and the soil solution and are therefore the first to come in contact with saline solutions and therefore the first to be damaged (Bernstein and Kafkafi, 2002). High salinity levels inhibit the root growth and development (Imada et al., 2015). The roots are responsible for detecting salt in the soil and this is known as salt perception. This perception is important for the functioning of roots and for the transmission of signals from the roots to the shoots so that the right adaption by the plant is made (Zhao et al., 2013). Plant roots have adapted to cope with salt stress by enhancing the amount of sodium exclusion and by reducing the amount of sodium entering, but this is only for moderate levels, too high levels will rapidly reduce root growth (Zhao et al., 2013).

Leaf area is one of the best ways to see if plants are under stress or not. Visually, smaller leaves usually indicate that plants are under stress. The leaf area of plants are also affected by stress i.e. drought, salinity or high/low pH. The leaf area of plants are expected to be smaller and the leaf area reduces in size as stress increases, this was found in studies done on various grasses were the salinity levels were high (Ortega and Taleisnik, 2003, Munns and Termaat, 1986). The lower leaf area is due to stress effecting the cell division process of plants, therefore cells do not divided properly and therefore there is a reduction in the photosynthetic area of the plant (Taleisnik et al., 2009). The lower photosynthetic area will in turn result in a lower production of leaves and therefore a lower total biomass production.

The most accurate way to measure leaf area is by making use of the Licor (LI-3100C) machine. The leaf samples are placed on a transparent belt that takes the leaf into the machine. The sample then pass a fluorescent light that causes the sample to be reflected, via three mirrors, on a camera in the machine. This design and mechanisms of the machine then gives you an accurate area of the sample. The machine is designed in such a way that the leaves are flattened out so as to give an accurate reading of the area.

1.10 Test species

Six of the most popular and commonly used species for rehabilitation of mined areas are *Cenchrus ciliaris*, *Chloris gayana*, *Cynodon dactylon*, *Digitaria eriantha*, *Eragrostis curvula* and *Medicago sativa* (Truter, 2015, Rethman, 2000, Mentis, 2006). The rehabilitation process or more accurately termed as the ‘bridging succession phase’, usually takes five years and incorporates various species (Truter, 2015, Rethman, 2000). In year one a combination of *E. tef* and *C. gayana* in a ratio of 9:1 is used, the *E. tef* provides the micro- climate for the *C. gayana* and species in the consecutive year (Dickinson, 2004, Truter, 2015). In year two the same combination is used just in different ratios, the ratio is 2: 8, *E. tef* to *C. gayana*. In year three the *E. tef* is completely left out and *E. curvula* or *D. eriantha* is used, depending on environment, in combination with *C. gayana*. The ratio used is 6 (*C. gayana*): 4 (*E. curvula* or *D. eriantha*) (Truter, 2015). In year four and five, the same species are used, the ratios only change. In year four it is 1:1 and in year five its 3 (*C. gayana*):7 (*D. eriantha* or *E. curvula*) (Truter, 2015).

The other three species used are also important in other circumstances and environments. *Cynodon dactylon* is also important due to its creeping growth habit as mentioned above. *Cynodon dactylon* is used on slopes or areas that are prone to erosion, therefore prevents excessive degradation due to erosion (Dickinson, 2004, Morgenthal and Van Rensburg, 2004). *Cenchrus ciliaris* is important in areas that receives low rainfall, and can be used in the place of *D. eriantha* or *E. curvula* in areas that needs to be rehabilitated (Dickinson, 2004). It is important to get the maximum germination and production from areas that are cultivated with *M. sativa* and it is important to improve its success in any way possible.

a) *Cenchrus ciliaris* (Foxtail buffalo grass)

Cenchrus ciliaris also known as blue buffalo grass is a perennial summer grass that is tufted with limited number of rhizomes (Dickinson, 2004, Marshall et al., 2012). It has a bluish green colour and the leaves are 25cm long and 1cm wide. It becomes unpalatable when mature and can reach lengths of 1.2 meters (Dickinson, 2004).

Cenchrus ciliaris is found in hot areas, does well in low rain fall areas in South Africa and is drought resistant due to its deep root systems, therefore reaching greater depths in search of water (Rao et al., 1996). The trait that makes *C. ciliaris* so popular is its remarkable resistance to drought and that its underground runners develop quickly (Nawazish et al., 2006). *Cenchrus ciliaris* is adapted to most soils but does not do well in light sandy soils and does well in calcareous soil. It will not tolerate waterlogged conditions and will not survive in very acidic soils (not lower than a pH 4.5 (KCl) (Dickinson, 2004, Marshall et al., 2012).

Cenchrus ciliaris seed is large compared to other seeds, therefore it is easy to sow. Seeding time is from February to end March. *Cenchrus ciliaris* will do well if P is added, therefore do well in soils high in P (Dickinson, 2004, Marshall et al., 2012). *Cenchrus ciliaris* is used for grazing by cattle and sheep and hay making, however to utilize the best quality of this species, *C. ciliaris* should not be allowed to reach maturity.

b) *Chloris gayana* (Rhodes grass)

Chloris gayana is a perennial grass (is only productive for 3-5 years) and can be stoloniferous and tufted. *Chloris gayana* grows in wet areas, along rivers and also in disturbed soil (Van Oudtshoorn, 1999). *Chloris gayana* is known to be very salt tolerant and can survive in very saline conditions, they do this by the salt glands located in the leaves that excrete excessive salts (Kobayashi and Masaoka, 2008, Kopittke et al., 2007, Ortega and Taleisnik, 2003).

Chloris gayana is popular because it produces large amounts of seed and establishes very fast and easy. It is also popular because of its creeping growth, as compared to *C. ciliaris* this species is not as good quality (Dickinson, 2004, Luna et al., 2002). It also does well in dry areas of South Africa and is suited to most soil types, but not drought tolerant (Dickinson, 2004). *Chloris gayana* is sensitive to very acidic soils and also alkaline soils, does best in soils with a pH of between 5.5-7.0 (KCl) (Dickinson, 2004). The seed sizes of *Chloris gayana* are smaller than *C. ciliaris*, from about 5 mm long and 1 mm wide. These hulls sometimes open up and then the seed can fall out.

Chloris gayana is not able to secrete Mg ions and will start secreting K ions when there are high levels of Mg which will affect the plants osmotic regulation (Kobayashi and Masaoka, 2008). Although *C. gayana* is salt tolerant, the production will be lower in saline soils compared to non-saline conditions, but will still have a higher production compared to other grass species (Luna et al., 2002). *Chloris gayana* is used mainly for grazing in hotter areas with higher rainfall, and it is a palatable species that can withstand trampling. *Chloris gayana* is used in areas with high salinity and alongside newly build roads (Van Oudtshoorn, 1999).

c) *Cynodon dactylon* (couch grass)

Cynodon dactylon is a summer sub-tropical or warm temperate perennial grass that has *rhizome*s or runners (stolons) and therefore it is a creeping grass (Dickinson, 2004, Speranza, 1995). This creeping growth habit makes *C. dactylon* very popular in the stabilizing and rehabilitation of mine dumps. *Cynodon dactylon* grows in all soils, establishes easily and does well in disturbed soils (Van Oudtshoorn, 1999, Manuchehri and Salehi, 2014). *Cynodon dactylon* is drought resistant and can invade areas if not managed properly (Dong and Kroon, 1994). *Cynodon dactylon* is not as nutritious and palatable as *C. ciliaris*, but remains green for longer periods and is cold resistant but not frost resistant (Van Oudtshoorn, 1999). *Cynodon dactylon* species grows best at a pH of between 5.0-6.5 (KCl) (Dickinson, 2004). The seed size of *C. dactylon* is very small, therefore the seed bed must be very fine to ensure enough seed contact with the soil, if not, germination rate will be lower.

d) *Digitaria eriantha* (Smuts finger grass)

Digitaria eriantha is a perennial subtropical grass species that can be tufted with stolon's and is spread throughout humid tropics. *Digitaria eriantha* grows in areas where rainfall is higher than 500 mm per year, but will not tolerate waterlogged conditions and drought (Dickinson, 2004, van Rooyen and Dannhauser, 1988, Hacker et al., 1993). Its common name is Smuts finger grass or common finger grass and it is mostly found in southern Africa (native to Angola, Botswana, south Africa, Mozambique and Namibia), but it's increasing in popularity in Australia, Argentina and USA (Dickinson, 2004) It is mainly used as a pasture grass that is used in extensive beef and sheep farming. *Digitaria eriantha* is sweet and keeps its palatability until late winter. It is also a good source of foggage and silage (Van Oudtshoorn, 1999, Tow et al., 1997, van Niekerk et al., 2008)

Digitaria eriantha is adapted to all soil types, especially gravel soil and also including those of lower potential and clay soils (Dickinson, 2004, Theunissen, 1997). The pH of the soil should not be lower than 4.5 (KCl), since this will limit growth. Growth will also be limited if levels of K and P in the soil is low (Dickinson, 2004, Hacker et al., 1993). *Digitaria eriantha* also has small seed, bigger than *C. dactylon* and just smaller than *C. gayana*. The seed bed also has to be very fine to ensure god seed/soil contact. Germination is affected in heavy clay soils and will drop in these soils. The seeding density should be increased in these clay soils (Dickinson, 2004, Van Oudtshoorn, 1999).

e) *Eragrostis curvula* (Weeping love grass)

Eragrostis curvula also known as weeping love grass and is a summer, perennial grass that has its origin in South Africa and is commonly found as a planted pasture in South Africa (Dickinson, 2004, Wan and Sosebee, 1998). *Eragrostis curvula* can grow in all soil types but does the best in sandy soils, well drained soils, and in areas where rainfall is higher than 650 mm per year, therefore it is not drought tolerant (Dickinson, 2004, Colom and Vazzana, 2001, Voigt et al., 2004). *Eragrostis curvula* is naturally found in areas where soil is disturbed and is also found in areas with intense grazed veld (Van Oudtshoorn, 1999, Wan and Sosebee, 2002), this grass is also tufted and flowers from August to June in South Africa. *Eragrostis curvula* is very tolerant of high levels of N fertilizers, up to 60 g Nm^{-2} and will grow fast in response to these high levels (Fynn and Naiken, 2009). When large amounts (10 tons) of hay is removed from the pasture, a minimum of 150 kg ha^{-1} of potassium is required to sustain this high yield (Dickinson, 2004).

Eragrostis curvula is sensitive to frost and will die at onset of frost and needs phosphorus to grow (15 mg P kg^{-1}). Phosphorus plays an important role in the process of photosynthesis and protein synthesis, therefore low levels of phosphorus will impede these processes and plants will not grow optimally (Gourley et al., 1993). The more P the plant receives the better it will grow due to higher photosynthesis and protein synthesis in the plant. Potassium is also important, levels of 100 mg K per kg are required (Dickinson, 2004). The pH is also important in *E. curvula* because it can tolerate low pH levels, but will not tolerate low levels of Ca and Mg (Dickinson, 2004, Cox et al., 1988). It can invade areas where veld is overgrazed or disturbed (Van Oudtshoorn, 1999).

Eragrostis curvula can be used for grazing but is preferred for hay making due to high quality of this grass is captured if bales are made at the right time (Van Oudtshoorn, 1999, Voigt et al., 2004). *Eragrostis curvula* has the smallest seed of all the species mentioned, therefore seed bed must be very fine otherwise very little germination will occur. Study done by (Morgenthal and Van Rensburg, 2004) on rehabilitated mines in Bethal, Mpumalanga, found that *E. curvula* and *C. gayana* dominated at rehabilitated sites and did even better when *Eragrostis tef* which was used as a nurse crop in the first year of establishment.

f) *Medicago sativa* (Lucerne/ alfalfa)

Medicago sativa is a perennial legume that is known for its taproots that can reach great depths in search of water in dry areas, therefore it is drought resistant (Dickinson, 2004).

Medicago sativa is usually sown under irrigation for haymaking however, there is an increase in dry land sowing (Dickinson, 2004). *Medicago sativa* does well in most soils, but the pH of the soil is important, pH below 5.0 (KCl) and above 6.5 (KCl) will affect growth and some can die (Dickinson, 2004). *Medicago sativa* establishment and germination is also affected by high levels of salt (Dickinson, 2004). *Medicago sativa* should be sown under cool conditions and very hot temperatures at planting should be avoided. *Medicago sativa* is sensitive to frost but is tolerate to frost after the four leaf stage (Dickinson, 2004). Phosphorus requirement is very high, therefore if the soil is high in P (more than 25 mg P kg^{-1}) it will result in larger yields (Dickinson, 2004). High levels of K and Ca are required for high yields of *M. sativa*, whereas Ca should be at levels of 2000 mg kg^{-1} and potassium (K) above 100 mg kg^{-1} (Dickinson, 2004). *Medicago sativa* seed size is just smaller than *C. ciliaris* with a smooth texture, it is therefore easier to sow these seed.

1.12 Seed coating treatment

The coating of seeds is not a new practice, and it has been applied for many years. In the 1960s, the adding of lime and other organic material to seed had already been used to aid in nodulation or adding agro-chemical applications to aid in the survival of seedlings (Brockwell, 1962). The coating of seeds can be beneficial with the exceptions of a few. The coating is found on the outer layer of the seed and therefore released directly in the soil around the seed. This is ideal for root development and for uptake by roots (Brockwell, 1962). The carriers of the chemicals added to seed coatings are very important. Inferior or carriers of lower quality will cause the whole coating to be of lower quality. The aspect of carriers in coating are constantly researched and new discoveries are still to be made.

There are four common coatings added to seed in South Africa:

a) Nutrients

Nutrients in seed coating are applied through fertilizers, and are only in very small amounts due to the limited ‘space’ around the outer layer of the seed. Fertilizers remain the easiest, fastest and most common way to optimize crops or pastures.

The producer can reduce costs while still producing high yields by only adding the right amounts of the nutrients required by the plant (Hardy et al., 2003, Van Oudtshoorn, 1999). Tissue analysis can be done to see the requirements or the needs of the plants, therefore the amount of nutrients that should be included in maintenance fertilizer can be determined

(Hardy et al., 2003). Soil analysis is also important to do on a regular basis to see what amount of nutrients are still left in the soil after plants have assimilated it and also as a result of nutrients lost due to leaching (Hardy et al., 2003). Tissue analysis can also determine deficiencies or toxicities levels due interactions of minerals in the soil which can affect the availability of nutrients (Hardy et al., 2003).

Farmers spend vast amounts of money each year to counter act the deficiencies of nutrients in the soil by using fertilizers. There is also a movement of farmers towards precision farming where only the deficient nutrients are added and at the right amount to supply the needs of a specific plant. The nutrients in the coating are only adequate to support the initial growth and root development, there after the plant uses nutrients in the soil. Nutrients in the soil are therefore still important and a fertilization plan is still needed (Van Oudtshoorn, 1999, Dickinson, 2004). The most common nutrients added in the coating are phosphorus (P), K, Ca and sulphur (S).

Micro nutrients are very important in the physiological development of the plant and especially in the development of the roots. These micro nutrients are required in small amounts and can therefore be supplied in the fertilizer or even better on the seed itself. High levels of these micro nutrients can cause toxic effects and this in turn can cause problems. One cause of such a toxicity is the immobility of micro nutrients in the plants (Gupta et al., 2001). Many crops are sensitive to deficiencies, however grasses to a lesser extent, therefore micro nutrients should always be included at the right amounts (Gupta et al., 2001).

Phosphorus is important in the establishment of pastures because phosphorus will support strong seedlings by encouraging tillering and root growth. This will enable them to be stronger competitors against weeds and other unwanted species (Scott and Blair, 1988). The amount of P required depends on the specific species or crops. The availability of P is also affected by soil pH and the clay content in the soil (Scott and Blair, 1988). Phosphorus is more important in legumes compared to grasses, and is required by the inoculants so that the inoculant is stimulated to colonise the roots of legumes to ensure efficient nodulation (Gourley et al., 1993).

Potassium is usually only required is small amounts and deficiencies are more of a problem than toxicities. When there is a deficiency of K, plants will have a lower tolerance to frost, drought and diseases (Berg et al., 2003). Potassium is needed for the cytoplasmic functioning of the plant, the more K there is, the faster the plant will grow due to greater cytoplasmic development (Leigh and Wyn Jones, 1984). Potassium is also very important for the plants metabolic processes, deficiencies will cause the plant to slowdown in growth and eventually die (Leigh and Wyn Jones, 1984).

Sulphur is taken up by plants in an inorganic form from the soil. Sulphur levels are low in soils near cities, sandy soils and soils with low organic matter, therefore this shows the importance of S in coating and fertilizers (Berg et al., 2003)

There are other nutrients that also play a role in development of plant and they are; Molybdenum (Mo), Zn, Cu, Mg, Boron (B) and Iron (Fe). Molybdenum is needed for protein synthesis and N fixation in plants. Deficiency of Mo is linked to low pH in soils, therefore lower pH will cause deficiencies in Mo (Gupta et al., 2001). Higher levels of Mo can be poisonous to animals so the right amount is very important in fertilizers and toxicity is also associated with high pH (Van Oudtshoorn, 1999). Molybdenum has synergistic effects with phosphorus and antagonistic effects with Cu and S (Van Oudtshoorn, 1999).

b) Pesticides

Pesticides have become a very important component in coated seeds. Pesticides in general include fungicides and insecticides that are important in agriculture (Van Oudtshoorn, 1999). Pesticides on seed, reduces the amount of pesticides added to the environment by conventional methods due to the fact that only a small amount is added to the outer layer of the seed (Van Oudtshoorn, 1999, Skinner et al., 1997). In addition to the reduction in environmental effects, the farmer saves money due to the reduction in pesticides. Fungicides and insecticides are the only pesticides added to coatings currently, however there is research being conducted to add herbicides to seed coatings (Kuchlan et al., 2010, Taylor et al., 1998).

As mentioned earlier chemical carriers are important and there is a small amount of them that can be added to the outer layer of seeds and not have a negative effect on the pesticides, depending on the pesticide used. The carrier is not the only important factor in pesticides but also the method of applying the coating (Scott, 1998, Taylor et al., 1998). Fluid seed treatment should not be used in a dry seed system. The carriers of the chemicals are just as important as the chemicals themselves (ISTA, 2003).

c) Inoculants

The main purpose of inoculation is for N fixation in legume crops and has been done for many years (Catroux et al., 2001). The aim of inoculants is therefore to increase the yield of leguminous plants independently from external sources of N. There are different combinations of inoculates and the right inoculant must be used for a specific environment and specific cultivar to achieve the desired result (Xavier et al., 2004). Liquid spraying, lime pelleting, peat pelleting and sowing porous granules with seed are the methods to inoculate leguminous plants, however, farmers often buy pre-inoculated seeds from various different companies (Herridge et al., 2002). Farmers can add inoculants themselves, but this is not recommended due to uneven coating and seeds that stick together.

When there is a lack of N in the soil, the rhizobia of the inoculant will attach to the root hairs and form nodules. Nitrogen is then taken from the air (fixed) and it is now available for the plants to utilize it. The N that is taken up through the air and a proportion is then used by the roots, with a proportion left in the soil (Catroux et al., 2001). This residual N in the soil will increase the crop yield that is planted the following year after the legume. Inoculants of higher quality will result in higher N levels fixed in the soil and in turn will result in higher production.

d) Polymers

Polymers are known as carriers and there are various types of polymers. The importance of carriers has already been discussed earlier. The main function of these carriers are to change the form and size of the seed, changing the point water permeability (ability of a porous media to transmit a fluid) of the seed and to be a carrier for nutrients, pesticides and inoculants (Vyn and Marua, 2001). It is important to know which polymer is used, if different components are added together they can have a negative effect on one another and the seed itself (Vyn and Marua, 2001). To limit or eliminate this problem more than one polymer can be used per treatment (Herman, 1991). Developing technology will enable companies to determine the most suitable polymer combination to suit a cultivar in a specific area, therefore eliminating many of the production challenges (Vyn and Marua, 2001).

1.12 Conclusion

Planted pastures are one of the easiest and most economical way to rehabilitate degraded land and mined areas where mining processes are finished. The amount of areas being mined and degraded land is increasing and these areas need to be rehabilitated and made productive by law. The most common planted pastures species that are used are (not limited to them); *C. dactylon*, *C. gayana, C. ciliaris, D. eriantha, E. curvula* and *M. sativa*. These mined areas and degraded areas have very poor soil structures, high and low pH and high salinity's. These soils also have high levels of other minerals for example: Al, Cu etc. The water holding capacity of these soils are also altered and affects the infiltration of water into them.

All the above mentioned factors shows how complex the rehabilitation process is and all these factors affects the germination rate of planted pastures. The germination rate is usually very low in these soils and contributes to a low establishment rate. Germination rate is not only affected by the environmental factors, but by the genetics of the seed itself. Coated seed were developed to increase the germination rate and establishment rate of these pastures. Coated seeds are a relatively new technology were the outer layer of the seed is coated, with nutrients, polymers insecticides, etc. The coating promotes the growth of the seedling by releasing the nutrients directly in the soil where the primary root can take it up and grow faster compared to uncoated seed. Therefore coated seed is hypothesized to benefit the seedling, enabling it to grow faster and in turn increase the establishment rate on degraded land and improving the success rate of the rehabilitation process in general in these areas.

1.13 References

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CHAPTER 2

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The effects of seed coating on germination and emergence of various pasture species in different growth substrates

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2.1 ABSTRACT

Mining throughout the world is increasing rapidly due to the increase in the demand for minerals such as coal, gold, etc. According to legislation in South Africa mined areas need to be rehabilitated and the most cost effective way is to use pastures. Conditions such as chemical and physical properties, in addition to the presence of potentially harmful elements (including high salinity's and pH) in the soil or substrates, can often restrict germination rate and root development. This complicates the establishment and rehabilitation process. The technology to coat seeds with inoculants, pesticides, nutrients, etc. have shown much potential in previous studies and can facilitate the establishment of grasses in these degraded soils or substrates (growth) environments. In this study the emergence of six species (coated and uncoated) were tested in substrates with different salinity's (0.05M , 0.1M and 0.15M) and pH (3, 5 , 7 and 9) levels. It was concluded that there was no significant difference in emergence percentages between *Digitaria eriantha, Eragrostis curvula* and *Medicago sativa. Cenchrus ciliaris* and *Cynodon dactylon* had higher emergence percentages in acidic soils, while *Chloris gayana* showed sensitivity to acidic and alkaline soils. The highest emergence for most of the species were when the soil moisture was at field capacity. Coated seed had a higher emergence percentage for *C. ciliaris*, *C. dactylon* and *E. curvula*, while uncoated seed had higher emergence rate for *C. gayana* and *D. eriantha*. There was no notable difference for *E. curvula*. In the salinity trials all the emergence percentage dropped as the salinity levels increased. *Cenchrus ciliaris*, *C. dactylon* and *E. curvula* are very sensitive to saline soils and it is therefore not recommended to use these species in similar situations. Coated *C. gayana* and *M. sativa* had a higher emergence percentage compared to the uncoated treatments of the

species at the different salinity's. Uncoated *D. eriantha* had a higher emergence percentage compared to the coated treatment. These results highlight the importance of coating treatments in some of the species in lower quality soils and are therefore important in rehabilitation.

Keywords: Coated seeds, emergence, germination, rehabilitation, pastures

2.2 INTRODUCTION

Germination starts when the seed takes up water which accelerates cellular activity and ends when the radicle breaks through the surrounding tissues and becomes visible (Fairey et al., 1999). The seedling growth is not part of germination and is considered a different phase of growth, this is referred to as emergence (Fairey et al., 1999). The germination process or phase differs in time between different species and cultivars, even in the same cultivar if the seed was coated. The higher the germination percentage of the seed that was planted, the higher the emergence will be and in turn the higher the establishment of a particular area will be, therefore seed companies strive for the highest possible germination percentage. Germination and emergence are different phases, but for this study the term emergence percentage will be used and will refer to both germination and emergence, when the radicle breaks through, up to and the seedling appears.

The amount of degraded land in South Africa is increasing. Currently there is more than a hundred thousand hectares that have been mined in South Africa (Neke and Du Plessis, 2004). The law of South Africa states that these areas need to be made productive again and the most cost effective way is by using pasture species (Mentis, 2006). These large areas that need to be rehabilitated just shows the importance of successful establishment. Mining activities produce by-products of tailings material, waste products and large slurry dams. These tailings materials or areas near mines are high in heavy metals that are above the normal threshold. These heavy metals include copper (Cu), zinc (Zn), lead (Pb), aluminium (Al), salts etc. (Shu et al., 2002). Degraded land caused by farming is also a major problem. Irrigation of crop lands causes an increase in soil salinity due to dissolved salts in the water. Constant tillage of the soil also causes top soil to be eroded causing lower quality soil to be exposed. Similarity, but to a larger extent, mining also changes the structure of the soil through past-mining landscaping practices (Chapman and Younger, 1995). The large mining vehicles causes soil compaction and contributes to the difficulty in establishment of vegetation (Chapman and Younger, 1995). Mines or areas that are rehabilitated are not fully functional ecosystems yet and needs time to develop into a fully functional ecosystem. Only then is an area considered to be fully rehabilitated (Morgenthal and Van Rensburg, 2004). To achieve a fully functional ecosystem is difficult and contributes to the difficulty of rehabilitation. The success of rehabilitation depends on the long term monitoring and management of the area and not only the once of planting of pasture species in year one. These are just some of the challenges that influence successful seedling germination and emergence faces. Higher germination, emergence and successful establishment will result in more successful revegetation and rehabilitation.

Water, oxygen levels in the soil, temperature and light are the normal requirements for seed to germinate (Hadas, 2004). A compacted growth substrate influences water, oxygen and temperature of the soil. These factors on their own are not the big problem, but the combined effects germination and emergence. The water quality is also important especially, for coated seed, as it is essential for all chemical reactions in the seed and the soil. Lower germination will result if the water is of lower quality (Hadas, 2004). Factors like seed bed quality, low quality seed and planting at the wrong time and also lowers emergence (Catroux et al., 2001).

High levels of salinity contributes to lower germination and emergence by affecting the osmotic balance in the plants (Munns and Termaat, 1986). Ion antagonism is a major problem in saline soils, reduces the uptake of other minerals, such as calcium (Ca) (Grattan and Grieve, 1999). The soil requirement of Ca, therefore increases with an increase in Na. A study done on the effects of salinity on kikuyu showed that increased the sodium chloride (NaCl) levels from 200mM (millimoles) to 400mM reduced the germination percentage from 70% to low 5% (Muscolo et al., 2013), which supports the severe effects of salinity.

The use of coated pasture seed was introduced to production systems to overcome some of these problems and to improve germination percentage and establishment success in pastures. Coated seed is used to improve production systems of pastures, improve cost savings and is becoming more popular in industry (Van Oudtshoorn, 1999, Detroz and Gago, 1991, Rehman et al., 2014). The process of coating the seed is a science that incorporates intellectual property and technologies from seed, pesticide and chemical companies. The seed coating process involves adding polymers, nutrients, fungicides, fillers and insecticides to the outer coating of the seed itself (Detroz and Gago, 1991, Porter and Scott, 1981). It is hypothesized that these coating treatments can improve the germination percentage of species, depending on the soil it is planted in, therefore in turn making the establishment of these species more successful. The coating of seeds will disintegrate when there is enough water, but will remain in dry conditions, therefore it is more drought resistant (Detroz and Gago, 1991). The main aim of coated seed is to overcome environmental challenges by improving the success of germination and to give a seed the best chance of survival.

Coated seed are not always better than uncoated seed and is only there to improve the survival of seed that germinates. Seed coatings were developed to increase the ease of handling, to have a heavier and more uniform seed (Halmer, 2004). Coated seed also germinates 12 hours later compared to uncoated seed in the same species (Nel, 2014). The seed coating technologies for seed are complex and seed companies are constantly changing and trying to improve the coating. The coating used is different for each species and generalization of coating cannot be made.

The hypotheses for this trial were:

- Coated seed will have an improved germination when compared to uncoated seed in different mining substrates
- Coated seeds germinate and emerge better in extreme pH's substrates (low/high).
- Coated seeds germinate and emerge better in saline substrates.
- Coated seed germinates and emerges better in wetter soils/substrates.

2.3 METHODOLOGY

This study was carried out in a growth chamber at Phytotron C on the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa. This study was conducted to determine the effect of seed coatings on the emergence percentage of different pasture species in different substrates. The trial was divided into two individual trials; an emergence study to evaluate the emergence potential (%) of selected species at different (1) salinity and moisture levels and an emergence study to evaluate the emergence potential (%) at (2) different pH and moisture levels. For both the studies five grass species and one legume species were used.

<u>1. Emergence trial evaluating the emergence percentage at different salinity levels</u>

The germination and emergence trial was conducted using coated and uncoated Smuts finger grass (*Digitaria eriantha)*, Weeping love grass (*Eragrostis curvula)*, Lucerne *(Medicago sativa)*, Couch grass *(Cynodon dactylon)*, Rhodes grass (*Chloris gayana)* and Foxtail Buffalo grass (*Cenchrus ciliaris).* These species are commonly used in mine rehabilitation and therefore used in these trials (Truter, 2015). The study was conducted in homogenized silica sand. There were multiple observations as can be seen in Table 2.1.

This table shows the days when observations were made and when the emerged seed was counted using ISTA guidelines (ISTA, 2003) for the first 2 counts and then 2 more counts were made at 5 days intervals. The two additional counts were used to ensure that the maximum emergence percentage was recorded and this extra time was given to account for expected delay of the coating (Nel, 2014) and the environmental conditions.

Table 2.1: Specifications of species in terms of expected germination and days after planting when observations are made.

	Days at which observations were taken			
Species	**First observation**	**Second observation**	**Third observation**	**Fourth observation**
Digitaria eriantha	4	10	15	20
Eragrostis curvula	5	15	20	25
Medicago sativa	4	10	15	20
Cynodon dactylon	7	21	26	31
Chloris gayana	7	14	19	24
Cenchrus ciliaris	7	28	33	38

Four hundred grams of homogenized silica sand was placed in small plastic containers (600 cm^2). *Digitaria eriantha*, *C. gayana* and *C. dactylon* were planted in the same container and were separated by wooden dividers. Coated and uncoated seeds were planted in separate containers. Coated and uncoated *C. ciliaris* were planted in the same container with a 4cm gap between them. *Medicago sativa* and *E. curvula* were planted in the same container, again separated by the wooden dividers with coated and uncoated seeds planted in different containers. Each treatment was replicated 4 times.

One hundred seeds of each species were planted per treatment. Three water treatments were applied, W1, W2 and W3, where W2 was the amount of water required for the soil to be at field capacity. Treatment W1 was 75% of treatment W2 (75% of field capacity) and treatment W3, 25% more than treatment W2 (125% of field capacity). The field capacity was determined using the WP4-T machine. The WP4-T is also known as the Decagon's WP4 Dew point Potential Meter. WP4 is used to measure water potential and is measured in MegaPascals. It measures water potential from 0 to -300 MPa with an accuracy of ±0.1 MPa (Devices, 2007). The machine has mirrors on the inside where water condensates on to. At a certain point there exists an equilibrium between the moisture in the sample and the moisture that condensates on these mirrors. This equilibrium gives the water potential reading that is used to determine field capacity (Devices, 2007).

The correct amount of water was added to the containers, weighed and then placed into plastic bags to prevent excessive water loss. Every 10 days the containers where removed, weighed and topped up with the correct amount of water to keep the water level the same, therefore keeping the amount of water constant throughout the trial. The growth chamber was set at a constant 25˚C, with a twelve hour lighting cycle. The light was constant and the containers were kept the same distance away from the light in each treatment.

The water treatments used in this study contained different salinity levels. There were three different salinity treatments made up of three different salts (NaCl, (Magnesium Sulphate) $MgSO_4$ and (Sodium Sulphate) Na_2SO_4) and for each treatment there were three concentrations (0.05M, 0.1M and 0.15M) of each salt treatment. The salts were added to deionized water to ensure that the concentrations remained the same throughout the trial. The salts that was evaluated in terms of their EC (electrical conductivity), using an EC meter. The EC of soil is the ability of soil to conduct a charge, a normal EC of between 4 to 8 ds m^{-1} enables plant to easily take minerals from the soil, and above this threshold plants are not able to absorbed minerals from the soil (Maas and Nieman, 1978). The minerals remain in the soil and the plants growth is impacted negatively. Therefore the concentrations of salts were not important but the EC of each salt was more important. The emergence percentage was determined in each study by counting the number of seeds that emerged. The emerged seed that were counted was not removed after each count, they were left to ensure that maximum emergence percentage was observed over the period.

2. Emergence trial evaluating the emergence percentage of species at different pH levels

This trial was conducted similarly to the 'salinity' trial with the exception of the water quality treatments. The water added to this trial had different pH (H_2O) (3, 5, 7(control) and 9) levels, made up of Hydrochloric Acid (HCl) and Sodium Hydroxide (NaOH). These solutions were added to deionised water and using a pH meter (Hanna HI 991300 model) to ensure the desired pH was achieved. These waters were made up three days prior to adding it to the trials to ensure the pH was correct.

The emergence percentage of the different treatments were determined in each study by counting the number of seeds that emerged. The seeds that were counted were not removed after each count.

Statistical analysis

This experimental study was a completely random design (CRD) in a growth chamber with four replications. As the emergence percentage (based on the day of best emergence for both pH and salts data) were skew with heterogeneous variances the Generalized Linear Mixed Model (GLMM). Analysis was used with the Binomial distribution for proportions (x/100 seeds) to test for differences between four pH levels, three water levels and two coating effects, as well as all their interactions, for each species separately at the 5% level.

Means in all trials were compared using Tukey's least significant difference test at the 5 % level of significance (Snedecor and Cochran, 1980)

Data was analysed using the statistical program GenStat® (Payne et al., 2014).

2.4 RESULTS AND DISCUSSION

i. <u>Emergence percentage of selected species to different salinity concentrations</u>

Cenchrus ciliaris

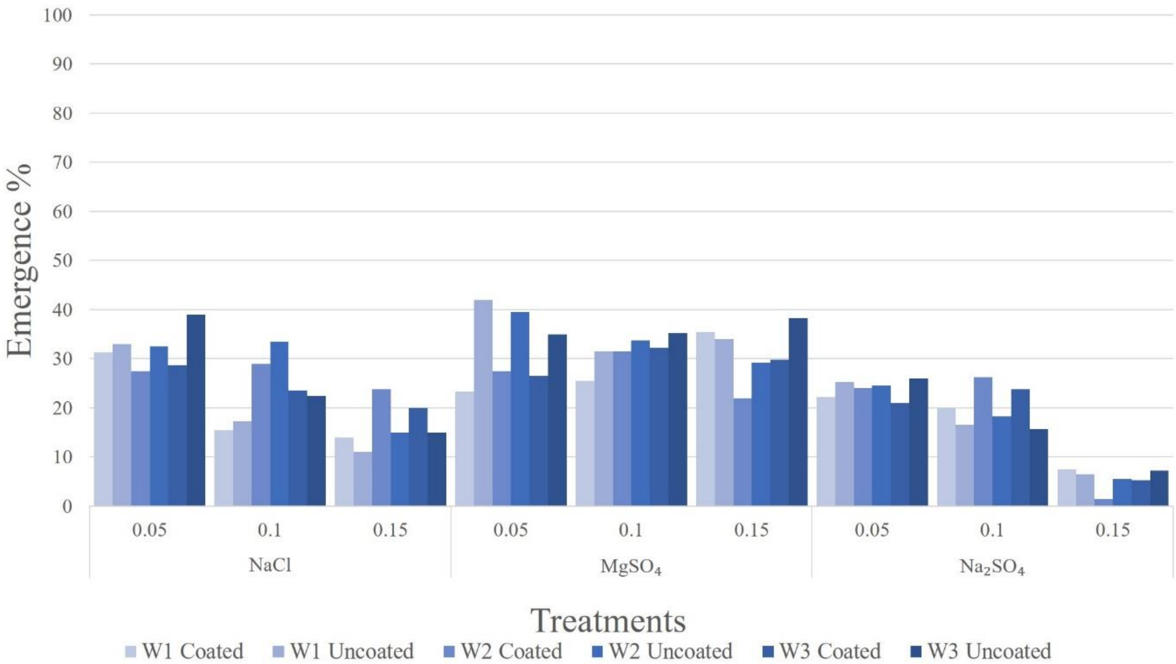

Figure 2.1: The emergence percentage of coated and uncoated *C. ciliaris* at three different salinity's (NaCl, $MgSO_4$ and Na_2SO_4) at three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Statistics were not included due to no significance*

The figure above shows the emergence percentage of *C. ciliaris* and how the interaction between growth substrate, water level and coating affects the emergence percentage of *C. ciliaris*. There was no significant influence in this interaction, Figure 2.1 was included to show the results that were obtained. There was, however a significant difference between the different salinity levels, coating treatments and the interaction between the growth substrate and coating ($P \leq 0.01$). Figure 2.1 clearly shows the difference in growth between the different salinity levels, although there is a smaller difference at $MgSO_4$. There is a clear decline in the emergence percentage as the concentrations of NaCl and Na_2SO_4 increase, this shows the sensitivity of *C. ciliaris* to salinity.

The drop in emergence percentage due to high salinities, is due to the change in osmotic potential outside the seed relative to the inside (change in water potential gradient) (Kaydan and Yagmur, 2008). The increase in salinity cause osmotic stress in the seed and the seed is unable to absorb enough water into the inner layer causing a lower emergence percentage (Kaydan and Yagmur, 2008). Coated seed had no benefit in increasing the emergence percentage of *C. ciliaris*. Uncoated seed had a much higher emergence percentage at all the different salinity's.

<u>*Chloris gayana*</u>

Chloris gayana is known to be very salt tolerant due to the glands on the leaves of the plant (Ortega and Taleisnik, 2003, Kobayashi et al., 2007), however in Figure 2.2 we can see that higher salinity levels had an effect on emergence percentage. Figure 2.2 shows the interaction between the growth substrate, water content and seed coating. This interaction showed no significant difference. There was a significant difference in the different substrates ($P \leq 0.01$), interaction between growth substrate and coating and the interaction between water content and seed coating, these differences will be discussed below. The seed coating effect on its own did not have a significant effect.

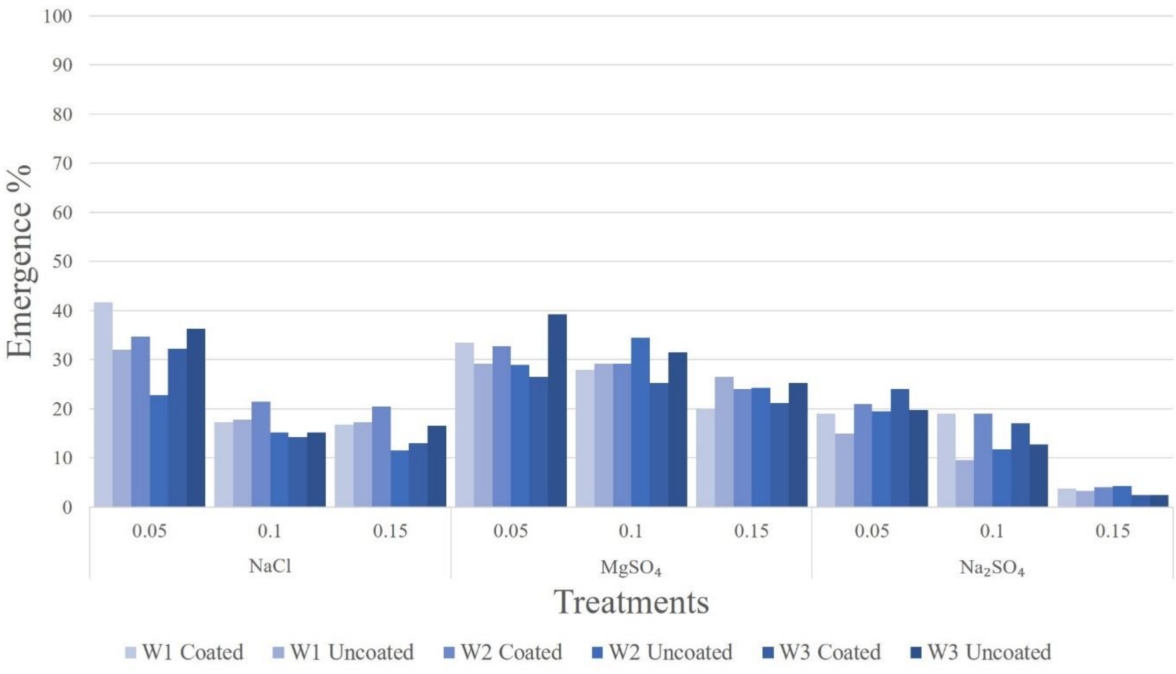

Figure 2.2: The emergence percentage of coated and uncoated *C. gayana* at three different salinity's (NaCl, $MgSO_4$ and Na_2SO_4) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Statistics were not included due to no significance*

As expected, the growth substrate (the different concentrations of three different salts) had a significant difference, there was a decrease in emergence percentage as the salinity levels increased, as is seen in Figure 2.3. The increases in salinity causes an osmotic stress in the seeds, preventing them from absorbing enough water and can also cause mineral toxicity in the seed due to mineral imbalances (Kaydan and Yagmur, 2008).

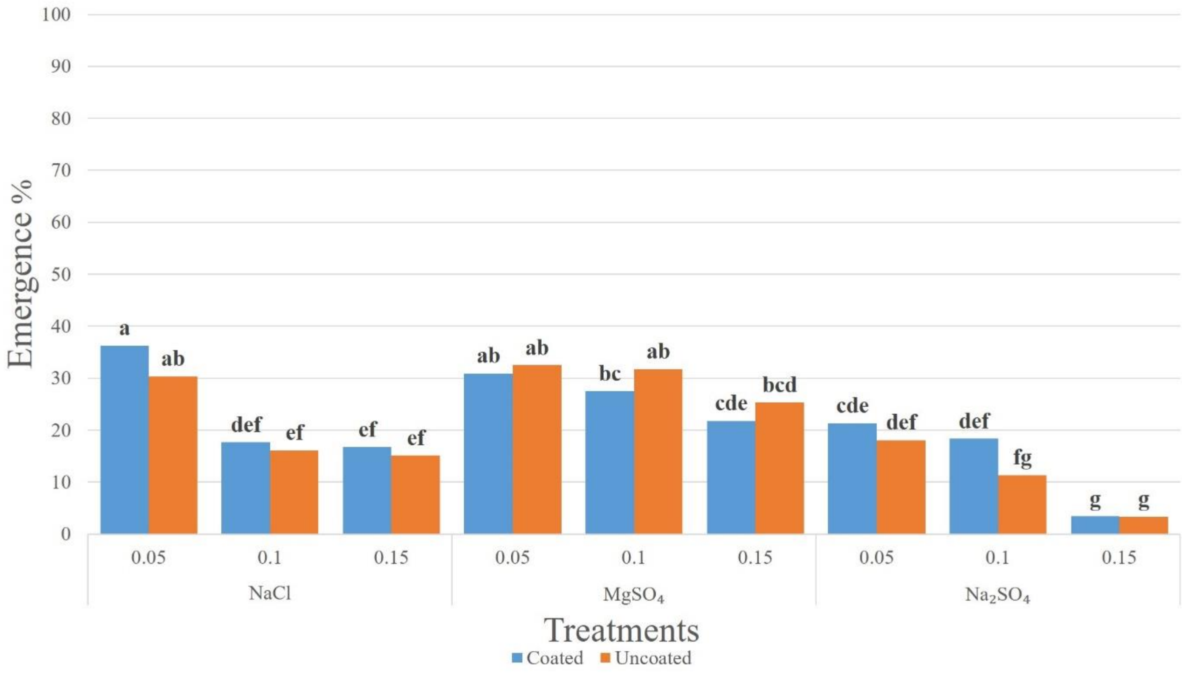

Figure 2.3: The emergence percentage of coated and uncoated *C. gayana* at three different salinity's (NaCl, $MgSO_4$ and Na_2SO_4)

**Values with the same letter are not significant and values with different letters are significantly different*

From Figure 2.3 it can be seen that the interaction effects between seed coating and growth substrate, that uncoated seed had a higher emergence percentage in the $MgSO_4$ treated growth substrate. Coated seed had a higher emergence percentage in all the growth substrates with Na_2SO_4 and NaCl. This difference can be as a result of the Mg or Na which could have an effect on the coating around the seed. Figure 2.4 also illustrates how Na had a big impact on emergence. In all the cases there were a lower emergence percentage, likely caused by the osmotic stress. High levels of Na can also cause toxic build-up in the seed (Kaydan and Yagmur, 2008, Tuteja, 2007). In this particular case coated *C. gayana* prefer substrates with NaCl and Na_2SO_4, with W1 and W2 water levels compared to uncoated seed.

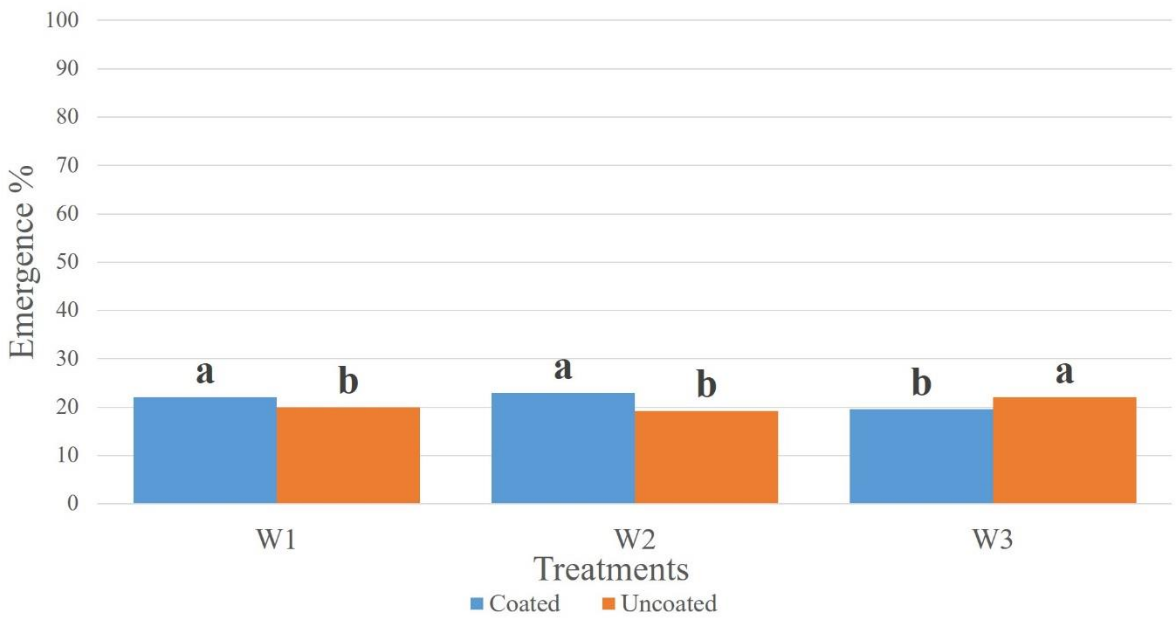

Figure 2.4: The emergence percentage of coated and uncoated *C. gayana* at three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Values with the same letter are not significant and values with different letters are significantly different*

Figure 2.4 shows the interaction effects of seed coating and water content. It is clear from this figure that coated seed had a higher emergence percentage at field capacity (W2) and uncoated seed had a higher emergence at W3. This suggests that at field capacity there was enough water to dissolve the coating and prevent the dissolved coating from washing away. At W3 uncoated seed had a higher emergence percentage, the coatings effect was likely revered.

Cynodon dactylon

Figure 2.5 shows the interaction between coating, water level and growth substrates (different salinity soils). There was a significant effect ($P \leq 0.01$) in the coating treatments, the different growth substrates and in the interaction between growth substrates and coating.

