

Nitrogen fixation of legumes in different growth mediums

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Abstract

South Africa has an array of mining commodities which all play an integral role in our everyday surroundings, income, and most importantly, in the economy of the country. These mining activities also produce vast amounts of discard material, better known as tailings material, which is stored in different ways after extraction has taken place. Usually, storage entails the construction of tailings storage facilities, normal discard or tailings dumps. The upper surfaces of these anthropogenic structures are usually unstable and are, in most cases, characterised by different forms of erosion. This can be due to the chemical and physical properties of the materials of which they are constructed, but mainly due to unstable construction geomorphology, steep slopes, which leads to poor water run-off management and subsequent instability. Therefore, these structures need to be actively managed in order to increase and maintain their stability. Grass establishment, as a stabilisation technique, is the most effective out of all of the techniques, but there are certain constraints regarding this method (Titshall *et al.* 2013). The most costly constraint is nutrient supplementation during aftercare phases. In order to minimize this cost, new and innovative technologies need to be explored, and trialled.

The contribution of soil biological processes in this regard was assessed, in order to minimise anthropogenic inputs. These biological processes refer to the fixation of atmospheric nitrogen by nodular root bacteria that grow on a group of plants referred to as legumes. These bacteria, also known as rhizobia, live in a symbiotic relationship with the host plant where they receive energy in the form of nutrients by trading nitrogen, which is an essential plant nutrient.

Nine different tailings materials from different commodities available from South African Mines were selected. For a control medium, a well-drained soil type with an apedel structure and a clay content of approximately 6% was selected in order to promote optimal natural growth. These materials were chemically and physically analysed in order to develop a more holistic understanding on a micro scale level, as well as to ascertain possible constraints in this regard.

Pot trials were selected as the experimental method in order to apply more specific control over root growth, plant development and growing conditions. The experimental data were collected over one growing season for both live forms. For this study, seven legume species were selected for establishment in the tailings materials in order to investigate their establishment potential in the growth mediums and their ability to fixate nitrogen.

Based on the data, specific species were identified as viable options to include in future tailings amelioration projects; it can be assumed that the nitrogen produced by these species will be available in the growth medium for uptake by neighbouring plants that lack this biological function. These plants will also play a vital role in the long-term sustainable development of vegetation in the anthropogenic growth mediums. *Sericea lespedeza* had the highest enrichment ability during this study.

Key words

mine tailings material, legumes, nitrogen fixation, establishment potential

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Glossary

Tailings Storage Facility: civil structures containing mine residue storage facilities for potential harmful waste products such as waste rock, cyanide-treated sand and slime, surplus mine water and discarded solutions, known as tailings material or mill tailings.

Tailings Material: ore milling overburden such as waste rock, cyanide-treated sand and slime, surplus mine water and discarded solutions, produced through the process of extracting valuable minerals.

Rehabilitation: repairing damaged ecosystems to the most functional state as governed by the biogeochemical potential of the landscape matrix. Not necessarily to pre-existing conditions, but can in some cases yield self-sustaining ecosystems, perhaps with occasional input (Jackson *et al.*, 2006).

Soil Texture: the relative proportions of sand, silt, or clay in the soil, thus determined by the size and type of particles that make up the soil (including the organic, but mostly referring to the inorganic material).

Soil Structure: the arrangement of soil particles into groupings. Soil structure has a major influence on water and air movement, biological activity, root growth and seedling emergence.

Legumes: a plant in the family Fabaceae (or Leguminosae), or the fruit or seed of such a plant. Legumes are notable in that most of them have symbiotic nitrogen-fixing bacteria in structures called root nodules.

Nitrogen Fixation: a process by which nitrogen (N_2) in the atmosphere is converted into ammonia (NH_3). Atmospheric nitrogen or molecular nitrogen (N_2) is relatively inert: it does not easily react with other chemicals to form new compounds. The fixation process frees up the nitrogen atoms from their diatomic form (N_2) to be used in other ways.

Rhizobium: soil bacteria that fix nitrogen after becoming established inside root nodules of legumes (Fabaceae). Rhizobia require a plant host; they cannot independently fix nitrogen.

Root Nodule: occur on the roots of plants (primarily Fabaceae) that associate with symbiotic nitrogen-fixing bacteria. Under nitrogen-limiting conditions, capable plants form a symbiotic relationship with a host-specific strain of bacteria known as rhizobia.

Plant Host: an organism that harbours a parasite, or a mutual or commensal symbiont, typically providing nourishment and shelter. In Botany, a host plant is one that supplies food resources and substrate for certain insects or other fauna.

Electrical Conductivity (EC): the ability of a material to transmit an electrical current. Usually refers to the potassium levels in the soil expressed as the salt content of the soil.

pH: the pH scale measures how acidic or basic a substance is. The pH scale ranges from 0 to 14. A pH of 7 is neutral. A pH less than 7 is acidic. A pH greater than 7 is basic.

Exchangeable Sodium Percentage (ESP): the amount of sodium (Na) held in exchangeable form and expressed as a percentage of the cation exchange capacity. These results are used to estimate the structural stability of the soil as Na^+ ions are likely to cause dispersion of soil particles.

Cation Exchange Capacity (CEC): the quantitative measure of the soil's ability to adsorb cations.

Abbreviations

Agrilasa: The Agri-Laboratory Association of Southern Africa

Al: Aluminium

CEC: Cation exchange capacity

EC: Electrical conductivity

ESP: Exchangeable sodium percentage

N₂: Nitrogen

NO₃: Nitrate

NH₄: Ammonium

P: Phosphate

pH (H₂O): pH (water)

pH (KCl): pH (potassium chloride)

PCA: Principal Component Analysis

TSF: Tailings storage facility

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Chapter 1: General Introduction

1.1. Problem statement and substantiation

Nitrogen fixation has been thoroughly studied and is a well-understood soil biological process. Therefore, numerous studies in this regard have been done in the past. As depicted in literature, the majority of these studies only focused on common commercial available plant species (usually one per study) and therefore the potential of “wild” legume species was still to be investigated. These research projects also focussed on naturally-occurring soils and not on anthropogenic growth mediums such as mine tailings material. Therefore, the simultaneous evaluation of seven different legume species in nine different tailings substrates and one control soil (under the same environmental conditions) has not been done in the past for tailings materials.

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1.2. Research aims and objectives

1.2.1. Aims

The central focus of this study is to evaluate the contribution of different legume species to plant / soil-available nitrogen within the different growth mediums. Apart from the chemical analysis, the study further aims to identify possible new legume species that can be utilized for the rehabilitation of tailings storage facilities (TSFs).

With regard to the above, this study (in due course) aims to improve the long term sustainability of land rehabilitation projects on TSFs.

1.2.2. Objectives

The specific objectives to achieve the abovementioned research aims include:

- Investigate the establishment potential of seven different legume species in nine different tailings materials and a control growth medium (virgin soil).

- Investigate the nitrogen-fixing ability of the established species in the different tailings materials.
- Determine whether the geochemical and physical constraints associated with tailings material influence the nitrogen-fixing ability of the legumes.
- Conduct a visual inspection to investigate the nodule formation on the roots.