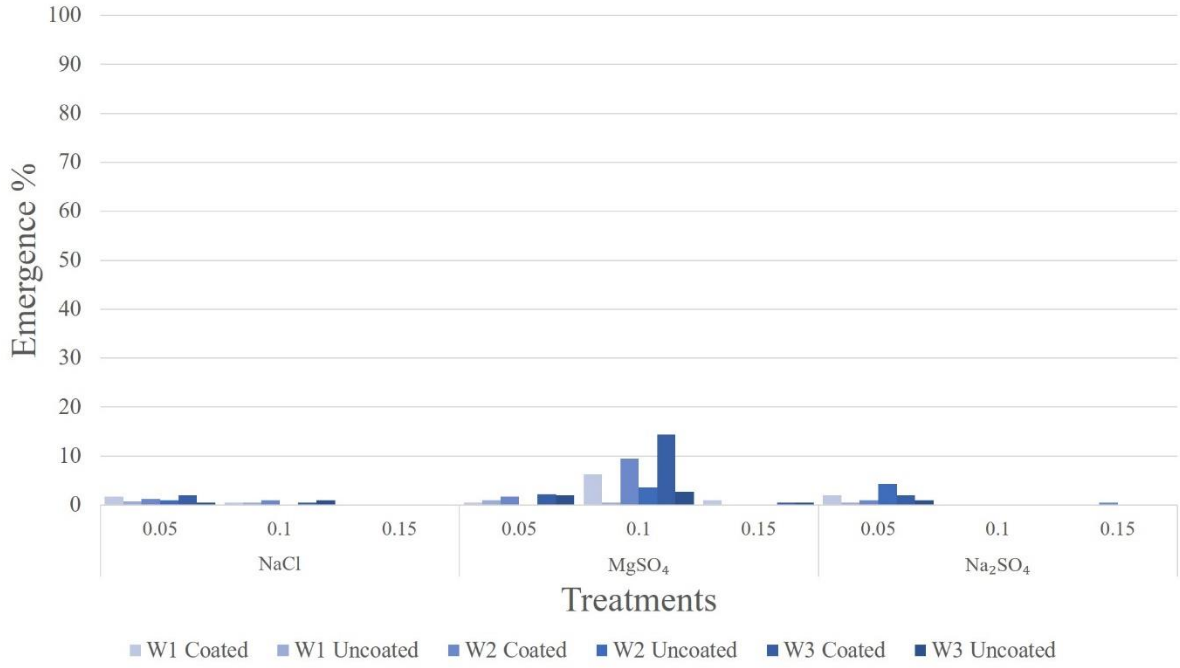

Figure 2.5: The emergence percentage of coated and uncoated *C. dactylon* at three different salinity's (NaCl, $MgSO_4$ and Na_2SO_4) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Statistics were not included due to no significance between all the interactions*

There was a significant difference between the different salinity levels, with soils containing $MgSO_4$ with emergence less than the other two substrates. There was a notably higher emergence percentage for 0.1M $MgSO_4$ treated soil. In both NaCl and Na_2SO_4 there was a decline in emergence percentage as the salinity concentration increased. Coated seed had a higher emergence percentage compared to uncoated seed for most treatments.

The interaction between coating and growth substrate shows there is a significant influence. This is especially true for $MgSO_4$ as mentioned above. The other treatments showed that the coated and uncoated seed had very similar emergence percentages. Therefore *C. dactylon* is very sensitive to high salinity levels and this means the hydrolytic enzymes found within these seeds are very sensitive to high salinity that in turn causes osmotic stress (Muscolo et al., 2003).

<u>*Digitaria eriantha*</u>

The statistical analysis of *D. eriantha* showed that there is a significant difference ($P \leq 0.01$) between the different growth substrates (salinity levels), the interaction between the growth substrate and the coating and the interaction between the growth substrate and water content.

There was no significant difference between the coatings. Figure 2.6 shows the interaction between growth substrate, water content and coating, there was no significant difference.

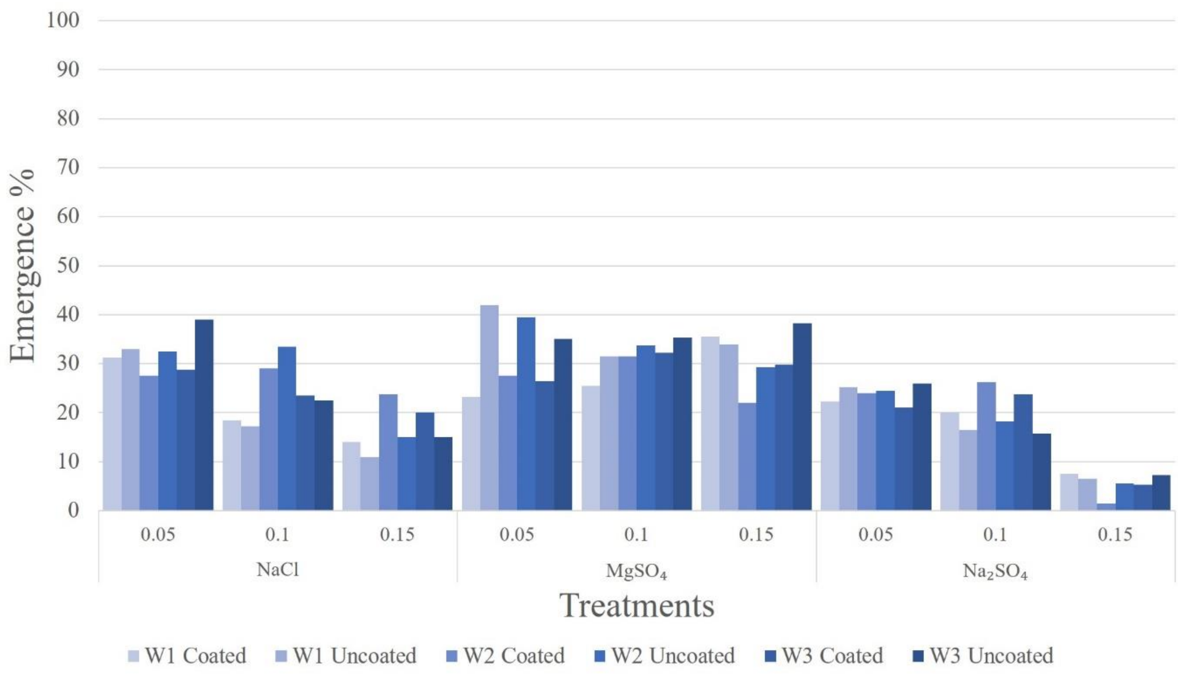

Figure 2.6: The emergence percentage of coated and uncoated *D. eriantha* at three different salinity's (NaCl, $MgSO_4$ and Na_2SO_4) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Statistics were not included due to no significance between the interactions*

There was a clear decline in emergence percentage as the salinity levels increased, except in $MgSO_4$ were the emergence remained relatively constant. *Digitaria eriantha* was more sensitive to Na_2SO_4 when compared to NaCl. It is safe to say that the higher salinity levels affected the emergence, but *D. eraintha* was able to still germinate at high saline levels. It can be hypothesized that the hydrolytic enzymes were affected, or their activity was only reduced, not halted and therefore there was a slightly lower water content change within the seed. Only at 0.15M Na_2SO_4 was the emergence percentage severely affected showing osmotic stress.

Figure 2.7 shows that the highest emergence percentage for $MgSO_4$ was for 0.05M at W2, therefore showing no real trend. As the salinity increases up to 0.15M, so does the emergence at W3 and vice versa for field capacity. NaCl shows a clear trend (Figure 2.7) with the emergence percentage decreasing at all the different water levels as salinity increases. Therefore too much or too little water caused a reduction in emergence percentage due to an altered water content affecting the water potential gradient. Similarly, the maximum emergence for Na_2SO_4 was at field capacity.

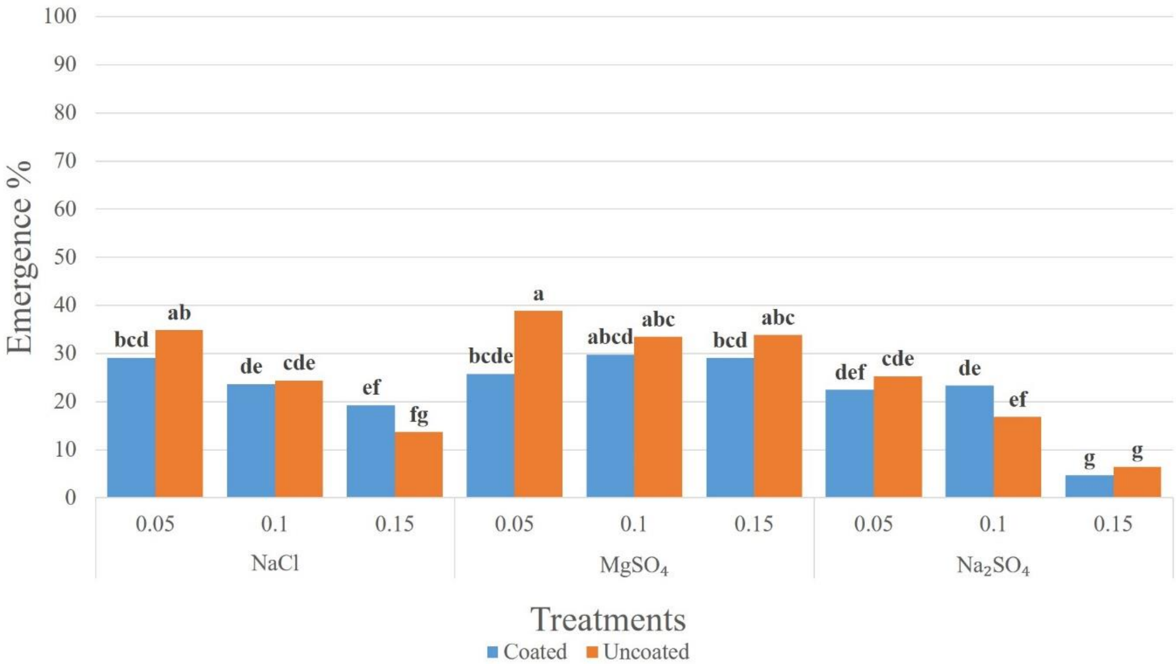

Figure 2.7: The emergence percentage of coated and uncoated *D. eriantha* at three different salinity's (NaCl, $MgSO_4$ and Na_2SO_4)

**Values with the same letter are not significant and values with different letters are significantly different*

The statistical analysis showed no significant difference between the seed coatings. At all the $MgSO_4$ treatments, uncoated seed had a higher emergence percentage compared to coated seed. At NaCl 0.05M and 0.1M uncoated seed also had a higher emergence percentage compared to coated seed, and only at the high salt concentration (0.15M) did coated seed have a higher emergence percentage. The treatment with Na_2SO_4 showed that where there is a low salinity level uncoated seed had a higher emergence percentage but as the salinity increased so did the emergence percentage of coated seed.

<u>*Eragrostis curvula*</u>

Figure 2.8 below shows the interaction between the growth substrate, seed coating and water treatments and there was no significant difference. The statistical analysis showed that there was a significant difference ($P \leq 0.01$) between the different growth substrates (different salinity levels), between coated and uncoated seed, and between the interaction between

growth substrate and coatings). There was a difference between the different water treatments, but it was not significant.

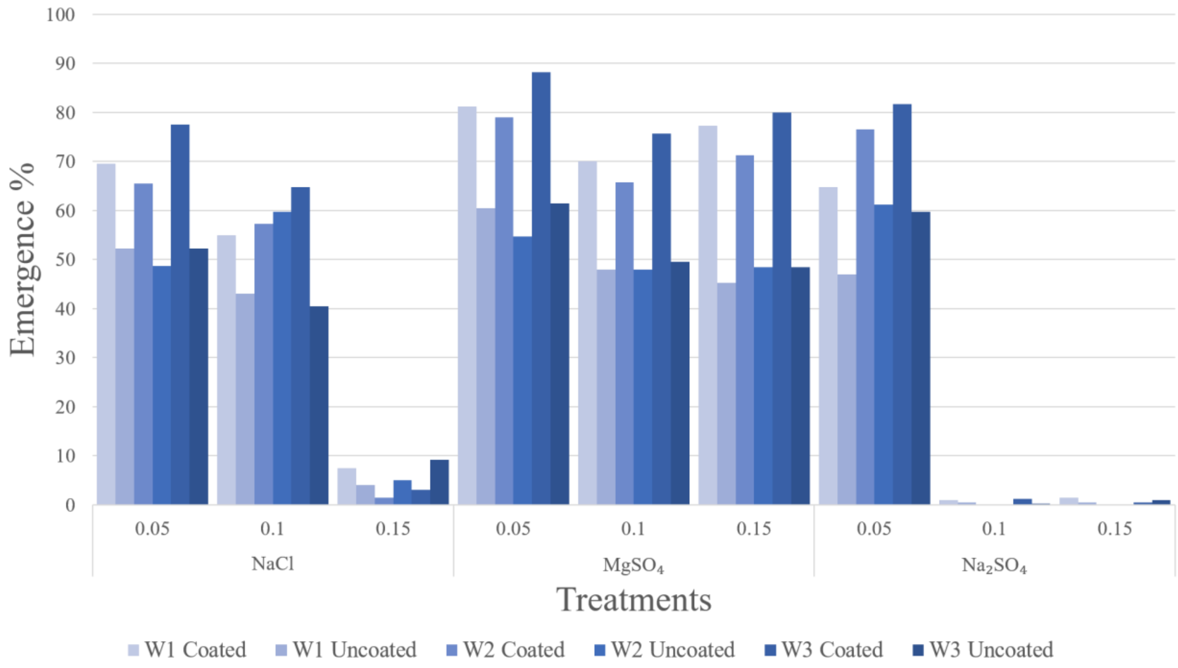

Figure 2.8: The emergence percentage of coated and uncoated *E. curvula* at three different salinity's (NaCl, $MgSO_4$ and Na_2SO_4) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Statistics were not included due to no significance between all the interactions*

From the data it is clear that *E. curvula* is very sensitive to high levels of salinity as this was confirmed in literature (Dickinson, 2004, Van Oudtshoorn, 1999). *Eragrostis curvula* is less sensitive to $MgSO_4$, due to it having a lower EC. This is not the case when Na is the dominant criteria. In both the Na_2SO_4 and NaCl treatments, the lowest concentration does not seem to effect the emergence percentage. There is a clear decline in emergence as the concentration increases. In the NaCl treatment there is a slight decline at 0.1M and a severe decline at 0.15M. In the Na_2SO_4 treatment there is a severe decline already at 0.1M.

The decline in emergence percentage is likely due to the high levels of Na in the substrate. The Cl in NaCl can lower the severity of Na at first but when the concentration increases, the effects become stronger (Bui, 2013). The high Na levels prevent the water from entering the seed and therefore no germination and emergence will occur (Kaydan and Yagmur, 2008, Muscolo et al., 2003).

Uncoated seed had a higher emergence percentage compared to coated seed in all the different treatments. The coating around the seed did contribute to the amount of seed that emerged. It seems that the coating had a slightly inhibitory effect on germination and emergence. It is hypothesized that the emergence percentage of *E. curvula* is attributed to the genetic ability of the seed itself and that the coating showed no benefit.

Medicago sativa

Figure 2.9 below shows the interaction between the growth substrate, coating and water treatments. The statistical analysis showed that there was a significant difference ($P \leq 0.01$) between the different growth substrates and the different water treatments.

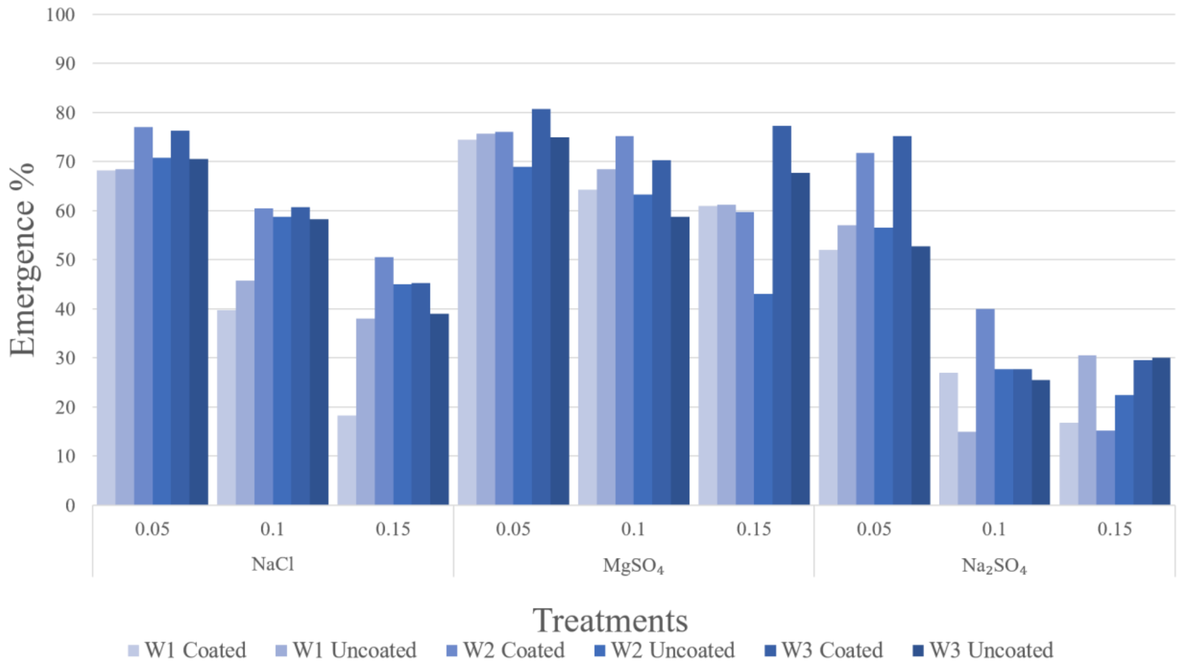

Figure 2.9: The emergence percentage of coated and uncoated *M. sativa* at three different salinity's (NaCl, $MgSO_4$ and Na_2SO_4) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Statistics were not included due to no significance between the interactions*

Medicago sativa is known to be sensitive to high salt concentrations and this is seen in the data and in Figure 2.9. In all the different salt treatments there was a decline in emergence percentage as the concentration of salt increased. The decline was however less significant in $MgSO_4$. From the data we can see that Na had a big effect on emergence percentage, and the emergence decreased as the level of Na increased. The high levels of Na cause osmotic stress and ion toxicity that in turn effects the water potential gradient, which in turn prevents the seed from taking in any water.

The highest emergence percentage was at W3 and the lowest at W1. This shows that the water content had a large effect and *M. sativa* usually performs well irrigated conditions, not waterlogged conditions. The use of coated seed of *M. sativa* where the substrates had a higher salinity level, showed no effect on emergence percentage.

ii. Emergence response of selected species to different pH's

Cenchrus ciliaris

The emergence percentage of *C. ciliaris* was very low during the whole study, with the maximum emergence being 10%. The data analysis showed that there is a significant difference between the different pH, water content and the coatings, $p \leq 0.001$. There was also a significant difference in the interaction between pH and coating, $p \leq 0.020$. There was also a significant difference in the interaction between pH, water content and coatings, $p \leq 0.021$. All of this is shown in Figure 2.10 below.

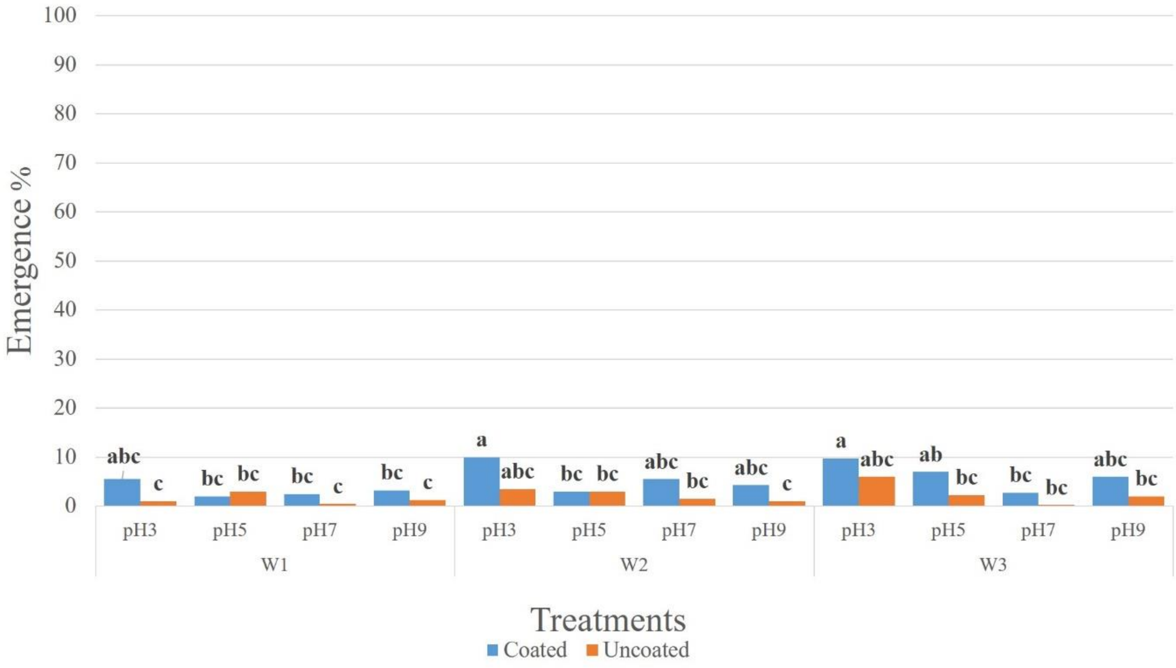

Figure 2.10: The emergence percentage of coated and uncoated *C. ciliaris* at four different pH (3, 5, 7 and 9) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Values with the same letter are not significant and values with different letters are significantly different*

The trend shown in Figure 2.10 shows that the lowest emergence percentage was at pH 7 and the highest at pH 3 for both coated and uncoated seed. No literature sources was found that explains this effect on pH on germination and emergence. Figure 2.10 shows that *C. ciliaris* are more adapted to an acidic environment. This higher emergence percentage of the acid and alkaline is hypothesized to trigger a mechanism in the seed or surrounding tissue or expose the seed to water. There is another hypothesis that the polarity of the water in the silica stimulate the surrounding tissue to expose the seed and stimulate the germination process. The difference in pH contributed the second highest variation and the coatings contribute the highest to the total variation. This higher variation shows that the coatings had a big impact on emergence percentage, coatings ensured that there was a higher emergence percentage.

From Figure 2.10 coated seed had an improved emergence percentage when compared to uncoated seed. The seed that was coated came from the same batch as that of uncoated seed and this shows that coated seed did have a higher emergence percentage in all the water treatments. The constituents of the coating stimulated germination, emergence and development. It can be hypothesized that the coating changed the water quality in the direct surrounding of the seed, influencing osmotic potential and mineral toxicities, by doing this it can increase the chances of successful emergence (Halmer, 2004). The osmotic potential of the soil can be changed, this in turn can cause minerals and nutrients to remain in the soil and not be taken up by the seed (Halmer, 2004).

The different water levels also contributed to the total variation, but to a much smaller extent. Figure 2.10 also shows that the emergence percentage was lower when the environment was drier. According to literature *C. ciliaris* is drought tolerant and the emergence percentage was expected to be unaffected in drier environments due to its drought tolerance (Van Oudtshoorn, 1999, Nawazish et al., 2006). It was also expected that the highest emergence would be at field capacity (W2) and lower at a higher water content (W3), due to the fact *C. ciliaris* is sensitive to waterlogged conditions (Dickinson, 2004, Anderson, 1974). The lower water content (W1) can have an impact on the osmotic potential of the seeds, therefore lowering the emergence percentage as seen in other studies done (Bewley and Black, 1994). The same is possible at the higher water content (W3), where the osmotic pressure is too high, affecting germination and emergence or it can be due to the drop in oxygen levels in the substrate. The interaction between water content and coating did not have a significant difference.

Chloris gayana

Chloris gayana is known to be very salt tolerant and more sensitive to alkaline soils and acidic soils (Taleisnik et al., 1997, Ortega and Taleisnik, 2003, Kobayashi et al., 2007). It is predicted that the emergence percentage will be lower at low and high pH values. According to the statistical analysis the coated treatment contributed to the most variation. There was a significant difference between the coatings ($P \leq 0.001$), pH ($P \leq 0.012$) and water content ($P \leq 0.001$), with pH contributing very little to the total variation. The interaction between the different pH and water content and the interaction between pH and coating also contributed a significant influence to the variation ($P \leq 0.001$), pH intensified the effects. There was also a significant difference in the interaction between pH, water content and coating ($P \leq 0.001$). All of the above can be seen in Figure 2.11 and will be discussed more in detail.

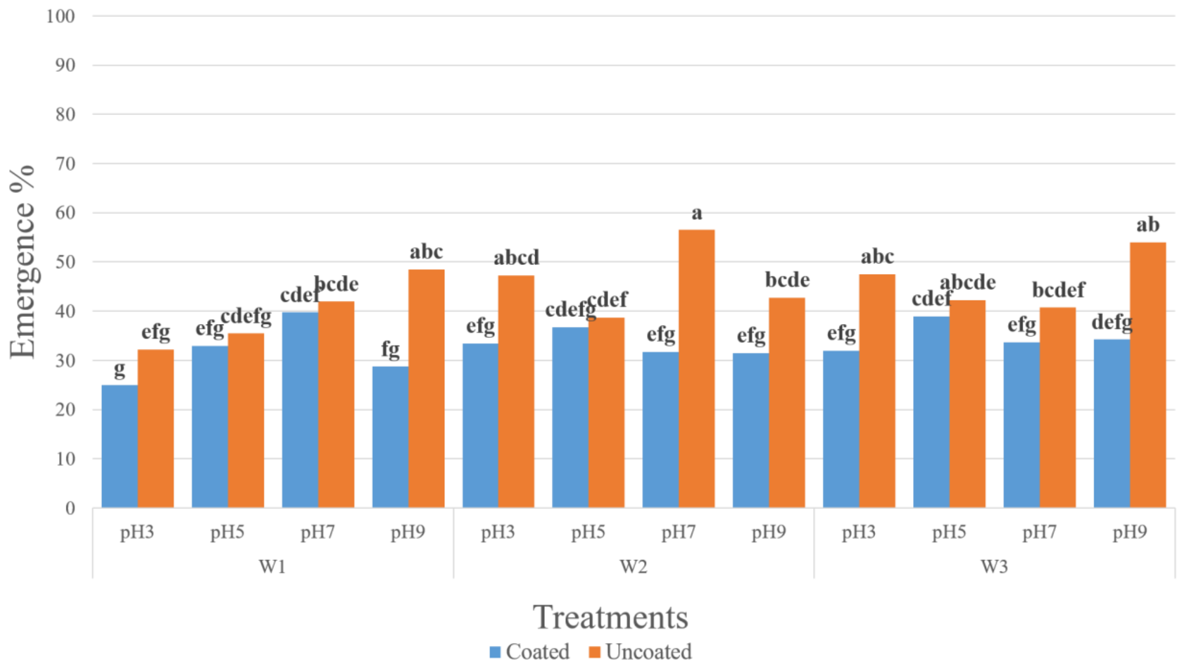

Figure 2.11: The emergence percentage of coated and uncoated *C. gayana* at four different pH (3, 5, 7 and 9) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Values with the same letter are not significant and values with different letters are significantly different*

The highest emergence percentage was at pH 7, W2 (seen in Figure 2.11) as one would expect and decreases slightly as the pH increases or decreases, with a slightly bigger effect at pH 3. The statistics showed that the highest emergence percentage was at W3 with field capacity treatment (W2, pH 9) having a slightly lower emergence percentage compared to W3. At W1 there was a low emergence percentage and this is likely due to coated seed not able to initiate germination properly as the coating seed weren't able to dissolve properly. Apart from the coatings not dissolving, *C. gayana* preferred wet soils and therefore the higher emergence where water was available (Dickinson, 2004).

The coated seed treatments contributed the most to the total variation with uncoated seed having the highest emergence percentage. The coating around the seed had a negative effect on the germination due to the free availability of water. This could be due to the coating not being able to dissolve properly. The emergence percentage dropped as the water level and the pH decreased. Once the water content decreased the effects of acidity were more severe. The effects of acidity on *C. gayana* are more severe in soils that are drier.

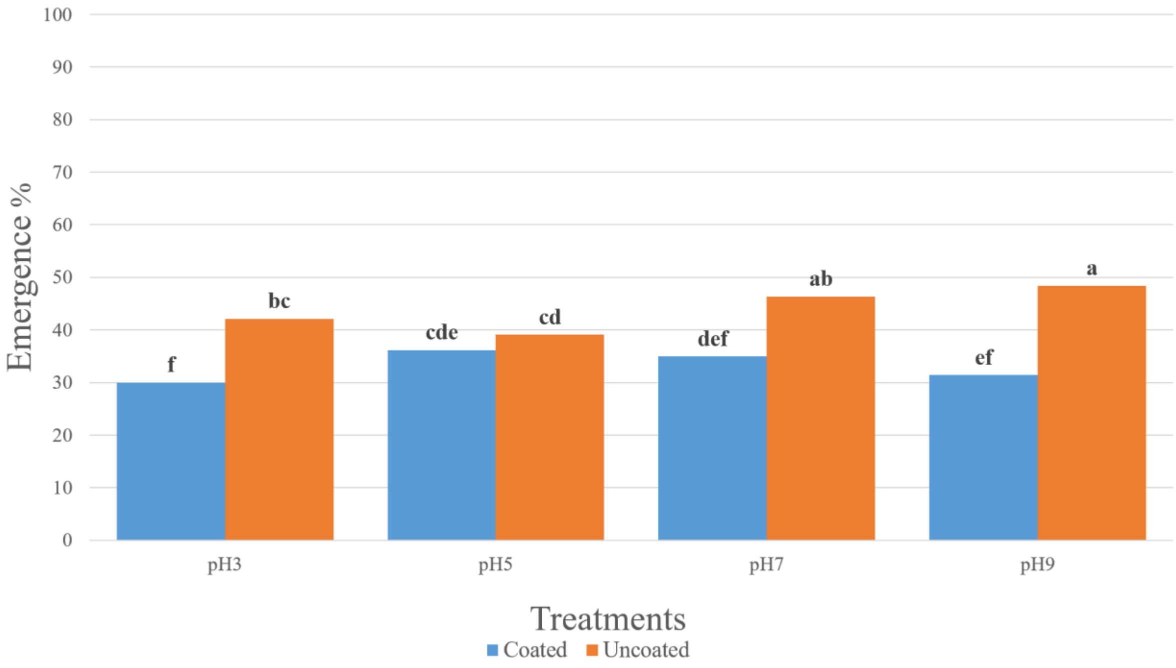

Figure 2.12: The emergence percentage of coated and uncoated *C. gayana* at four different pH (3, 5, 7 and 9)

**Values with the same letter are not significant and values with different letters are significantly different*

Figure 2.12 shows the trend between coated and uncoated seed at the different pH levels. The maximum emergence percentage for uncoated seed was at pH 7 while the maximum for coated seed was at pH 5, however the lowest uncoated emergence percentage was still higher than the highest coated seed emergence percentage.

Cynodon dactylon

The emergence percentage of *C. dactylon* was very low throughout the study and *C. dactylon* is known to have low germination, but due to its creeping growth habit the low germination percentage is of lesser concern (Van Oudtshoorn, 1999). Figure 2.13 below shows how the interaction between coating, water content and pH levels has an effect on the emergence. Firstly the pH contributed significantly to the variation ($P \leq 0.001$), with the highest emergence percentage being at pH 3 and the lowest at pH 7. It seems from this data that *C. dactylon* prefers acidic soils. No literature sources were found to support or refute these findings, further anatomical and physiological studies are needed.

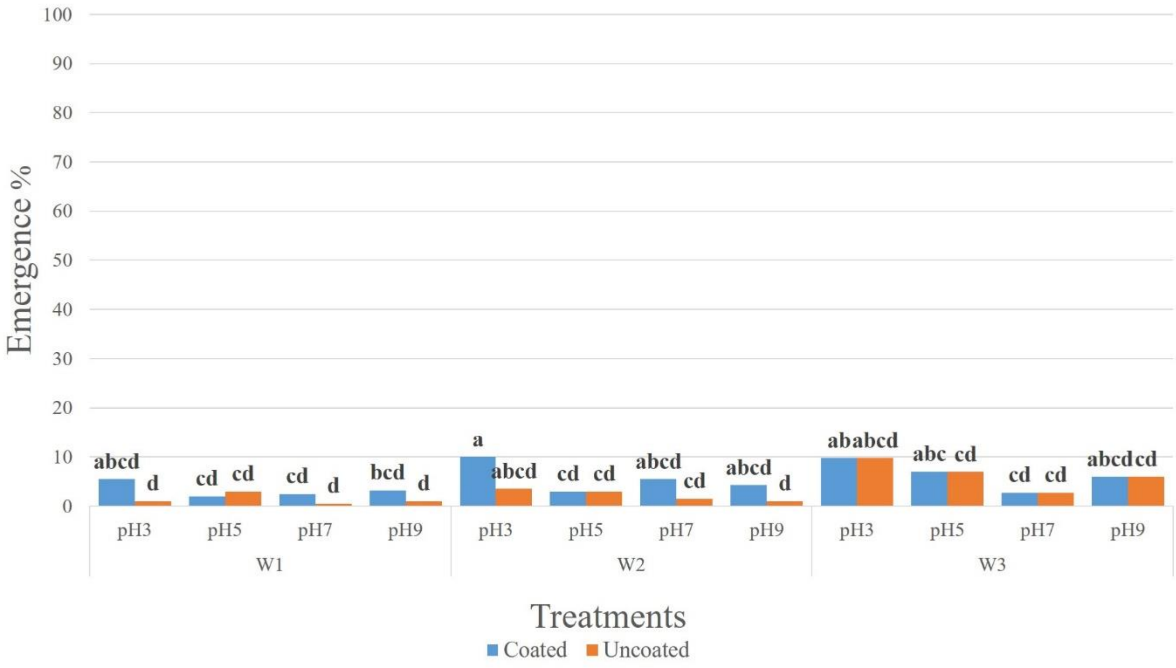

Figure 2.13: The emergence percentage of coated and uncoated *C. dactylon* at four different pH (3, 5, 7 and 9) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Values with the same letter are not significant and values with different letters are significantly different*

Coating and water content also contributed to the total variation ($P \leq 0.001$), with pH and seed coating contributing to the most variation. The overall emergence percentage was higher at W3 compared to W1 and W2 (Figure 2.13), and this shows that the water content had an effect on emergence percentage. The lower water level effected the osmotic potential of the seed and it was not able to absorb enough water, therefore lowering the emergence percentage (Bewley, 1997).

As previously mentioned, seed coating had a major effect on seed resulting in a higher emergence percentage as compared to uncoated seed. The coating around the seed protected the seed when the environment was drier, as seen in Figure 2.13. There was a significant interaction between the seed coating and the pH ($P \leq 0.017$) levels. At W3, the emergence percentage of coated and uncoated seed were very similar, and this can be due to the higher abundance of water. Therefore *C. dactylon* benefited from the seed coating.

Digitaria eriantha

Digitaria eriantha is known to be sensitive to acidic soils, and it is also sensitive to waterlogged conditions (Dickinson, 2004). These factors did not seem to effect the emergence of coated and uncoated *D. eriantha* as seen in Figure 2.14.

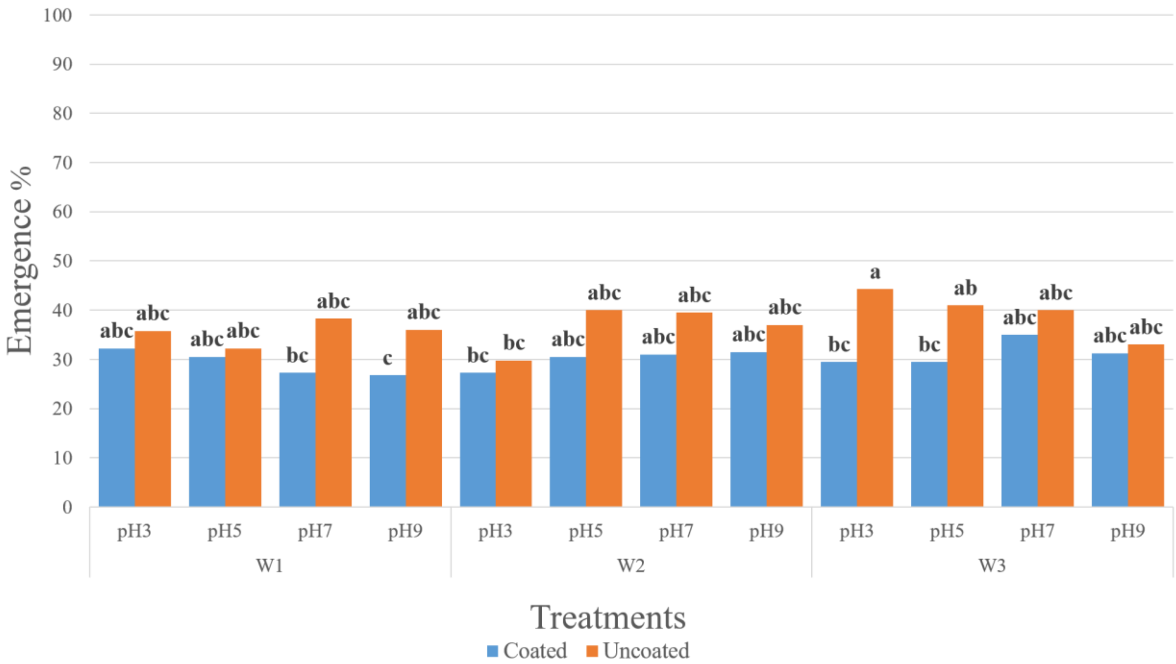

Figure 2.14: The emergence percentage of coated and uncoated *D. eriantha* at four different pH (3, 5, 7 and 9) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Values with the same letter are not significant and values with different letters are significantly different*

The different pH levels did not contribute to the difference in variation ($P \geq 0.05$). The water content however had a significant effect ($P \leq 0.021$). The coating treatment contributed to the highest difference in variation in emergence percentage ($P \leq 0.001$). Uncoated seed had the highest emergence percentage compared to coated seed, therefore the coating did not perform as expected. Coated *D. eraintha* seed showed no clear benefits with respect to emergence percentage at different pH levels.

Eragrostis curvula

The statistical analysis of *E. curvula* only yielded the coating treatment as a significant influence in the data ($P \leq 0.001$). The pH, water content and the interaction between the three did not have a significant interaction ($P \geq 0.05$). The coated seed had a higher emergence percentage compared to uncoated seed. The coating provided enough support and nutrients in all the different treatments to have a superior emergence percentage. Figure 2.15 shows the results of *E. curvula* for the different treatments.

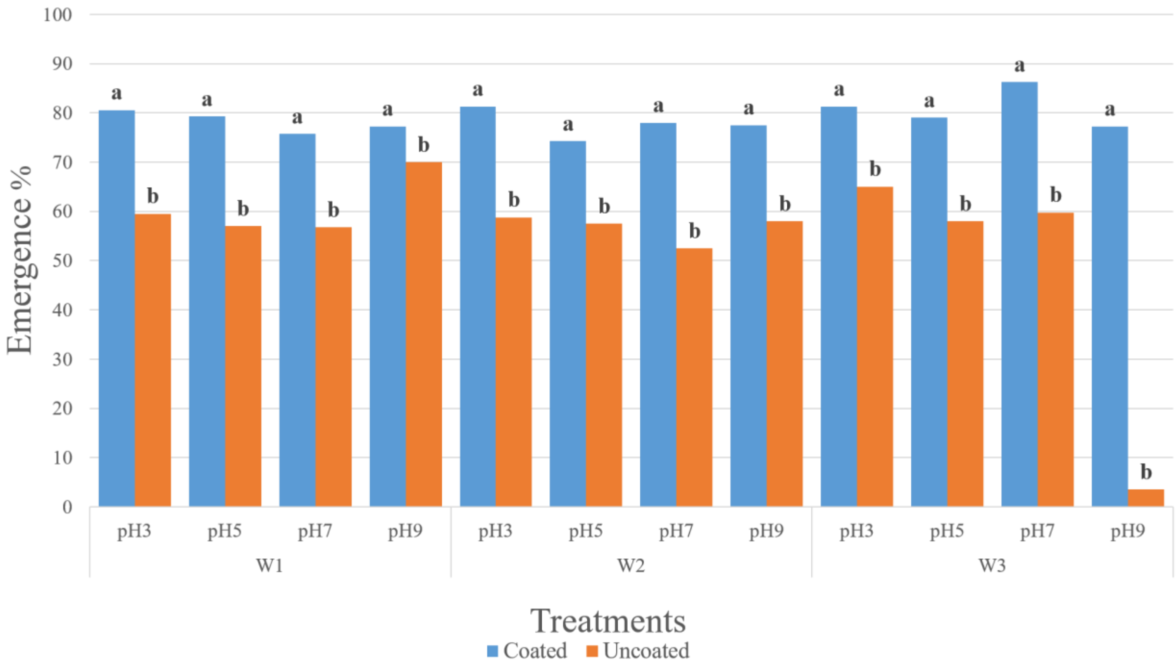

Figure 2.15: The emergence percentage of coated and uncoated *E. curvula* at four different pH (3, 5, 7 and 9) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Values with the same letter are not significant and values with different letters are significantly different*

Medicago sativa

Medicago sativa is known to be very sensitive to alkaline and acidic soils (Dickinson, 2004), while statistical analysis showed the contrary. The statistical analysis showed that the different pH treatments did not contribute to the difference in variation ($P \geq 0.456$), but the interaction between pH and water content showed a significant variation ($P \leq 0.001$). The coating and the water content also showed a significant difference ($P \leq 0.001$) and all of this is seen in Figure 2.16. There is no significant differences between coated uncoated seed emergence.

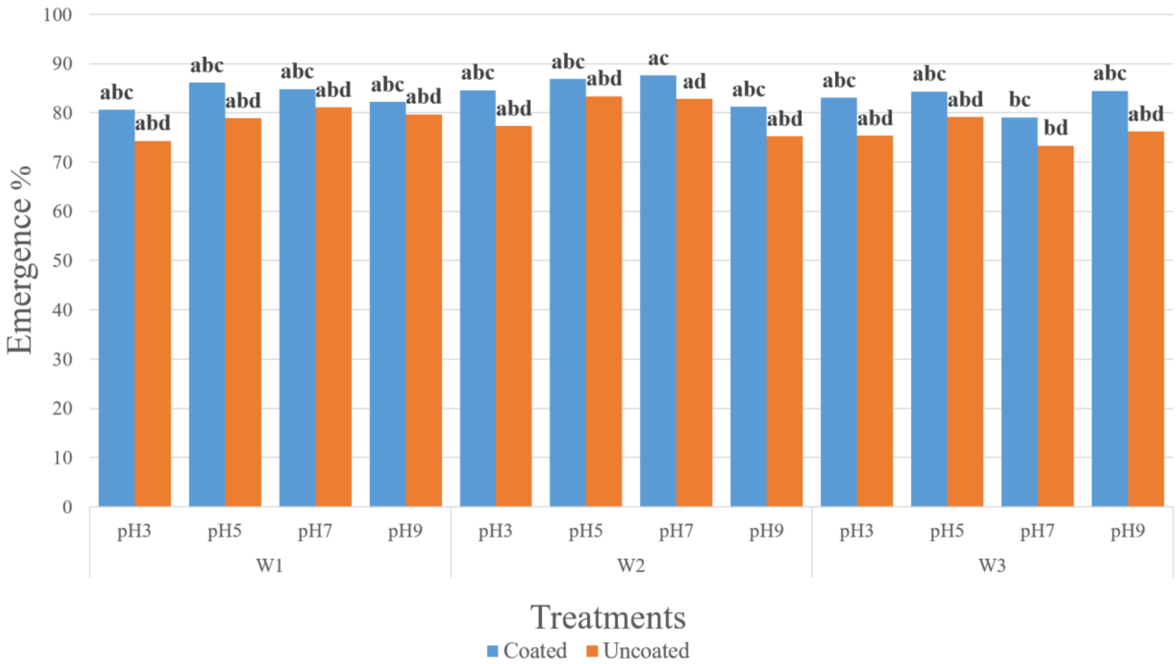

Figure 2.16: The emergence percentage of coated and uncoated *M. sativa* at four different pH (3, 5, 7 and 9) and three different water treatments (75% of field capacity (W1), field capacity (W2) and 125% of field capacity (W3))

**Values with the same letter are not significant and values with different letters are significantly different*

The highest emergence percentage was at field capacity (W2) and the lowest at W3. At W1 the emergence percentage was higher than that of W3 and lower compared to field capacity. This shows that *M. sativa* is very sensitive to the water content in the soil. The water potential range in *M. sativa* seed is very low, and if it does not fall in this range, the emergence will be affected as noted in this trial (Bewley and Black, 1994).

The coating contributed significantly to the difference in emergence percentage as already mentioned. Coated seed had a higher emergence percentage in all the different treatments compared to uncoated seed as seen in Figure 2.16. The coating around the seed definitely had an advantage by improving germination and emergence.

2.5 CONCLUSION

Salinity affected all the species. In all the species there was a decline in emergence as the concentration of the salts added were increased. Na_2SO_4 had the largest effect on emergence due to the high EC values, above 10 dms^{-1} (normal range of salinity in the field is between 4 and 10 dms^{-1} (EC)). *Cenchrus ciliaris*, *C. dactylon* and *E. curvula* were very sensitive to high salinity levels and is generally not recommended in soils with high salinity's. There can be exceptions to this statement as these species can be adapted in some soils with high salinity's. The different water treatments did not have an effect on most of the species in the salinity treatments, except for *C. ciliaris and C. dactylon* that had improved emergence at higher water levels.

Coated seed had a higher emergence percentage compared to uncoated seed in the *C. gayana, C. dactylon* and *M. sativa* treatments, therefore coated seed of these species are more adapted in soils with higher salinity levels. Uncoated seed had a higher emergence percentage in the *C. ciliaris*, *E. curvula* and *D. eriantha* treatments, but *C. ciliaris* and *E. curvula* should not be used in soils with high salinity levels due to their sensitivity to salinity.

The different pH levels of water applied to the growth substrates did not have a significant effect on *D. eriantha*, *E. curvula* and *M. sativa*. *Cenchrus ciliaris* and *C. dactylon* (both hulled seed) had a higher emergence percentage in acidic soils and lower emergence percentage in soils with a neutral pH (pH 7). *Chloris gayana* had a higher emergence percentage at pH 7 and was more sensitive to acid and alkaline soils. In most of the species the highest emergence percentage was found at field capacity except for *D. eriantha* and *C. dactylon* where the emergence percentage increased as the water level increased. Coated seed had a higher emergence percentage in *C. ciliaris*, *C. dactylon* and *E. curvula* treatments. Uncoated seed had a higher emergence percentage for C. *gayana* and *D. eriantha* treatments. There was no difference between coated and uncoated seed in *E. curvula* treatments. Therefore seed coatings is not always beneficial and can sometimes also have no effect. These findings were made on small scale trials and these findings need to be confirmed on bigger field studies before any real recommendations can be made.

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CHAPTER 3

Prepared according to African Journal of Range and Forage Science guidelines

The effects of seed coating on the germination and emergence of various grass species in different mined substrates

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3.1 ABSTRACT

Degraded mined land and the amount of degraded farmland is on the increase. These unfavourable growth conditions, can often restrict germination and root development. These restrictions complicates the establishment and therefore revegetation process. The presence of potentially harmful elements, high salinity's and high pH in the soil or substrate contributes to the problem of rehabilitation. The technology to coat seeds with pesticides, nutrients, etc. has shown much potential in previous studies and can facilitate the establishment of grasses in these chemically and/or physically degraded soils or substrates. In this study the germination rate of *Cynodon dactylon, Chloris gayana, Cenchrus ciliaris, Digitaria eriantha, Eragrostis curvula* and *Medicago sativa* (coated and uncoated) were tested in nine different mined substrates. This research concluded that coated seed benefitted the establishment of *C. ciliaris, E. curvula* and *M. sativa*. Coated and uncoated seed had very similar germination rates in *C. gayana*. Uncoated seed had the highest germination for *C. dactylon* and *D. eriantha*. These were the overall observations of the study, but there were a few exceptions to these observations. This study's results highlight the importance of coating treatments for some of the species, especially in soils of lower quality and are therefore important in land rehabilitation practices.