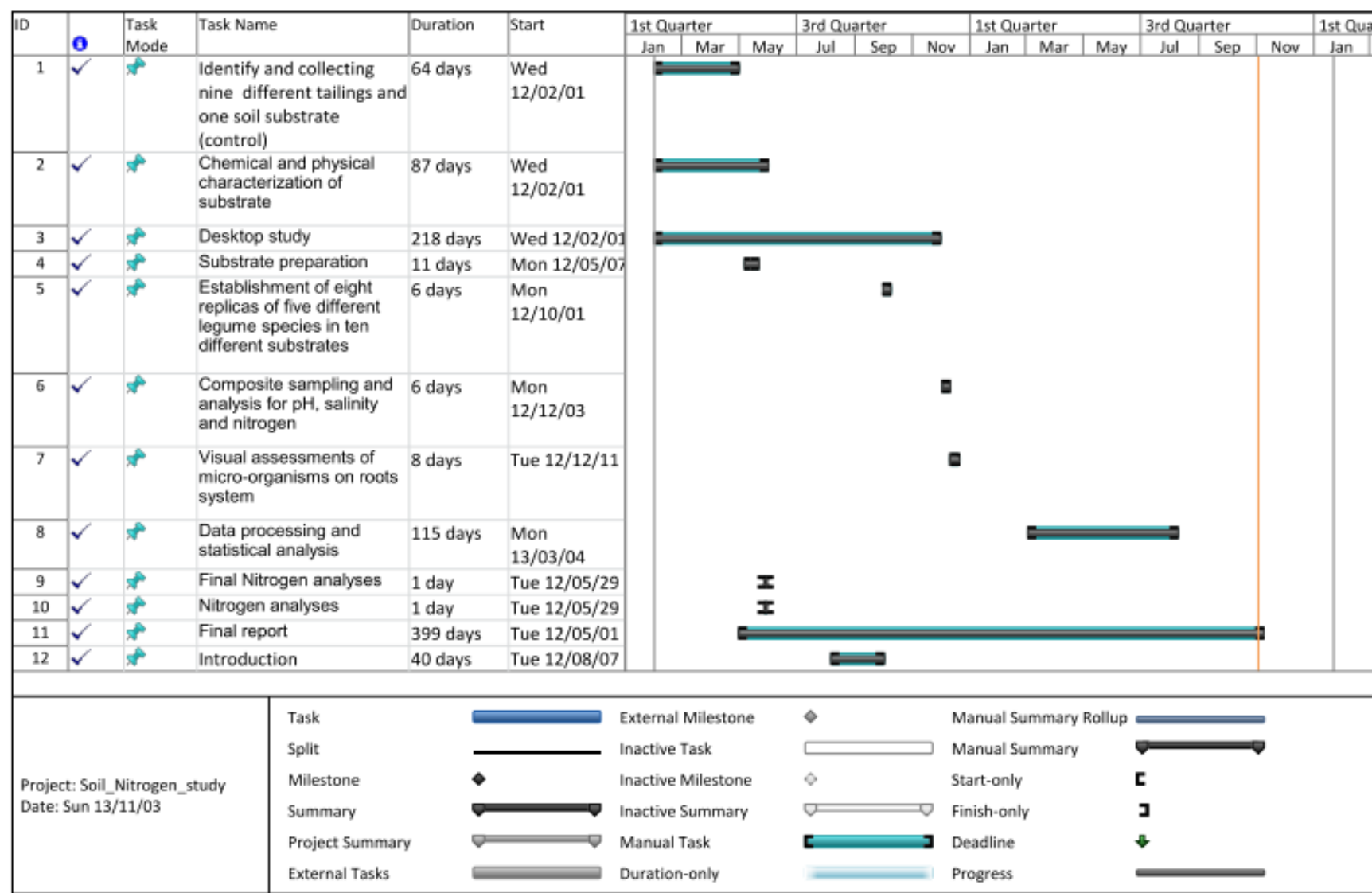
1.3. Project management

A key factor to the success of this study was by means of proper and well-organised project management as well as coordinated task management. The progress of the study was done by means of a monthly progress report provided in Table 1.1.

Table 1.1: THRIP project management report for the study period

Milestone / Task	Description	% Completed	Status
1. Identify and collect nine different tailings and one soil substrate (control)	Collection of soil and tailings material	100	Completed
2. Chemical and physical characterization of substrate	10 samples collected: 9 tailings & 1 Control	100	Completed
3. Desktop study	Gathering of literature and literature study	100	Completed
4. Substrate preparation	Amelioration of substrates with fertilizer and compost	100	Completed
5. Establishment of eight replicas of five different legume species in ten different substrates	Seeding and transplanting (five forbs) (400 individual plant specimens)	100	Completed
6. Composite sampling and analysis for pH, salinity and nitrogen	Three samples monthly	100	Completed
7. Visual assessments of micro-organisms on root system	Three times a month	100	Completed
8. Data processing and statistical analysis		100	Completed
9. Nitrogen analyses		100	Completed
10. Final nitrogen analyses		100	Completed
11. Final report		100	Completed

Before the project commenced, a project Gantt chart was created in order to assist with the planning of all of the actions that needed attention. The Gantt chart that was created is presented in Figure 1.1.