Keywords: Degraded mined land, coated seed, germination and emergence, rehabilitation

3.2 INTRODUCTION

Mining activities produce by-products, tailings materials, waste products which contribute to large slurry dams. These tailings materials are high in heavy metals (above the normal threshold) for example; copper (Cu), zinc (Zn), lead (Fe), aluminium (Al) etc. (Shu et al., 2002). The mining of metals and consequently the disturbance of the soil structure due to the physical extraction process causes the soil quality to decline. The decline in soil quality makes in very difficult for plants to survive and these areas are left barren (Shu et al., 2002).

Coal is one of the most important fossil fuels mined in the world and is regarded as one of the pillars of most countries' economies. The demand for coal is on the increase and therefore it is very difficult to move away from coal to generate electricity. There are large areas where the natural vegetation is removed and cleared to mine coal. These areas that are mined are required to be rehabilitated once mining is completed (Mentis, 2006). The conventional way to revegetate mined land is by planting grass species. The problem however is that germination rate of these species are very low in these mined substrates.

Water, air, composition of the soil, temperature and light are the normal requirements for seed to germinate under environmental conditions (Hadas, 2004). The soil or substrate that the seed is planted in, should not be too compacted which will prevent germination. It is not the factors on their own but the interaction between them that effects germination. The water quality of water is also important especially for coated seed, as water is responsible for all chemical reactions in the seed and the soil, and therefore a lower germination rate will result if the water is of lower quality (Hadas, 2004). Factors like seed bed not being fine enough, low quality seed, planting at the wrong time and seed quality (dead seed, sterile seed and seed dormancy) contributes to a lower germination rate (Catroux et al., 2001).

The chemical reactions in the water are important, also in addition to the interaction between pH, salinity of the water and the metals found within the soil. The pH is affected by the amount of hydrogen ions within the soils. The pH within water and soil alone will in most cases not have an effect on the germination or growth of species, but the interaction of the pH and metals with each other (Dong et al., 1995). The sanity of the soil also effects the germination. Salinity is measured in EC (electrical conductivity), and the higher the EC, the higher the salinity of the soil. Germination rate decreases as the salinity of a growth medium increases. The higher salinity in the soil causes the water to stay in the soil and prevent water from moving into seed and roots, resulting in no or lower germination rate.

High salinity levels cause the effects of high pH to be more severe. The pH of the soil on its own is not a major problem, but is very important in the release of some minerals, making minerals more available to plants or preventing the release of some minerals (Mattigod and Page, 1983). The pH of the soil determines the amount of minerals in the soil that will be made available for the plant to use for growth. A study was conducted were the uptake of aluminium was measured in soils with low pH, and it was found that the uptake of Al increased as the pH decreased (Dong et al., 1995). The uptake increased dramatically as the pH (H_2O) dropped below 4.4 (Dong et al., 1995). The pH also affects the amount of nitrogen (N) that was taken up by the plants. The highest amount of ammonia (NH_4) that was taken up was at a pH (H_2O) between 6.5 and 8.5 and the lowest was were the pH was less than 6.5 and more than 8.5 (Jampeetong et al., 2013).

The use of coated pasture seed was introduced to production systems to overcome some of these problems and to improve germination and establishment in pastures. The process of coating the seed is a science that incorporates information and technologies from seed, pesticide and chemical companies. The seed coating process involves adding polymers, nutrients, fungicides, fillers and insecticides to the outer coating of the seed itself (Detroz and Gago, 1991). It is hypothesized that these coating treatments can improve the germination rate of species, depending on the soil it is planted in, therefore in turn making the establishment of these species more successful. The coating of coated seeds will disintegrate when there is enough water, but will remain intact when there is not enough water, therefore it is more drought resistant (Detroz and Gago, 1991). Additives are added to the outer layer of the coating of these coated seeds, and are slow releasing so that the activity lasts longer and seed is supported for longer and therefore better chance of surviving (Detroz and Gago, 1991). The main aim of coated seed is to overcome most of the environmental challenges by improving the success rate of germination and to give a seed the best chance of survival.

Coated seed are not always successful and is only there to improve the survival rate of seed that germinates. Seed coatings were developed to increase the ease of handling, to have a heavier and more uniform seed (Halmer, 2004). Coated seed also germinates 24 hours later compared to uncoated seed in the same species (Nel, 2014). The seed coating technologies for seed are complex and seed companies are constantly changing and trying to improve the coating. The coating used is different for various species and a general of coating cannot be made.

The hypotheses of this study were:

- Coated seed will have a better emergence percentage compared to uncoated seed in different mining substrates.
- Coated seed germinates and emerges better in wetter soils/substrates.
- Seedlings emerged from coated seed in drier soil/substrates are more vigorous.

3.3 METHODOLOGY

This study was carried out in a growth chamber at Phytotron D on the Hatfield experimental farm, Pretoria, South Africa. This study was done to determine the effect of seed coatings on the germination percentage of different pasture species in different mined substrates. Ten individual trials were conducted, one trial for each of the different substrates used. The germination percentage was determined in each of the trials. For each of the trials five grass species and one legume species were used. The species used were *Chloris gayana*, *Cenchrus ciliaris, Cynodon dactylon, Digitaria eriantha, Eragrostis curvula* and *Medicago sativa*. All the species had two seed treatments, uncoated and coated. The same cultivars where used for both coated and uncoated treatments for each of the different species used. The seed was supplied by Advance Seed and fresh batch of seed was used for each trial. The coating applied to the seed contained nutrients, fertilizers, pesticides, polymers, lime and in the case of *M. sativa* it contained an inoculant (rhizobia).

The germination percentage and emergence trial using coated and uncoated *D. eriantha*, *E. curvula*, *M. sativa*, *C. dactylon*, *C. gayana* and *C. ciliarus* was conducted in different substrates with multiple observations as presented in Table 3.1. Table 3.1 shows the days when observations were made and when the emerged seed is counted using ISTA guidelines (ISTA, 2003) for the first 2 counts and then 2 more counts were made with 5 days intervals. The two additional counts were used to ensure that the maximum emergence was recorded.

Table 3.1: Specifications of species in terms of expected germination and days after planting when observations are made.

	Days at which observations were taken			
Species	**First observation**	**Second observation**	**Third observation**	**Fourth observation**
Digitaria eriantha	4	10	15	20
Eragrostis curvula	5	15	20	25
Medicago sativa	4	10	15	20
Cynodon dactylon	7	21	26	31
Chloris gayana	7	14	19	24
Cenchrus ciliaris	7	28	33	38

Four hundred grams of each substrate was placed in small plastic container ($600cm^2$). *Digitaria eriantha*, *C. gayana* and *C. dactylon* were planted in the same container separated by wooden dividers, and coated and uncoated seeds were planted in separate containers. Coated and uncoated *C. ciliaris* was planted in the same container with a 4 cm gap between them. *Medicago sativa* and *E. curvula* were planted in the same container separated by wooden dividers, and coated and uncoated seeds were planted in different containers. The different substrates used are shown in Table 3.2.

Table 3.2: Nine different substrates produced from mining activities with a red sandy loam soil as a control

Gypsum,	Fluorspar
Gold <2% pyrite	Coal tailings
Gold>2% pyrite	Andulusite
Platinum tailings	Control (red sandy loam soil)
Kimberlite	

One hundred seeds of each species were planted per treatment. Three water treatments were applied, W1, W2 and W3, where W2 was the amount of water required for the soil to be at field capacity. Treatment W1 was 75% of treatment W2 (75% of field capacity) and treatment W3, 25% more than treatment W2 (125% of field capacity). The field capacity was determined using the WP4-T machine. The WP4-T is also known as the Decagon's WP4 Dew point Potential Meter. WP4 is used to measure water potential and is measured in MegaPascals. It measures water potential from 0 to -300 MPa with an accuracy of ±0.1 MPa (Devices, 2007). The machine has mirrors on the inside were water condensates on to. At a certain point there exists an equilibrium between the moisture in the sample and the moisture that condensates on these mirrors. This equilibrium gives the water potential reading that is used to determine field capacity (Devices, 2007).

Table 3.3 shows the amount of water each substrate should get for each water treatment. The correct amount of water was added to the containers, weighed and then placed into plastic bags to prevent excessive water loss. Every 10 days the containers where removed, weighed and topped up with the correct amount of water to keep the water level the same, to keep the amount of water constant throughout the trial. The growth chamber was set at a constant 25˚C, with a twelve hour lighting cycle. The light was constant and the containers were kept the same distance away from the light in each treatment.

Table 3.3: Water applications required by each treatment to achieve the specific water content of different substrates.

	125% OF FIELD CAPACITY		FIELD CAPACITY		75% OF FIELD CAPACITY	
	Water content (g/400g)	Water potential (Pa)	Water content (g/400g)	Water potential (Pa)	Water content (g/400g)	Water potential (Pa)
Control (sandy loam soil)	43.0	-3.0	34.0	-33.0	26.0	-242.0
Gypsum	60.0	-4.0	45.0	-33.0	30.0	-221.0
Gold <2% pyrite	64.0	-4.0	51.0	-33.0	38.0	-235.0
Gold>2%	144.0	-6.0	115.0	-33.0	86.0	-177.0

pyrite						
Platinum tailings	109.0	-6.0	87.0	-33.0	65.0	-185.0
Kimberlite	139.0	-5.0	111.0	-33.0	83.0	-225.0
Fluorspar	140.0	-6.0	112.0	-32.0	84.0	-178.0
Andulusite	98.0	-5.0	78.0	-34.0	59.0	-224.0
Coal tailings	201.0	-4.0	161.0	-32.0	121.0	-239.0

The emergence percentage of each treatment was determined by a physical count of emerged seedlings each time. The seed that emerged each time was not removed when observations were made and left every time, they were only removed at the end of the trial. Germination and emergence are different phases, but for this study the term emergence percentage will be used and will refer to both germination and emergence, when the radicle breaks through, up to and the seedling appears.

Statistical analysis

This trial was established as a completely random design (CRD) with four replications. As the emergence percentages were not skew with homogeneous variances ANOVA factorial analysis was used to test for differences between the effects of ten mediums, three water levels and two coating effects, as well as all their interactions, for each species separately at the 5% level.

Means in all trials were compared using Tukey's least significant difference test at the 5 % level of significance (Snedecor and Cochran, 1980).

Data were analysed using the statistical program GenStat® (Payne et al., 2014)

3.4 RESULTS AND DISCUSSION

The substrates used in this study were submitted to a laboratory for a complete chemical and physical analysis, as is seen in the Table 3.4 and Table 3.5 below.

Table 3.4: The chemical composition of the different substrates used as growing mediums in this trial.

		Different substrates								
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**
pH (KCl)		4.1	4.1	5.3	6.4	8	7.6	7.9	5.5	3.8
pH (H20)		4.1	3.9	5.4	6.1	6.6	9.7	5.7	6	2.5
EC	mSm^{-1}	13	199	193	422	205	121	146	11	390
SO4	mg kg^{-1}	12	159824	1674	447	143	32	122	20	709
P (Bray 1)	mg kg^{-1}	4	252	1	0	0	5	1	3	1
K	cmol kg^{-1}	0.16	0.051	0.076	0.115	0.076	1.846	0.061	0.222	0.064
	mg kg^{-1}	62	20	30	45	31	720	24	87	25
Ca	cmol kg^{-1}	0.441	27.976	8.965	10.213	1.964	9.753	10.144	1.956	11.186
	mg kg^{-1}	88	5595	1793	2043	393	1951	2023	392	2237
Mg	cmol kg^{-1}	0.406	0.142	0.776	2.158	0.407	1.934	1.325	3.352	1.747
	mg kg^{-1}	49	17	94	261	49	234	160	406	211
Na	cmol kg^{-1}	0.004	0.05	0.039	0.51	3.746	0.095	0.199	0.086	0.012
	mg kg^{-1}	1	12	9	117	68	862	46	20	3
Al	cmol kg^{-1}	0.31	0.62	0.04	0	0	0	0	0	21.28
Al	%	22.5	275.5	3.2	0.1	0	0	0	0	2718.3

Key: S1: Red Sandy Loam; S2: Gypsum; S3: Gold < 2% Pyrite; S4: Gold > 2% Pyrite; S5: Platinum Tailings; S6: Kimberlite; S7: Fluorspar; S8: Andulusite; S9: Coal Tailings

Table 3.5: The physical analysis of the substrates used in this trial

	Very coarse sand %	Coarse sand %	Medium sand %	Fine sand %	Very fine sand %	Silt %	Clay %
Red Sandy loam	0.5	4.1	25.3	38.2	24.5	2.9	4.3
Gypsum	0	0.3	0.5	0.9	76.6	14.7	6.9
Gold < 2% Pyrite	0.2	0.6	2.9	33.9	33.5	24.2	4.6
Gold > 2% Pyrite	0.1	0.6	6.5	45.7	33.2	11.8	2.1
Platinum Tailings	0	0.3	8.7	48	33.4	7.4	2.1
Kimberlite	34.6	33.2	13.7	7.7	3.6	2.9	4.3
Flourspar	0.1	2.3	16	36	31.3	12.2	2.2
Andulusite	2.2	4	3.4	2.4	4.1	68.6	15.2
Coal Tailings	1.1	6	13.1	21.2	18	20.9	19.7

Cenchrus ciliaris

The statistical analysis indicated that there was a significant difference ($p \leq 0.01$) between the different growth substrates. There was a significant difference ($p \leq 0.01$) between the coatings in all the mined substrates. There was no significant difference between the different water treatments in Gold > 2% pyrite- and Andulusite substrate, however there was a significant difference in all the other substrates. There was a significant ($p \leq 0.01$) difference in the interaction between coating and water content in Kimberlite-, Fluorspar- and Coal discard substrate. Figure 3.1 and Figure 3.2 illustrates the interaction between coating and water treatments of *C. ciliaris* in the different mined substrates. Figure 3.1 shows the substrates that are more acidic and Figure 3.2 the substrates that are less acidic, more alkaline.

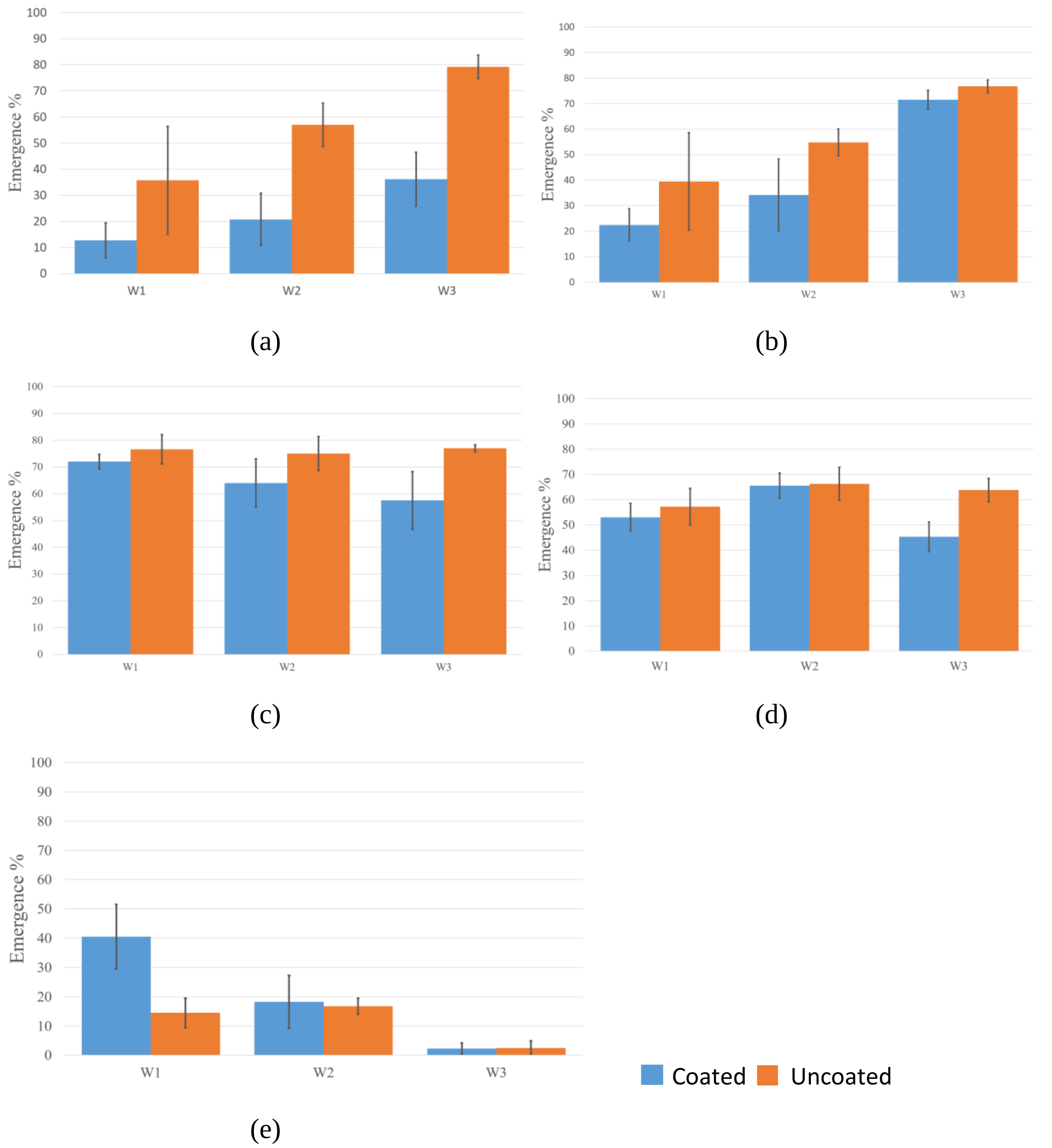


Figure 3.1: The emergence percentage of coated and uncoated *C. ciliaris* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gold < 2% Pyrite-, **c)** Gold > 2% Pyrite-, **d)** Platinum tailings- and **e)** Coal Discard substrate.

Red sandy loam clearly shows that there is much higher emergence percentage for uncoated seed compared to coated seed and this trend is also found in Gold < 2% pyrite substrate. Uncoated seed also had a higher emergence in Gold > 2% pyrite and Platinum tailings. Coated seed had a higher emergence in the coal discard.

In both the control (sandy loam soil) and gold < 2% pyrite, water had a big effect, with the emergence percentage increasing as the water level increased. The emergence percentage for coated seed in Gold > 2% pyrite substrate decreased as the water level increased, in contrast to uncoated seed. In the platinum tailings substrate, the highest emergence occurred at field capacity (W2) for both coated and uncoated seed. There was a significant drop in emergence for coated seed as the water level dropped or increased, which confirms that the platinum tailings substrate should be as close to W2 as possible for highest emergence percentage. In the coal discard substrate the highest emergence percentage was at W1, therefore less water in this substrate is preferred.

The emergence decreased for coated seed in Gold > 2% pyrite substrate as the water level increased. This can be due to the substrate having a higher EC value (199 mS m^{-1} compared to 13 mS m^{-1}) compared to red sand loam that can affect the coatings as they are dissolved as the water level increased. The EC of soil is the ability of soil to conduct an electrical charge between different minerals, a normal EC of between 4 to 8 dS m^{-1} (400 to 800 mS m^{-1}). An EC of above 4 dS m^{-1} is considered saline, enables plant to easily take minerals from the soil, and above this threshold plants are not able to absorbed minerals from the soil (Maas and Nieman, 1978, Setia et al., 2011). The minerals remain in the soil and the plants growth is impacted negatively.

Gold > 2% pyrite substrate is also high in Ca and Na which can both have an effect on the coating. High levels of Na causes imbalances of other minerals in plant (Geilfus and Mühling, 2014), it is hypothesized that the same imbalances can occur in the coating of the seed. The emergence percentage in the coal discard substrate was very low, however the coated seed had a higher emergence at W1. It is also hypothesized that this low water level along with the coating protected the seed for a long enough time to germinate, the coating around the seed took a longer time to disintegrate. The coal discard substrate had a very low pH, it was high in S and Al, therefore not the best growth substrate. The low water level was just enough water for the seed to germinate without washing away.

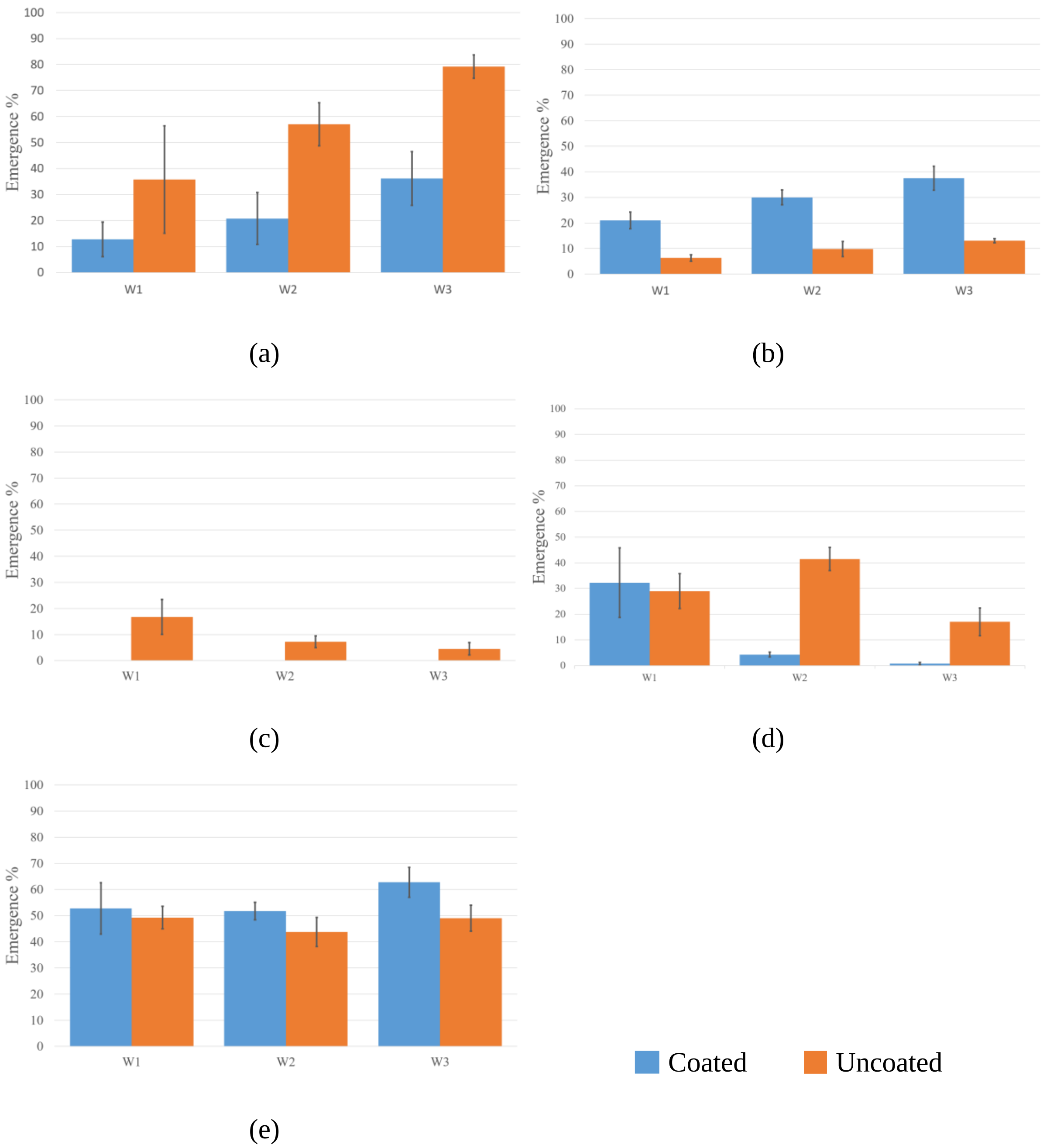


Figure 3.2: The emergence percentage of coated and uncoated *C. ciliaris* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gypsum-, **c)** Kimberlite-, **d)** Fluorspar- and **e)** Andulusite substrate.

Coated seed had a higher emergence percentage in the Gypsum- and Andulusite substrate. The emergence in Kimberlite substrate was very low, but uncoated seed had a higher emergence percentage in this substrate as well as in the Fluorspar substrate. Water content also played a big role in Gypsum substrate, as the water level/content increased, so did the emergence. The opposite happened in the kimberlite substrate, there was a drop in emergence as the water level increased. Uncoated seed had an overall higher emergence in Fluorspar substrate, the highest was at W2 and decreased when water levels decreased. The emergence decreased dramatically when the water level was too high. Coated seed only had a good emergence at W1. There was no real trend in the Andulusite substrate; the emergence percentage increased as the water levels increased and decreased, at W2 the emergence was the lowest for both coated and uncoated seed. These emergence observations will be discussed in detail in the coming paragraphs.

It is clear from both these figures that the different substrates had a huge effect on the emergence percentage and this was also confirmed by the statistical analysis. Previous studies concluded that *C. ciliaris* had a slightly higher emergence at lower pH's compared to higher pH's (Pretorius, 2016). These studies also found that *C. ciliaris* is sensitive to soil with high salinity's levels. Red sandy loam was used as the control in this study. Gypsum-, Kimberlite-, Fluorspar- and Coal discard substrates had a low emergence percentage as compared to the Red sandy loam substrate which acted as the control.

In Table 3.4 and Table 3.5 the pH was low but as mentioned earlier the pH does not really affect *C. ciliaris* germination and emergence. The EC value of Gold > 2% pyrite substrate is higher (199 $mS.m^{-1}$ compared to 13 $mS.m^{-1}$) compared to that of the control; however an additional contributing factor is the high S and P contents of the substrate. Gypsum substrate had the highest level of S and P compared to all the other substrates. Gypsum substrate also had a high level of Ca and Al. High levels of P are not necessarily a problem in plant growth, whereas low levels are of more of a concern (Logan et al., 2000, Gourley et al., 1993). High levels of Al reduces the uptake of Ca, K and Mg (Rout et al., 2001), therefore the plant will have imbalances due no uptake from the soil of minerals and will eventually have a poor growth rate.

Kimberlite substrate is high in K, Mg and Na (can cause toxicity in plants) (Tuteja, 2007). High levels of Na and Cl can cause imbalances of other minerals in plants, for example K and Ca (Geilfus and Mühling, 2014). The biggest cause of the low emergence percentage in Kimberlite substrate is not because of the chemical composition of the substrate, even though it had a small effect, but it was the physical nature of the substrate. As seen in table 3.5, Kimberlite substrate has a high percentage of very coarse and coarse texture. This coarse texture prevent adequate contact between the substrate and the seed. This in turn caused a low germination and emergence, this was the biggest contributor to the low emergence percentage rate in Kimberlite substrate (Nel, 2014). The EC of Fluorspar substrate is also higher (146

$mS.m^{-1}$ compared to 13 $mS.m^{-1}$) as compared to that of the red sandy loam soil control and is hypothesized that it could have had an effect on the emergence. The only other difference is that Fluorspar substrate had a higher Mg level. Higher Mg levels in plants promote a higher photosynthetic rate and results in a higher growth rate (Fischer et al., 1998).

Coal discard substrate also had a very low emergence percentage. The two major causes of the low emergence is the very low pH (2.5 (H_20)) and the high level of Al (Dickinson, 2004, Rout et al., 2001). It was mentioned that the germination is not that sensitive to low pH, but this is a very low pH and the low pH exacerbates the effects of Al (Dong et al., 1995). Therefore the cause of the low emergence percentage is due to a combination of the low pH and the high levels of Al. High levels of Al causes damage in the cells by effecting the cell division process in the roots and shoots, therefore killing the plants (Rout et al., 2001). The major effect of Al toxicity is that there is an inhibition of root growth, especially in the root tips, they turn brown and then die (Rout et al., 2001).

The highest emergence percentage was found in the Red sandy loam- and Gold > 2 % pyrite substrate. The pH of Gold > 2 % pyrite substrate was optimal (6.4 (H_20)) and had a very high EC value (422 $mS.m^{-1}$). It also had a high Na content and this can be the reason for the higher EC value. When considering the physical properties of the substrate, it has a very high percentage of fine and very fine sand, this ensures that there is enough contact with the seed.

Platinum tailings substrate had a good emergence percentage with coated and uncoated seed having similar emergence percentages, except uncoated had a higher emergence at W3. The emergence was very constant and slightly lower than that of red sandy loam soil. The platinum tailings substrate has a fine physical texture that ensures adequate contact with the seed as to ensure optimal germination and emergence. Gold < 2% pyrite substrate had a very similar emergence compared to the red sandy loam soil. Gold < 2% pyrite substrate only had elevated Ca levels, and *C. ciliaris* prefers high levels of Ca (Dickinson, 2004). Gold <2% pyrite substrate had a fine texture with high percentage of sand, this fine texture ensured enough contact was made with the seed surface. Seed planted in the Andulusite substrate had a lower emergence percentage compared to that of red sandy loam soil. This substrate also had a very fine texture, similar to that of the Gold < 2% pyrite substrate. This substrate also had high levels of Mg, which plays a less important role in germination and emergence, however it is important in photosynthesis (Fischer et al., 1998, Mayland, 1990).

Cynodon dactylon

The statistical analysis showed that there was no significant difference between the seed coating treatments in all the substrates except in Andulusite substrate. There was a significant difference ($p < 0.05$) between the interaction between the substrates and the water content in Andulusite substrate, there was no significant difference in the other substrates. There was a significant difference in the different water treatments in Gold > 2% pyrite-, Platinum tailings, Fluorspar tailings-, Andulusite- and Coal discard substrate. Figure 3.3 and Figure 3.4 below shows the interaction between seed coating and water treatments in the different substrates which *C. dactylon* is grown in. Figure 3.3 shows the acidic substrates and Figure 3.4 the alkaline substrates.

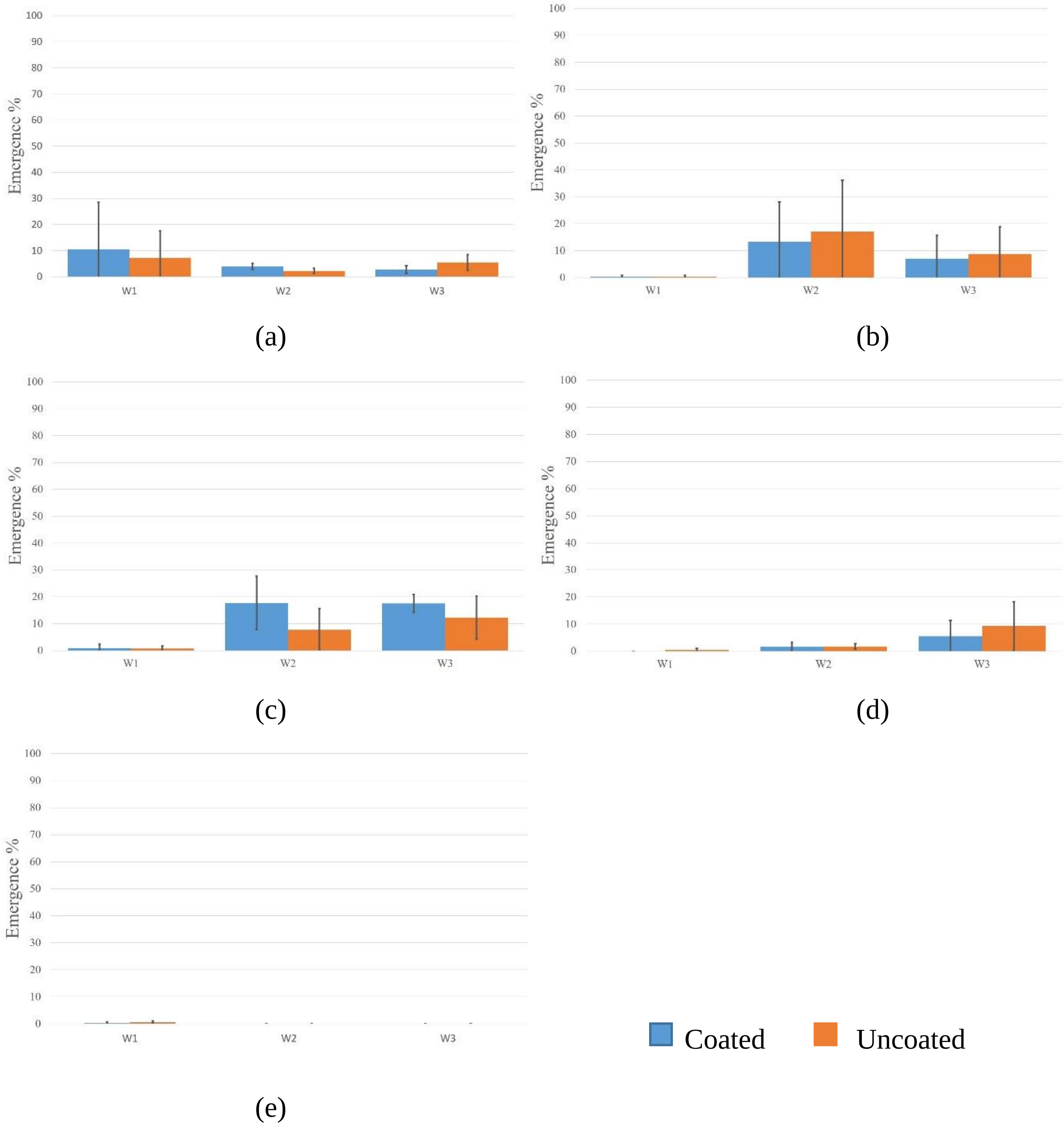
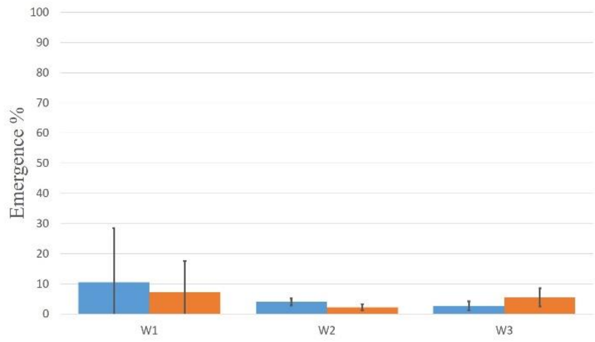


Figure 3.3: The emergence percentage of coated and uncoated *C. dactylon* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gold < 2% Pyrite-, **c)** Gold > 2% Pyrite-, **d)** Platinum tailings- and **e)** Coal Discard substrate.

As previously mentioned, the seed coating treatments did not significantly differ, however it is clear that coated or uncoated seed can be used in any of the different substrates. The water content of the substrate had a significant impact on emergence percentage. In Red sandy loam substrate it is clear that the highest emergence was at W1 (even though the statistical anylis did not show it) and then declines as the water level increases. In the Gold > 2% pyrite substrate the emergence percentage was the same for coated seed at both W2 and W3 treatment. In Gold > 2% pyrite substrate it is clear that uncoated seed had a slightly higher emergence at water treatment W3 as compared to W2, with almost nothing emerging at treatment W1. In the Platinum tailings substrate the highest emergence percentage was noted at water treatment W3. There was very low emergence at W2, and there was no emergence at treatment W1. There was almost no emergence in the coal discard substrate.



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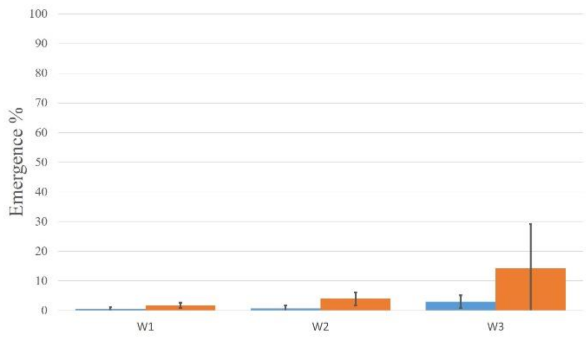

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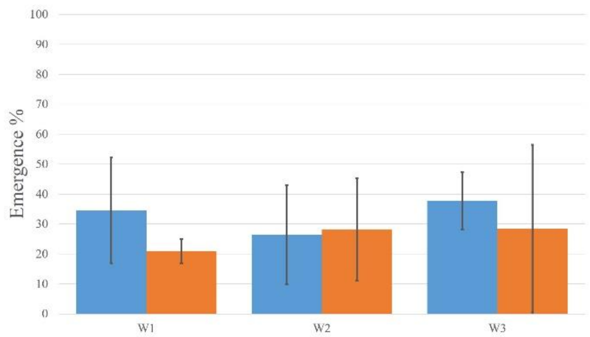

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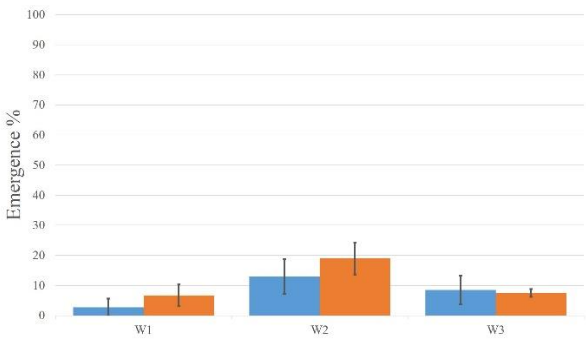

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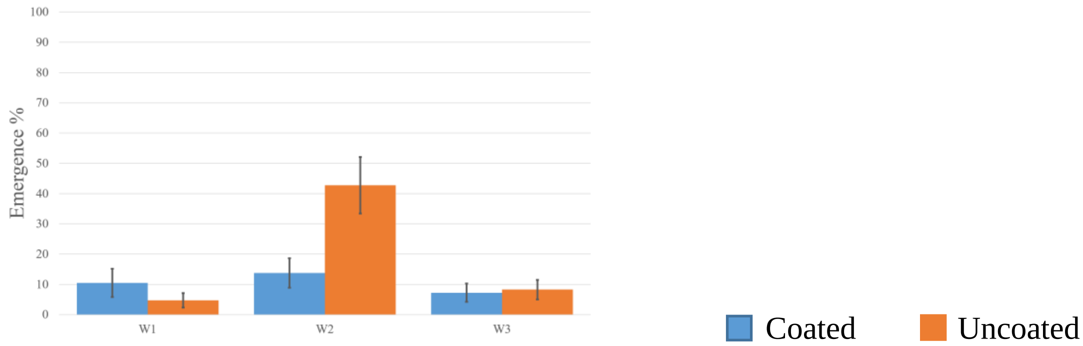


(e)

Figure 3.4: The emergence percentage of coated and uncoated *C. dactylon* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gypsum-, **c)** Kimberlite-, **d)** Fluorspar- and **e)** Andulusite substrate.

The emergence was low in the Gypsum substrate, with the highest emergence percentage obtained for water treatment W3, for uncoated seed. Coated seed emergence percentage was very low, the statistical analysis showed there was no significant difference, but from Figure 3.4 uncoated seed had a higher emergence compared to coated seed. The emergence percentage in the Kimberlite substrate does not show any trends and none of the treatments were significantly different. The highest emergence percentage was at treatment W3 for coated seed and for uncoated seed it was in treatment W2 and W3. The lowest germination rate was in treatment W1 for both seed coating treatments. In both Fluorspar- and Andulusite substrates, the highest emergence percentage was at W2 for uncoated seed, only Andulusite substrate was significant.

The highest mean emergence percentage was in the Andulusite substrate while slightly lower in Kimberlite substrate. Table 3.4 indicates that the Andulusite substrate had a slightly higher level of K as compared to the red sandy loam substrate. The pH was also closer to neutral and also had a lower level of Ca compared to the Red sandy loam soil. There was also a higher level of Na and Mg compared so red sandy loam substrate, with high levels of Mg not having any effect on germination and emergence (Fischer et al., 1998). All these factors could have contributed to a higher emergence percentage. Andulusite substrate has a very fine physical texture, which ensures a good contact between the seed and the substrate (Van Oudtshoorn, 1999, Nel, 2014).

The Kimberlite substrate had a very high K, Ca, Mg and Na content. High levels of K are not detrimental to plants and are often common in soils (Ashley et al., 2006). High levels of Ca can cause imbalances in other minerals which affects the transport and availability of nutrients that in turn effects germination, emergence and growth (Tuteja, 2007). These high Ca levels are still within adequate ranges (Fertilizer Society of South, 2007). The Kimberlite substrate has a coarse texture and this prevents sufficient contact made by the outer layer of the seed with the substrate. This however does not explain the results obtained for *C. dactylon.*

Red sandy loam substrate acted as the control and this substrate is closest to the ideal and/or normal growing conditions, with the chemical and physical properties most optimal (Fertilizer Society of South, 2007). The emergence results however do not reflect the latter statement as they are very low. Gold < 2% pyrite- and Gold > 2% pyrite substrates have low emergence percentages and are very similar. Both these substrates have high EC values and elevated S levels. They are both low in K and as already mentioned low levels can affect germination, emergence and growth negatively (Ashley et al., 2006). The Gold > 2% pyrite substrate has high levels of Mg and Na. Gold < 2% pyrite substrate has adequate levels of Mg and Na but the emergence still remains low. The only similarities between Gold < 2% pyrite- and Gold > 2% pyrite substrate is the low K levels, which can be the cause of the low emergence percentages. The physical texture are also very similar; both have a fine texture, which is adequate for growth.

Platinum tailings- and gypsum substrate have a very low emergence, with coal discard substrate having almost no emergence. The Platinum substrate has a high pH, but as already established, pH does not have an effect on germination and emergence (Pretorius, 2016). The Platinum substrate is low in K and as mentioned above, low levels of K can affect germination, emergence and growth negatively, especially in the roots (Ashley et al., 2006). The Platinum tailings substrate is also low in Mg while the other mineral levels found in Platinum tailings substrate are sufficient (Fertilizer Society of South, 2007). Magnesium and K are most likely to have caused the low emergence percentage in platinum tailings substrate. The physical texture of the Platinum tailings substrate is also adequate with a good fine sand fraction to support good seed contact.

Gypsum substrate is very high in S, Ca and Al, with very low levels of Mg and K. High levels of S can cause other micro nutrients and metals to be less available in the soil (Dawood et al., 1985, Rout et al., 2001). The negative effects of K and Mg have already been discussed before. High levels of Ca can cause imbalances in other minerals that affects the transport and availability of nutrients that in turn effects emergence and growth (Tuteja, 2007). High levels of Al causes damage in the cells by effecting the cell division process in the roots and shoots, therefore killing the plants (Rout et al., 2001). The major effect of Al toxicity is the inhibition of root growth, especially in the root tips, they turn brown and then die (Rout et al., 2001). The coal discard substrate is not an ideal growth substrate, with a very low pH, very high Al, S content and a very high EC. With all these factors it is difficult to ascribe the low emergence percentage to only one factor.

Chloris gayana

Chloris gayana is known to be very salt tolerant and can survive in very saline conditions, since they have salt glands located in the leaves that excrete excessive salts (Kobayashi and Masaoka, 2008, Kopittke et al., 2007, Ortega and Taleisnik, 2003). *Chloris gayana* is not able to secrete Mg ions and will start secreting K ions when there are high levels of Mg, which will then affect the plants osmotic regulation (Kobayashi and Masaoka, 2008). Although *C. gayana* is salt tolerant, the production will be lower in saline soils as compared to non-saline conditions, but will still have a higher production compared to other grass species (Luna et al., 2002).

The statistical analysis of *C. gayana* showed there were significant differences ($P \leq 0.05$) between the coatings in Kimberlite-, Fluorspar- and Andulusite substrates. Andulusite substrate also showed significant difference in the different water treatments. Coal discard substrate had significant interaction in the coating treatments, water treatments and in the interaction between coating and water treatments. Figure 3.5 and Figure 3.6 below shows the interaction between seed coating and water treatments in the different substrates which *C. gayana* is grown in. Figure 3.5 shows the acidic substrates and Figure 3.6 the alkaline substrates.

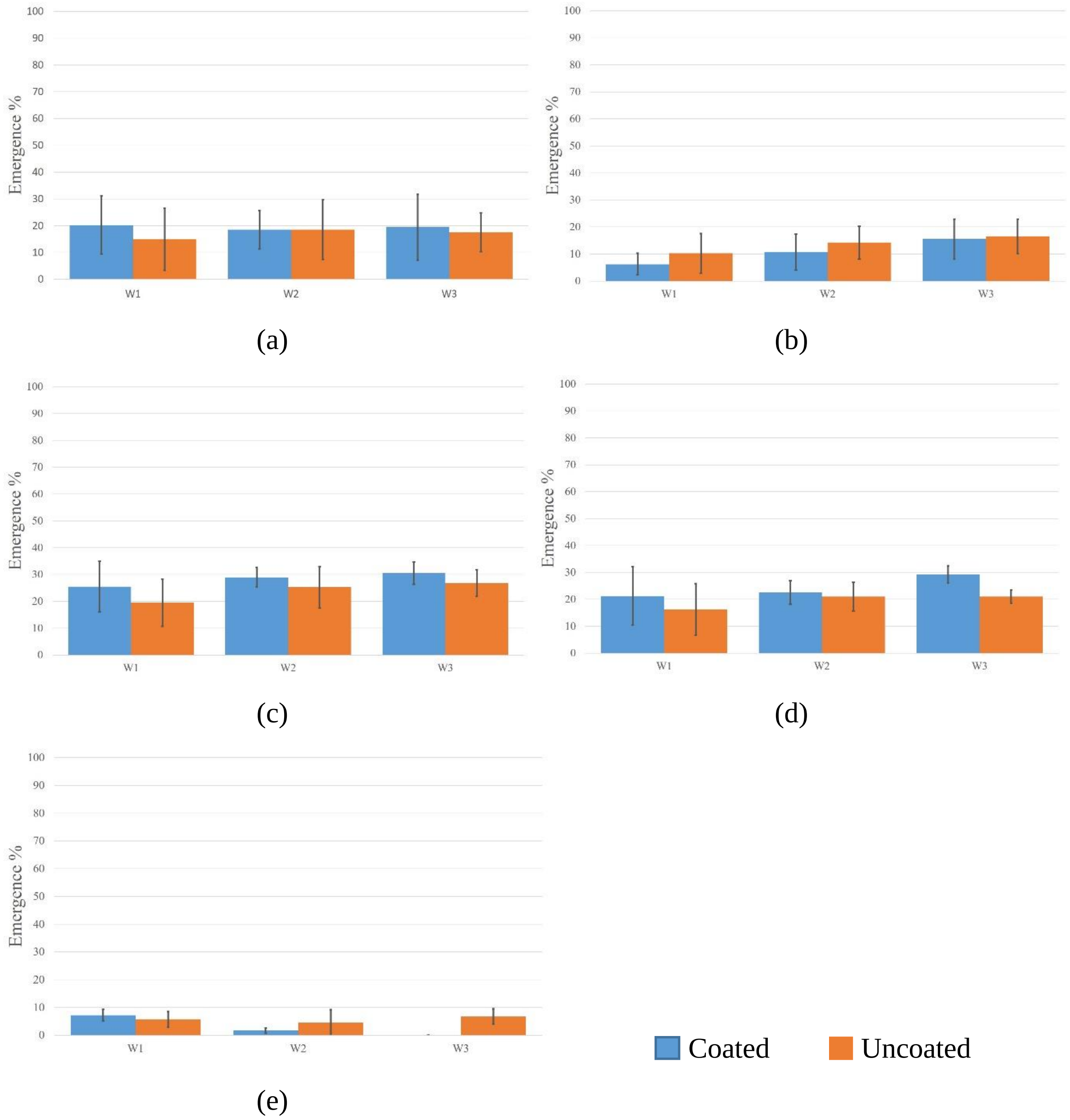


Figure 3.5: The emergence percentage of coated and uncoated *C. gayana* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gold < 2% Pyrite-, **c)** Gold > 2% Pyrite-, **d)** Platinum tailings- and **e)** Coal Discard substrate.