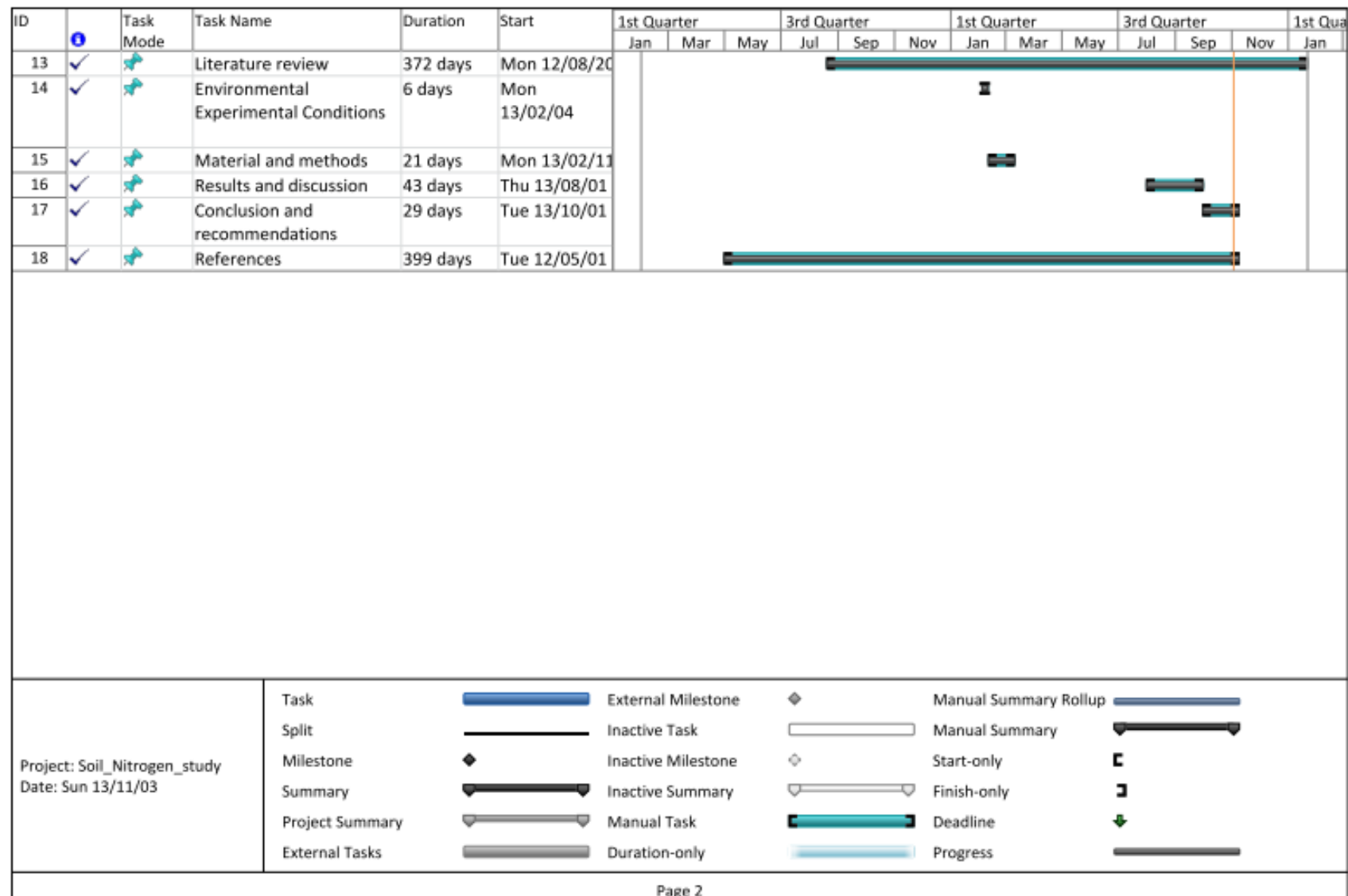


Figure 1.1: GANTT chart to illustrate project management through time management and coordination.

1.4. Dissertation structure

Below follows a short outline of the content of each of the ensuing chapters:

- Chapter 2 provides an insight into existing information from previous studies regarding nitrogen fixation and the application thereof to this study.
- Chapter 3 reviews the location and general experimental conditions for this study.
- Chapter 4 outlines the basic experimental design of this project as well as sampling, laboratory and analytical procedures.
- Chapter 5 presents the results as well as the discussion of the project findings.
- Chapter 6 collates all of the information from the previous chapters to make bias recommendations regarding practical applications in practice, as well as the value it can add.
- Chapter 7 presents the literature reference list.

Chapter 2: Literature Review

2.1. Introduction

This chapter serves to summarise and highlight the key issues regarding nitrogen-fixing bacteria and their role within the growth medium. This chapter also outlines the terminology in order to understand the context in which many, and often confusing, phrases are used.

The initial part of this chapter outlines the general properties and occurrence of nitrogen in nature. The next section explains the relationship between legume plant species and the bacteria responsible for the fixation process. The following section focusses on the vital role the latter bacteria play within the growth medium. Within the next section, emphasis is placed on the limiting and controlling factors for bacterial growth within the growth medium.

2.2. Properties, natural occurrence and importance of nitrogen and the bacteria fixating it

Nitrogen (N) is a colourless inorganic compound that occurs naturally in the atmosphere as N_2 (Kotz et al. 2006; Belnap, 2001); at 78% by volume, nitrogen is also the most abundant component of air (Winegardner, 1995). The four main constituents of vascular plants are mainly carbon, hydrogen, oxygen and nitrogen; therefore, nitrogen plays an integral part in plant growth (Stewart, 1966). As stated by Belnap (2001), Winegardner (1995) and Hopkins and Dungai (2010), the N_2 form is unusable by vascular plants and first needs to be reduced or “fixed” to ammonia (NH_3), ammonium (NH_4^+) or nitrate (NO_3^-). In order to form NH_3 , N_2 needs to react with hydrogen (Kotz et al. 2006); for this process to occur in the soil, prokaryotic organisms are needed (Belnap, 2001). Nitrogen is an important element within the soil ecosystem (Prescott et al. 2008) and, other than water, is the most important plant growth element (Winegardner, 1995). Nitrogen is an essential component of chlorophyll (each chlorophyll molecule contains one magnesium and four nitrogen atoms), amino acids, nucleotides and vitamins (Winegardner, 1995; Nabors, 2004).

Leguminous plants are distributed throughout the world and are ranked as the biggest angiosperm family, the Fabaceae, which consists of 17 000 to 19 000 species (Shtark et al. 2010; Sprent, 1999; Sy et al. 2000). According to Balser et al. (2010) and Zahran (2001), nitrogen-fixing plants play an integral part during the natural succession phase within semi-arid ecosystems as they have great influence on nitrogen input into the surrounding soil environment. Zahran (2001) further states that, along with the occurrence of these species, greater species diversity can be observed, as well as increased soil fertility and structural quality. The bacteria responsible for this process also play a vital role in ecosystem functioning, and therefore they can serve as indicators of land-use change and ecosystem health (Balser et al. 2010).

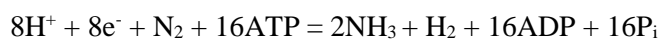
2.3. Symbiosis between legumes and nitrogen-fixing bacteria

Symbiotic root-fungus association research goes as far back as 1877 and the term ‘mycorrhiza’ was first published in 1885 by Franck (Paul, 2007). According to Hopkins and Hüner (2004), there are various associations between different plant species and bacteria, such as parasitism, symbiotic relationships and mutualistic associations. Symbiotic relationships include the inhabitation of a plant by a micro-organism (Paul, 2007). The plant represents the host and the microbial component the microsymbiont (Hopkins and Hüner, 2004). Symbiosis implies that both of the affected organisms are positively affected by the relationship. In order for the symbiotic relationship to commence, the bacteria need to locate the host plant; the plant facilitates this process by producing a product from its root hairs that attracts the rhizobial bacteria (Stewart, 1966). In the case of mycorrhizal fungi and the host plant, the fungi attain photosynthetic carbon from the plant; in turn, the plant has better water and nutrient uptake (Paul, 2007). Due to this symbiotic association, enlarged multicellular structures (known as nodules) form on the roots (Hopkins and Hüner, 2004; Stewart, 1966). These nodules mainly emerge laterally on the roots in a spherical or club-shaped form (Stewart, 1966). The three main species (known as rhizobia) are associated with legume species, namely *Rhizobium*, *Bradyrhizobium* and *Azorhizobium* (Hopkins and Hüner, 2004).