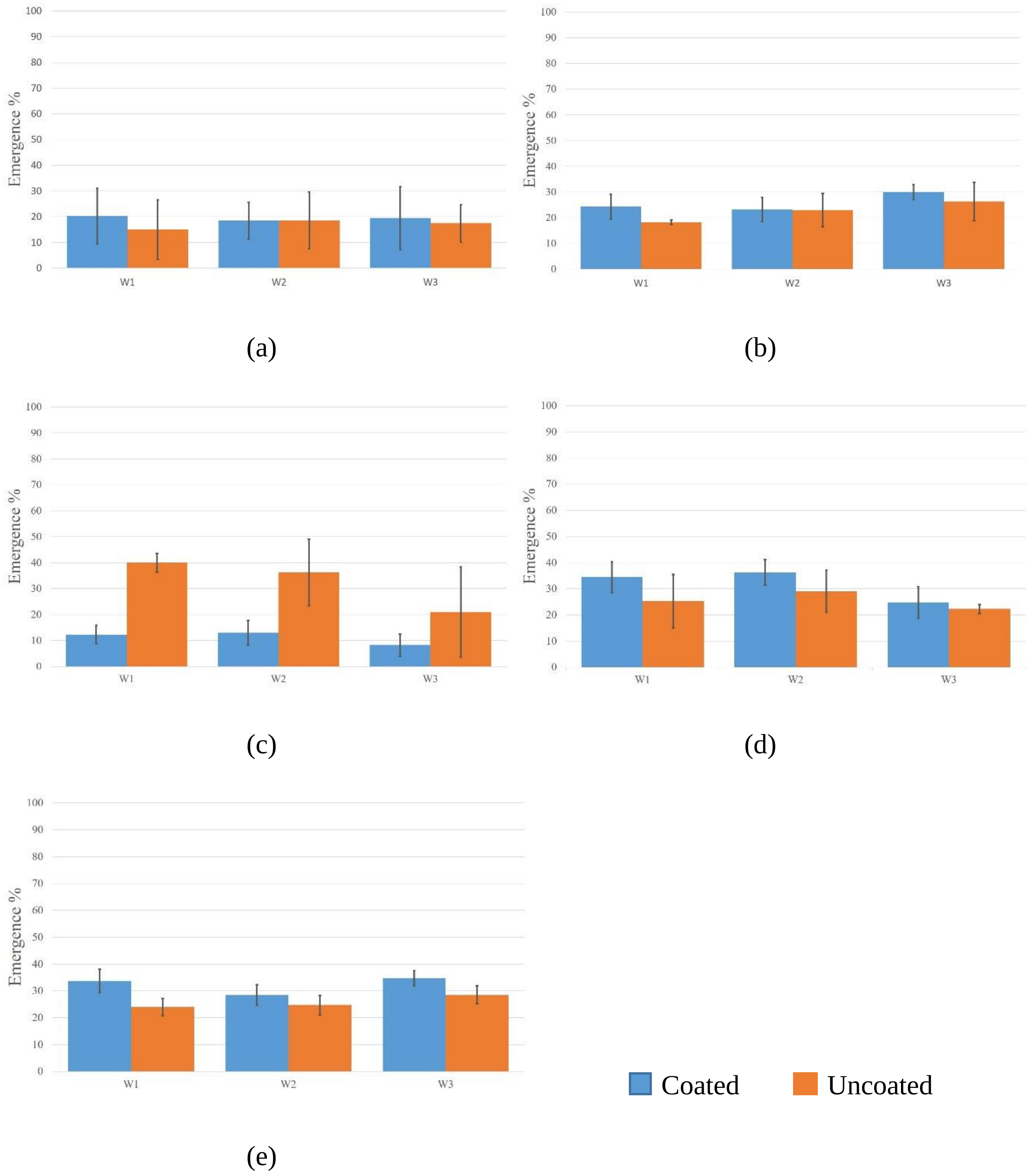


Figure 3.6: The emergence percentage of coated and uncoated *C. gayana* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gypsum-, **c)** Kimberlite-, **d)** Fluorspar- and **e)** Andulusite substrate.

There was no difference between the coated and uncoated *C. gayana* seed planted in the red sandy loam substrate. The Gold < 2% pyrite substrate had a lower emergence compared to the red sandy loam substrate, this was not due to the physical texture as the texture are very similar. The Gold < 2% pyrite substrate had an elevated S level and a lower K level, therefore the lower emergence was due to these to minerals. There was no difference in emergence percentage between coated and uncoated seed.

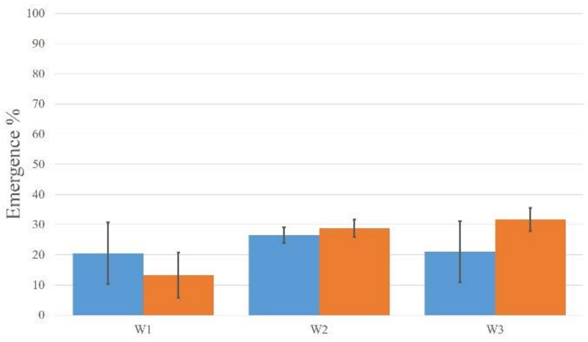
The Gold > 2% pyrite substrate had a higher emergence percentage compared to red sandy loam substrate, this can be due to the higher Ca, Mg and Na levels. Higher EC values and Na values are negatively associated with growth (Tuteja, 2007). Calcium and Mg ratios are also important for growth, if the Mg levels are too high the ratio will be altered, the plant growth and plant functioning is impacted negatively (Machette, 1986). The Ca value is high, but it is still in the acceptable range (Fertilizer Society of South, 2007). The statistical analysis for Gold > 2% pyrite substrate showed that there was no significant difference between coated and uncoated seed. Figure 3.5 shows that coated seed had a higher emergence percentage compared to uncoated seed. In the Platinum tailings substrate, coated seed had a higher emergence as compared to uncoated seed; where uncoated seed had the same emergence percentage as red sandy loam substrate, although the statistical analysis showed there was no significant differences. All the mineral levels are within an acceptable range (Fertilizer Society of South, 2007), with the only difference being a higher pH and a higher EC. The pH does not have an effect on germination and emergence, therefore the higher EC values are beneficial to coated seed. The texture of the Platinum tailings substrate is similar to that of red sandy loam substrate, fine texture that promotes germination and emergence.

The emergence of *C. gayana* in coal discard substrate is also low, as in the previous two species. Coal discard substrate is not an ideal growth substrate, with very low pH, very high in Al, S and a very high EC. It is difficult to determine what caused the low emergence, but it is clear that *C. gayana* is not suited for this substrate. The Gypsum substrate also had a higher emergence, the reason for the higher emergence is unknown at this point. This substrate is very high in S, P, Ca an Al, but low in K, and all these factors affect growth negatively, (Rout et al., 2001, Tuteja, 2007) however the emergence in the Gypsum substrate is high. The only factor that can be beneficial is the low Na content, because high levels of Na can be toxic to plants (Yang et al., 2008, Tuteja, 2007). The texture of the Gypsum substrate is similar to that of red sandy loam soil. There was no difference in emergence percentage between coated and uncoated seed treatments, but there was a slight increase in emergence as the water level increased.

The Kimberlite substrate showed a big difference in emergence percentage between the coated and uncoated seed treatments. This big difference can possibly be ascribed to the physical texture of the substrate which had a negative effect on the coated seed (Van Oudtshoorn, 1999). Uncoated seed had a higher emergence as compared to red sandy loam soil. This substrate is also high in Na, K and Mg, and these two minerals could have had a negative effect on the seed coating. Sodium causes imbalances in other minerals, for example K and Mg (Tuteja, 2007), it is hypothesized that the same imbalances is caused in the coating that lowers the emergence. Fluorspar- and Andulusite substrates showed a similar trend, having slightly higher emergence as compared to Red sandy loam soil, with the coated seed having a higher emergence percentage. These two substrates have similar mineral compositions, with Fluorspar substrate having a low level of K and the Andulusite substrate having high levels of Mg, however none of these factors affected growth.

Digitaria eriantha

There was a significant difference ($p<0.05$) between the different water treatments in Red sandy loam-, Gypsum-, Gold < 2% pyrite- and Gold > 2% pyrite substrate according to the statistical analysis. There was also a significant difference between the different coating treatments in Kimberlite-, Andulusite- and Coal discard substrate and in the interaction between coating water treatment in Gypsum. Figure 3.7 and Figure 3.8 below shows the interaction between seed coating water treatments in the different substrates which *D. eriantha* is grown in. Figure 3.7 shows the acidic substrates and Figure 3.8 the alkaline substrates



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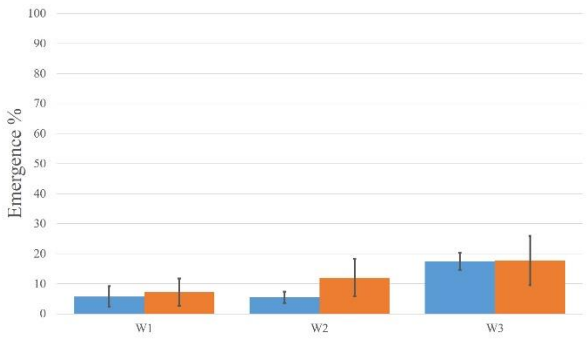

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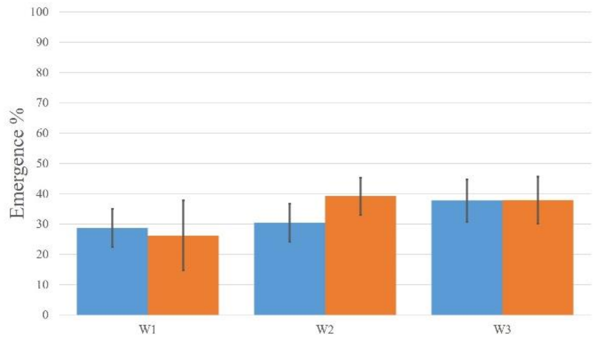

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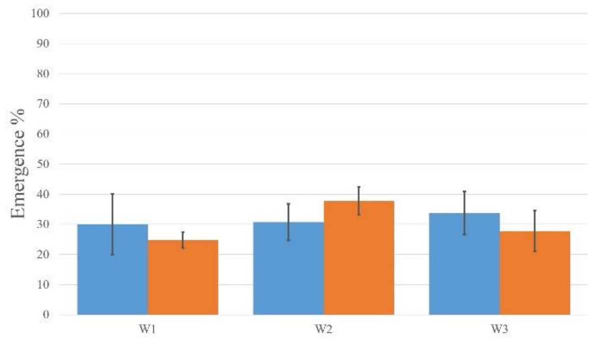

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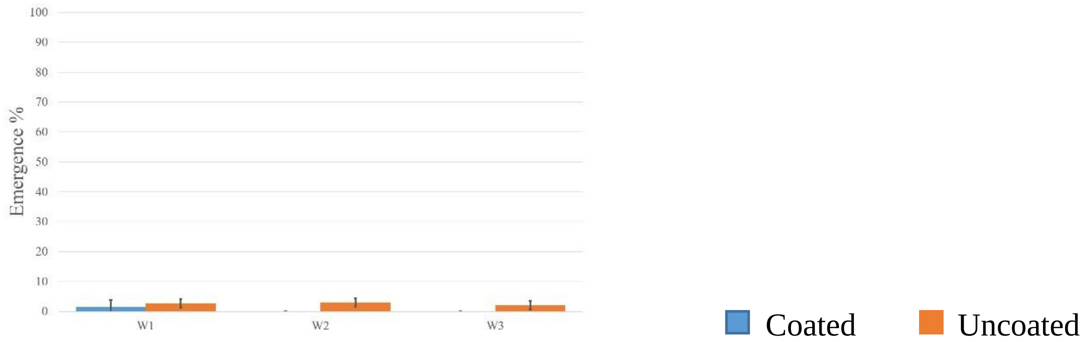

(e)

Figure 3.7 The emergence percentage of coated and uncoated *D. eriantha* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gold < 2% Pyrite-, **c)** Gold > 2% Pyrite-, **d)** Platinum tailings- and **e)** Coal Discard substrate.

The lowest emergence percentage for Gold < 2% pyrite-, Gold > 2% pyrite- and Gypsum substrate is at W1 and increases until maximum emergence is reached at W3. In Platinum substrate the highest emergence was at W2 for uncoated seed even though the statistical analysis showed there was no significance. In Red sandy loam substrate uncoated seed had a higher emergence percentage compared to coated seed, but it was not significant. The Kimberlite substrate had almost no emergence for coated seed and the emergence percentage declined as the water content increased for uncoated seed, this was the same for coated seed in the Fluorspar substrate. The highest emergence percentage was at W2 for uncoated seed in Fluorspar substrate, but there was no significant difference in any of the treatments in Fluorspar substrate. Coal discard substrate had a very low emergence percentage.

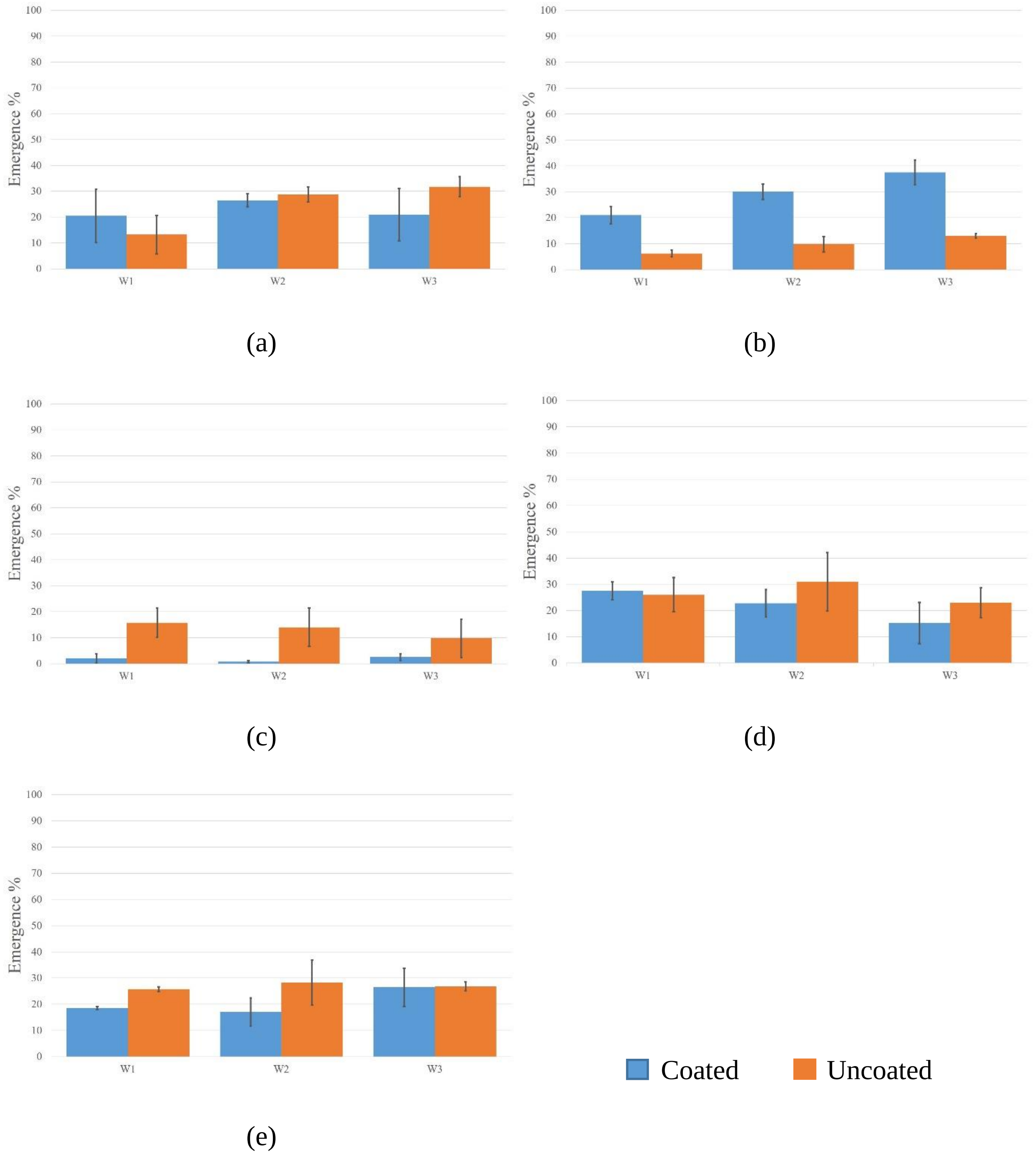

Figure 3.8: The emergence percentage of coated and uncoated *D. eriantha* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gypsum-, **c)** Kimberlite-, **d)** Fluorspar- and **e)** Andulusite substrate.

The emergence percentage in most of the substrates are very similar and are almost the same as that of Red sandy loam. There were only three substrates where the emergence was very low; Gold < 2% pyrite-, Coal discard- and Kimberlite substrate. The cause of low emergence in the Gold pyrite substrates is not clear. The low emergence could be ascribed to the higher S levels, that causes imbalances in other minerals, or the very low K levels, reduces root growth (Ashley et al., 2006, Dawood et al., 1985). The negative factors (very low pH levels and very high Al levels) that cause the low emergence in Coal discard substrate have also been discussed (Rout et al., 2001, Dong et al., 1995) . Uncoated seed in the Kimberlite substrate had a much higher emergence percentage compared to coated seed that had almost no emergence; this was similar to *C. gayana*. The data obtained to date indicates that the physical texture is regarded as the cause for the big difference in emergence percentages. The high Mg (promotes photosynthesis) and Na (causes mineral imbalances in plants) can also have an impact on the emergence (Fischer et al., 1998). A hypothesis for the low emergence in coated seed is the texture of the substrate, all the minerals that was around the seed was washed away and had no benefit to the seed.

There was a clear benefit in using seed that is coated in Gypsum substrates, it was the only substrate were coated seed had a significantly higher emergence percentage. Uncoated seed had a higher emergence percentage in Kimberlite-, Andulusite- and Coal discard substrate, using coated seed in these substrates did not show any benefit.

Eragrostis curvula

The data analysis of *E. curvula* showed that there was a significant difference ($p < 0.05$) between the different water treatments in Red sandy loam-, Gypsum-, Gold > 2% pyrite-, Platinum- and Kimberlite substrate. There was a significant difference between the different coating treatments in Gold < 2% pyrite-, Platinum tailing-, Fluorspar-, Andulusite- and Coal discard substrate. The interaction between coating and water content in Platinum- and Fluorspar substrate also showed a significant difference. Figure 3.9 and Figure 3.10 below shows the interaction between seed coating water treatments in the different substrates which *E. curvula* is grown in. Figure 3.9 shows the acidic substrates and Figure 3.10 the alkaline substrates.

From both these figures it can be seen in almost all the different substrates, coated seed had a higher emergence percentage compared to uncoated seed. In the Gypsum substrate, coated and uncoated seed had almost the same level of emergence percentage, but water played a significant effect, and the highest emergence was at field capacity. In the Kimberlite substrate the uncoated seed had a slightly higher emergence compared to coated seed, this difference was not significant. The water played a significant role, with the highest emergence percentage at W1 and the decreasing as the water level increased. The highest (significant) emergence in Gold > 2% pyrite substrate and Platinum tailings substrate was at W1 and also decreased as the water level increased. The opposite was true for Red sandy loam substrate, the emergence percentage increased as the water level increased.

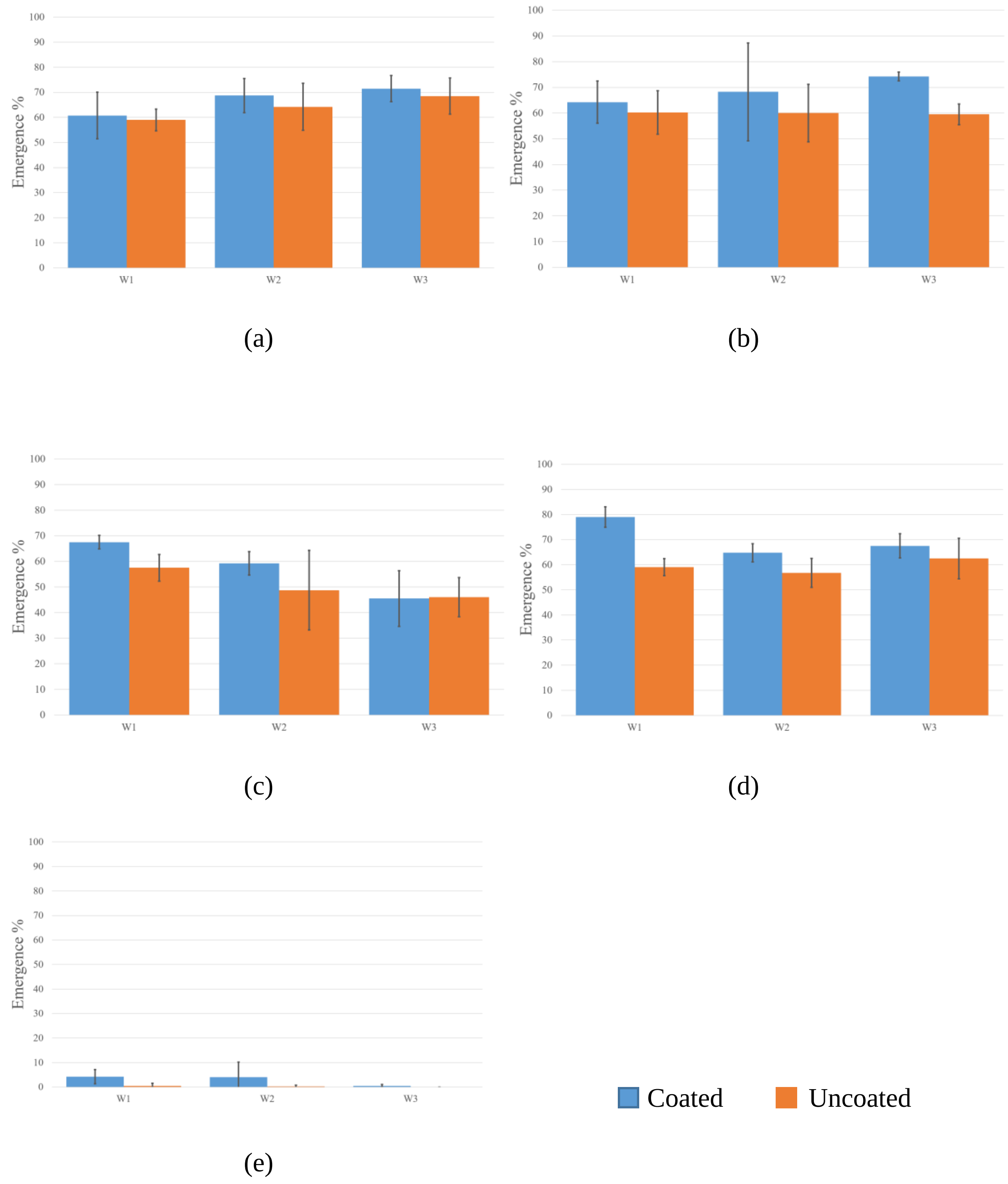

Figure 3.9: The emergence percentage of coated and uncoated *E. curvula* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gold < 2% Pyrite-, **c)** Gold > 2% Pyrite-, **d)** Platinum tailings- and **e)** Coal Discard substrate.

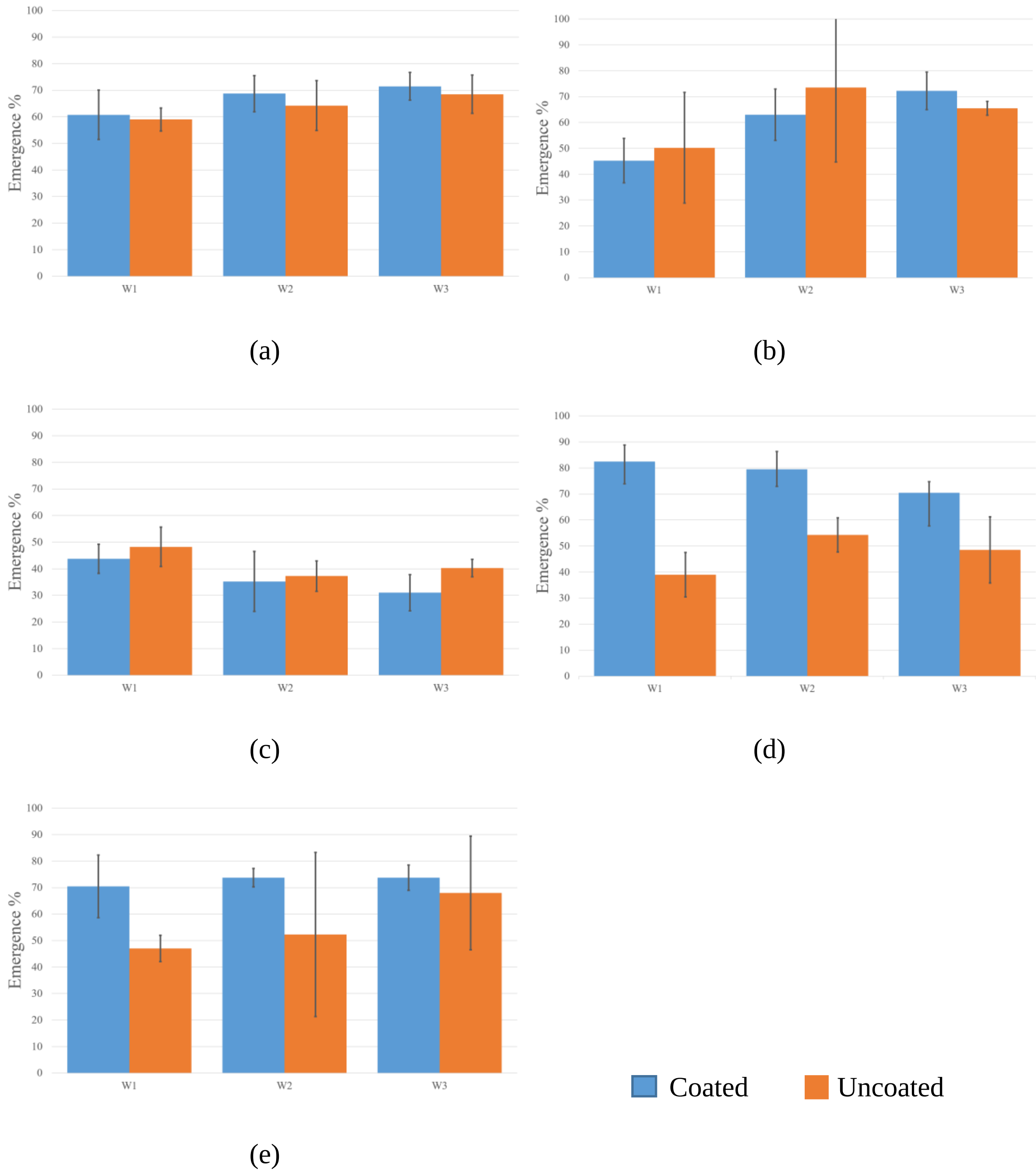

Figure 3.10: The emergence percentage of coated and uncoated *E. curvula* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gypsum-, **c)** Kimberlite-, **d)** Fluorspar- and **e)** Andulusite substrate.

The emergence percentage of *E. curvula* was very similar in most of the substrates, with a few exceptions. Platinum tailings- and Fluorspar substrates (planted to coated seed), had a higher emergence percentage as compared to the red sandy loam substrate. The Platinum substrate had a high pH, but as we already showed in our previous studies, pH did not affect germination and emergence of *E. curvula*. The minerals found within the Platinum substrate are all within the normal range of recommendation (Fertilizer Society of South, 2007), therefore the cause of the higher emergence is due to the coating. The coating around the seed provided enough nutrients and support to ensure a higher germination and emergence. This was similar for the Fluorspar substrate, the only difference is that the levels of K was low in the Fluorspar substrate, and low levels of K effects the emergence of *E. curvula* negatively (Dickinson et al., 2007). The coating definitely had an effect on emergence percentage in the fluorspar substrate; the seed coating could have reduced the effects of the low levels of K.

The emergence percentage in the Coal discard substrate for this species was very low, as in all the other cases, however some of the coated seed germinated and emerged, while the uncoated seed did not. The coatings provided a slight benefit. The low pH and the high Al level could have also been responsible for the low emergence. The Kimberlite substrate with a high K level compared to Red sandy loam, did not have a negative effect on emergence due to it being in the normal acceptable range (Fertilizer Society of South, 2007). The Kimberlite substrate also has a high Na and EC level, however the EC value is not that high, therefore it is highly possible that the low emergence percentage is due to the physical texture. Seed need to make enough contact with substrates, but the seed of *E. curvula* is very small and the texture of Kimberlite substrate is very course. This causes that there is not enough contact made between substrate and seed causing the low emergence percentage. Coated seed planted to Gold < 2% pyrite substrate had a significantly higher emergence compared to uncoated seed, therefore the coating provided a benefit to the seed in this substrate

Medicago sativa

According to the statistical analysis there was a significant difference ($p < 0.05$) between the different water treatments in Red sandy loam-, Gypsum-, Gold < 2% pyrite-, Gold > 2% pyrite- and Coal discard substrates. The coating treatments showed a significant difference in the Kimberlite-, Andulusite- and in Coal discard substrates. There was also a significant difference in the interaction between water treatment and coating in coal discard substrate. Figure 3.11 and Figure 3.11 below shows the interaction between seed coating water treatments in the different substrates which *M. sativa* was grown in. Figure 3.11 shows the acidic substrates and Figure 3.12 the alkaline substrates

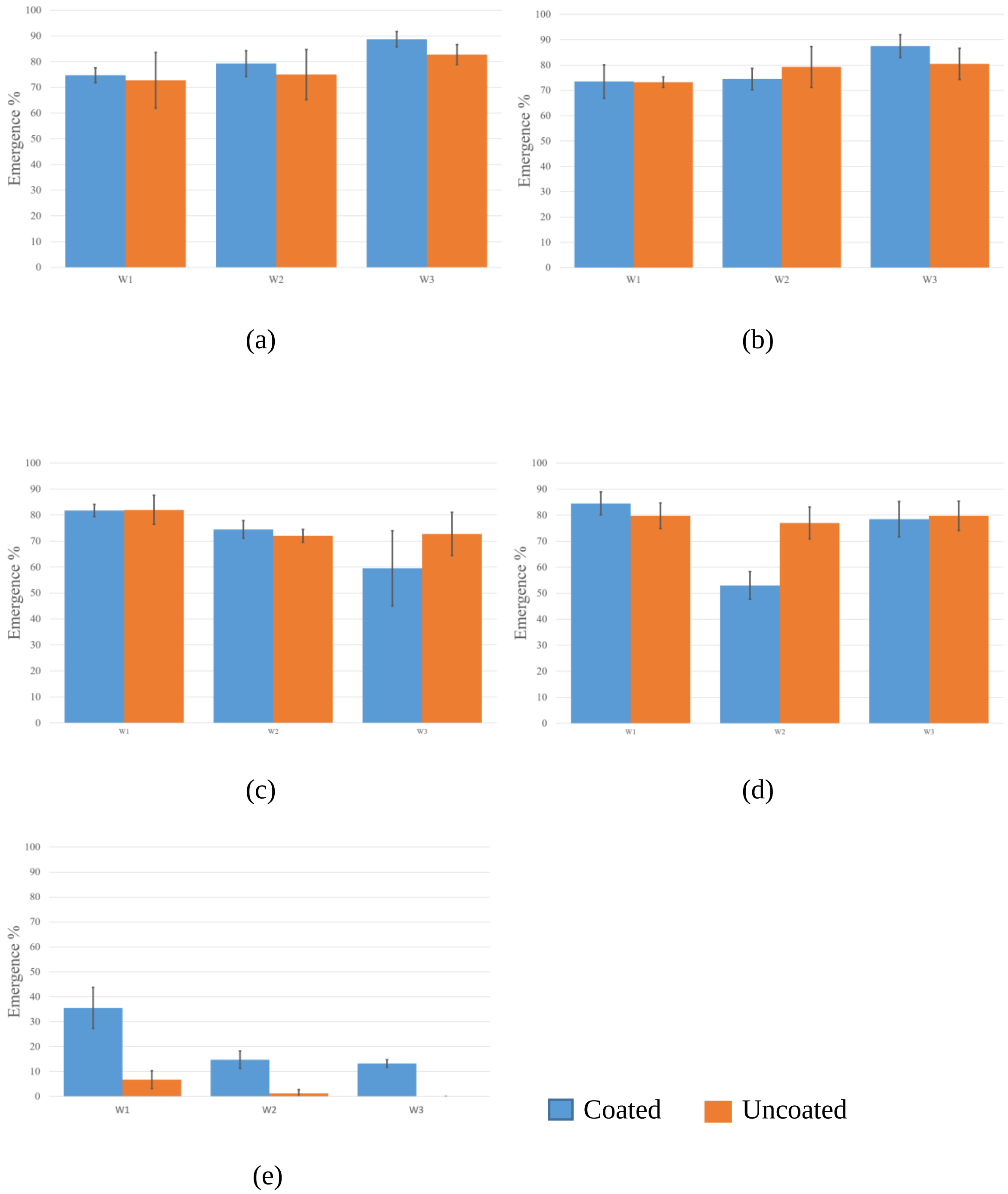

Figure 3.11: The emergence percentage of coated and uncoated *M. sativa* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gold < 2% Pyrite-, **c)** Gold > 2% Pyrite-, **d)** Platinum tailings- and **e)** Coal Discard substrate.

From both figures it can be seen that coated seed had a slightly higher emergence percentage in Red sandy loam-, Gypsum- and Fluorspar substrates, this was however not significant. Uncoated seed had a higher emergence percentage in the Kimberlite- and Andulusite substrates, which was significantly different. Coated seed had a much higher germination and emergence percentage in Coal discard substrate compared to uncoated seed, the highest of all the different species, but it is important to note that the seed that germinated died soon thereafter. There was no difference between coated and uncoated seed in Gold > 2% pyrite substrate.

The seed coating around the seed could have protected the seed from the harsh conditions in Coal discard substrate for a period of time. The coated seed germinated and died while there was almost no emergence for uncoated seed, this shows that the seed coating had a benefit initially. In the Kimberlite substrate it is true that the physical texture, once the coated seed dissolved it was washed away due to the coarse texture, and therefore served no purpose. Why the uncoated seed had a higher emergence percentage in Andulusite substrate is not certain.

The emergence increased in Red sandy loam-, Gypsum- and Gold < 2% pyrite substrate as the water level increased. The highest emergence percentage was at W3 and the lowest at W1. The opposite is true for Gold > 2% pyrite- and Coal discard substrate where the emergence decreased as the water level increased, the highest emergence was at W1 and the lowest at W3. It is therefore important to look at each substrates chemical and physical nature in addition to the area's environmental conditions to establish if the rainfall is also adequate. The substrate again played an important role in the emergence percentage. The emergence was low in the Coal discard substrate like in all the other species mentioned above. The emergence percentage was much higher for *M. sativa* in coal discard substrate in comparison to the other species, but the seedlings died soon after germination. There is a clear benefit of using coated seed, with coated seed having a higher emergence percentage. As already mentioned, Coal discard have a very low pH and high levels of Al, the low pH makes the effects of Al worse, and this causes the lower emergence percentage.

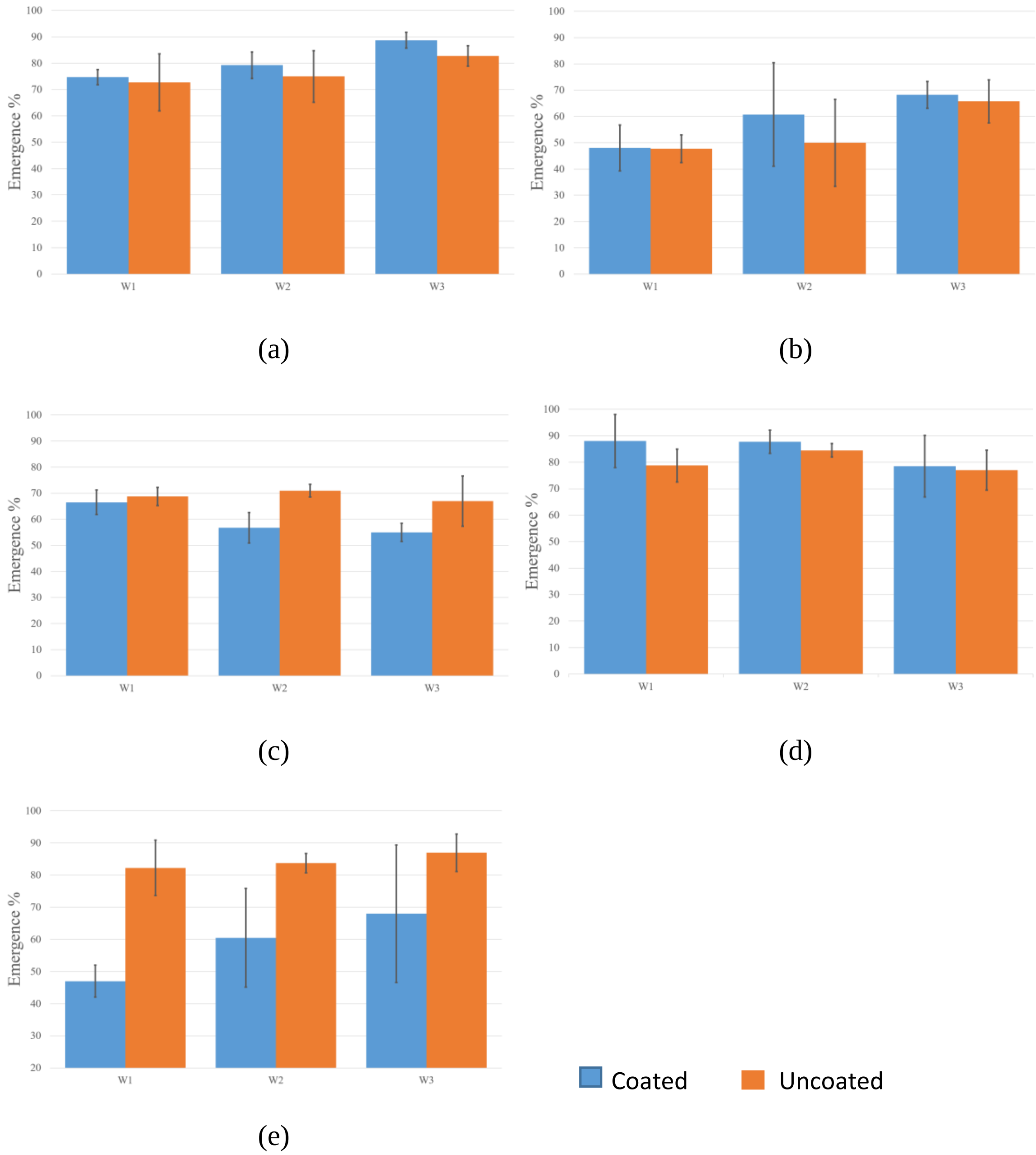

Figure 3.12: The emergence percentage of coated and uncoated *M. sativa* treated with three different water treatments, W1 (75% of field capacity), W2 (field capacity) and W3 (125% of field capacity) in **a)** Red sandy loam-, **b)** Gypsum-, **c)** Kimberlite-, **d)** Fluorspar- and **e)** Andulusite substrate.

The emergence percentage in the Gypsum substrate is also lower compared to that of Red sandy loam substrate. This can be due to the very high levels of S in Gypsum substrate. The levels of K and Mg is also very low (Fertilizer Society of South, 2007, Dickinson, 2004), and this can cause the lower levels of emergence. Potassium and Mg are both very important in photosynthesis, low levels will reduce photosynthesis and growth. The Kimberlite substrates' emergence percentage was also lower as compared to Red sandy loam soil, due to its coarse texture. Andulusite substrate also had a lower emergence percentage, for coated seed. The Andulusite substrate had a very low level of Ca in regards to Mg. For normal plant functioning and growth are dependent on the right ratio of ca to mg (at least 2:1) (Machette, 1986). The level of calcium must be higher than that of Mg to ensure adequate growth and functioning (Machette, 1986). The level of Mg must never be lower than that of Ca, and the low emergence is due to the low ratio of Ca: Mg.

3.5 CONCLUSION

The study yielded a large amount of interesting results, with more questions arising from it then questions being answered. The aim of the study was to find out if there was a benefit of using coated seed and if water levels affected germination and emergence.

The study found that coated seed had a higher emergence for *C. ciliaris* in Gypsym-, Andulusite- and Coal discard substrates, *C. gayana* in Fluorspar- and Andulusite substrate. Coated seed had a definitely advantage in *E. curvula* with higher emergence percentage in Gold < 2% pyrite-, Platinum-, Fluorspar, Andulusite and Coal discard substrates, compared to uncoated seed. Uncoated seed in all the different species had a higher emergence percentage in kimberlite due to Kimberlites very coarse texture. The texture prevented and seed to substrate contact. The study also found that for *M. sativa*, coated seed only had a higher emergence percentage in coal discard substrate, but soon after emergence the plants died. The low pH and high aluminium levels prevented anything from growing in the Coal discard substrate.

It was also found that uncoated seed had a higher emergence for *C. ciliaris* in Red sandy loam- Gold < 2% pyrite and in Gold > 2% pyrite substrate, for *C. dactylon* in Andulusite- and Coal discard substrate and for *D. eriantha* in Andulusite- and coal discard substrate. There were no differences in emergence percentages between coated and uncoated seed for *C. cilliaris* in Gypsum-, Platinum- and Fluorspar substrate and for *C. dactylon* in any of the substrates, except Kimberlite and Andulusite. There was also no differences between coated and uncoated seed for *C. gayana* Gypsum, Red sandy loam-, Gold < 2% pyrite-, Gold > 2% pyrite- and Platinum tailings substrates. In *D. eriantha* and *M. sativa* there was no difference in emergence percentages between coated and uncoated seed in Gypsum-, Gold < 2% pyrite-, Gold > 2% pyrite-, Platinum discard- and Fluorspar substrate. *Eragrostis curvula* there was no difference between coated and uncoated seed in Gypsum- and Gold > 2% pyrite substrate.

The water content also played an important role. In most of the substrates *C. cilaris* had a higher emergence percentage as the water level increased, except in Gold > 2% pyrite-, Kimberlite- and Fluorspar substrate. *Cynodon dactylon* and *D. erientha* showed that there was little difference in emergence between different water treatments, only in Red sandy loam-, Gypsum-, Gold < 2% pyrite- and Gold > 2% pyrite substrate had a higher emergence when the water levels increased. In *M. sativa* the emergence increased as the water level increased in Red sandy loam-, Gold < 2% pyrite and Gypsum substrates. The emergence decreased as the level of water increased in Platinum tailings-, Fluorspar-, Kimberlite- and Gold > 2% pyrite substrates. This just shows the importance of the right water content that should be applied to the right substrate.

The study also found, as with previous studies, that high levels of Al in combination with low pH, had a severe negative effect on germination and emergence. The low pH causes the impact of Al to be more severe. High sodium levels also had a negative impact on growth, by causing imbalances of other minerals in the seed and in turn lower the emergence percentage. The texture also had an impact on the emergence, for example Kimberlite had a coarse texture and there was a lower emergence percentage while in Red sandy loam that had a fine texture there was a higher emergence due to seed having more contact with the substrate.

There is a vast amount of information that came out of this study, but it is safe to say that there is a place for coated seed and for improving the germination and emergence of seeds. It is however important to know physical and chemical properties of the substrates that the seed are going to be planted in. The right saturation or field capacity level of a substrate should also be known before anything is planted in a specific substrate. One needs to fully know the properties of the substrates before one can make recommendations of which seed to use, coated or uncoated. Coated seed is just one part of trying to improve the germination and emergence rate of pasture, but this study has made it clear that there are various other aspects that needs to be taken into consideration. There is no right or wrong answer to if coated seed improves the germination and emergence of seed. Each case must be looked at individually and be treated differently, therefore findings should not be generalized, due to coated seed being area and environment specific.

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CHAPTER 4

Prepared according to African Journal of Range and Forage Science guidelines

The effects of seed coating on the development of *Chloris gayana, Eragrostis curvula* and *Medicago sativa* in response to different salinity growth conditions

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4.1 ABSTRACT

Mining activities, especially improper mining activities causes unfavourable growth conditions that can often restrict germination, emergence and root development complicating the establishment and rehabilitation process. Seed coating technology has shown much potential in previous studies and can facilitate the establishment of grasses and legumes in unfavourable growth conditions. In this study *Chloris gayana, Eragrostis curvula* and *Medicago sativa* were used. The species were planted in silica sand placed in 400 mm tall and 150 mm wide pvc pots. Irrigation water treatment were tested, namely water with different salinity's (0.05 M and 0.09 M NaCl). In *C. gayana* and *M. sativa* the root production was unaffected by using coated seed, the production only decreased as the salinity's increased. *Eragrostis curvula* showed an increased in root production when coated seed was used. The root diameters were slightly larger in *C. gayana* and *E. curvula* when planted to coated seed. Only in *C. gayana* was it found that using coated seed, the highest amount of roots was found deeper in the soil as compared to uncoated seed. Plants from coated seed also showed no effect in the size of leaves, except for *E. curvula* were leaves from coated seed were larger compared to uncoated seed. The leaf area decreased as the salinity increased in all three species. The dry matter production of these

species was also unaffected by using coated seed. The dry matter production did however decreased when the salinity concentrations increased.

Keywords: unfavourable growth conditions, root development, rehabilitation, coated seed, grasses

4.2 INTRODUCTION

The process of growth and development in plants are highly variable and adaptable. The processes involved that enable the plant to adapt, starts with germination and continues when the plant is fully grown and producing seed itself (Veenendaal et al., 1996). Growth parameters are therefore measured differently for different species, depending on the growth form, adaptive mechanisms and ultimate use of the plant. The growth of grasses is usually measured by determining the biomass of the roots, stems, and leaves and the vigour by measuring the height of the plant at certain intervals. For creeping grasses, the growth rate should include the number of rhizomes and stolon's produced. The growth process is influenced by genetic makeup of the plant, and the effects the environment have on these genetic factors (Hartmann et al., 1981). Therefore plant growth is the increase in the size of the plant due to an increase in cell number, a factor of both cell division and cell enlargement (Hartmann et al., 1981). The development of the plants is defined by how the plant goes through processes or stages throughout its life cycle. This includes processes like pre-germination (seed dormancy), germination, tiller development, flowering, etc., and is fully discussed in the literature review, chapter 1 (Skinner and Moore, 2007).

Genetic factors influencing growth and development of the plant will be optimally expressed under ideal conditions (environment); genetics and environment interaction control the production potential. Plant breeders are constantly trying to improve the genetics by breeding stronger or more adapted plants. Environmental factors can include light, temperature (this is especially important for this study, due to the use of sub-tropical species), water quality, soil quality etc. (Hartmann et al., 1981). Warm to very warm temperatures are conducive to active growth in plants (Hartmann et al., 1981) and the minimum night temperature must be more than 16˚C. The daylight length (length of the day) needs to be adequate, as flowering is induced by short day lengths (Fairey et al., 1999). There is however an exception to this, since some crops are unaffected by day length. Age of the seed is also important,- as the germination is often lower in seed younger than six months (due to the maturing processes) while seed older than six months becomes mature and lose some of the germination inhibitors (Fairey et al., 1999). The environment is one aspect of pasture production that cannot be controlled, especially where infrastructure or water is the limiting factor. It is for this reason we breed species that are more adapted to these harsh environments.

The use of coated seed to support this primary root development in the early stages of emergence could be a positive solution to overcome limiting factors. The primary root that is supported or have more nutrients available in early life will result in a larger and healthier plant later in its life cycle.

Seed coatings are not always successful and is only there to improve the survival rate of seed that germinate (Pretorius, 2016). Seed coatings were developed to also increase the ease of handling, to have a heavier and more uniform seed (Halmer, 2004). In some species, coated seed germinates 24 hours later in comparison to uncoated seed. The technologies involving seed coating is complex and is constantly changing and improving the coating. The coating used for different species differs and a generalisation of coatings cannot be made. The nutrients found in these coatings can support the primary root system development in early stages of the plants life cycle. The nutrients are released in the soil surrounding the seed and is then taken up by the roots when needed.

Salinity in soils or in water is known to reduce the growth of plants by effecting the osmotic potential in the plant. High levels of sodium interfere with nutrient balances, osmotic regulation in plants and can change the structure of the soil (Radhakrishnan et al., 2006). The increase in salts reduce plant growth by affecting the osmotic effect of the plants (Munns and Termaat, 1986).

The hypotheses of this trial were:

- Species grown from coated seeds will have a higher root mass in the different growth substrates compared to uncoated seed
- Species grown from coated seeds will have bigger root diameters in the different growth substrates compared to uncoated seed
- Roots of species grown from coated seeds will reach greater depths in different growth substrates compared to uncoated seed
- Species grown from coated seeds will have a greater total leaf area in the different growth substrates compared to uncoated seed

- Species grown from coated seed had a higher dry matter production compared to uncoated seed in different substrates

4.3 METHODOLOGY

A study on the effects of coated seed on different pasture species in response to different growth conditions, compared to uncoated seed was conducted in a greenhouse, Phytotron C at the Hatfield experimental farm, Pretoria, South Africa. The objective of this study was to evaluate the effect of coated seed on leaf area, dry matter production and root production in these different pasture species and to compare coated and uncoated seed in growth media with a range of salinity levels. The test species used were *Chloris gayana, Eragrostis curvula* and *Medicago sativa*. These species were chosen because *C. gayana* and *E. curvula* is widely used a mine rehabilitation and also used in farming practice. *Medicago sativa* is one South Africa's most economically important legume crops. These three species generally have very high germination and emergence rates. All the species had two seed treatments, namely uncoated and coated. The same seed batches where used for both coated and uncoated treatments in each of the different species used, therefore excluding genetic differences from this study. The seed was supplied by AGT Foods Africa Pty Ltd (Advance Seed) and a new batch of seed were used for the study, to prevent any old seed from being used to ensure maximum germination and emergence was possible. The commercial (AgriCOTE®) coating applied to the seed contained nutrients, fertilizers, pesticides, polymers, lime and in the case of *M. sativa* it contained inoculants (rhizobia).

In this study, an inert growth substrate (silica sand) was used to which water of different salinity concentrations was added. Two different concentrations of Sodium Chloride (NaCl) water (0.05M and 0.09M) was made up. A third water treatment (municipal water) was also added to serve as a control. These concentrations had an EC (electrical conductivity) of 4.55 ds.m^{-1} and 8.2 ds.m^{1} respectively. The EC of soil is the ability of soil to conduct a charge, a normal EC of between 4 to 8 ds.m^{-1} enables plant to easily take minerals from the soil, and above this threshold plants are not able to absorbed minerals from the soil (Maas and Nieman, 1978). The upper limit of salinity for most plants is 0.05M and 0.09M is considered very saline in most environments (Munns and Termaat, 1986). A diluted Hoagland solution was added to the water and this solution also contributed to the salinity of the water. Therefore it was important to first add the Hoagland solution and then only the NaCl water to ensure that the desired EC was achieved. The Hoagland solution contained Nitrogen (N), Phosphorus (P), Potassium (K), Magnesium (Mg), Sulphur (S), Iron (Fe), Zinc (Zn), Copper (Cu) and Boron (B). In most environments salts have a build-up effect in soils, therefore a logging factor of 20% was used when the water was added to the treatments. A logging factor is when you add additional water to saturated soil, so that the extra water can leach out. The EC of the water that leached out was measured to determine what the build-up effect was to ensure that the soils did not become too saline. The logging factor was sufficient to ensure that there was no build-up of salt in the soil.

Figure 4.1: The outlay of the trial

Self-made containers were made of PVC cylinders with a diameter of 160 mm. The containers were 400 mm high and were divided into four stacked sections of 100 mm each. Coated and uncoated seed of each species were planted in separate containers. There were four replications of each treatment. The amount of water applied to each container was determined by filling a container with sand and the container was then filled with water until saturated and then weighed. The container was then placed in an oven (35˚C) for three days and weighed again at the end of the three days. This was repeated three times and from this the water loss and the amount of water that should be given to the pots every third day was calculated. Therefore the amount of water per unit of sand was determined. The level of saturation was determined by using the WP4-T machine (Devices, 2007, Nel, 2014).

The WP4-T is also known as the Decagon's WP4 Dew point Potential Meter. WP4 is used to measure water potential, given in MegaPascals (MPa) (Devices, 2007). It measures water potential from 0 to -300 MPa with an accuracy of ±0.1 MPa. The machine has mirrors on the inside were water condensates on to (Devices, 2007). At certain point there exists an equilibrium between the moisture in the sample and the moisture that condensates on these mirrors. This equilibrium gives the water potential reading that is used to determine field capacity (Devices, 2007).

Once the seed was planted, water was added until the soil reached saturation and then the whole pot, including water, sand and seed was weighed. The containers were then covered with plastic for a week to prevent excessive water loss and to give the seed enough time to germinate. Containers were weighed every third day to determine the water loss. The weight was subtracted from the starting weight of the container and this gave the amount of water that was lost in three days. The containers were topped up to the same weight with water every third day. The trial ran for five months.

There were three dry matter harvests during the study period. All the species were harvested at the same time, the first two occurred every 8 (8 weeks after germination) weeks and the last harvest occurred at the end of the trial (6 weeks after the second harvest). The stems were cut at the same height. For the first and second harvest they were cut at 10 mm above ground and the last harvest they were cut 1cm above ground to ensure that all the plant material can be dried and weighed. The stems and leaves were placed in brown bags and dried at 65˚C for three days. During the last harvest, the leaf area of each treatment was calculated using a Licor (LI-3100ºC) leaf area meter machine before drying.

After the leaf area was determined the samples were dried. The leaf material was weighed after drying and the biomass recorded.

Upon completion of this study, each 100 mm section of the containers were destructively removed (Figure 4.2a and Figure 4.2b). The roots of each section of the container were harvested, washed and placed in brown bags. The roots were then placed in an oven and dried at 65˚C for two days. The weight of the roots in each section was recorded. The root diameter was also determined by using a Vernier calliper. Three roots were taken at random and the diameter was recorded at a level of 40 mm from the base of the plant for each treatment (Geilfus and Mühling, 2014).

(a) (b)

Figure 4.2: The pot a) before it is destructed and b) were it is busy being destructed and the roots removed

<u>Statistical analysis</u>

This trial was established as a completely random design (CRD) in a glasshouse. A two factor ANOVA was used to test for differences between three salinity concentrations and two coating treatments as well as their interaction, for each species separately at a 5% significance level. The residuals from the analyses were acceptably normal with homogeneous treatment variances. Some pH and salt treatments trials were measured at three root depths; 0-10cm, 11-20cm and 21-30cm. These data were analysed by CRD split-plot ANOVA to test for differences between pH/salts, seed coating and root production per depth, as well as all their interactions, for each species separately. Means in all trials were compared using Tukey's least significant difference test at the 5% level of significance (Snedecor and Cochran, 1980).
Data were analysed using the statistical program GenStat® (Payne et al., 2014)

4.4 RESULTS AND DISCUSSION

a) Above ground parameters

Chloris gayana

Leaf Area (cm^3)

The leaf area, measured in cm^3 of plants and pastures is affected by stress, be it drought, salinity or high pH levels (Taleisnik et al., 2009). The leaf area of plants are expected to be smaller as the area reduces when the stress levels increase (Munns and Termaat, 1986, Ortega and Taleisnik, 2003). The lower leaf area is due to stress effecting the cell division process of plants, therefore cells do not divide properly and subsequently reduce in the photosynthetic area of the plant (Taleisnik et al., 2009). The lower photosynthetic area will in turn result in a lower production of grasses and therefore a lower total DM production.

Seed coatings cannot reduce the effects of salinity on leaf area but it can give the seedling a better chance of survival due to nutrients added in the coating. Figure 4.3 shows the leaf area of *C. gayana* at three different levels of salinity. The data analyses show that there were no significant differences in seed coating treatments and the interaction between seed coating and salinity level for *C. gayana*. There was no significant difference for the different salinity levels of *C. gayana* according to the statistical analysis. There is however a trend significance especially for leaves that came from coated seed at 0.09M. The leaves that came from coated seed shows a definite larger leaf compared to those that came from uncoated seed.

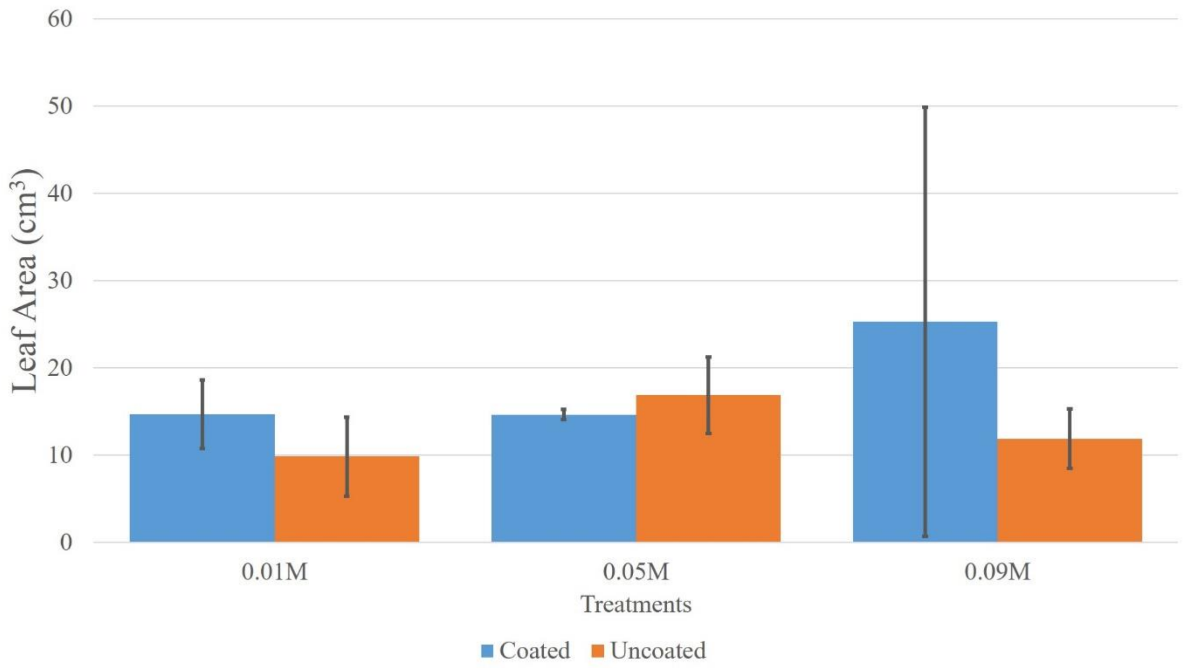

Figure 4.3: The mean Leaf Area (cm^3), with standard deviation of *C. gayana*, planted to coated and uncoated seed, at three different salinity levels (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Dry matter production ($g.plant^{-1}$)

Pastures that are planted in favourable conditions, high rainfall, adequate sunlight, fertile soil and enough fertilizers, are expected to have a much higher production. Pastures that are planted in more stressful conditions, poor soils, droughts, etc., are in turn expected to have a lower dry matter production and a lower overall production. Dry matter production is the most important factor in pastures and farmers aim for higher dry matter production. The environmental conditions for most farming enterprises are not optimal and there is a need to limit the effect of environmental conditions on production.

Figure 4.4 below shows the DM production ($g.plant^{-1}$) of *C. gayana* at three different salinity levels. The data analyses show that there were no significant interactions between the seed coatings and the interaction between the seed coating and different salt concentrations for *C. gayana*. There was a significant difference between the different salinity levels ($P \leq 0.024$) for *C. gayana*. The dry matter production decreased as the salt concentration increased.

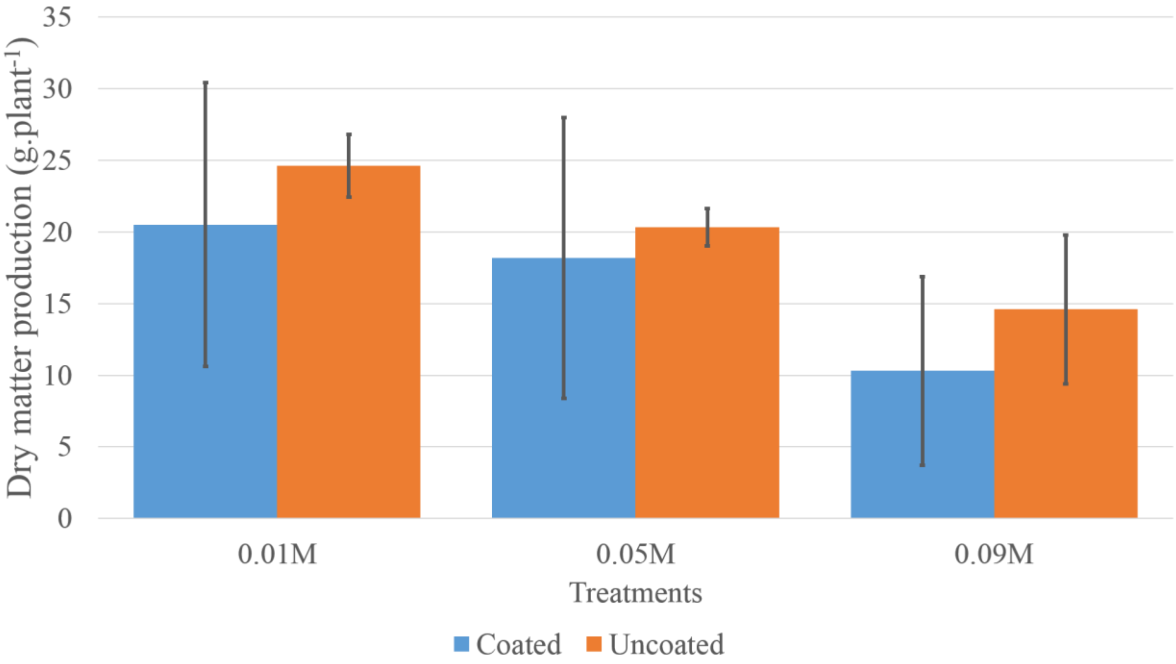

Figure 4.4: The mean dry matter production ($g.plant^{-1}$), with standard deviation, of *C. gayana*, planted to coated and uncoated seed, at three different salinity levels (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

The increase in salt concentrations reduce plant growth by affecting the osmotic potential of the plants (Munns and Termaat, 1986). Too high levels of salinity will cause very low phloem mobility and this in turn will cause low levels of Ca which ultimately results in plant death (Grattan and Grieve, 1999). High levels of Na and Cl in very saline soils causes an imbalance of other minerals in plants, for example; K and Ca. This affects the partitioning, transport and availability of nutrients in plants which affects the growth and germination of the whole plant because it causes imbalances (Tuteja, 2007). These imbalances can cause the lower DM production seen in the data and Figure 4.4. Data presented in figure 4.4 confirms what is stated in literature, but does however show that coatings had no significant effect on DM production in soils with high salinity levels. The data therefore does not support the hypothesis that coated seed will improve DM production of *C. gayana* at high levels of salinity.

Correlation between leaf area (cm^3) and dry matter production ($g.plant^{-1}$)

The correlation between leaf area and dry matter production was not done statistically, but it is interesting to note this correlation (Figure 4.5). The figure clearly shows that the leaf area is directly correlated to the dry matter production. The increase in salinity had an effect on the size of the leaf, resulted in a smaller leaf and this in turn caused the dry matter production to decrease.

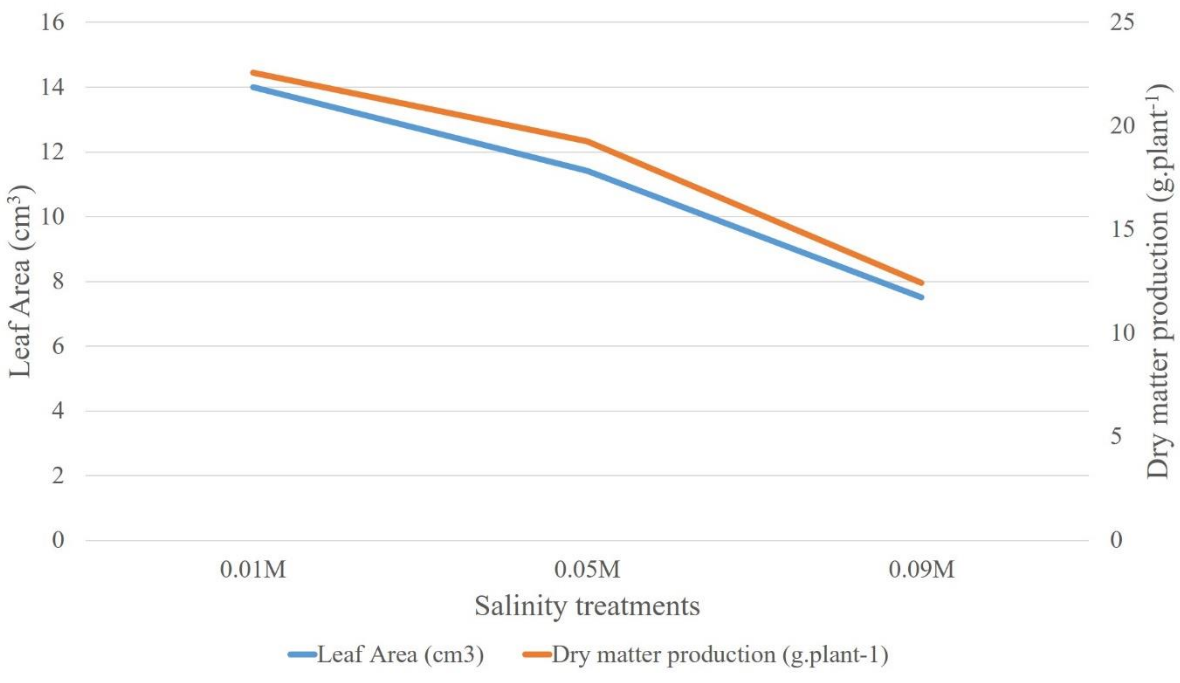

Figure 4.5: The correlation between leaf area (cm^3) and dry matter production ($g.plant^{-1}$) for *C. gayana* at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Eragrostis curvula

Leaf Area (cm^3)

Figure 4.6 shows the leaf area of *E. curvula* at three different salinity levels. The data analyses show that there is only a significant difference in the interaction between the different salinity concentrations and coating in *E. curvula*, ($P \leq 0.001$).

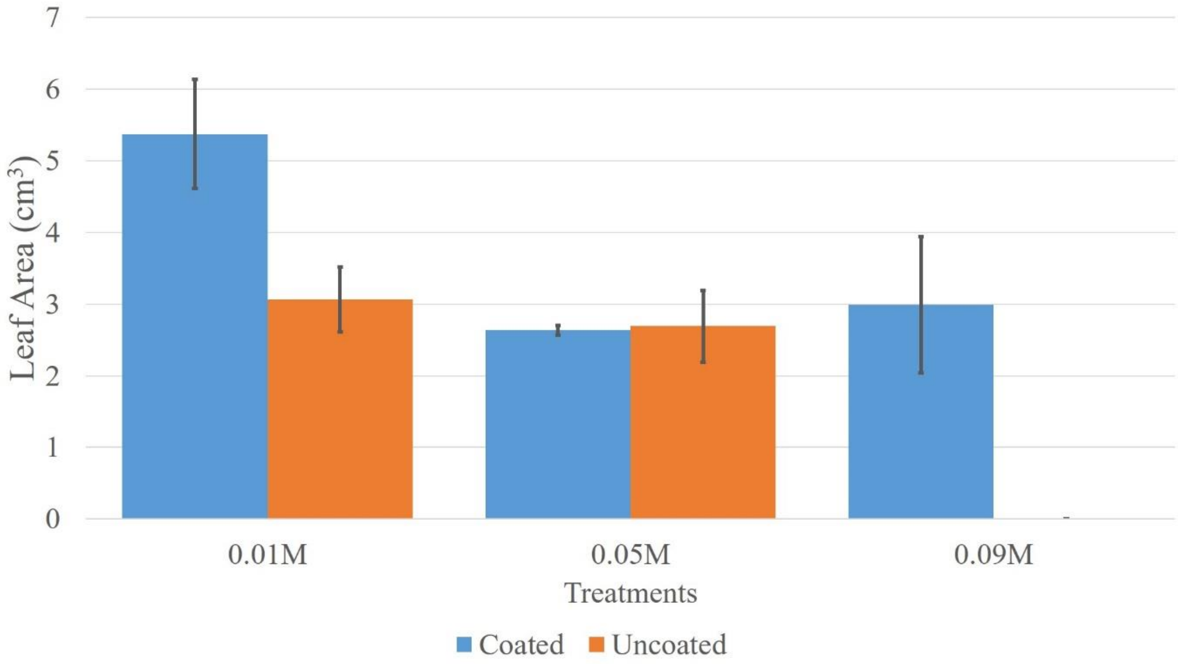

Figure 4.6: The mean Leaf Area (cm^3), with standard deviation of *E. curvula*, planted to coated and uncoated seed, at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Figure 4.6 shows that there was a significant difference at 0.01M NaCl and that plants from coated seed had a bigger leaf area compared to plants from uncoated seed. This will result in more photosynthesis and therefore more growth. When the salinity level increases no difference between plants from coated and uncoated seed was noted. At 0.09M concentration plants from coated and uncoated seed cannot be compared, due to uncoated seed not germinating. Interesting to note that the leaf area at 0.09M was larger than that of 0.05M, this can be due to plant channelling more energy into leaf production to maximise photosynthesis and to try and overcome the high salinity in the soil.

Figure 4.6 shows that there is a benefit in using coated seed at a 0.01M salinity concentration. The leaf area was considerably larger for plants that germinated from coated seed compared to plants from uncoated seed. There was not enough NaCl to interfere or to react with the coating therefore the benefits of the coating is maintained. The high salinity concentrations (0.05 and 0.09M) could have had an effect on the coating.

Dry matter production (g.plant^{-1})

Figure 4.7 below shows the dry matter production of *E. curvula* influenced by different salinity levels. The data analysis illustrates that there was no significant difference for *E. curvula* in any of the treatments; and this can be ascribed to no germination for coated seed at 0.09M NaCl. Looking at Figure 4.7 it can be seen that the salinity had an effect on the DM production of *E. curvula*. This is likely as a result of clear decline in production as the salinity increased. *Eragrostis curvula* is sensitive to high levels of salts (Dickinson, 2004, Ghebrehiwot et al., 2006) and this is also clear in Figure 4.7.

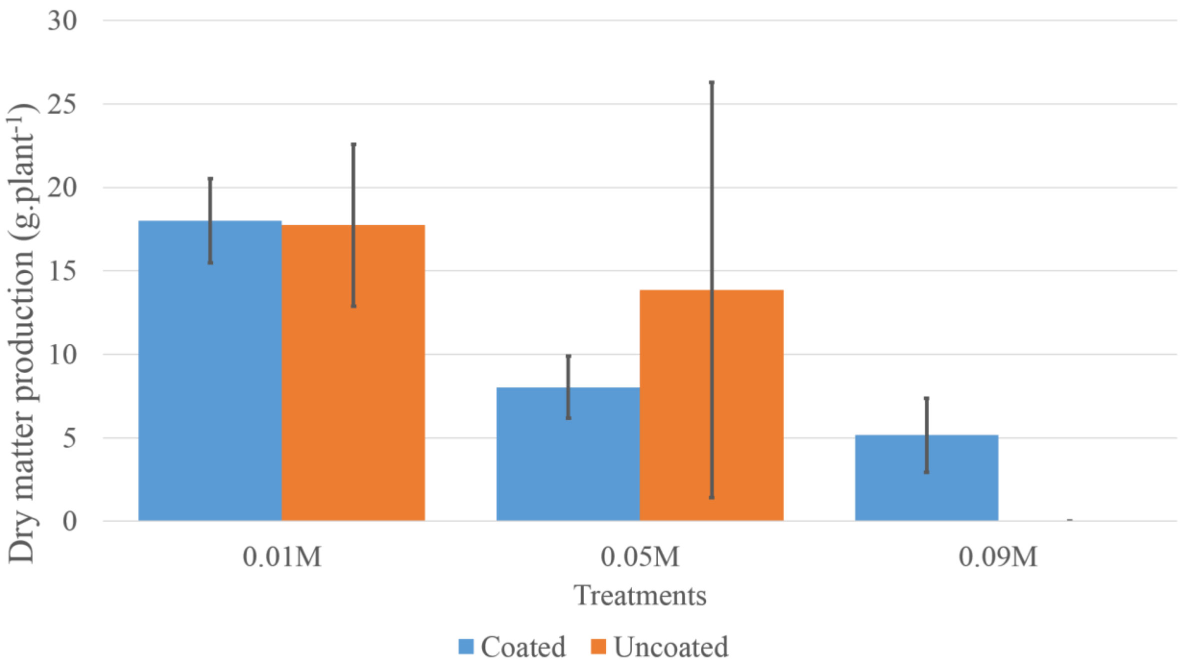

Figure 4.7: The mean dry matter production (g.plant^{-1}), with standard deviation, of *E. curvula*, planted to coated and uncoated seed, at three different salinity levels (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Correlation between Leaf area (cm^3) and dry matter production (g.plant^{-1})

The correlation between leaf area and dry matter production was not done statistically, but is shown in Figure 4.8. As in the case with *C. gayana*, the figure clearly shows that the leaf area is directly correlated to the dry matter production. The dry matter production decreased rapidly when there was an increase in salt concentration that caused the leaf area of *E. curvula* to decrease.

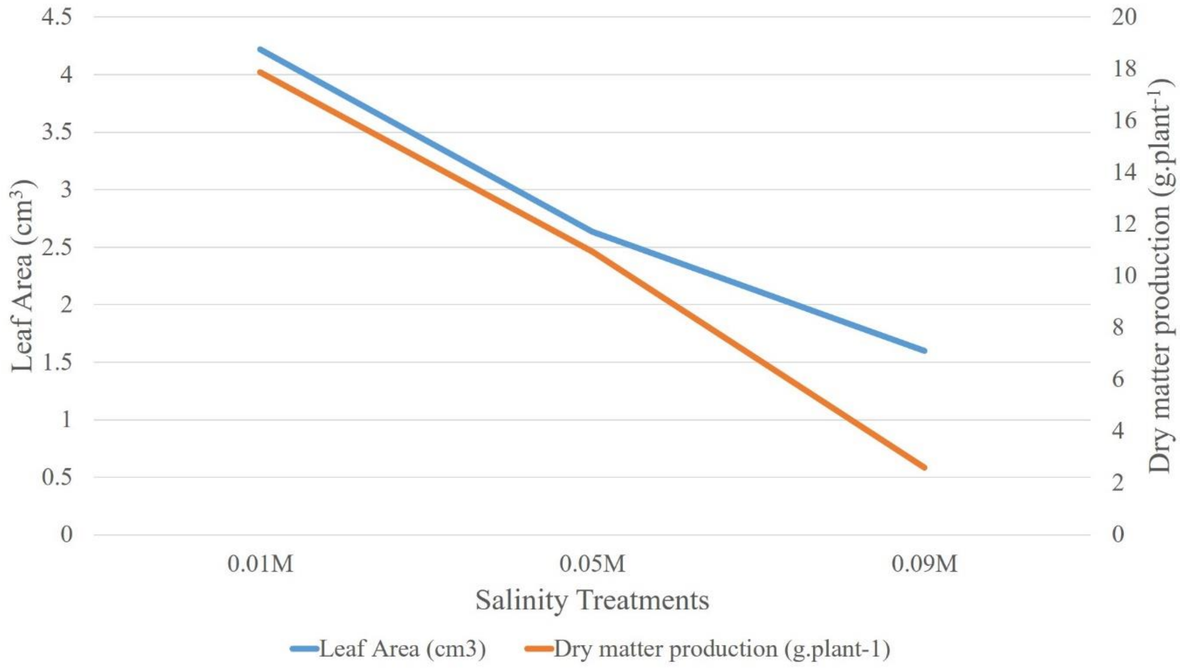

Figure 4.8: The correlation between leaf area (cm^3) and dry matter production ($g.plant^{-1}$) for *E.curvula* at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Medicago sativa

Leaf Area (cm^3)

The leaf area (cm^3) of plants and pastures are also affected by stress, i.e. drought, salinity or high pH levels. The leaf area of plants are expected to be smaller as the stress (higher salinity concentrations) increases, as was found in studies done on various grasses where the salinity levels were high (Ortega and Taleisnik, 2003, Munns and Termaat, 1986). The lower leaf area is due to stress effecting the cell division process of plants. The altered cell division process causes cells to not properly divide and therefore there is a reduction in the photosynthetic area of the plant (Taleisnik et al., 2009). The lower photosynthetic area will in turn result in a lower production due to improper photosynthesis of grasses and therefore a lower total production.

As previously mentioned, salinity severely effects leaf area and in turn reduces dry matter production. Coatings cannot reduce the effects of salinity on leaf area but it can give the seedling a better chance of survival due to nutrients added in the coating. Figure 4.9 shows the leaf area of *M. sativa* at three different salinity's. The data analysis shows that there is only a significant effect between the different salts on *M. sativa* ($P \leq 0.010$). There was no significant difference in coating and the interaction between the coating and different salinity concentrations.

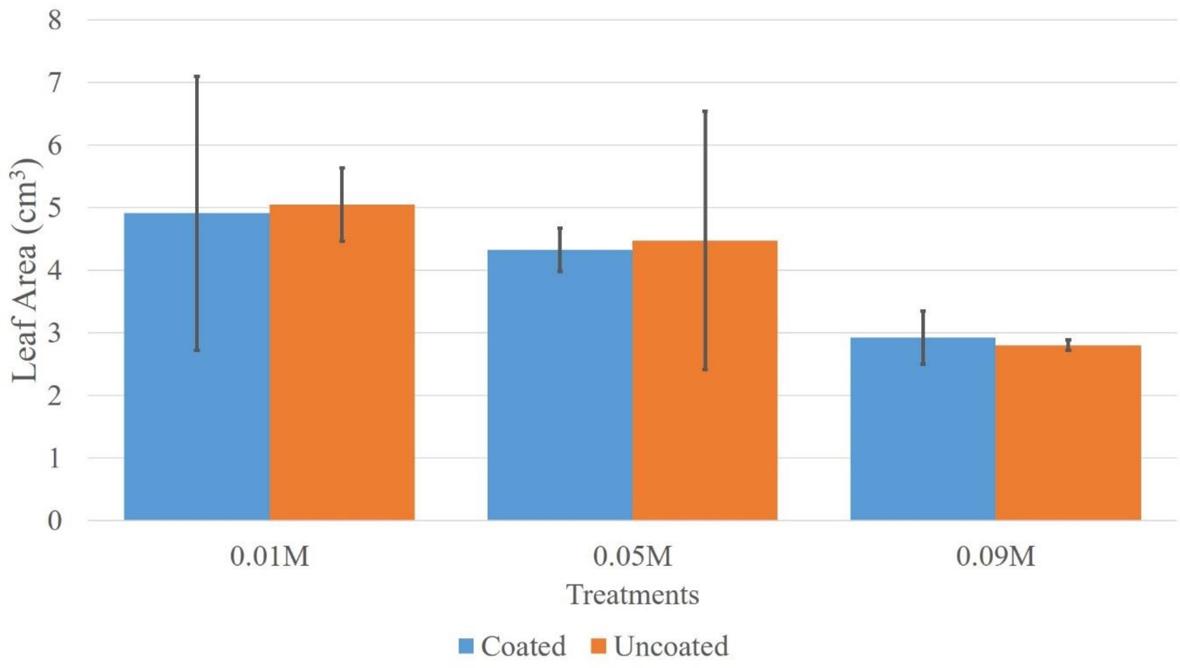

Figure 4.9: The mean Leaf Area (cm^3), with standard deviation, of *M. sativa*, at three different salinity levels (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Figure 4.9 illustrates that salinity had an effect on *M. sativa*'s leaf area, as was concluded by other studies done on leaves of other plants (Munns and Termaat, 1986, Taleisnik et al., 2009). *Medicago sativa* is also known to be sensitive to high salinity's (Dickinson, 2004), therefore it was expected to have smaller leaves at higher salinity's as seen in Figure 4.9. As mentioned earlier, the higher salinity levels effect the cell division process, therefore not functioning properly and in turn reducing the photosynthetic area of plants (Taleisnik et al., 2009). This is clearly the reason for the results obtained and presented in Figure 4.9.

Dry matter production ($g.plant^{-1}$)

Figure 4.10 below shows the dry matter production ($g.plant^{-1}$) of *M. sativa* as influenced by salinity concentrations. The data analysis showed that there was no significant interaction between the seed coating and the different salinity concentrations in *M. sativa*. There was a significant difference between the different salinity's, $P \leq 0.001$ in *M. sativa.*

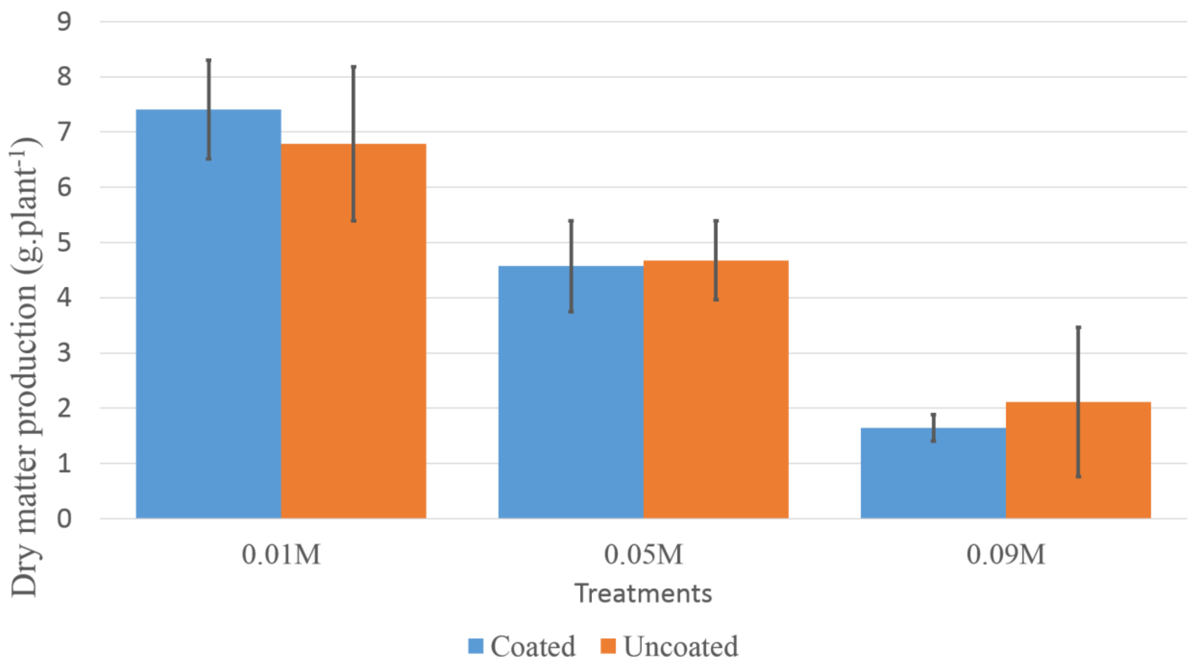

Figure 4.10: The mean dry matter production (g.plant^{-1}), with standard deviation of *M. sativa*, planted to coated and uncoated seed, at three different salinity levels (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

The production of *M. sativa* decreased quite remarkably as the salinity concentration increased. This was expected due to *M. sativa's* sensitivity to high salinity concentrations (Dickinson, 2004, Guerrero-Rodríguez et al., 2011).

The increase in the salt concentrations reduce plant growth by affecting the osmotic potential of the plants (Munns and Termaat, 1986). Too high concentrations of salt will cause very low phloem mobility and this in turn will cause low levels of calcium and this can result in plant death (Grattan and Grieve, 1999). High levels of Na and Cl in very saline soils causes an imbalance of other minerals in plants, for example; K and Ca. This effects the partitioning, transport and availability of nutrients in plants and the growth and germination of the whole plant which results in imbalances (Tuteja, 2007). All this can cause a lower dry matter production seen in earlier data, at the high salinity concentrations. The data and Figure 4.10 confirms what is stated in literature, and illustrated that seed coatings had no significant effect on the plant production in these soils that had high salinity concentrations. This data therefore does not support the hypothesis that coated seed will improve dry matter production at high salinity concentrations.

Correlation between Leaf area (cm^3) and dry matter production ($g.plant^{-1}$)

The correlation between leaf area and dry matter production was included, as with the case with the previous species. This correlation was also not done statistically. The correlation in *M. sativa* is similar to the previous two species. The leaf area decreased as the salinity increased that in turn caused the dry matter production to decrease.

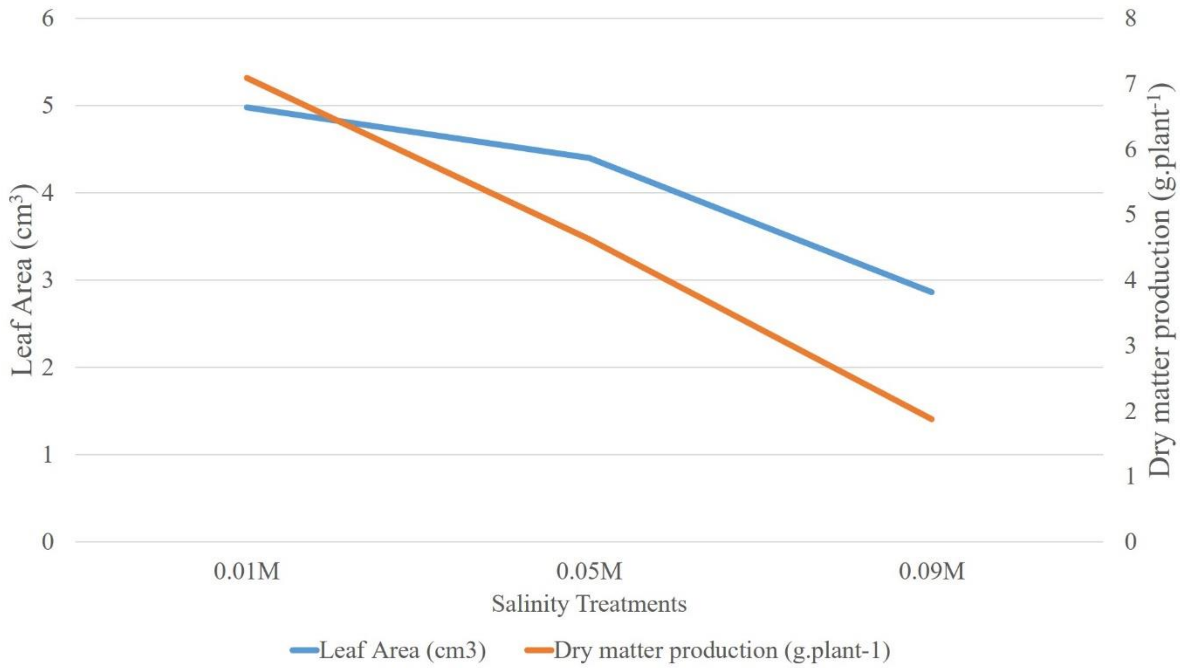

Figure 4.11: The correlation between leaf area (cm^3) and dry matter production ($g.plant^{-1}$) for *M. sativa* at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

b) Below ground parameters

Chloris gayana

Root production ($g.plant^{-1}$)

The coating around the surface of the seed is expected to buffer the seed against the effects of salinity to some extent. The seedling from coated seed is expected to have larger amount of roots, compared to seedlings from uncoated seed, because the coating produces favourable conditions in the soil after germination, therefore it is favourable for root production. The total root production of the pastures are expected to be lower as the salinity levels increases in the soil (Munns and Termaat, 1986).

The increase in salt concentration in the growth substrate reduces plant growth by affecting the osmotic effect of the plants (Munns and Termaat, 1986). Too high levels of salinity will cause very low phloem mobility and this in turn will cause low levels of calcium (Ca) and this can result in plant death (Grattan and Grieve, 1999). High levels of Na and Cl in very saline soils causes an imbalance of other minerals in plants, for example; K and Ca. This affects the partitioning, transport and availability of nutrients in plants which affects the growth and germination of the whole plant because of the imbalances created (Tuteja, 2007). Roots are more affected than any other part of the plant. The high levels of salt concentration causes a low osmotic potential of the soil solution that in turn results in a lower amount of water that is taken up by the plants. Ion imbalance and ion toxicity is also a problem caused by high levels of salinity (Setia et al., 2013). Plants that are healthier and without stress have thicker roots compared to roots of plants that are under stress. Figure 4.12 below shows the mean root production, in g.plant^{-1}, of *C. gayana* at three different salinity's.

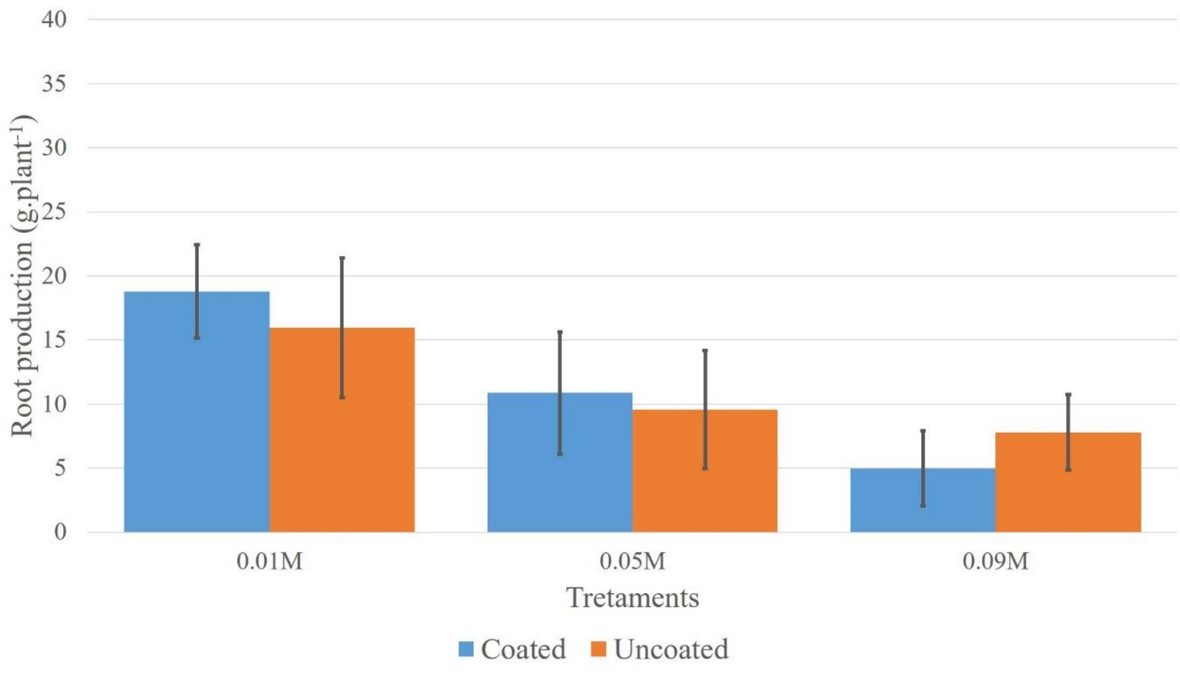

Figure 4.12: Mean root production (g.plant^{-1}) with standard deviation of *C. gayana,* planted to coated and uncoated seed, at three different salinity levels (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

In *C. gayana* there was no significant difference between the different seed coating and the interaction between the salinity level and the seed coating. It was expected that the coating around the seed will contribute significantly and these seedlings will have a larger amount of roots. There was a significant difference between salinity levels ($P \leq 0.001$) where the salinity levels contributed 62.64% of the total variation. This is due to *C. gayana* being more salt tolerant compared to most other pasture species (Dickinson, 2004, Kobayashi and Masaoka, 2008).

Figure 4.12 shows a clear trend, where a decline in the root production as the salinity levels increase is observed. *Chloris gayana* can survive very saline conditions; due to assistance from the salt glands located in the leaves that excrete excessive salts (Figure 4.13) (Kobayashi and Masaoka, 2008, Kopittke et al., 2007, Ortega and Taleisnik, 2003). The production of *C. gayana* will be lower in saline soils compared to non-saline conditions, but will still have a higher production as compared to other sub-tropical grass species (Luna et al., 2002) as seen in Figure 4.12. It is clear from Figure 4.12 and the data analysis that the seed coating had no effect on the initial root production of *C. gayana*.

Figure 4.13: Showing the excretion of salt crystals from the salt glands located on *C. gayana's* leaves

Root diameter (mm)

Plants that are healthier and without stress have thicker roots compared to roots of plants that are under stress (Zhao et al., 2013, Imada et al., 2015). Plants that are under severe stress, i.e. exposed to high pH or salinity levels, are expected to have thinner roots (Imada et al., 2015).

The reason being that energy reserves are used for the adaption to the stress environment while healthy plants build the reserves in the roots and crown. Studies conducted in Japan on various plants, found that the root thickness increased as the plant stress increased under moderate stress. Other studies done on legumes showed a contradictory result, the thickness of roots increased and the length decreases as salinity increased (Franco et al., 2011).

The data analysis shows there is no significant difference between the root diameter at different salinity levels, seed coating and the salinity concentrations and seed coating interaction in *C. gayana*. It was expected that roots from plants under stress will be thinner and roots thicker from plants that are not stressed. The data analysis did not show any significance, but the figure below shows a trend significance. Roots from coated seed had a larger diameter at the low salinity concentrations, compared to roots from uncoated seed.

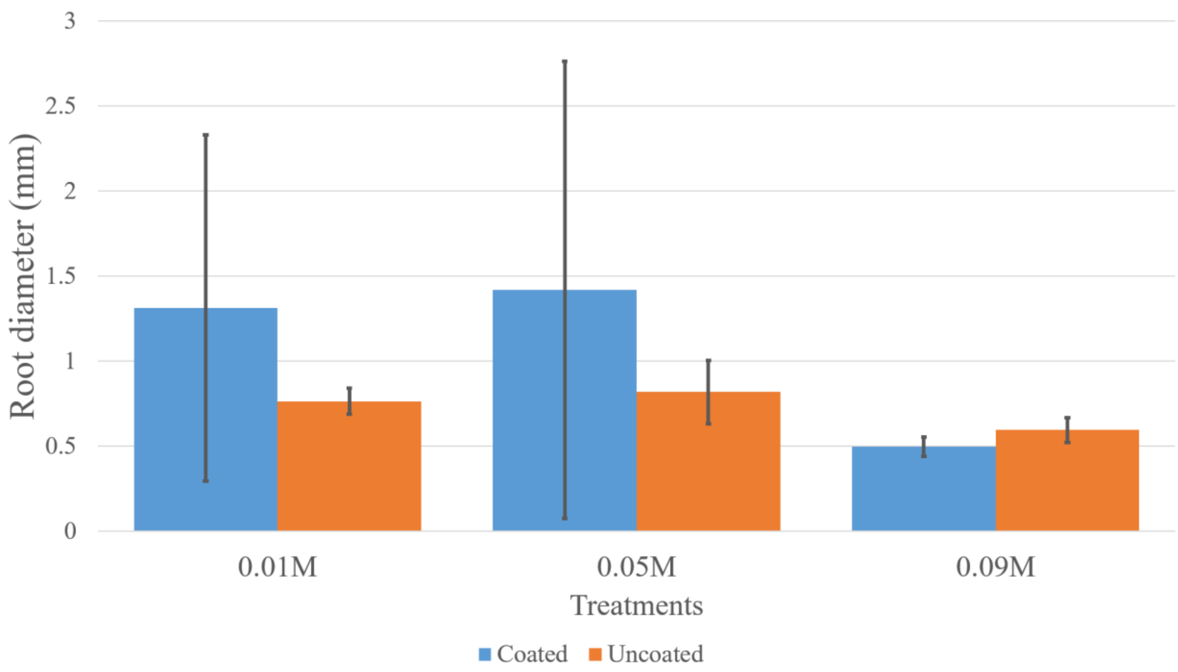

Figure 4.14: The mean root diameter (mm) with standard deviation of *C. gayana*, planted to coated and uncoated seed, at three different salinity levels (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

<u>Root production (g.plant^{-1}) at various root depths</u>

Figure 4.15 below shows the mean root production of *C. gayana* at three different depths (0-10, 11-20 and 21-30 cm) at three different salinity levels (0.01M, 0.05M and 0.09M NaCl). The data analyses show that there is a significant difference in root production at the concentration of salts used ($P \leq 0.002$) and in the interaction between the seed coating and the

different root depths (P ≤ 0.006). There was no significant difference between the different seed coatings, root production per depth, salinity levels and seed coating interactions and salt root depth interaction.