In order to understand nitrogen fixation, the process of root infection needs to be understood. According to broad-based literature, this matter has been studied extensively; however, in order to gain a clear understanding, only the main steps will be clarified. According to Hopkins and Hüner (2004), Van Elsas et al. (2006), Paul (2007) and Gibson (1971), the four principal stages of infection are as follows (refer to Figure 2.1):

1. Multiplication of the rhizobia, colonization of the rhizosphere and then attachment to epidermal and root hair cells (Phase A in Figure 2.1).
2. Curling of the root hair due to stimulation by the rhizobia is followed by digestion of the root hair wall by the bacteria and formation of an infection thread that elongates towards the root cortex (Phase B in Figure 2.1).
3. Nodule initiation through the infection thread that branches and penetrates numerous cortical cells (Phase C in Figure 2.1).
4. The final stage is the release of rhizobia into the host cells and their differentiation into specialized nitrogen-fixing cells (Not shown in Figure 2.1).

As stated by Shubert et al. (1977), the main products of natural nitrogen fixation are H_2 and NH_3 ; in order for the abovementioned bacteria to produce these products, the following reaction within the bacteroids is required:



The produced NH_3 then reacts with H^+ under a physiological pH of 7.3 in order generate NH_4^+ by the following reaction (Hopkins and Hüner, 2004):

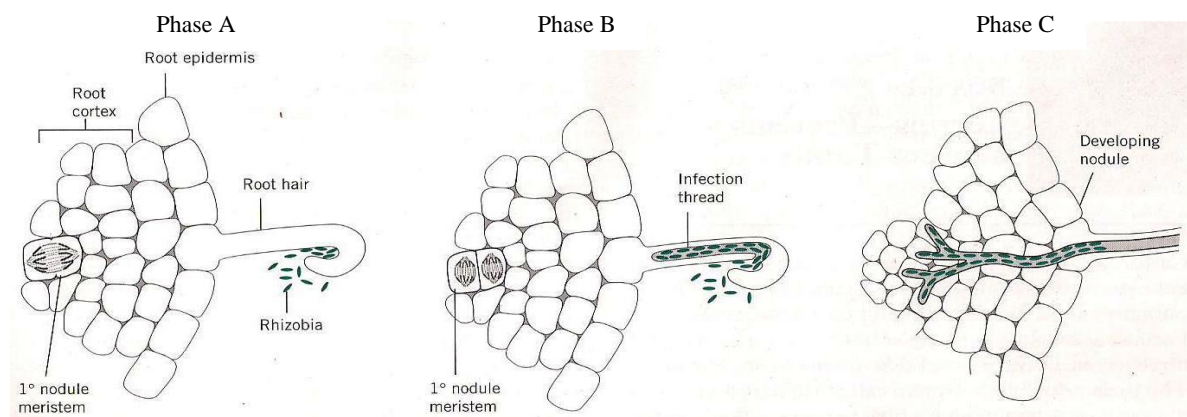


Figure 2.1: Schematic illustration of the plant root infection process leading to nodule formation (figure adopted from Hopkins and Hüner, 2004)

2.4. Role and biochemistry of nitrogen-fixing bacteria

The anthropogenic process of producing nitrogen fertilizers is extremely energy-consuming and due to the current global energy crisis, the use of naturally nitrogen-fixing plants is becoming a necessity (Schubart and Evans, 1976). As stated earlier, nitrogen from the atmosphere must be converted to NO_3^- or NH_4^+ . For this process to take place, nitrogen-fixing bacteria on legumes (Gibson et al. 1977) first need to convert nitrogen gas into NH_3 by means of the nitrogenase enzyme (Hopkins and Dungait, 2010). The NH_3 then binds a H^+ ion from the soil solution to become NH_4^+ , which can be taken up by plants (Nabors, 2004). Therefore, plants that have these associations consume less nitrogen from the soil or growth medium, but increase the nitrogen levels within the medium (Nabors, 2004; Zahran, 2001; Mulder et al. 2002). The degree of nitrogen fixation in leguminous plants is directly dependent on the characteristics of the host plant and associated rhizobial strain (Lie, 1971).

According to Drinkwater et al. (1998), Zahran (2001), Nabors (2004), and Marschner and Rengel (2010), the bacteria associated with a single legume plant can produce between 1 and 3 grams of fixed nitrogen; therefore, a legume crop of ten thousand plants per hectare can produce 150-300 kg of fixed nitrogen from that specific hectare per growing season. According to Elsas et al. (2006), nitrogen-fixing organisms are responsible for 60 percent of the earth's total fixed nitrogen. The important role of legume species is further emphasised by Palm and Sanchez (1991) and Hopkins and Dungai (2010) where they state that nitrogen from biological sources like nitrogen-fixing rhizobia is the only source of additional

nitrogen in developing countries where inorganic fertilizers are too expensive. Marschner and Rengel (2010) support this statement by stating that in certain cereal species and sugarcane genotypes, the correct species selection contributed to available nitrogen to such an extent that very little to no inorganic nitrogen was required.

The bacteria are also regarded as one of the factors that have the greatest influence on soil aggregation due to the release of glomalin (glycoprotein) (Bronick and Lal, 2004). According to Paul (2007) and Zahran (2001), a good rhizobial community helps with the establishment of certain key species; they also indirectly increase the soil organic matter and enhance the formation of hydrostable soil aggregates.

The abovementioned factors emphasise the important role of these biota in the tailings substrates due to the low levels of organic matter and stable aggregates found in these materials.

A positive factor that may play a possible role in these tailings materials is the fact that rhizobial-bearing plants are better able to take up ions in nutrient-deficient soils due to an increase in the available root surface (Lie, 1971). Lie (1971) also states that the presence of rhizobium mobilizes insoluble phosphate due the production of organic acids, and therefore the uptake of phosphorus is enhanced.

The regeneration of rhizobial bacteria is of utmost importance in these hostile growth mediums, particularly as it relates to sustained incidence. The occurrence of rhizobial bacteria within a rehabilitated medium over time can serve as indicator that the rehabilitation of the soil status was successful (Straker, et al. 2007). With plant die-off and subsequent root decay, each nodule that consists of a number of bacteroid packets, which further consists of 4 to 6 bacteria inside; these bacteria are released into the soil, and subsequently nitrogen as well as decay takes place (Tate, 2000; Zahran, 2001).

2.5. Factors influencing nitrogen fixing bacteria growth

The structure and activity of soil microbiota is highly dependent on the status of the soil habitat (Balser et al. 2010; Lie, 1971). According to Bronick and Lal (2004), soil structure plays an integral role in supporting fauna and flora which are dependent on it as a microhabitat. The soil habitat can further be described as a complex matrix of physical structure, aggregates and pores, as well as composition in the form of particle size distribution (Balser et al. 2010). Soil chemical and physical properties can present certain constraints with regard to nodule formation and persistence; the main factors include pH, metal interactions, salinity and moisture (Tate, 2000). Stewart (1966) state that high light intensities can place certain levels of constraint on nitrogen fixation. Balser et al. (2010) also states that factors like effective soil depth, particle size and stable aggregates play an important role in the soil microbial community structure and composition. Bronick and Lal (2004) state that soil bacteria are mainly associated with clay and polysaccharides in micro-aggregates.

Stewart (1966) and Sprent (1999) state that inorganic nitrogen plays a vital role in the assimilation process of elemental nitrogen; therefore, nodules which grow in the presence of inorganic nitrogen are less efficient at assimilating nitrogen, although their presence and abundance is high. Marschner et al. (1999) and Stewart (1966) state that higher inorganic nitrogen fertilizer levels have a direct negative effect on nitrogen-fixing bacteria and their ability to colonize plant roots. Okon et al. (1976) found that the additional application of NH_4^+ entirely subdued nitrogenase. The extensive use of nitrogen fertilizers also enhances the grass components' competition levels; therefore, this component can outcompete the legume component (Rethman and Tanner, 1995).