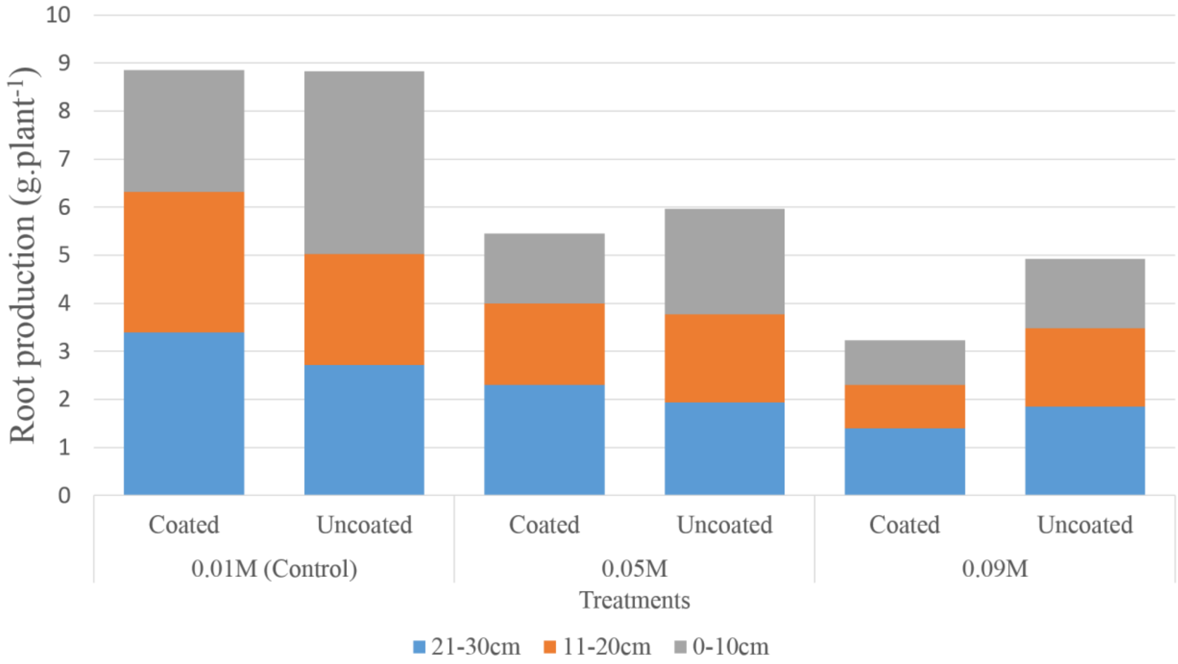

Figure 4.15: The mean root production ($g.plant^{-1}$) of *C. gayana* planted to coated and uncoated seed, at three different depths (0-10cm, 11-20cm and 21-30cm) of three species at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

The mean root production of *C. gayana* follows the pattern where the highest root production was found in the top 10 cm and the lowest in the last 30 cm (21-30cm). Figure 4.16 below shows plants from uncoated seed had the highest production of roots in the top 10 cm compared to plants from coated seed. Plants from coated seed had a higher root production at a 21-30 cm depth compared to plants from uncoated. This indicates that the plants from coated seed produced at stronger primary root that developed faster and reached a greater depth. Plants from uncoated seed developed slower and established most of the roots in the top 10 cm of the growth substrate. There was a steady increase in root production for plants from coated as the depth increased unlike uncoated seed where there is no real trend, therefore coated seed had an effect on root production at various depths. The root production increased steadily as the roots reached greater depths.

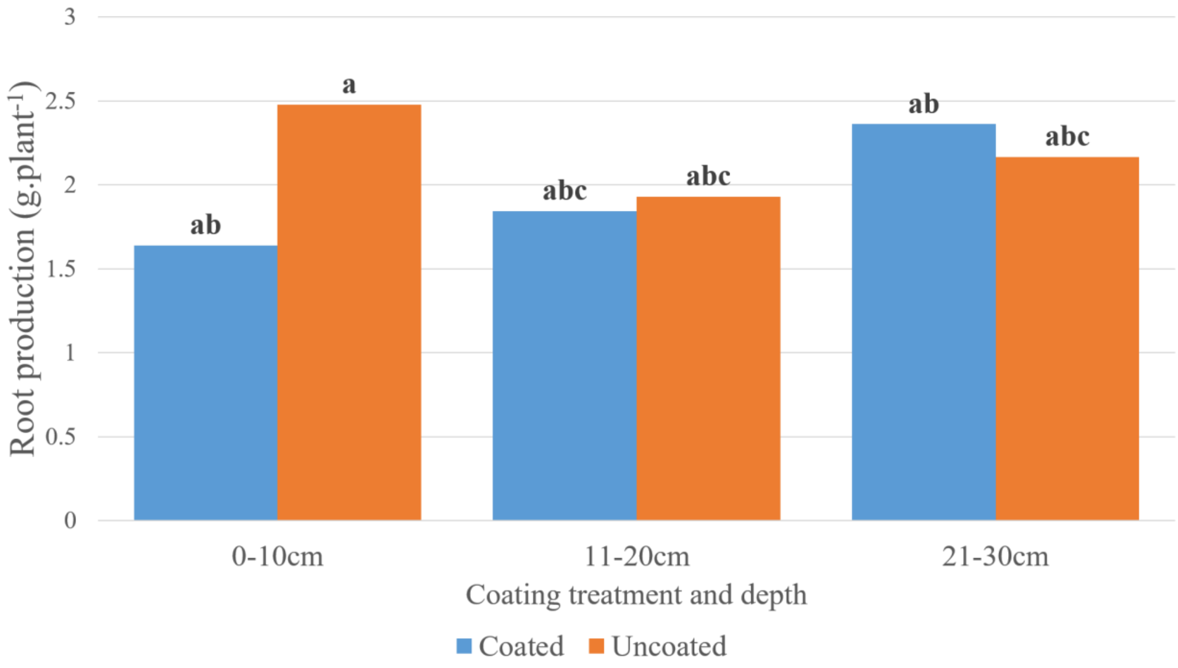

Figure 4.16: The interaction between coatings and mean root production (g.plant^{-1}) at different depths (0-10cm, 11-20cm and 21-30cm) in *C. gayana*

Eragrostis curvula

Root production (g.plant^{-1})

Figure 4.17 shows the mean root production of *E. curvula* at three different salinity levels. At salinity levels of 0.09M, none of the uncoated seed germinated. The statistical analysis showed there was only a significant difference ($P \leq 0.001$) in the interaction between the seed coating and the salinity concentrations.

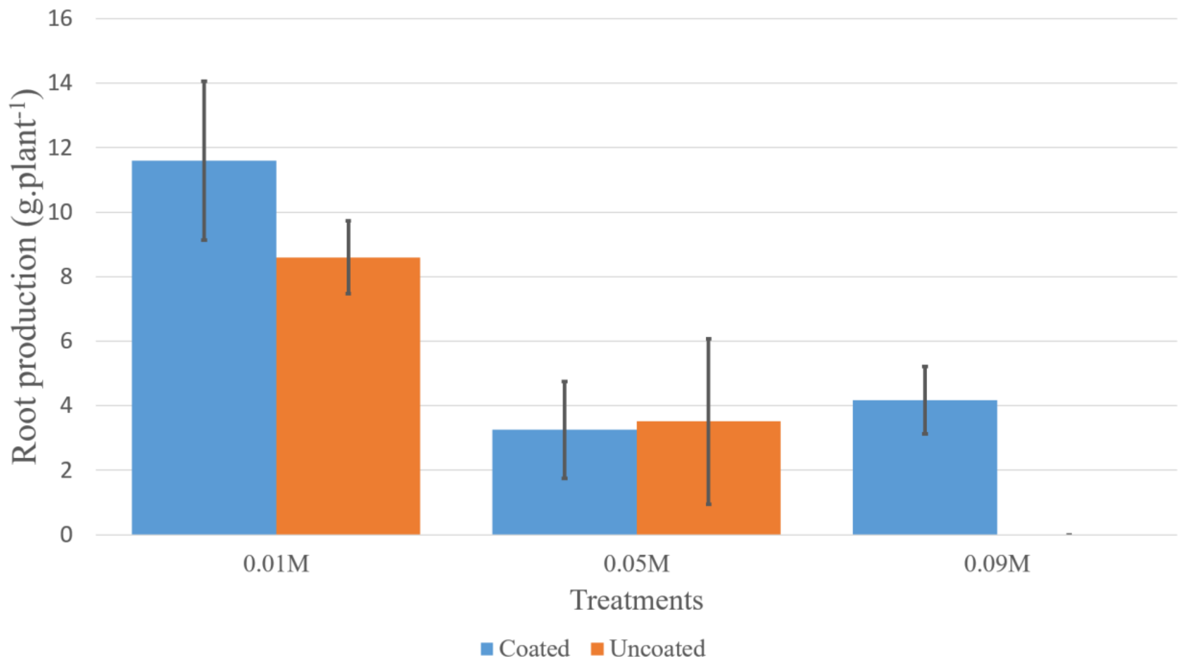

Figure 4.17: The mean root production (g.plant^{-1}) with standard deviation of *E. curvula* plants, planted to coated and uncoated seed at three salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Figure 4.17 shows that *E. curvula* is sensitive to high levels of salinity, where the root production is very high at 0.01M, but declines as the salinity level increases. *Eragrostis curvula* is known to be sensitive to high levels of salinity (Dickinson, 2004, Van Oudtshoorn, 1999). Interesting to note, the level of root production (g.plant^{-1}) at 0.09M that is higher compared to the level at 0.05M, which can be ascribed to the very low germination levels at 0.09M. The coating had a higher production compared to uncoated seed, illustrating that a seed coating is beneficial for *E. curvula*.

Root diameter (mm)

Plants that are healthier and without stress have thicker roots compared to roots of plants that are under stress. Plants that are under stress, i.e. exposed to high pH or salinity levels, are expected to have thinner roots (Imada et al., 2015). The reason being that energy reserves are used for the adaption to the stress environment while healthy plants build the reserves in the roots and crown.

The mean diameter of *E. curvula* at three different salinity levels are shown in Figure 4.18 below. There were no significant differences found for *E. curvula*. Coating and salinity did not have an effect on the root diameter according to the statistics, but there is a trend significance. The coated seed had a slightly larger root diameter.

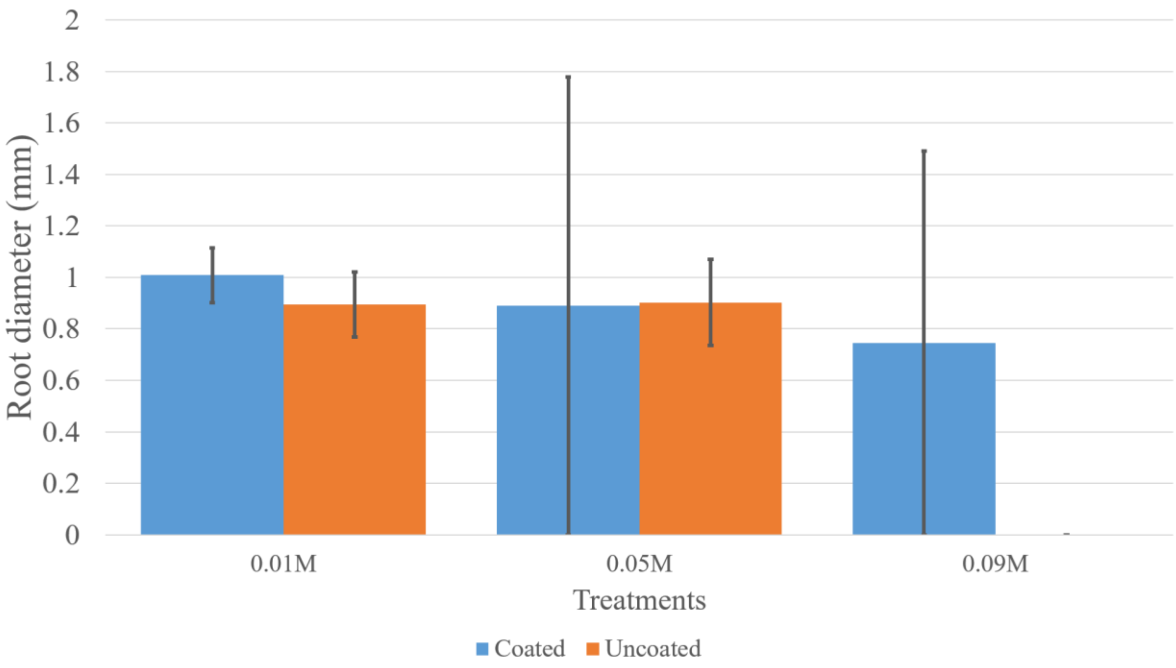

Figure 4.18: The mean root diameter (mm) with standard deviation of *E. curvula* planted to coated and uncoated seed at three salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Root production (g.plant^{-1}) at various depths

Figure 4.19 shows the mean root production (g.plant^{-1}) at various depths at three different salinity concentrations. The data analysis showed that there is only a significant difference between the concentration of salts and the interaction between the different concentration of salts, seed coating and different depth, $P \leq 0.001$ and $P \leq 0.050$ respectively.

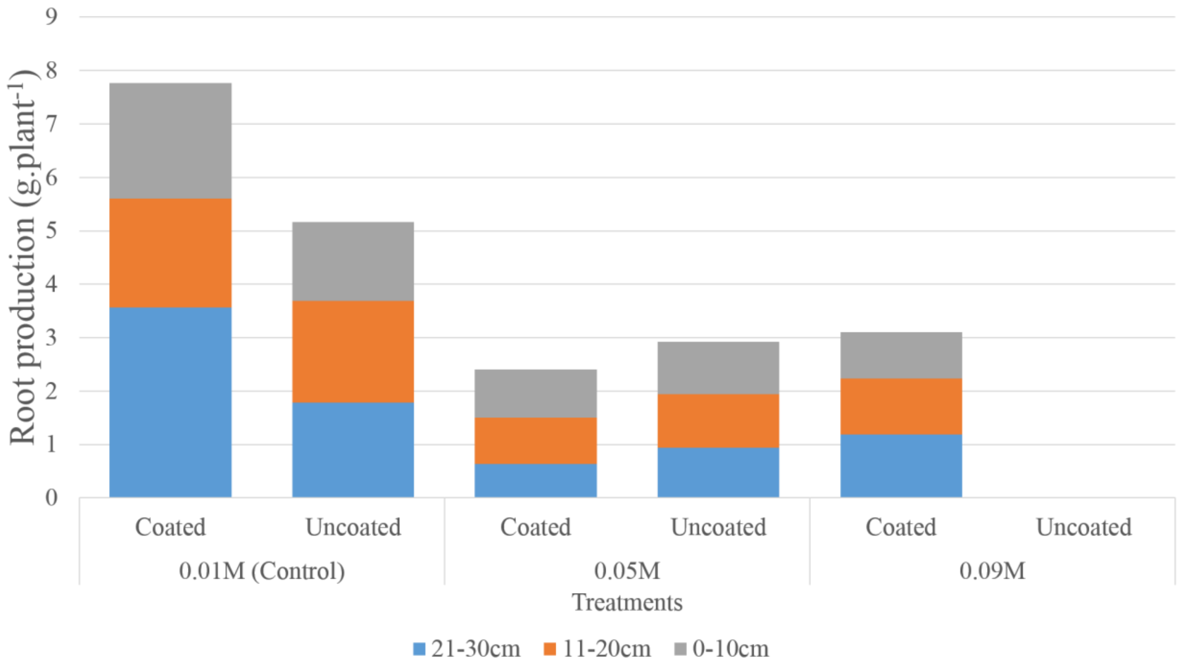

Figure 4.19: The mean root production (g.plant^{-1}) of *E, curvula* planted to coated and uncoated seed, at three different depths (0-10cm, 11-20cm and 21-30cm) and at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Figure 4.19 shows the effect of salinity on root production at different levels. It is clear from that *E. curvula* is very sensitive to high salinity levels. There is a dramatic decline in root growth with a slight increase in salinity. There is a significant difference ($P \leq 0.050$) in the interaction between salt concentrations, seed coating and root depth as seen in Figure 4.20. The highest root production was for 0.01M NaCl at all three the depths. The highest production was for coated seed at 21-30 cm. The primary root system developed faster and could reach greater mass and depth, while uncoated seed did not; this is only for seed at low salinity levels.

The root mass of the coated seed at 0.05M NaCl was significantly lower compared to that of uncoated seed, but the results for 0.09 M salinity treatment was different, this is likely due to genetic difference between the plants. In the treatment where 0.09M was used, the most root production occurred at 0-10cm and the least at 21-30cm. The uncoated seed showed that the highest root production was found at 11-20 cm for both 0.05 and 0.09 M NaCl. Therefore coated seed had the highest root mass when stress was small (low salinity concentration), 0.01M NaCl.

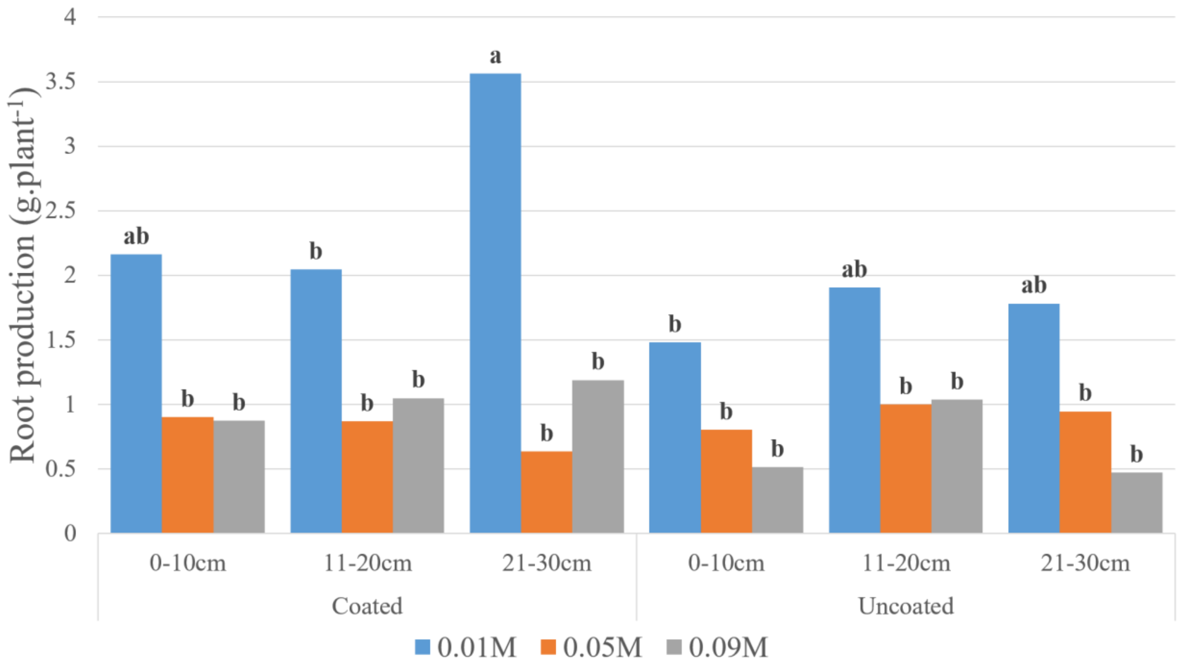

Figure 4.20: The mean root production (g.plant^{-1}) of *E. curvula*, planted to coated and uncoated seed, at three different depths (0-10cm, 11-20cm and 21-30cm) and at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

<u>*Medicago sativa*</u>

<u>Root production (g.plant^{-1})</u>

As with the other pasture species, if not more, the total root production of *M. sativa* are expected to be lower as the salinity levels increases in the soil (Munns and Termaat, 1986). The plants from coated seed is expected to produce a slightly larger amount of roots, compared to plants from uncoated seed. The coating produces favourable conditions in the soil after germination, therefore being favourable for root production. Figure 4.21 below shows the mean root production of three species at three different salinity concentrations. The data analysis of *M. sativa* found that there is no significant difference between the seed coating treatments and the interaction between the salinity concentrations and the seed coating. There was a significant difference between the salinity concentrations ($P \leq 0.001$) and contributed 86.62% of the total variation in *M. sativa*.

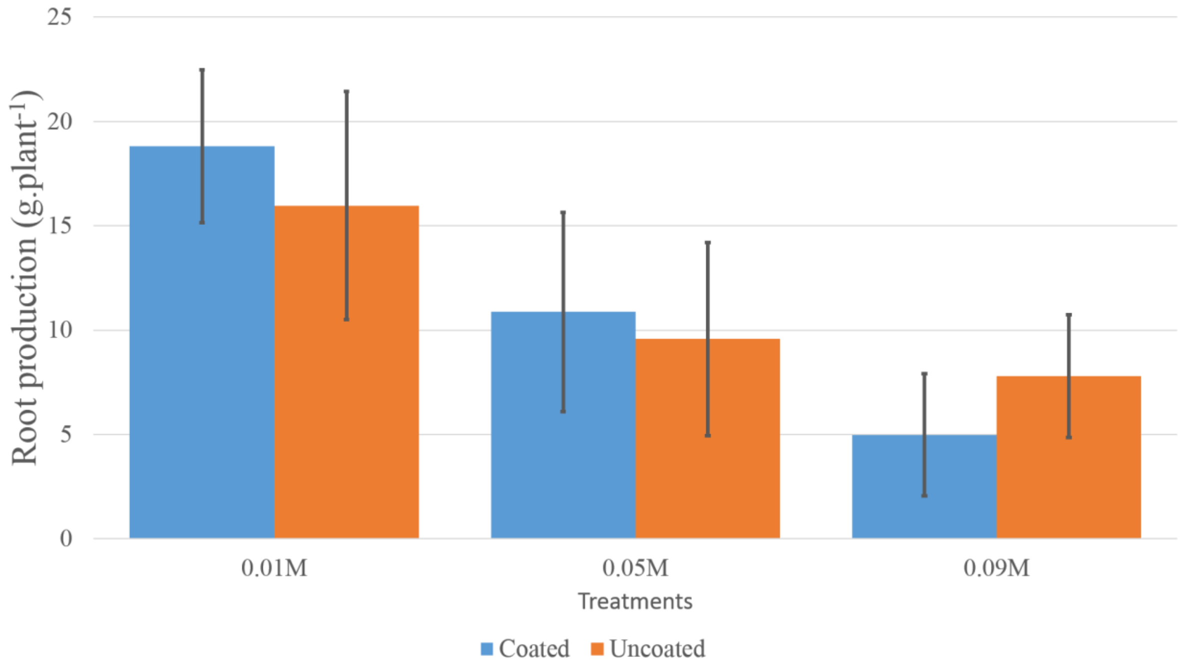

Figure 4.21: The mean root production (g.plant^{-1}) with standard deviation of *M. sativa* planted to coated and uncoated seed at three salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Figure 4.21 shows a decline in the root production as the salinity levels increase. The effect of salinity is significant on *M. sativa* growth (86.62%) and this is due to *M. sativa* and most other legumes being sensitive to high levels of NaCl (Dickinson, 2004). It was also interesting to note that there were no nodules found in *M. sativa* at 0.05M and 0.09M, however nodules were only found at 0.01M. This clearly shows the effect high salinity have on the plant. It is clear from Figure 4.21 and the data analysis that the coating had no effect in *M. sativa* on the root production.

<u>Root diameter (mm)</u>

The data analysis shows there is no significant difference between the salt concentrations, seed coating and the salt concentrations and seed coating interaction for *M. sativa* (Figure 4.22) Coating and salinity did not have an effect on the root diameter. This is contradictory to some literature that states that roots under stress usually have thinner roots (Zhao et al., 2013, Imada et al., 2015). It is not necessary the case for all roots that are under stress as in the case of some legumes were the roots become thicker and shorter (Franco et al., 2011).

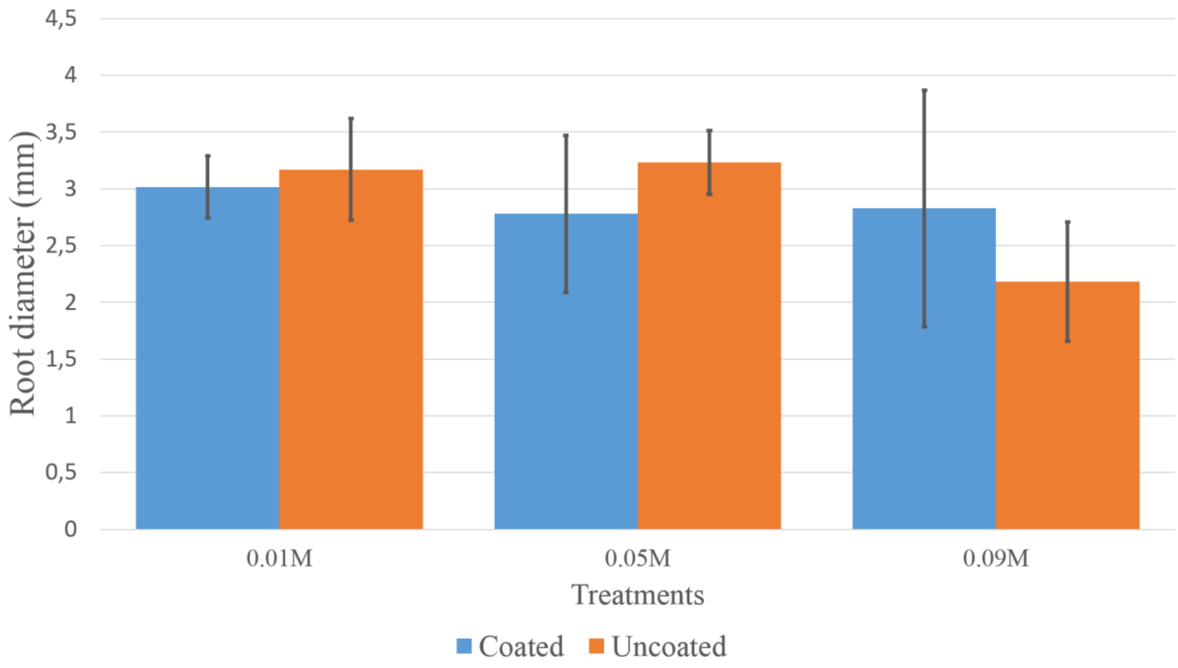

Figure 4.22: The mean root diameter (mm) with standard deviation of *M. sativa* planted to coated and uncoated seed, at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

<u>Root production (g.plant^{-1}) at various depths</u>

Figure 4.23 shows the mean root production (g.plant^{-1}) of *M. sativa* at three different depths (10, 20 and 30 cm) and at three different salinity concentrations (0.01M, 0.05M and 0.09M NaCl). The data analysis showed that the different root depths and salt concentrations had significant differences at $P \leq 0.01$. The interaction between salt concentrations and root depth showed significant differences at $P \leq 0.01$. Seed coating and the interaction between seed coating, salinity and root depth) had no significant difference.

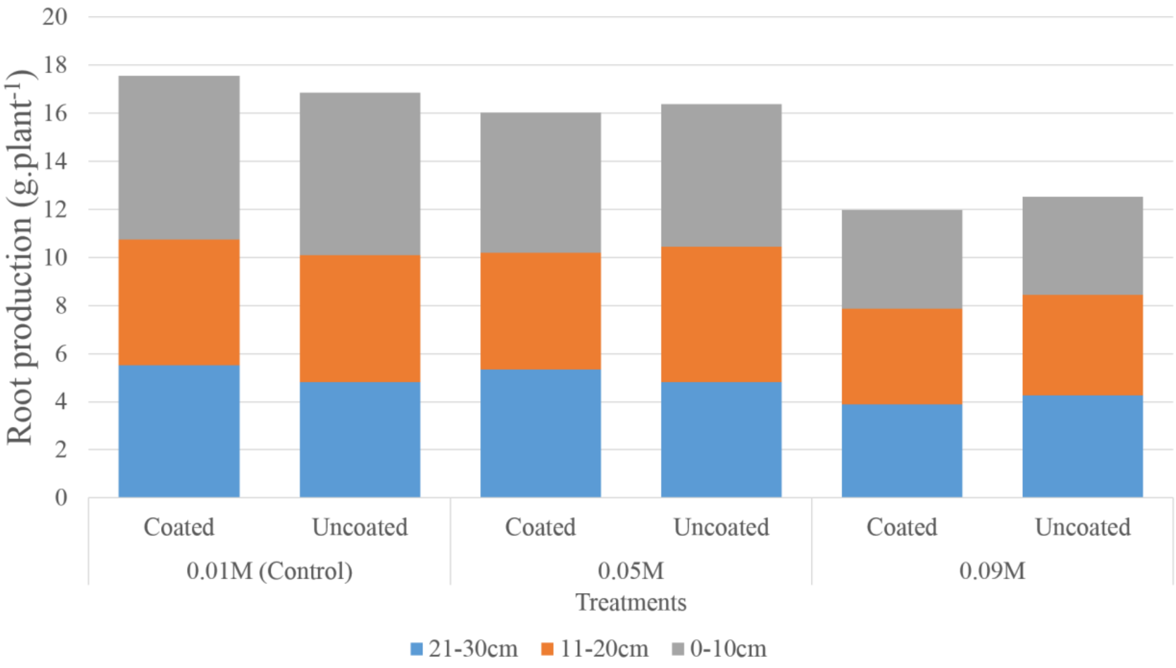

Figure 4.23: The mean root production (g.plant^{-1}) of *M. sativa* planted to coated and uncoated seed, at three different depths (0-10cm, 11-20cm and 21-30cm) and at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

Figure 4.23 shows that the higher salinity concentrations effected the root growth at the different root depths. At 0.09M NaCl concentration there, was low root production in the different root depths as compared to the low salinity (0.01M) concentration. The depth the roots reached also had a big effect on the amount of roots produced. The majority of roots were found in the first top 10 cm. At a depth of 0-10cm the most fibrous roots were noted and the taproot was at its thickest, therefore the large root production was found in the first 10cm. The deeper the root depth the less fibrous roots are found, at depth of 11-20 cm. The lowest amount of roots were found in the last 30 cm (21-30cm), where there was a small amount of fibrous roots together with the tap root, becoming thinner and thinner. Therefore the lowest amount of roots were noted for the 0.09M NaCl treatment at a depth of 21-30 cm.

In Figure 4.24, the decline in root growth as the depth increases can clearly be seen. Most mines that are rehabilitated only have between 20 and 30 cm of top soil that is placed above the underlying tailings (Truter, 2015). This alone is unfarouble for Lucerne growth. The data shows that in soils that have almost no salinity (0.01M), the biggest amount of roots accumulate in the first 10 cm and this will be beneficial to the growth of the plant. The higher salinity's showed a different trend, at 0.05M the highest amount of roots are in the first 10cm with slightly lower root production at 20 cm (11-20 cm) and 30 cm (21-30 cm). At 0.09M the growth was very low at all three the different depths. The root production for 0.01M and 0.05M, at 11-20 cm and 21-30 cm is very similar, this shows that salinity stress affects growth in the first 10 cm. The top 10 cm is were the most nodules were found and this contributed to the higher root production at 0.01M. The nodules were physically observed during the weighing of the roots and there was no nodules found at the higher salinity's. The top 10 cm is also where most of the fibrous roots were found, therefore the high salinity not only effected the nodules but also reduced the number of fibrous roots in *M. sativa.*

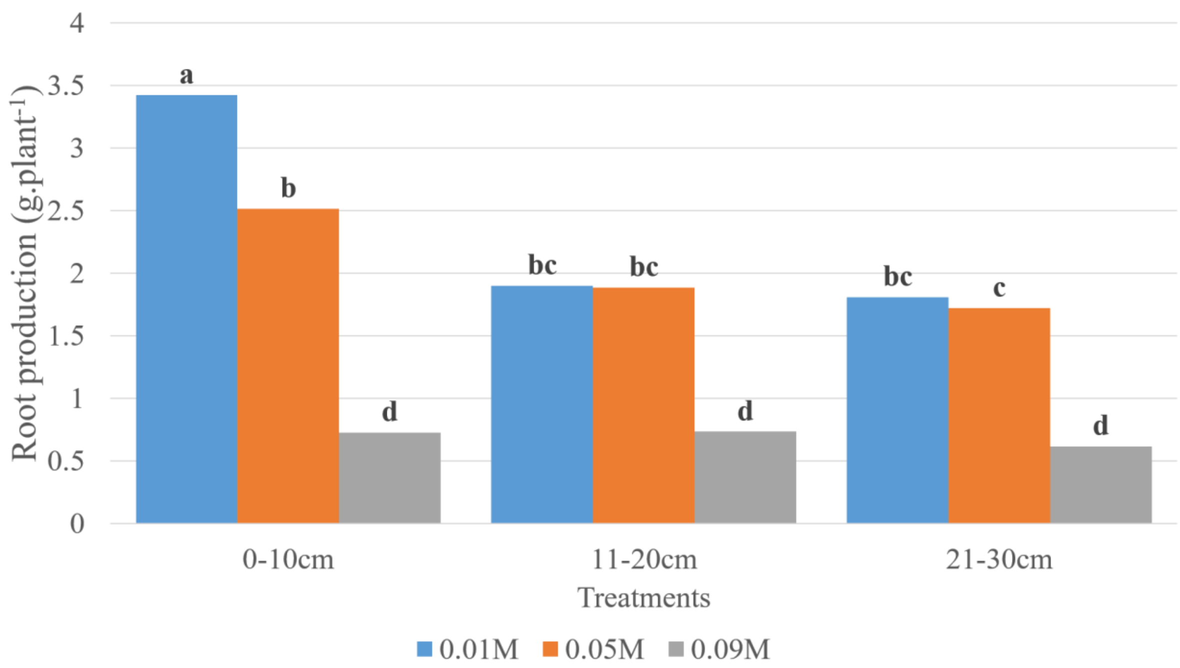

Figure 4.24: The mean root production (g.plant^{-1}) of *M. sativa*, planted to coated and uncoated seed, at three different depths (0-10cm, 11-20cm and 21-30cm) and at three different salinity concentrations (0.01M NaCl (control), 0.05M NaCl and 0.09M NaCl)

4.5 CONCLUSION

Coated seed did not have an effect on the fully grown plant in comparison to the results found in the emergence trials. In *C. gayana* coated seed had a significant effect on the root production at various depths. It was found that plants from coated seed produced more roots at deeper depths, the lowest production was at 0-10cm and the highest production was at 21-30cm. Roots that emerged from coated *C. gayana* seed had a slightly thicker root, the data showed it was not significant but the figures showed there was a trend significance. Coated *Eragrostis curvula* seed resulted in a higher leaf area compared to uncoated seed. The root production and root diameter was also slightly higher compared to uncoated seed. The overall production of *E. curvula* also decreased as the salinity concentrations increased.

There was a reduction in root production, leaf area and dry matter production for *C. gayana* when the salinity concentration increased. *Chloris gayana* is known to be salt tolerant but the production still decreased and coated seed did not contribute significantly. *Medicago sativa* is very sensitive to high salinities and this was again proven in this study. There was a clear reduction in root production, leaf area index and dry matter production when the salinity concentrations of the treatments were increased. *Medicago sativa* should not be considered for production in areas with high salinity levels, the production will definitely be impacted.

The overall benefits of coated seed when looking at production is very small and of no benefit, but is important to remember even though there was no increase in production for coated seed compared to uncoated seed, the coating of seed is of huge benefit to the handling process and planting. The seed of *C. gayana* is 'fluffy' and can easily be blown away by wind and get stuck in a planter. Coating *C. gayana* seed will add weight to the seed and therefore improves the handling of the seed. The physical and chemical aspects of coated seed should always be kept in mind, not only the production aspect.

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CHAPTER 5

Prepared according to African Journal of Range and Forage Science guidelines

The effects of seed coating on the development of *Chloris gayana, Eragrostis curvula* and *Medicago sativa* in response to different pH growth conditions

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5.1 ABSTRACT

The increase in unfavourable growth conditions caused by improper mining activities, that causes both chemical (including high salinity's and pH) and physical changes, in addition to the presence of potentially harmful elements in the soil/substrate, often restrict germination and root development which complicates the establishment and rehabilitation process. Coating seeds facilitate the establishment of grasses in these unfavourable growth conditions. Three species were used in this study namely, *Chloris gayana*, *Eragrostis curvula* and *Medicago sativa.* The species were planted in silica sand placed in 400 mm tall and 150 mm wide pots. Different water application treatments were tested, namely water with different pH levels (3, 5, 7 (control) and 9). *Choris gayan*a, *E. curvula* and *M. sativa* planted to coated seed showed no benefits in root production, root diameter, dry matter production and leaf area compared to uncoated seed. Roots from coated *C. gayana* seed reached greater depths while roots from uncoated seed were more concentrated in the first 0-10cm. Coated *E. curvula* seed had an effect on the depth roots reached. Roots from coated *M. sativa* seed had roots that reached greater depths compared to uncoated seed where the majority was found in the top 0-10cm. The benefit of using coated seed is very limited in mature plants, but it is important to note that the coated seed has other benefits.

Keywords: Coated seeds, rehabilitation, leaf area, dry matter production, root production

5.2 INTRODUCTION

Growth parameters are measured differently for different species, depending on the growth form, adaptive mechanisms and ultimate use of the plant. Parameters important in forage production, include biomass production and quality factors such as leaf to stem ratio. For rehabilitation, the root production also becomes an important parameter.

Various factors that affect the growth and development of plants are grouped into two categories; genetics and environment (Hartmann et al., 1981). Genetic factors are the internal mechanisms of the plant and will be optimally expressed under ideal conditions (environment), genetics and environment interaction. Plant breeders are constantly trying to improve the genetics by breeding stronger or more adapted plants. Environmental factors influencing the genetic expression include light, temperature (this is especially important for this study, with the use of sub-tropical species), water quality, soil quality etc.

Soil found in some of the mined areas are often high in metals such as; Aluminium (Al), Zinc (Zn) etc. and also have extreme pH and salinity levels (Shu et al., 2002). This complicates the rehabilitation process and influences the establishment of the selected grass species. The pH in combination with the high levels of Al can inhibit germinationan. This is also true for high levels of salt (Mattigod and Page, 1983). Apart from germination being affected, if the seed is able to germinate, these factors can also have a negative effect on the primary root and shoot that is produced. These soils are often shallow and hold little reserves of minerals to support the growth of the primary root.

The one suggestion is to use coated seed to support this primary root development in the early stages of emergence. The primary root that is supported or have more nutrients available in early life will result in a larger and healthier plant later in its life cycle.

Coating seeds is done by adding growth stimulates, polymers, nutrients, fungicides, fillers and insecticides to the outer coating of the seed itself (Detroz and Gago, 1991). These treatments can improve the germination rate of species, depending on the soil in which it is planted in, therefore it can make establishment of these species more successful. The coating of coated seeds will disperse / dissolve when there is enough water, but will remain intact when there is not enough water, preventing germination in drought conditions, therefore making seed more drought resistant (Detroz and Gago, 1991). The main aim to coat seeds is to overcome or lower the effects of most of the environmental challenges and to give a seed the best chance of survival.

High salinity levels causes the effects of high pH to be more severe (Mattigod and Page, 1983). The pH of the soil on its own is not a major problem, but is very important in the release of some minerals, making minerals more available to plants or preventing the release of some minerals (Mattigod and Page, 1983). A study was done where the uptake of Al was measured in soils with low pH, it was found that the uptake of Al increased as the pH decreased. The uptake increased dramatically as the pH dropped below 4.4 (Dong et al., 1995). The pH also affects the amount of nitrogen that was taken up by the plants. The highest amount of ammonia that was taken up was at a pH of between 6.5 and 8.5 and the lowest amount of ammonia was were the pH was more than 6.5 and 8.5 (Jampeetong et al., 2013).

The hypotheses of this trial were:

- Species grown from coated seeds will have a higher root mass in the different growth substrates compared to uncoated seed
- Species grown from coated seeds will have bigger root diameters in the different growth substrates compared to uncoated seed
- Roots of species grown from coated seeds will reach greater depths in different growth substrates compared to uncoated seed
- Species grown from coated seeds will have a greater total leaf area in the different growth substrates compared to uncoated seed
- Species grown from coated seed had a higher dry matter production compared to uncoated seed in different growth substrates

5.3 METHODOLOGY

A study on the effects of seed coating on different pasture species compared to uncoated seed was conducted in a greenhouse, Phytotron C at the Hatfield experimental farm of the University of Pretoria, Pretoria, South Africa. The objective of this study was to evaluate the effect of seed coating on leaf area, dry matter production and root production in these different pasture species and to compare the use of coated and uncoated seed in growth substrates with a range of different water pH levels. The test species used were *Chloris gayana*, *Eragrostis curvula* and *Medicago sativa*. These species were chosen because they are widely used in mine rehabilitation and also used in farming practice. All the species had two seed treatments, namely uncoated and coated. The same seed batches where used for both coated and uncoated treatments in each of the different species used, therefore excluding genetic differences from this study. The seed was supplied by Advance Seed and according to the quality standards according to the plant improvement act of 1976 (Act 36 of 1936), to prevent any old seed from being used to ensure maximum germination was possible. The commercial (AgriCOTE®) coating applied to the seed contained nutrients, pesticides, polymers, lime and in the case of *M. sativa* it contained inoculants (rhizobia).

An inert growth substrate (silica sand) was used and the water added had different pH levels. The four pH levels used were 3, 5, 7 (that will act as the control of this trial) and 9. The different pH levels were made up using hydrochloric acid (HCl) and sodium hydroxide (NaOH). Before the different pH waters were made and applied, a Hoagland solution was added to deionized water. The Hoagland solution contained Nitrogen (N), Phosphorus (P), Potassium (K), Magnesium (Mg), Sulphur (S), Iron (Fe), Zinc (Zn), Copper (Cu) and Boron (B). This Hoagland solution had an effect on the pH of water. After the Hoagland solution was added to the water, then only were the different amounts of HCl and NaOH added to the water and the pH then measured by using a pH meter. The water was allowed to stand for three days to ensure the pH was constant. The water was added to the treatments if the pH didn't change after three days.

Self-made containers were made of PVC cylinders with a diameter of 160 mm. The containers were 400 mm high and were divided into four stacked sections of 100 mm each. Coated and uncoated seed of each species were planted in separate containers. There were four replications of each treatment. The amount of water applied to each container was determined by filling a container with sand and the container was then filled with water until saturated and then weighed. The container was then placed in an oven (35˚C) for three days and weighed again at the end of the three days. This was repeated three times and from this the water loss and the amount of water that should be given to the pots every third day was calculated. Therefore the amount of water per unit of sand was determined. The level of saturation was determined by using the WP4-T machine (Devices, 2007, Nel, 2014).

The WP4-T is also known as the Decagon's WP4 Dew point Potential Meter. WP4 is used to measure water potential, given in MegaPascals (MPa) (Devices, 2007). It measures water potential from 0 to -300 MPa with an accuracy of ±0.1 MPa. The machine has mirrors on the inside were water condensates onto (Devices, 2007). At certain point there exists an equilibrium between the moisture in the sample and the moisture that condensates on these mirrors. This equilibrium gives the water potential reading that is used to determine field capacity (Devices, 2007).

Once the seed was planted, water was added until the soil reached saturation and then the whole pot, including water, sand and seed was weighed. The containers were then covered with plastic for a week to prevent excessive water loss and to give the seed enough time to germinate. Containers were weighed every third day to determine the water loss. The weight was subtracted from the initial weight of the container and this gave the amount of water that was lost in three days. The containers were topped up to the same weight with water every third day. The duration of the trial was five months.

There were three dry matter harvests during the study period. All the species were harvested at the same time, the first two occurred every 8 weeks and the last harvest occurred at the end of the trial (6 weeks after the second harvest). The stems were cut at the same height, for the first and second harvest they were cut at 10 cm above ground and the last harvest they were cut 1cm above ground to ensure that all the plant material can be dried and weighed. The stems and leaves were placed in brown bags and dried at 65˚C for three days. During the last harvest, the leaf area of each treatment was calculated using a Licor (LI-3100^{o}C) leaf area meter machine before they were dried.

After the leaf area was determined the samples were dried. The leaf material was weighed after drying and the biomass recorded.

Upon completion of this study, each 100 mm section of the containers were destructively removed. The roots of each section of the container were harvested, washed and placed in brown bags. The roots were then placed in an oven and dried at 65˚C for two days. The weight of the roots in each section was recorded. The root diameter was also determined by using a Vernier calliper. Three roots were taken at random and the diameter was recorded at a level of 40 mm, measuring from top of the root moving down, for each root (Geilfus and Mühling, 2014).

<u>Statistical analysis</u>

This trial was established as a completely random design (CRD) in a glasshouse. A two factor ANOVA was used to test for differences between four pH levels and two coating treatments as well as their interaction, for each species separately at a 5% significance level. The residuals from the analyses were acceptably normal with homogeneous treatment variances.

The pH and salt treatments trials were measured at three root depths; 10cm, 20cm and 30cm. These data were analysed by CRD split-plot ANOVA to test for differences between pH and salts interaction, seed coating and root production per depth, as well as all their interactions, for each species separately. Means in all trials were compared using Tukey's least significant difference test at the 5 % level of significance (Snedecor and Cochran, 1980).
Data were analysed using the statistical program GenStat® (Payne et al., 2014)

5.4 RESULTS AND DISCUSSION

a) Above ground parameters

i. *Chloris gayana*

Leaf Area (cm^3)

The leaf area of plants and pastures are also affected by stress, be it drought, salinity or high pH levels. The leaf area of plants are expected to be smaller or reduced as the plants stress increase, this was found in studies done on various grasses were the salinity levels were high (Munns and Termaat, 1986, Ortega and Taleisnik, 2003). The smaller leaf area's are due to stress affecting the cell division process of plants, therefore cells do not divided properly and therefore there is a reduction in the photosynthetic area of the plant (Taleisnik et al., 2009). The lower photosynthetic area in turn equates to lower production of grasses and therefore a reduced total production.

Information on the effects of pH on leaf area is very limited and the data in Figure 5.1 below shows the mean leaf area of *C. gayana* at four different pH levels. The data analysis of *C. gayana* showed that there were no significant differences between different pH concentrations, seed coating and the pH*seed coating interaction, therefore using coated seed had no impact on leaf area.

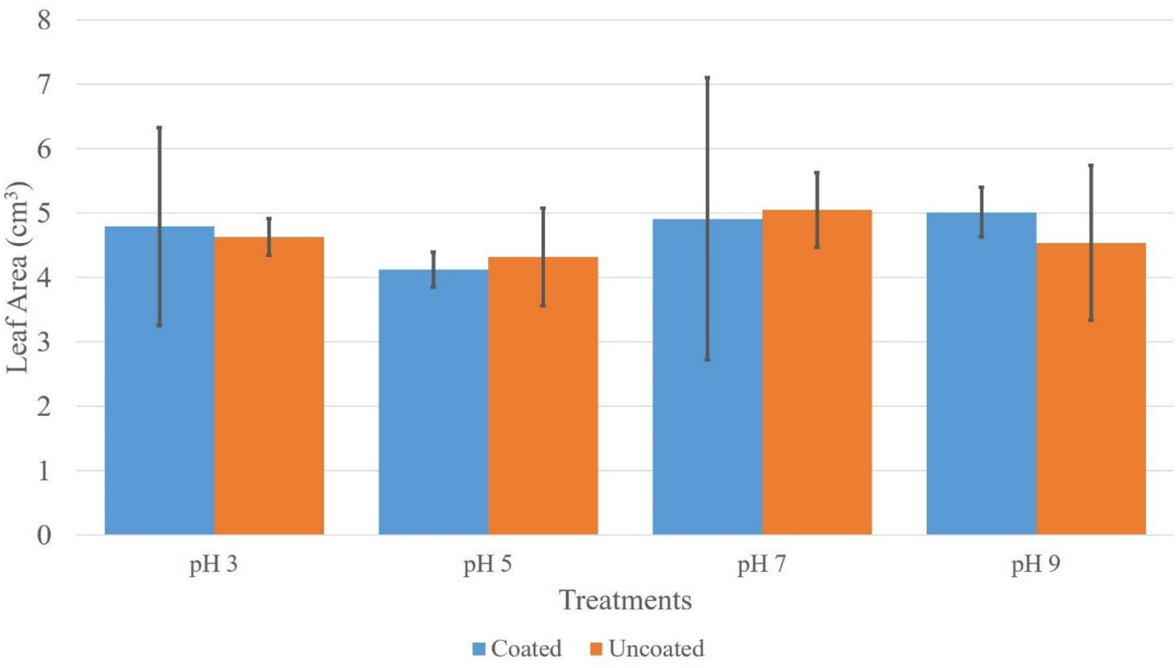

Figure 5.1: The mean Leaf Area (cm^3), with standard deviation, of *C. gayana* planted to coated and uncoated seed at four different pH (3,5,7(control)) and 9) levels.

Dry matter production ($g.plant^{-1}$)

Figure 5.2 below shows the dry matter production ($g.plant^{-1}$) of *C. gayana* at the four different pH levels. The data analysis showed that there was no significant difference between coatings and the interaction between pH and coating in *C. gayana*. There was a significant difference ($P \leq 0.029$) between the different pH treatments for *C. gayana*.

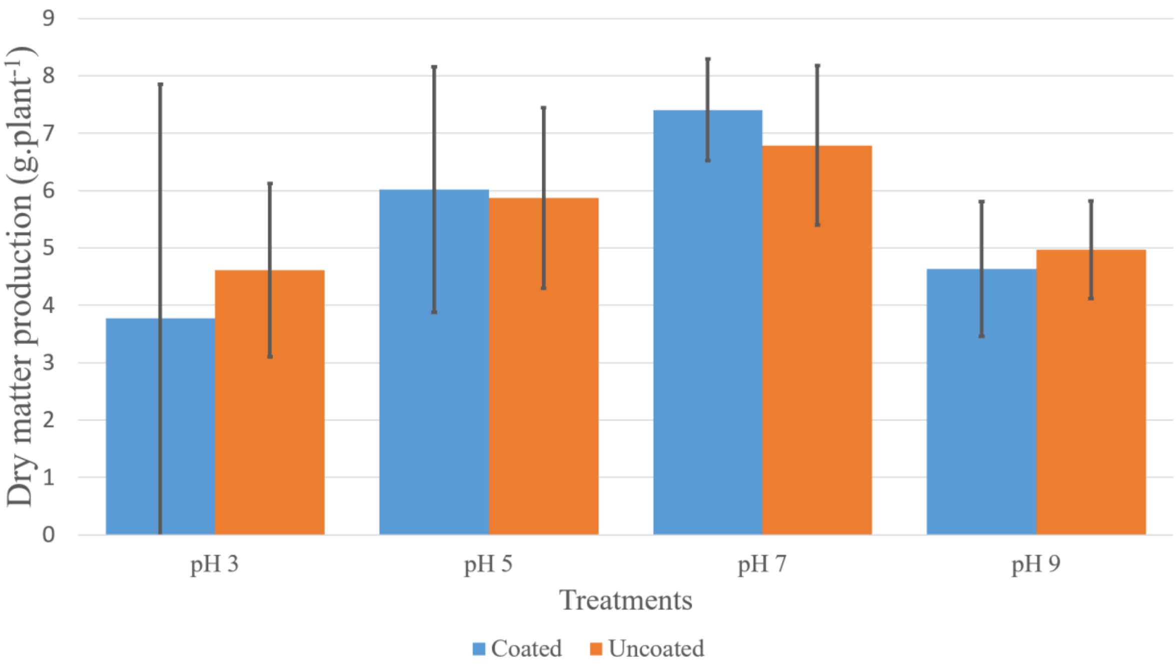

Figure 5.2: The mean dry matter production (g.plant^{-1}), with standard deviation, of *C. gayana* planted to coated and uncoated seed at four different pH (3,5,7(control)) and 9) levels

Dry matter production is the most important aspect to farmers, to a lesser extent in mine rehabilitation. It is clear from Figure 5.2 that coated seed did not improve the dry matter production of *C. gayana*, there was no difference between coated and uncoated seed. At pH 7 coated seed had a slightly higher dry matter production compared to uncoated seed, this was not significant.

<u>Correlation between leaf area (cm^3) and dry matter production (g.plant^{-1})</u>

The figure below shows a correlation between leaf area and dry matter production. At pH 3, pH 5 and pH 7, as the leaf area increase so do the dry matter production. There is an negative correlation at pH 9, the dry matter production decreased as the leaf area increased. The hypothesis is that at very high pH, *C gayana* compensates to survive by increasing the size of the leaves and reducing the amount of leaves causing the dry matter production to decrease.

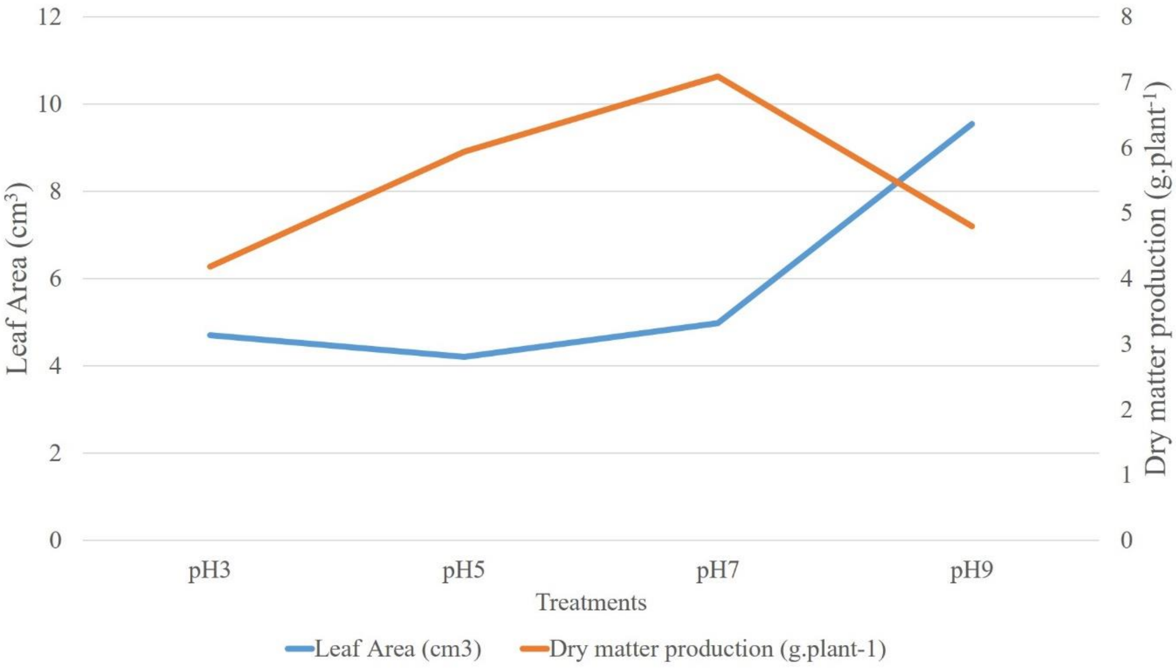

Figure 5.3: The correlation between leaf area (cm^3) and dry matter production ($g.plant^{-1}$) for *C gayana* four different pH (3, 5, 7 (control)) and 9) levels

ii. *Eragrostis curvula*

Leaf Area (cm^3)

Information on the effects of pH on leaf area is very limited. The data analysis showed there was no significant interaction between the different pH and coating treatments in *E. curvula*. There was however a significant difference in the pH and seed coating interaction (Figure 5.4) and contributed 46.67% of the total variation.

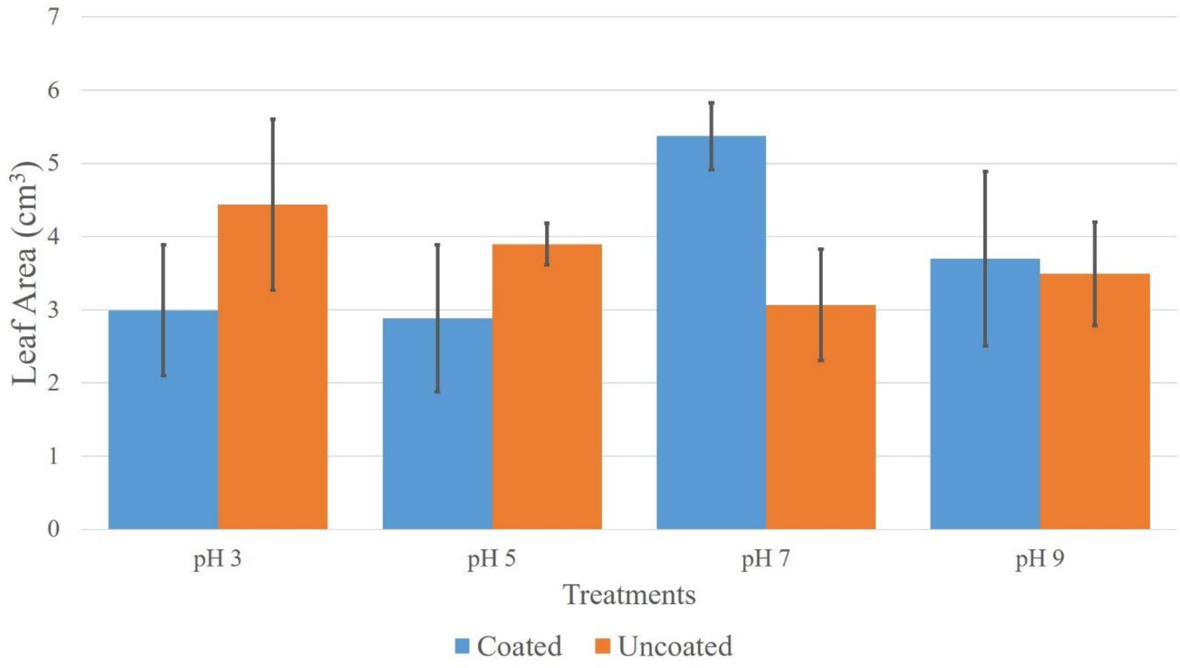

Figure 5.4: The mean Leaf Area (cm^3), with standard deviation, of *E. curvula* planted to coated and uncoated seed at four different pH (3,5,7(control)) and 9) levels

In acidic soils the seed coating did not have an effect on leaf area, the leaves from uncoated seed were larger at pH 3 and 5 (Figure 5.5). At pH 7, that is a neutral pH, coating had an effect and leaves from coated seed were much larger compared to uncoated seed. At pH 9 the coating had a slightly larger leaf area, therefore coatings have little or no effect in an acidic or alkaline soil. The coating was only successful at the neutral pH, the low pH had a negative effect on the coating therefore altering the growth of the leaves.

Dry matter production ($g.plant^{-1}$)

The data analysis showed that there was a significant difference ($P \leq 0.022$) between the different pH levels in dry matter production of *E. curvula* (Figure 5.6). There was no significant differences between the coating treatments, and in the interaction between coating and pH. Figure 5.5 below shows this interaction.

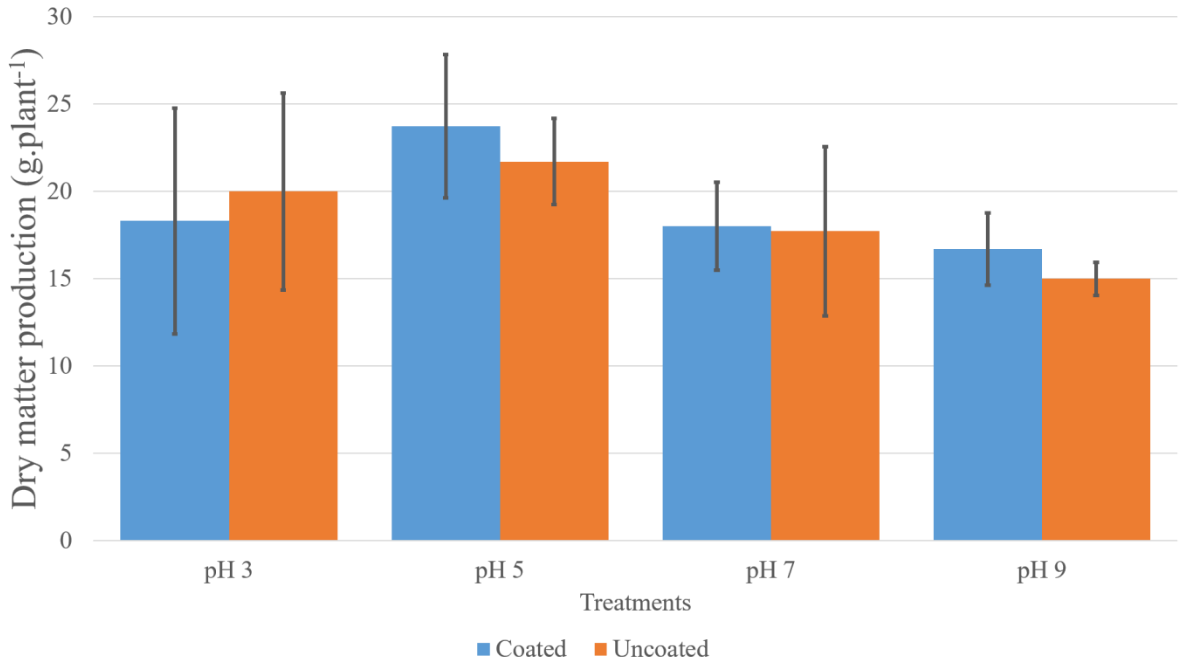

Figure 5.5: The mean dry matter production (g.plant^{-1}), with standard deviation, of *E. curvula* planted to coated and uncoated seed at four different pH (3,5,7(control)) and 9) levels

The highest dry matter production was at pH 5 and the lowest at pH 9. This shows that *E. curvula* does better in more acidic soil and is more sensitive to alkaline soil (Figure 5.6). The aim of this study was to examine if seed coatings will have an effect on dry matter production at different pH levels.

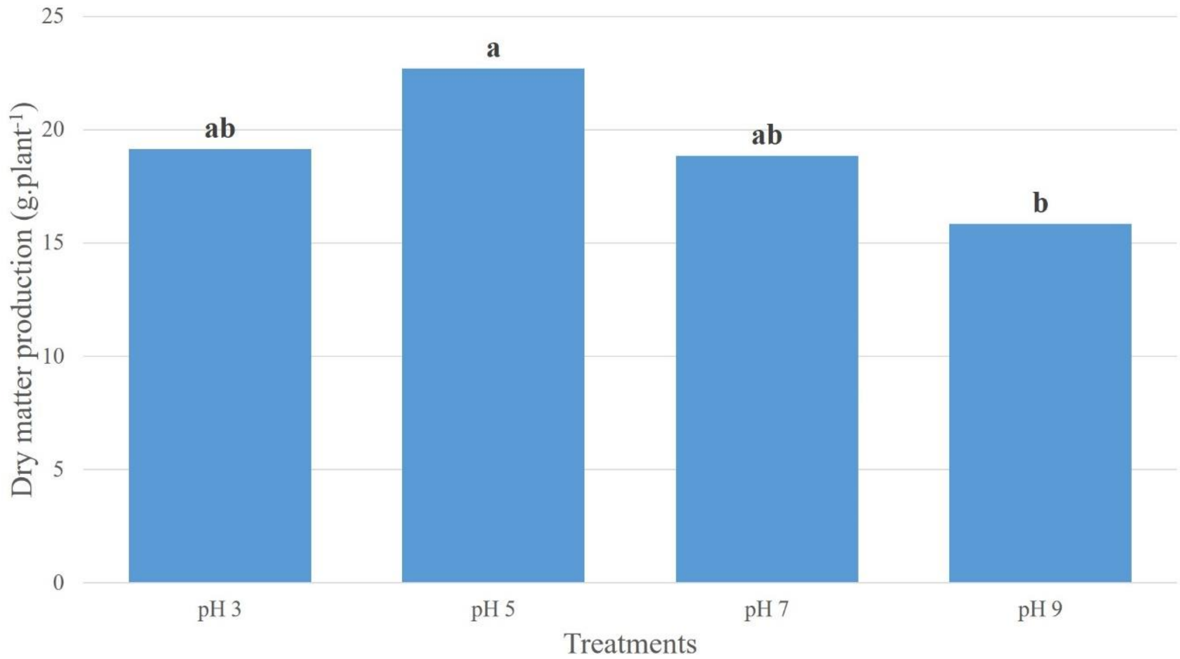

Figure 5.6: The mean dry matter production (g.plant^{-1}) of *E. curvula*, four different pH (3, 5, 7(control)) and 9) levels

**Values with the same letter are not significant and values with different letters are significantly different*

Correlation between leaf area (cm^3) and dry matter production ($g.plant^{-1}$)

The correlation between leaf area and dry matter production is shown in Figure 5.7 below. The leaf area decreases slightly at pH 5 and pH 9, increases at pH 7. The dry matter production is the highest at pH 5 and decreases as the pH decreases and increases; therefore there is no correlation between leaf area and dry matter production. It is also clear from this figure that the highest dry matter production was at pH 5, this is the ideal pH for *E. curvula*.

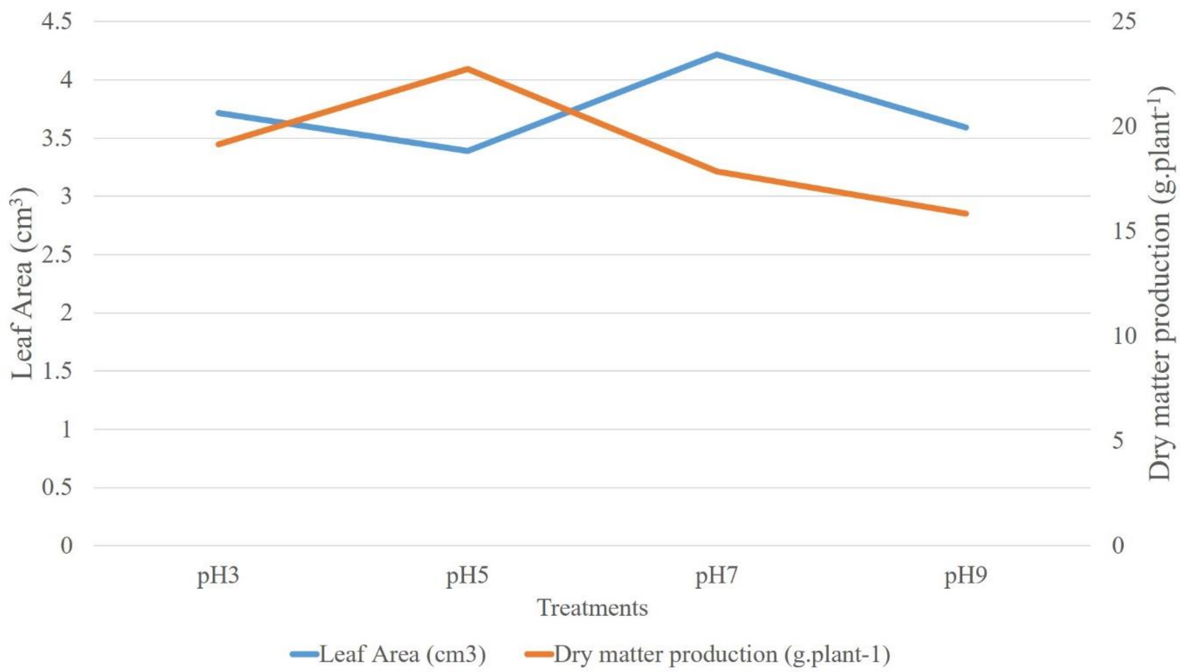

Figure 5.7: The correlation between leaf area (cm^3) and dry matter production ($g.plant^{-1}$) for *E. curvula* four different pH (3, 5, 7 (control)) and 9) levels

iii. *Medicago sativa*

Leaf Area (cm^3)

There was a significant difference between the different pH treatments in *M. sativa,* according to the data analysis. There was no significant interaction between seed coating, and the pH and seed coating interaction. The mean leaf area (cm^3) of *M. sativa* at four different pH levels can be seen in Figure 5.8.

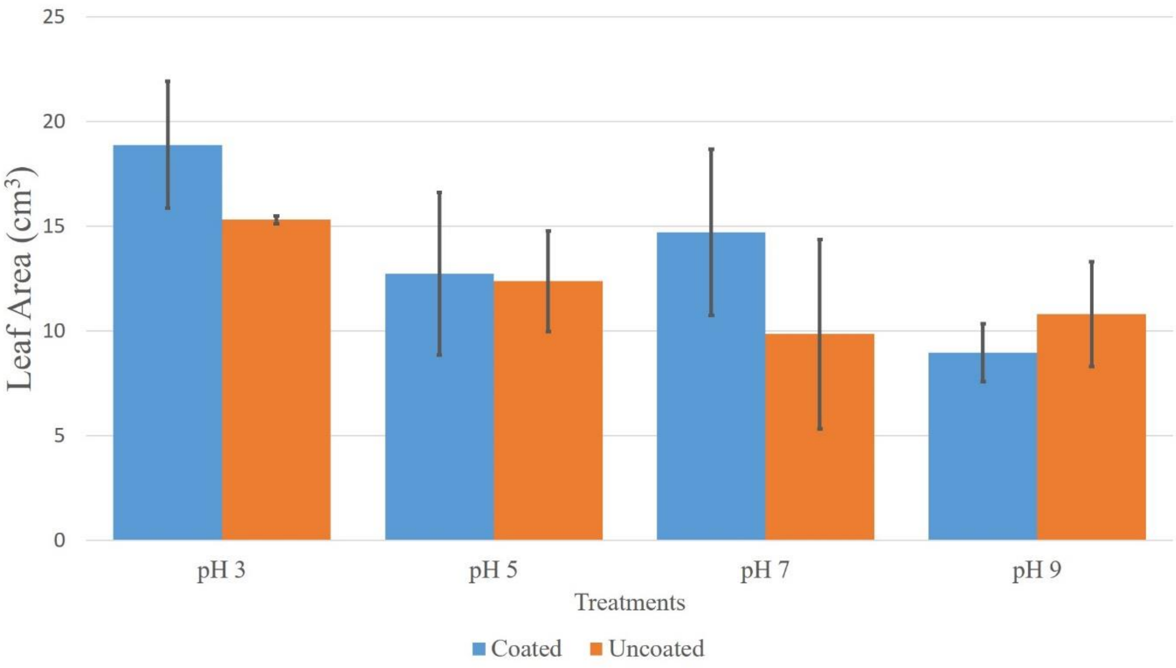

Figure 5.8: The mean Leaf Area (cm^3), with standard deviation, of *M. sativa*, planted to coated and uncoated seed at four different pH (3,5,7(control)) and 9) levels

Coated seed showed no effect on the leaf area of *M. sativa* according to the statistical analysis, but Figure 5.8 above shows that coated seed had a larger leaf area at pH 3 and pH 7. This was not statically significant but shows that there is definitely an influence by coated seed.

Dry matter production (g.plant^{-1})

The data analysis showed that there was no significant difference between the different coating treatments and the interaction between the seed coating and pH for *M. sativa* treatment. There was a significant difference ($P \leq 0.01$) between the different pH levels in *M. sativa* and the dry matter production of *M. sativa* at four different pH levels is shown in Figure 5.9.

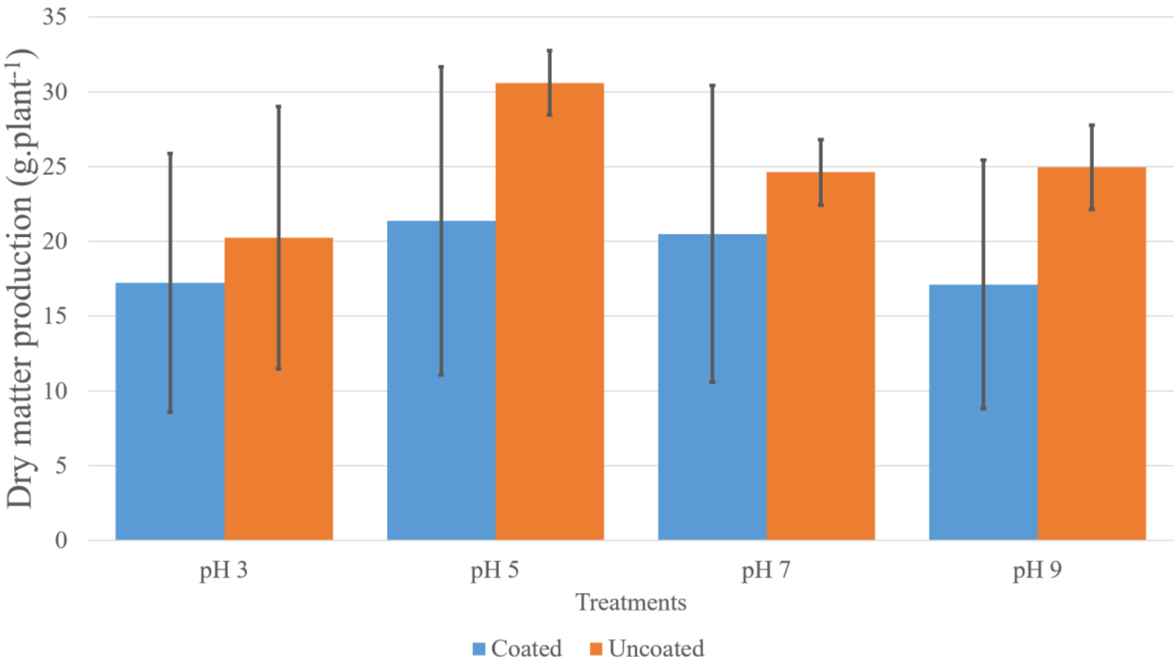

Figure 5.9: The mean dry matter production (g.plant^{-1}) with standard deviation of *M. sativa*, planted to coated and uncoated seed at four different pH 3,5,7(control) and 9) levels

The dry matter production of *M. sativa* planted to uncoated seed was much higher compared to coated seed. The coating did not have a significant impact on the dry matter production and showed no benefit. Figure 5.9 shows that pH has an effect on dry matter production, and as already mentioned previously, *M. sativa* is very sensitive to acidic soil and alkaline soil (Dickinson, 2004), which is confirmed by the data obtained. At pH 5, the production was the highest, but the production dropped as the pH increased or decreased. The lowest production was at pH 3 and therefore *M. sativa* is more sensitive to acidic soils than alkaline soils.

<u>Correlation between leaf area (cm^3) and dry matter production (g.plant^{-1})</u>

There was a negative correlation between leaf area and dry matter production at pH 3 up to pH 5, the dry matter production increased as the leaf area decreased, therefore smaller leaves but larger amount of leaves (Figure 5.10). The dry matter production decreased as the pH levels increased, from pH 5 up to pH 9. This decrease in production was associated with a decrease in the leaf area. There was a positive correlation between leaf area and dry matter production at pH 5, pH 7 and pH 9.

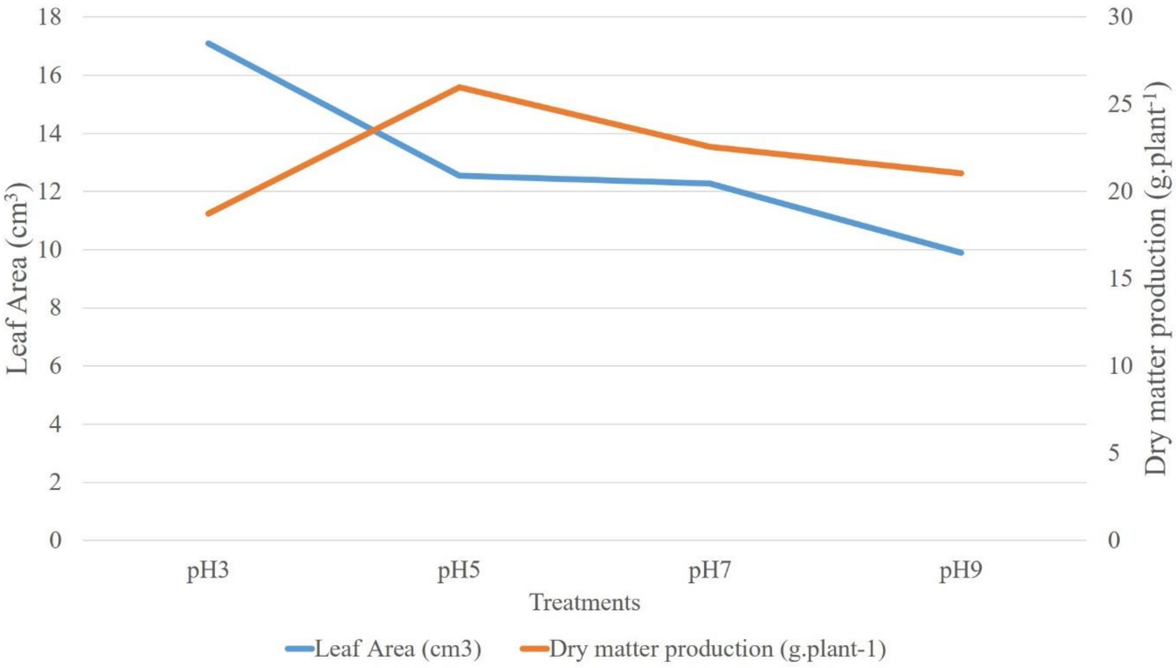

Figure 5.10: The correlation between leaf area (cm^3) and dry matter production ($g.plant^{-1}$) for *E. curvula* four different pH (3, 5, 7 (control)) and 9) levels

b) Above ground parameters

i. Chloris gayana

Root production ($g.plant^{-1}$)

The coating of the seed is hypothesized to increase the amount of roots produced compared to uncoated seed, therefore coated seed is hypothesized to have a higher amount of roots. The higher amount is expected due the more favourable conditions (nutrients and inoculants) the coating provided in the germination and the seedling phase. The primary root will develop faster in coated seed, compared to uncoated seed, due to the nutrients in the coating. The data analysis for *C. gayana* however showed that there was no significant difference between coating and the interaction between seed coating and pH. There was a significant difference between the different pH concentrations.

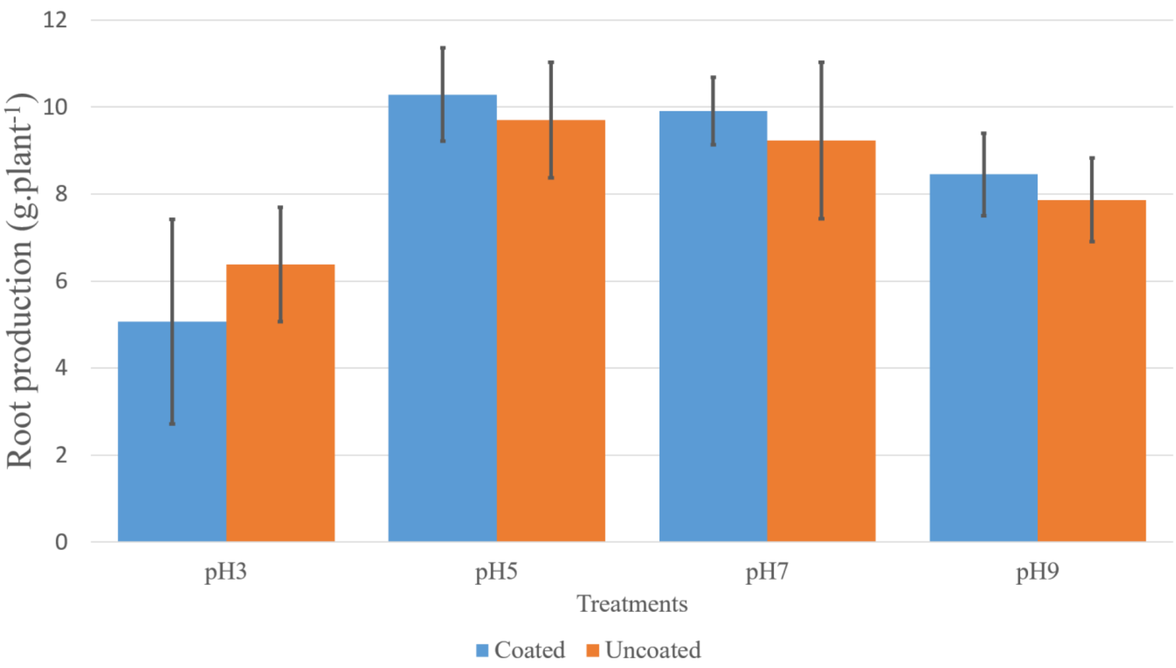

Figure 5.11: The mean root production (g.plant^{-1}) with standard deviation of *C. gayana*, planted to coated and uncoated seed at four different pH (3,5,7(control)) and 9) levels.

At pH 5, 7 and 9 the root production of *C. gayana* planted to coated seed had a higher production compared to uncoated seed, this was however not statistically significant. The root production was much lower at higher acidity (pH 3) levels and the production increased as the pH did (pH 5 and 7), but decreased as the pH became too high (pH 9).

<u>Root diameter (mm)</u>

Figure 5.12 shows the mean root diameter in mm for *C.gayana*. The data analysis found that only the different pH concentrations had a significant effect on root diameter. The coating and the pH and coating interaction had no significant difference in the root diameter of *C. gayana*.

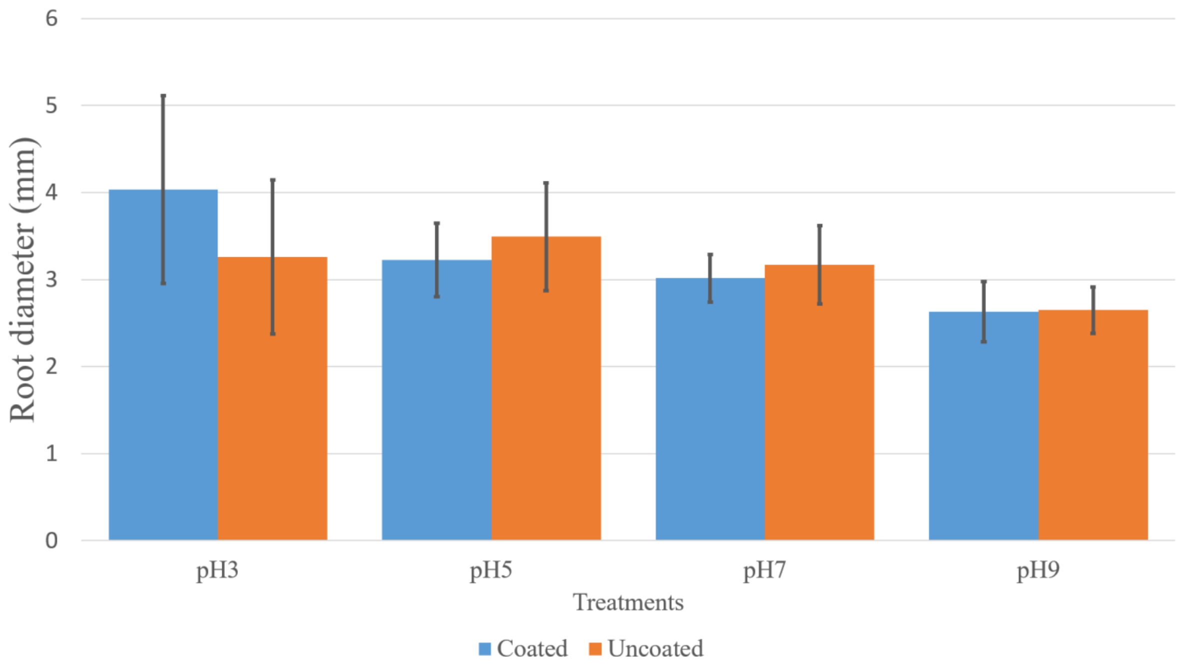

Figure 5.12: The mean root diameter (mm) with standard deviation of *C. gayana* planted to coated and uncoated seed at four different pH (3, 5, 7 (control)) and 9) levels.

The data analysis shows there is no significance difference between different coating treatments. It is important to note that root diameters are measured in mm and one millimetre in roots can be a big difference. At pH 3 *C. gayana* planted to coated seed had a thicker root compared to those planted to uncoated seed. At pH 5 and 7 roots from uncoated seed had a thicker diameter compared to roots from coated seed. At pH 9 there is no difference between roots from coated and uncoated seed. The overall trend for both coated and uncoated seed shows that the diameter of roots decrease as the pH increased, this shows when roots are under stress in low pH levels, the roots become thicker.

Root production (g.plant^{-1}) at various depths

Figure 5.13 below shows the mean root mass of *C. gayana* at various depths and the effect pH had on the total root production. The data analysis showed that there is no significant difference ($P \geq 0.05$), between the different pH's, coatings and the interaction between pH and coating interaction. There was a significant difference between the root production per depth ($P \leq 0.006$), in addition to an interaction between pH and root production per depth ($P \leq 0.043$) and the interaction between coating and root production per depth ($P \leq 0.031$), as seen in Figure 5.13, Figure 5.14 and Figure 5.15 respectively.

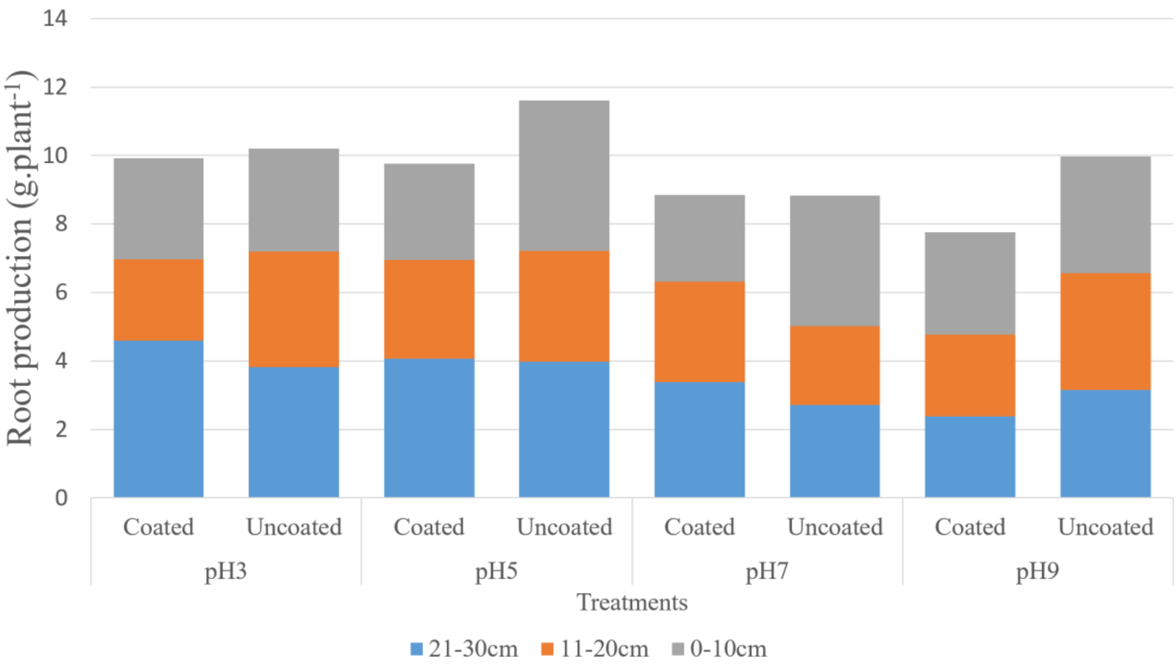

Figure 5.13 The mean root production (g.plant^{-1}) of *C. gayana* planted to coated and uncoated seed at three different depths (0-10cm, 11-20vm and 21-30cm), at four different pH (3, 5, 7 (control)) and 9) levels.

**Statistics were not included due to insignificance between all the interactions*

As seen in Figure 5.13 and Figure 5.14 there was a significant difference in the interaction between pH and root production per depth, $P \leq 0.043$. At pH 3 it is clear that the highest root production was found in the lowest depth. The first 10 and 20 cm had a lower production and this shows that the acidity had an effect. The root tips developed in such a way to seek better conditions, therefore growing deeper and deeper. Looking at pH 5 and pH 7, it shows a similar trend where the least production was found in the middle depth, 11-20 cm. At pH 5 the highest production was in the last 30 cm (21-30cm) and then in pH 7 the first 10cm (0-10cm). The highest root production was also found in the first 10 cm at pH 9. This is very important to note, especially during the rehabilitation process of mined land. As mentioned earlier the subsoil in rehabilitated mined land is very shallow (as little as 20 cm). Most of the production occurred at 21-30cm and this can alter the production if the subsoil in mines is only 20 cm deep (Truter, 2015).

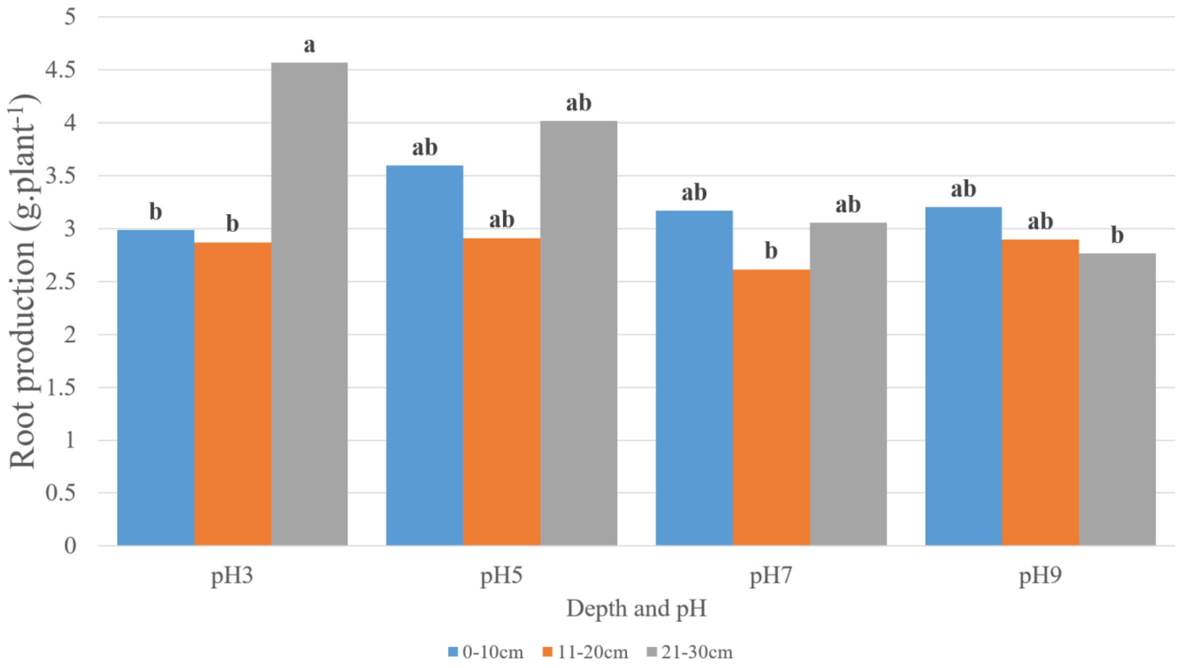

Figure 5.14: The interaction between substrate depth (0-10cm, 11-20cm and 21-30cm) and pH as it effects root production (g.plant^{-1}) of *C. gayana*

**Values with the same letter are not significantly different and values with different letters are significantly different*

Figure 5.15 below shows the effect the seed coating had on the root production at three different substrate depths. Plants from coated seed had the highest root production at 21-30 cm depth and the least at 11-20 cm. The coating around the seed provides enough support to enable the primary root to develop faster and to reach greater depths. In contrary, plants from uncoated seed had the highest root mass in the first 10 cm and the least at 11-20 cm. This confirms an earlier statement that seed coating produces a heathier seedling with more roots that can reach deeper depths. Plants from uncoated seed produced slightly lower amount of roots at 21-30 cm and produced more roots overall.

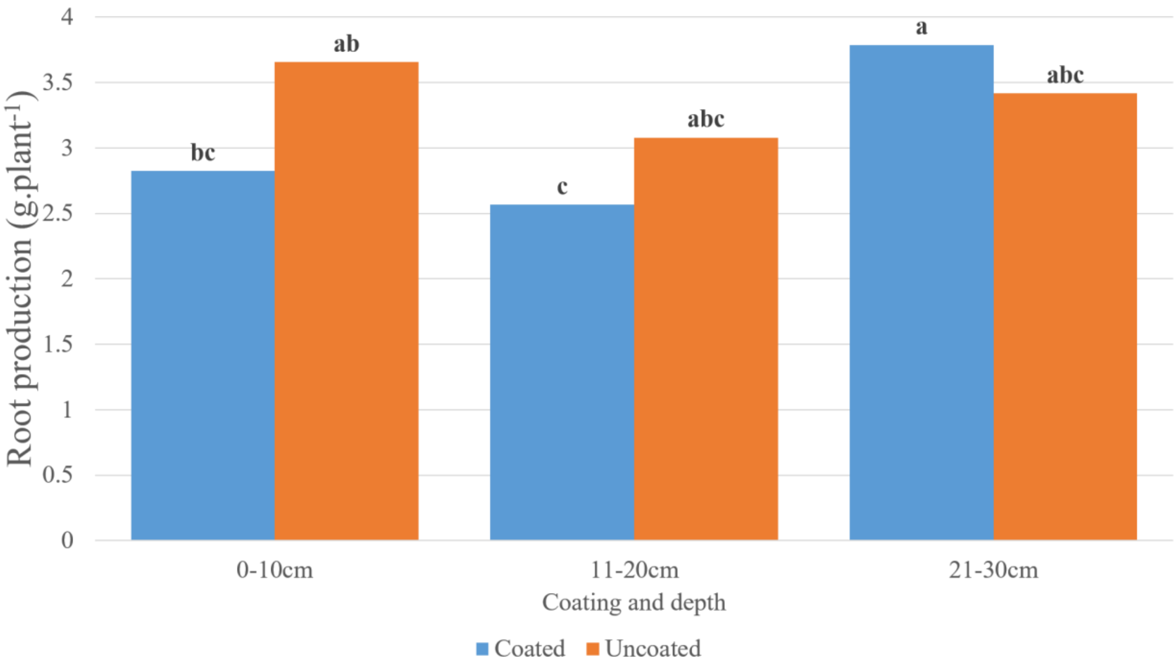

Figure 5.15: The interaction between seed coating, affecting root production (g.plant^{-1}) in *C. gayana* at 3 different depths (0-10cm, 11-20cm and 21-30cm)

**Values with the same letter are not significant and values with different letters are significantly different*

ii. *Eragrostis curvula*

Root production (g.plant^{-1})

The data analysis showed that root production in *E. curvula* were unaffected by the different levels of pH (Figure 5.16). The data analysis also showed there was no significant differences between the the different coatings and interaction between pH and seedcoating). *Eragrostis curvula* is known to be tolerant to acidic or alkaline soils (Dickinson, 2004), this was however not the case for the root production of this study.

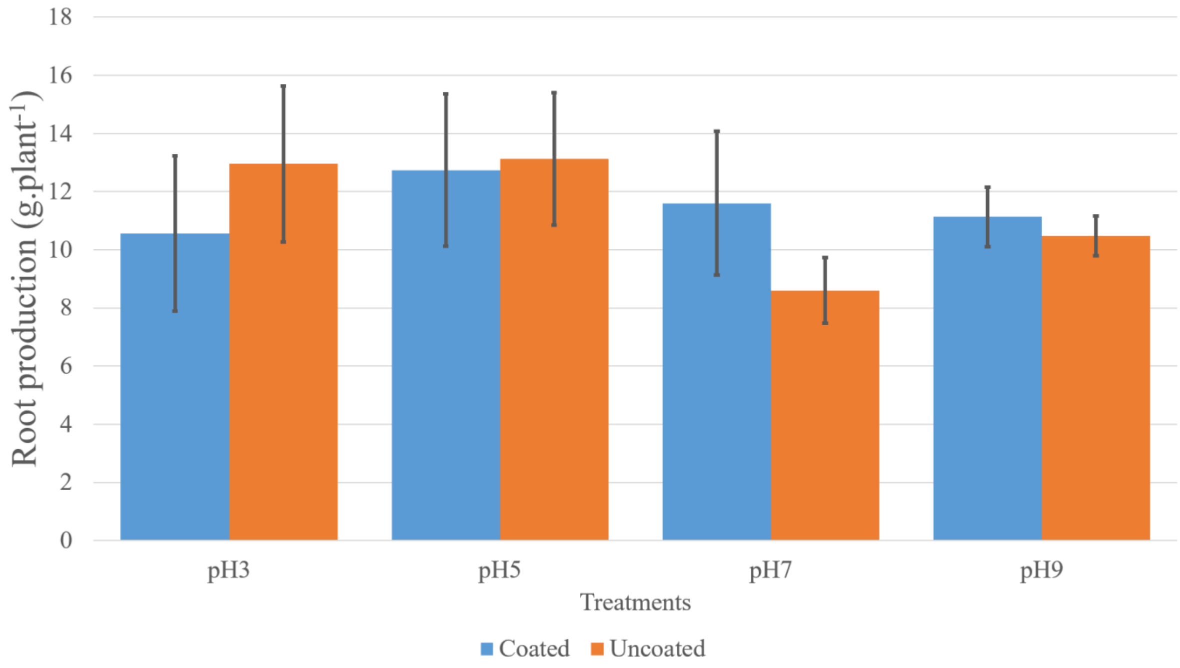

Figure 5.16: The mean root production (g.plant^{-1}) with standard deviation of *E. curvula* planted to coated and uncoated seed at four different pH (3, 5, and 7(control)) and 9) levels.

<u>Root diameter (mm)</u>

Figure 5.17 shows the mean root diameter in mm of *E. curvula*. The data analysis found that pH, coating and the pH*seed coating interaction had no significant difference in the root diameter of *E. curvula*. The P value of *E. curvula* was very low for pH and seed coating, (P = 0.063 and P = 0.068 respectively), but it was still higher than 0.05 and therefore not significant.

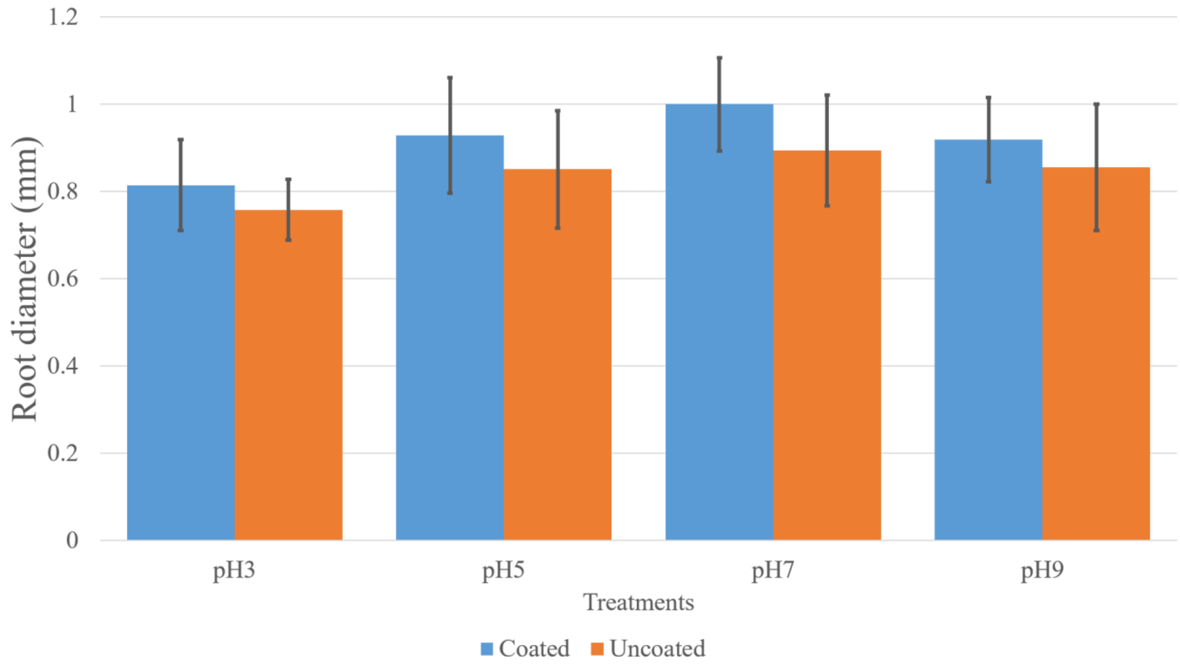

Figure 5.17: The mean root diameter (mm) with standard deviation of *E. curvula* planted to coated and uncoated seed at four different pH (3, 5, 7 (control)) and 9) levels.

If we look at Figure 5.17 it can be seen that there is a difference between coated and uncoated seed (trend significance). The roots from coated seed had a larger diameter and these thicker roots are hypothesized to give the plant a better chance of survival and better growth under these conditions. The data showed there is no significance but root diameters are so small and even millimetres is a big difference. Coated *E. curvula* plants had thicker roots at all four the different pH levels, but these are just observations made and the data analysis cannot be ignored.

<u>Root production (g.plant^{-1}) at various depths</u>

Figure 5.18 below shows the mean root mass of *E. curvula* at various root depths and the effect substrate pH had on the total root production. The data analysis showed that there is no significant difference ($P \geq 0.05$) between pH and seed coating, the interaction between pH and depth and the interaction between coating and depth. The only significant difference was between the different depths ($P \leq 0.032$) seen in Figure 5.19. Therefore seed coatings had no beneficial effect on the root growth of *E. curvula*.

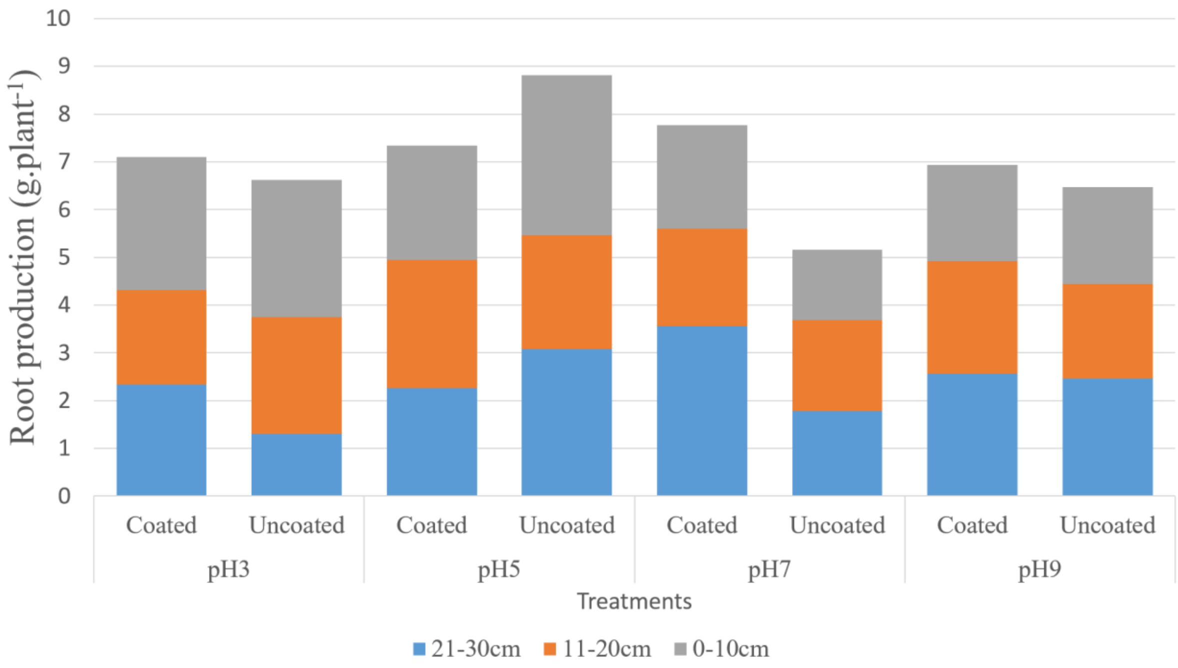

Figure 5.18: The mean root production (g.plant^{-1}) of *E. curvula* planted to coated and uncoated seed at three different depths (0-10cm, 11-20cm and 21-30cm), at four different pH (3, 5, 7 (control) and 9) levels.

**Statistics were not included due to no significance between all the interactions*

Figure 5.19 shows that the biggest root mass was found at 21-30 cm. This can be due to *E. curvula's* sensitivity to acidity and alkalinity (Dickinson, 2004).

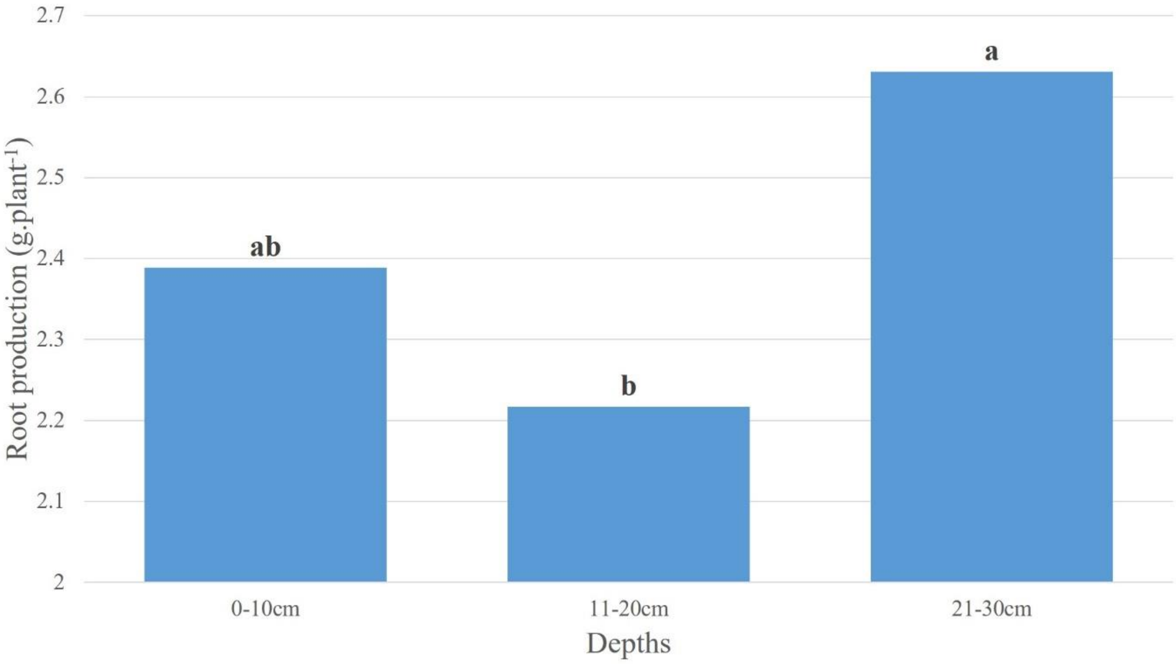

Figure 5.19: The root production (g.plant^{-1}) of *E. curvula* at three different depths (0-10cm, 11-20cm and 21-30cm)

**Values with the same letter are not significantly different and values with different letters are significantly different*

iii. *Medicago sativa*

Root production (g.plant^{-1})

There was a significant difference in root production for *M. sativa* between the different pH levels ($P \leq 0.001$). The seed coating and the pH and seed coating interaction had no significant difference for *M. sativa*. The difference in pH contributed 87.85% of the total variation in the observations made, as seen in Figure 5.20 below.

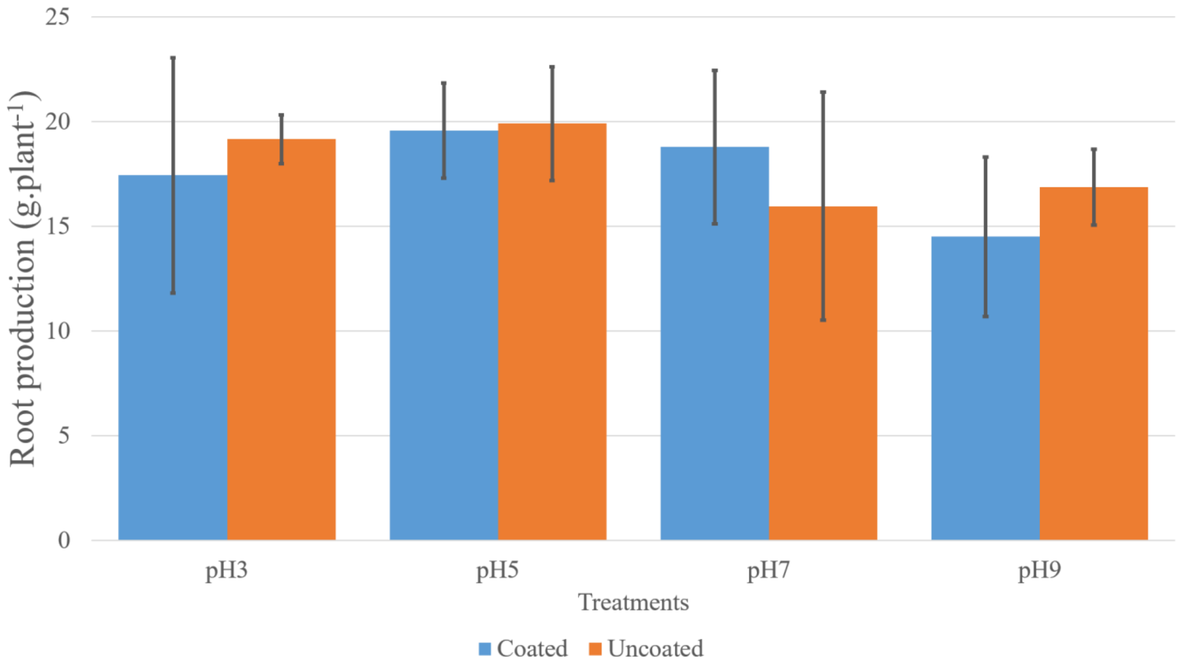

Figure 5.20: The mean root production (g.plant^{-1}) with standard deviation of *M. sativa* planted to coated and uncoated seed at four different pH (3, 5, 7(control) and 9) levels.

Figure 5.20 shows that root production of *M. sativa* is affected by a low pH as well as a high pH. There was a slight reduction in root production at pH 3 and a larger decline in production at pH 9. Study done on *Picea abies* (Norwegian spruce) found that acidic soils can reduce the meristematic activity in root tips and this can lead to a decreased penetration into the soil and a decrease in the fine root growth (Puhe, 2003). This in turn will result in a lower number of roots produced and also results in lower dry matter production. Therefore soils with a high acidity or alkalinity will cause damage to the the root tips and root meristems that will in turn cause lower root mass and production.

<u>Root diameter (mm)</u>

The seed coating, pH and the interaction between pH and seed coating did not have a significant effect on *M. sativa* growth. Figure 5.21 shows that there is no significant difference between coated and uncoated seed however root diameters are small and even millimetres is a significant observational difference. There was a significant trend at pH 7 and 9, where the coated seed had thicker roots compared to roots from uncoated seed. Therefore a more acidic soil can have a negative effect on the coating or the coating provides no benefit in acidic soils.

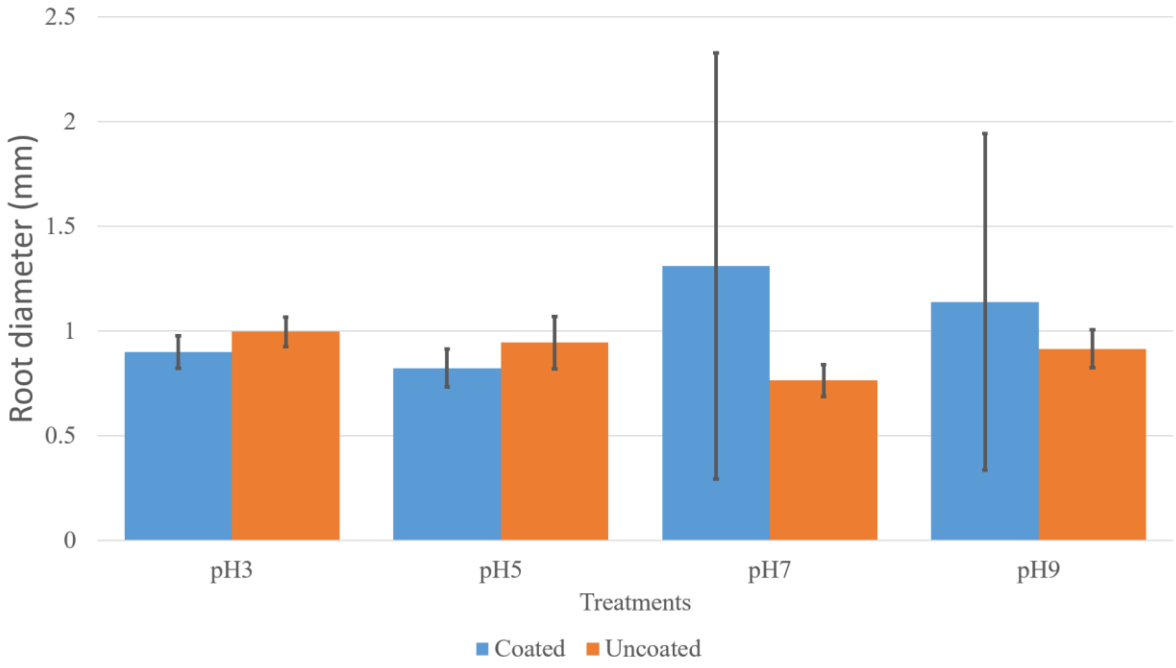

Figure 5.21: The mean root diameter (mm) with standard deviation of *M. sativa* planted to coated and uncoated seed at four different pH (3, 5, 7(control) and 9) levels.

Medicago sativa is very sensitive to acidic and alkaline soils and will have a lower production is these soils (Dickinson, 2004, Klos and Brummer, 2000). The roots are damaged at the meristems when the soils are too acidic and this can possibly the reason for the slightly thinner roots in acidic soils. Legumes under stress produce coarser and shorter roots to prevent any damage to the root system (Franco et al., 2011). Legumes that are under salinity and alkaline stress will try to conserve energy and therefore shorter and thicker roots will be produced, the number of nodules will also be reduced (Franco et al., 2011, Ashraf and Iram, 2005). When plants are under water stress the opposite is true, more energy will be available for root growth to enable roots to search for water, the roots will be thinner and longer (Franco et al., 2011).

Root production (g.plant^{-1}) at various depths

The data analysis showed that there is a significant difference between the different pH levels ($P \leq 0.001$), root production per depth ($P \leq 0.001$), the interaction between pH and depth ($P \leq 0.001$) and interaction between seed coating and depth ($P \leq 0.024$).

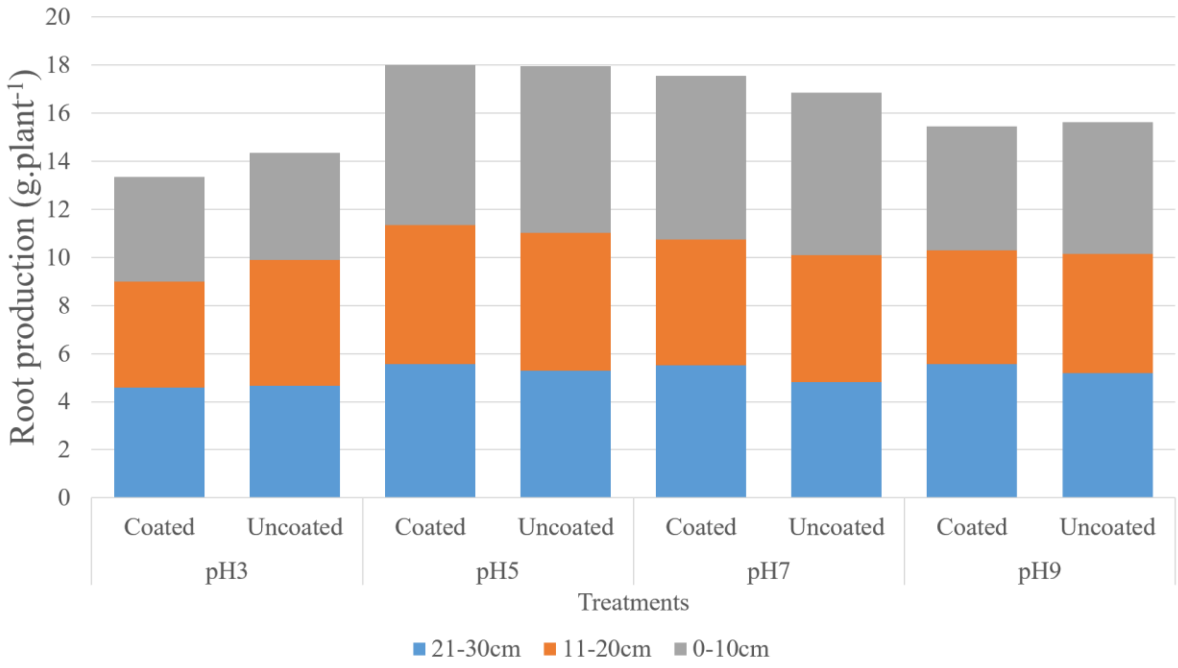

Figure 5.22: The mean root production (g.plant^{-1}) of *M. sativa* planted to coated and uncoated seed, at three different depths (0-10cm, 11-20cm and 21-30cm) at four different pH (3, 5, 7(control)) and 9) levels

**Statistics were not included due to no significance between all the interactions*

Figure 5.23 shows the root production of *M. sativa* at four different pH and three different depths. The difference in root production between the different pH levels are very significant, $P \leq 0.01$. The figure shows that the root production is poor at pH 3. The biggest majority of roots were concentrated in the first 0-10 cm (Figure 5.24). These findings are confirmed in literature that states when legumes are under stress the roots are much shorter and thicker (Franco et al., 2011, Hetrick, 1991). At the deeper depth of 11-20 cm there was a lower root production and the amount of root hairs decreased and slightly lower at 21-30cm.

This shows that when there is high acidity the fibrous roots are thinned out and other roots move downward in search of better conditions. There is a clear contradiction in the root production found in pH 3 and that of pH 5 and 7. The root production found at pH 9 is also low, but is more than that of pH 3, therefore this shows that the roots of *M. sativa* are less sensitive to higher pH compared to lower pH. In favourable conditions, for example at pH 5 and pH 7, the majority of roots are found in the first 0-10 cm. This is where the majority of fibrous roots were found and less were found at deeper levels. Roots that are under water stress will have thinner and longer roots as to enable them to search for water, plants that are under alkaline and pH stress will have thinner and shorter roots (Hetrick, 1991, Franco et al., 2011, Ashraf and Iram, 2005).

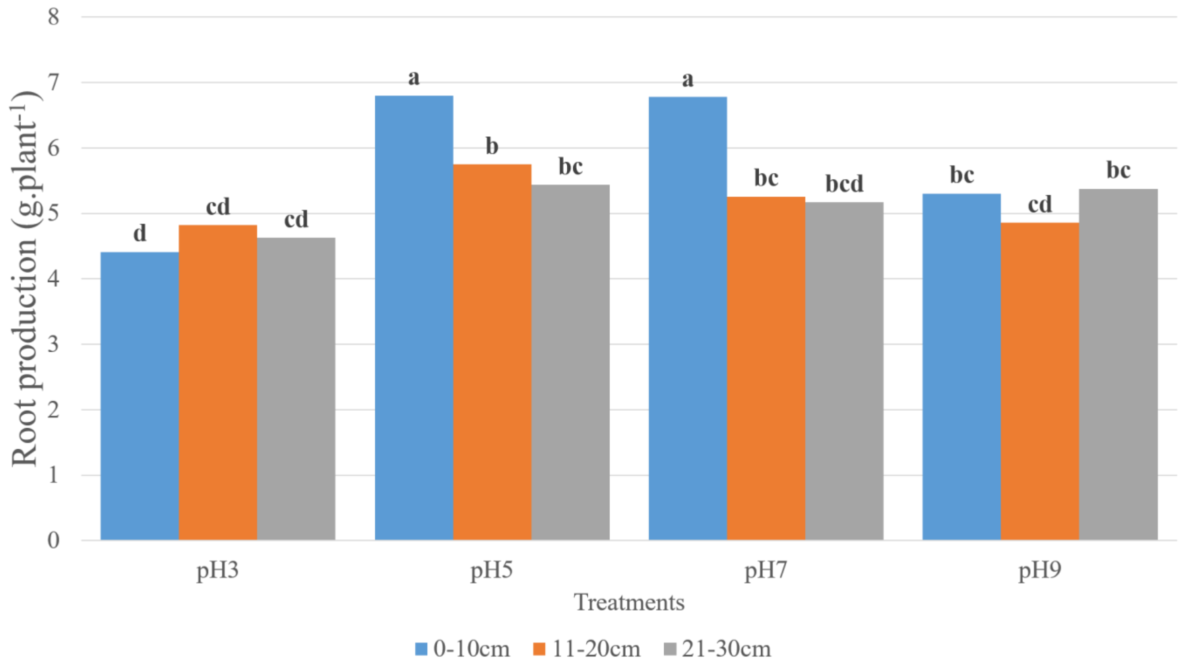

Figure 5.23: The interaction between mean roots mass (g) of *M. sativa* planted to coated and uncoated seed, at three different depths (0-10cm, 11-20cm and 21-30cm) at four different pH (3, 5, 7(control) and 9) levels.

**Values with the same letter are not significant and values with different letters are significantly different*

There is a significant difference in the interaction between coating and the different depths, $P \leq 0.024$. Uncoated seed shows a clear trend, there is a higher root mass in the first 0-10 cm and then a gradual decrease at the 21-30 cm depth. Uncoated seed also had a higher production at 11-20 cm depth. Coated seed does not show a clear trend. There is a reduction in production from the first 0-10 cm to the following 11-20 cm and then an increase again at 21-30 cm. The top 0-10 cm is where most of the fibrous roots are found and there will be less deep down. The increase in roots at 21-30 cm is due to the roots that became root bound in the pots which were not deep enough, however treatment differences were noted irrespective of the conditions created but shallow pots which is evident for all treatments.

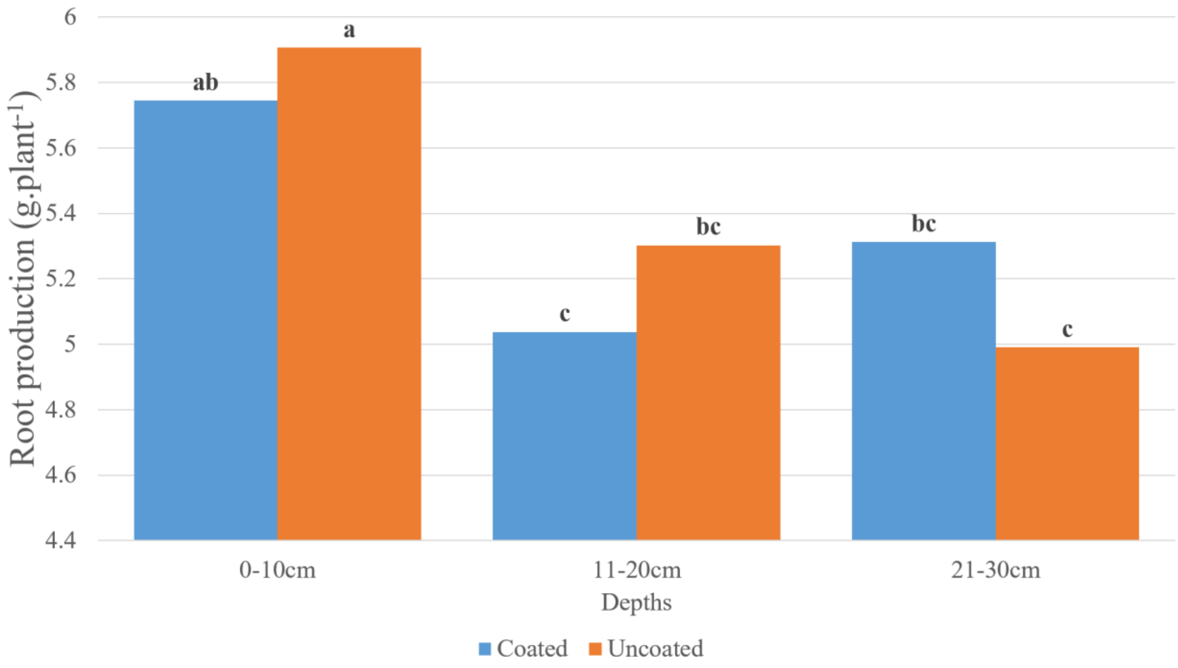

Figure 5.24: The interaction between coating treatment of *M. sativa* and 3 different depths (0-10cm, 11-20cm and21-30cm)

**Values with the same letter are not significant and values with different letters are significantly different*

5.5 CONCLUSION

This study showed that coated seed play a less of important role in mature plants as was hypothesized. It was assumed that plants that emerged from coated seed will grow much better due to germinating in a better environment by producing a stronger primary root. Coated *C. gayana* plants showed that the majority of the roots were produced at deeper depths, 21-30 cm, and plants from uncoated seed had the majority of seed in the top 0-10 cm. Using coated seed had no influence on leaf area, dry matter production, root diameter and root production. The dry matter production and root production was the lowest at pH 3 and pH 9 but the most at pH 5 and pH 7.

Eragrostis curvula showed similar results to that of *C. gayana*. Coated *E. curvula* seed did not result in a higher dry matter production, higher root production or a larger root diameter. There was however a larger leaf area produced for coated seed at pH 7 and pH 9 and larger leaf area for uncoated seed at pH 3 and pH 5. The roots produced from coated seed also showed no significance, the majority of roots were found at 21-30cm for both coated and uncoated seed.

Coated *M. sativa* only showed differences in the depth roots reached, roots from coated seed had a higher production at 21-30 cm and roots from uncoated seed at 0-10 cm. There was no benefit in using coated seed again out of a production point of view, and coated seed did not enable *M. sativa* plants to produce a higher dry matter or to produce larger leaves. It is still important to note that coated seed has other benefits especially if we look at *C. gayana*. The seed is very 'fluffy' and is difficult to handle and to plant it. The wind also moves these seeds easily. The coating of seed adds weight to the seed and therefore eases the planting and handling process. Apart from an improved handling perspective it also contains pesticides that prevent insects from eating the seed. It is therefore important to not only look at a production point of view when using coated seed.

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CHAPTER 6

General conclusions and recommendations

The effects of seed coating on the emergence of various pasture species in different growth substrates

According to literature seed coating can improve the germination process by providing favourable conditions for the seed to germinate in. In the study where different pH levels were used it can be concluded that coated *Cenchrus ciliaris, Cynodon dactylon* and *Eragrostis curvula* seed had a significant higher emergence compared to uncoated seed. Uncoated *Chloris gayana* and *Digitaria eriantha* seed had a significant higher emergence percentage compared to coated seed. There was no significant difference between coated and uncoated *E. curvula* seed.

The study also showed that pH did not have an effect on the emergence of *D. eriantha, E. curvula* and *M. sativa,* therefore there was no difference in emergence percentage between the substrate with a pH of 3, 5, 7 and 9. Contradictory to literature, which states that germination and emergence should decrease as acidity increases, the emergence of *C. ciliaris* and *C. dactylon* increased as the salinity increased and decreased as the pH became more neutral. *Chloris gayana* had a higher emergence percentage at pH 7 and was more sensitive to acid and alkaline soils. Water played an important role with the highest emergence percentage for *E. curvula*, *M. sativa*, *C. ciliaris* and *C. gayana* was at field capacity, the emergence decreased as the water level increased or decreased. The emergence percentage for *D. eriantha* and *C. dactylon* increased as the water level increased above field capacity.

Higher salinity levels caused the emergence percentage to drop in all the different species. Na_2SO_4 had the biggest effect on emergence percentage due to the high EC values, above 10 dms^{-1} (normal range of salinity in the field is between 4 and 10 dms^{-1} (EC)). *Cenchrus ciliaris, C. dactylon* and *E. curvula* were very sensitive to high salinity concentrations. The different water treatments did not have an effect on most of the species, except for *C. ciliaris and C. dactylon* that had higher emergence percentage at higher water levels.

Coated *C. gayana, C. dactylon* and *M. sativa* seed had a higher emergence percentage compared to uncoated seed, therefore coated seed of these species are more adapted in soils with higher salinity levels. Uncoated *C. ciliaris, E, curvula* and *D. eriantha* seed had a higher emergence percentage in soils with higher salinity levels.

The effects of seed coating on the emergence of various grass species in different mined substrates

It was concluded that coated seed had a higher emergence for *C. ciliaris* in Gypsum-, Andalusite- and coal discard substrates, *C. gayana* in Fluorspar- and Andalusite substrate. This higher emergence was due to the fine texture of these substrates. The fine texture ensure that the coating breaks down properly and the nutrients in the coating remains near the seed. The fine texture also provides favourable conditions for growth. Coated seed had a definite advantage for *E. curvula* with higher emergence percentage in Gold < 2% pyrite-, Platinum-, Fluorspar-, Andalusite- and Coal discard substrates, compared to uncoated seed. Uncoated seed for all the different species had a higher emergence percentage in Kimberlite due to Kimberlites very coarse texture. The texture prevented a good seed and substrate contact. The study also found that for *M. sativa,* coated seed only had a higher emergence percentage in Coal discard substrate, but soon after emergence the plants died. The low pH and high Al levels prevented anything from growing in the coal discard substrate.

It was also found that uncoated seed had a higher emergence for *C. ciliaris* in Red sandy loam- Gold < 2% pyrite and in Gold > 2% pyrite substrate, and for *C. dactylon* in Andalusite- and Coal discard substrate and for *D. eriantha* in Andalusite- and Coal discard substrate. There were no differences in emergence percentages between coated and uncoated seed for *C. ciliaris* in Gypsum-, Platinum- and Fluorspar substrate and for *C. dactylon* in any of the substrates, except Kimberlite- and Andalusite substrate. There was also no differences between coated and uncoated seed for *C. gayana* in Gypsum, Red sandy loam-, Gold < 2% pyrite-, Gold > 2% pyrite- and Platinum tailings substrates. In *D. eriantha* and *M. sativa* there was no difference in emergence percentages between coated and uncoated seed in Gypsum-, Gold < 2% pyrite-, Gold > 2% pyrite-, Platinum tailings- and Fluorspar substrate. For *Eragrostis curvula*, there was no difference between coated and uncoated seed in Gypsum- and Gold > 2% pyrite substrate.

The water content also played an important role. In most of the substrates *C. ciliaris* had a higher emergence percentage as the water level increased, except in Gold > 2% pyrite-, Kimberlite- and Fluorspar substrate. *Cynodon dactylon* and *D. eriantha* showed that there was little difference in emergence between different water treatments, only in Red sandy loam-, Gypsum-, Gold < 2% pyrite- and Gold > 2% pyrite substrate had a higher emergence when the water levels increased. In *M. sativa* the emergence increased as the water level increased in Red sandy loam-, Gold < 2% pyrite and Gypsum substrates. The emergence decreased as the level of water increased in Platinum tailings-, Fluorspar-, Kimberlite- and Gold > 2% pyrite substrates.

The study also found as with previous studies that high levels of Al in combination with low pH, had a severe negative effect on germination and emergence. The low pH causes the impact of Al to be more severe. High sodium levels also had a negative impact on growth, by causing imbalances of other minerals in the seed and internally lowered the emergence percentage. The texture also had an impact on the emergence, for example Kimberlite had a coarse texture and there was a lower emergence percentage, while, in Red sandy loam with a fine texture there was a higher emergence due to seed having more contact with the substrate.

The effects of seed coating on the development of* Chloris gayana, Eragrostis curvula *and* Medicago sativa *in response to different salinity growth conditions

It was concluded that the effects of coated seed were very small and in most cases there was no effect at all. The only considerable effect for *Chloris gayana* was for the root production at various depths. It was found that plants from coated seed produced more roots at deeper depths, the lowest production was at 0-10cm and the highest production was at 21-30cm. Roots that emerged from coated *C. gayana* seed had a slightly thicker root, the data showed it was not significant but the figures showed there was a trend significance. There was no significant differences when using coated *C. gayana* and *M. sativa* seed in root production, root diameter, leaf area and dry matter production. Coated *Eragrostis curvula* seed resulted in a higher leaf area compared to uncoated seed. The root production and root diameter was also slightly higher compared to uncoated seed. The overall production of *E. curvula* also decreased as the salinity concentrations increased.

There was a reduction in root production, leaf area and dry matter production for *C. gayana* when the salinity concentration increased. *Chloris gayana* is known to be salt tolerant but the production still decreased and coated seed did not contribute significantly. *Medicago sativa* is very sensitive to high salinities and this was again proven in this study. There was a clear reduction in root production, leaf area and dry matter production when the salinity concentrations of the treatments were increased. *Medicago sativa* should not be considered in areas with high salinity levels, the production will definitely be impacted.

The effects of seed coating on the development of* Chloris gayana, Eragrostis curvula *and* Medicago sativa *in response to different pH growth conditions

It was expected that the pH and coating will have an influence on the growth of pastures, however, the study concluded that pH did not have a big effect on growth and that coated seed also did not improve emergence conditions. Coated *C. gayana* plants showed that the majority of the roots were produced at deeper depths, 21-30cm, and plants from uncoated seed had the majority of seed in the top 0-10cm. Using coated seed showed no impact in leaf area, dry matter production, root diameter and root production. The dry matter production and root production was the lowest at pH 3 and pH 9 but the most at pH 5 and pH 7.

Eragrostis curvula showed a similar trend to that of *C. gayana*. There was no significant advantage in this study to use coated *E. curvula* seed. Coated seed did not result in a higher dry matter production, higher root production or a larger root diameter. There was however a larger leaf area produced when coated seed was used at pH 7 and pH 9 and larger for uncoated seed at pH 3 and pH 5. The roots produced from coated seed also showed no significance, the majority of roots were found at 21-30cm for both coated and uncoated seed. Coated *M. sativa* only showed differences in the depth roots reached, roots from coated seed had a higher production at 21-30cm and roots from uncoated seed at 0-10cm. There was no benefit in using coated seed again out of a production point of view, and coated seed did not enable *M. sativa* plants to produce a higher dry matter or to produce larger leaves in this short term phytotron study.

Recommendations

Most recommendations made are based on research conducted in a controlled environment and can differ in field conditions, however, it is important to note that all conditions were controlled to identify the true effect on different elements on germination, emergence and growth. The use of coated seeds are very complex and to make recommendations on the use of coated seed is not a simple yes or no answer. It is important to remember that coated seed does not improve the germination of the seed, it only improves the survival rate of seedlings that have emerged from the seed. It is also important to know the environment that the seed is going to be planted in. Coated seed can have a benefit in one substrate but the same coated seed can have no benefit in a different substrate. Coated seed only breaks down where there is enough moisture, coated seed will not germinate in very dry environments. It is beneficial to use coated seed for *C. ciliaris, C. dactylon* and *E, curvula*. There was little benefit to use coated seed for *C. gayana* and *D. eriantha*. The emergence for coated *D. eriantha* is lower compared to uncoated seed. There is no difference between coated and uncoated *Eragrostis curvula* seed. It is not recommended to use *M. sativa*, *C. ciliaris* and *E. curvula* in any environments with high salinity concentrations due to their sensitivity. These recommendations are for seed that were planted in an inert substrate and will change if they are planted in different substrates or ameliorated substrates. One needs to fully understand the processes in the substrates before recommendations can be made on which seed to use, i.e; coated or uncoated. Coated seed is just one part of improving the germination rate of pasture, but this study has made it clear that there are various other aspects that needs to be taken into consideration. There is no right or wrong answer to if coated seed improves the germination rate or emergence conditions of seed. Each case must be looked at individually and be treated differently, therefore findings should not be generalized, due to coated seed being area and environmentally specific.

From the first studies it was found that the highest overall germination and emergence was found in *E. curvula*, *M. sativa* and *C. Gayana*, it was therefore decided to use these three species in the next studies as they showed great potential. The pot trials proved to be more challenging than was expected, the inert silica substrate that was used had a too coarse texture causing the moisture to be evaporated quickly. It is therefore recommended if this trial was to be repeated a finer substrate should be used.

The studies however showed that coating is of little benefit when looking at mature plants (dry matter production and root production) in pot studies, but it is important to remember that coated seed has a physical benefit, not only chemically. Coated *C. ciliaris, C. gayana* and *D. eriantha* may have a lower emergence percentage compared to uncoated seed, but due to the ‘fluffiness’ of the seed it is very difficult to plant the seed, therefore it is recommended to use coated seed because it improves the process of planting. By coating the seed, weight is being added to the seed and therefore wind and insects don’t carry them away. The physical and chemical aspects of coated seed should always be kept in mind, not only the production aspect. Therefore there is a definite place for coated seed and the use of coated seed in any environment.

As already mentioned this was a small scale trial and was done in a greenhouse where all the environmental conditions were kept constant. It is recommended that this trial be up scaled and done under natural environment. The species that is recommended for the larger scale would be *C. gayana, E. curvula* and *M. sativa*, as these species had the greatest germination and emergence in the germination trials in the growth chambers. These three species also had high dry mater production in the greenhouse but it is important to determine the production under natural conditions. There arose more questions out of these trials and therefore shows that these trials needs to be up scaled.

Annexure A

Table A.1: The chemical composition of the different mined substrates used a growing substrates (results were obtained by laboratory testing)

		Different substrates								
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**
pH (KCl)		4.1	4.1	5.3	6.4	8	7.6	7.9	5.5	3.8
pH (H20)		4.1	3.9	5.4	6.1	6.6	9.7	5.7	6	2.5
EC	Sm^{-1}	0.013	0.199	0.193	0.422	0.205	0.121	0.146	0.011	0.390
SO4	mg kg^{-1}	12	159824	1674	447	143	32	122	20	709
P (Bray 1)	mg kg^{-1}	4	252	1	0	0	5	1	3	1
K	cmol kg^{-1}	0.16	0.051	0.076	0.115	0.076	1.846	0.061	0.222	0.064
	mg kg^{-1}	62	20	30	45	31	720	24	87	25
Ca	cmol kg^{-1}	0.441	27.976	8.965	10.213	1.964	9.753	10.144	1.956	11.186
	mg kg^{-1}	88	5595	1793	2043	393	1951	2023	392	2237
Mg	cmol kg^{-1}	0.406	0.142	0.776	2.158	0.407	1.934	1.325	3.352	1.747
	mg kg^{-1}	49	17	94	261	49	234	160	406	211
Na	cmol kg^{-1}	0.004	0.05	0.039	0.51	3.746	0.095	0.199	0.086	0.012
	mg kg^{-1}	1	12	9	117	68	862	46	20	3
Al	cmol kg^{-1}	0.31	0.62	0.04	0	0	0	0	0	21.28
Al	%	22.5	275.5	3.2	0.1	0	0	0	0	2718.3

Key: S1: Red Sandy Loam; S2: Gypsum; S3: Gold < 2% Pyrite; S4: Gold > 2% Pyrite; S5: Platinum Tailings; S6: Kimberlite; S7: Fluorspar; S8: Andalusite; S9: Coal discard

Annexure B

Table B.1: The physical analysis of mined substrates (results were obtained by doing laboratory tests)

	Very coarse sand %	Coarse sand %	Medium sand %	Fine sand %	Very fine sand %	Silt %	Clay %
Red Sandy loam	0.5	4.1	25.3	38.2	24.5	2.9	4.3
Gypsum	0	0.3	0.5	0.9	76.6	14.7	6.9
Gold < 2% Pyrite	0.2	0.6	2.9	33.9	33.5	24.2	4.6
Gold > 2% Pyrite	0.1	0.6	6.5	45.7	33.2	11.8	2.1
Platinum Tailings	0	0.3	8.7	48	33.4	7.4	2.1
Kimberlite	34.6	33.2	13.7	7.7	3.6	2.9	4.3
Fluorspar	0.1	2.3	16	36	31.3	12.2	2.2
Andalusite	2.2	4	3.4	2.4	4.1	68.6	15.2
Coal Tailings	1.1	6	13.1	21.2	18	20.9	19.7

Annexure C

Table C.1: The acid digestible metals (mg.kg^{-1}) of the different mined substrates (rsults were obtained by doing laboratory testing)

	S1 mg.kg^{-1}	S2 mg.kg^{-1}	S3 mg.kg^{-1}	S4 mg.kg^{-1}	S5 mg.kg^{-1}	S6 mg.kg^{-1}	S7 mg.kg^{-1}	S8 mg.kg^{-1}	S9 mg.kg^{-1}
Be	0.17	0.00	0.15	0.13	0.05	0.17	0.07	0.74	1.02
B	0.13	0.04	0.06	0.07	0.08	0.07	0.03	0.13	0.10
Al	5268	328	4178	4651	25190	13890	1348	18550	2928
P	339	1787	410	423	349	729	287	555	484
Ti	60.8	16	37.6	64.8	94	1551	19.4	462.6	216.8
V	13.3	1.2	5.6	5.9	8.3	16.4	2.4	31.6	12.6
Cr	70.4	7.9	25.5	33.8	668.3	303	11.3	39.7	21.1
Mn	128	2.5	191.8	439.9	153.4	312.4	6538	119.7	52.6
Fe	8457	1334	7182	15130	17680	23170	17070	26900	8368
Co	3.86	0.82	12.52	28.08	61.87	29.30	1.82	9.94	3.72
Ni	20.9	3.4	32.9	45.9	269.4	471.3	8.7	21.7	9.1
Cu	5.4	2.8	23.9	30.0	133.3	24.1	2.4	24.4	12.5
Zn	4.4	2.5	39.5	54.0	16.2	25.5	14.9	24.6	35.8
As	1.1	6.4	33.0	59.8	0.5	0.8	8.2	20.8	2.2
Se	0.37	4.10	0.52	1.03	0.49	0.23	0.78	0.98	1.41
Sr	0.92	1472	6.44	7.67	54.40	115.6	9.52	26.39	108.2
Mo	0.19	0.16	0.78	1.05	0.37	0.25	0.48	0.98	0.80
Pd	0.19	7.69	0.22	0.29	0.32	0.53	0.24	0.70	0.85
Ag	0.06	0.07	0.13	0.13	0.10	0.10	0.12	0.13	0.18
Cd	0.00	0.00	0.09	0.20	0.01	0.07	0.11	0.01	0.11
Sb	0.22	0.44	0.33	0.46	0.20	0.18	0.34	0.28	0.23
Ba	13	72	15	25	18	251	18	59	166
Pt	0.14	0.12	0.12	0.12	0.21	0.12	0.12	0.12	0.13
Au	0.21	0.33	0.26	0.30	0.20	0.19	0.18	0.23	0.18
Hg	0.00	0.11	0.02	0.03	0.00	0.00	0.00	0.00	0.02
Pb	2.0	6.3	14.7	26.0	26.8	4.1	12.6	23.7	17.0

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U	0.33	0.60	10.69	44.57	0.19	0.53	0.23	3.06	1.43

Key: S1: Red Sandy Loam; S2: Gypsum; S3: Gold < 2% Pyrite; S4: Gold > 2% Pyrite; S5: Platinum Tailings; S6: Kimberlite; S7: Fluorspar; S8: Andalusite; S9: Coal discard