According to Tate (2000), Holding and Lowe (1971), and Stewart (1966), the majority of legume species occurs on soils with pH values from 5 upward, and not exceeding 8. Therefore, any tailings material with a pH lower than 5 is not desirable for legume establishment and, subsequently, rhizobial infection. Although certain legume species can grow at pH values lower than 5, the rhizobia and their infection potential are limited to the abovementioned pH range (Holding and Lowe, 1971; Tate, 2000).

The effect of pH can either have a direct or indirect impact on the bacterial growth. The bacteria are influenced by the higher amount of H^+ ions in the growth medium. As an indirect effect, the higher acidity causes increased metal solubility within the soil and this, in turn, leads to poor growth within the rhizobial colony (Tate, 2000) as well as the host plant. Although certain plants can grow in mediums with extreme pH values, nodulation on these plants are absent. This is an indication that the nodulating organisms are more sensitive to low pH values than the host plant (Stewart, 1966). Sprent (1999), and Keyser and Munns (1979) found that aluminium toxicity and acidity had a greater effect on rhizobial growth than did manganese toxicity and calcium deficiency. Sprent (1999) further states that these pH extremes also influence mineral availability and therefore nutrient deficiencies occur under the latter circumstances (see Figure 2.2 for nutrient availability at different pH values). According to Okon et al. (1976) and Sprent (1999), pH (H_2O) values above 7.8 also inhibit nodulation and nitrogen fixation. According to Sprent (1999), molybdenum is an essential element in the nitrogen fixation process because it is one of the constituents of nitrogenase.

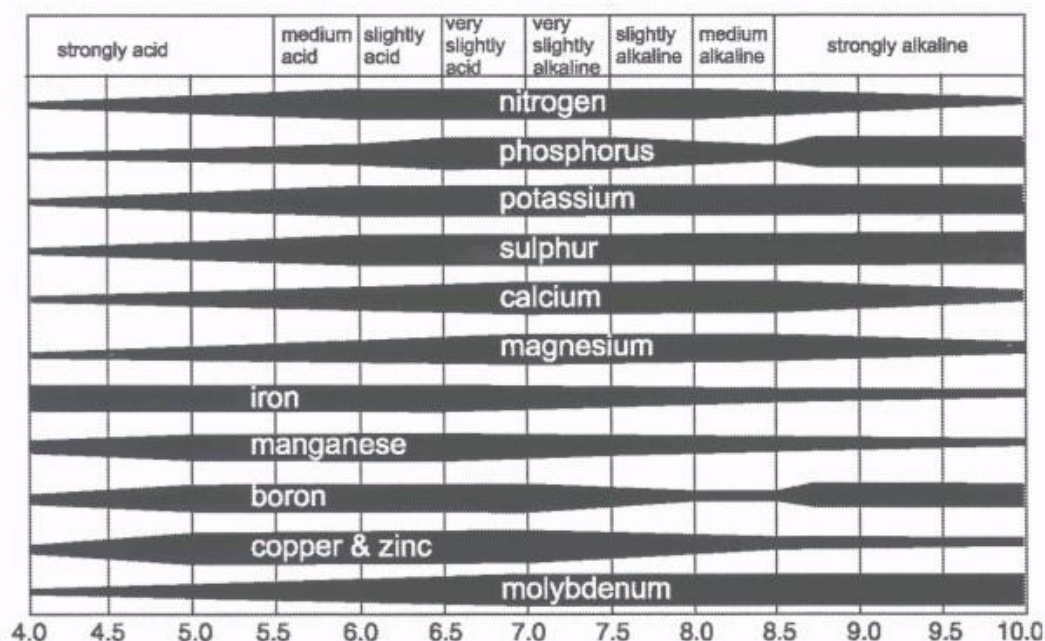


Figure 2.2: The effect of soil pH on nutrient availability to plants (Gardner, 1995)

Water stress is well known as a factor that affects nodule formation, longevity and the associated nitrogen fixation rate (Sprent, 1971). Sprent (1971) and Sprent (1999) also state that nodules can tolerate moderate water stress although their metabolic processes slow down; this can be reversible when favourable conditions arise. Regarding the effect of soil moisture on rhizobial growth, Tate (2000) and Zahran (2001) state that certain bacterial strains can grow in arid conditions and that there are strains that can only grow in tropical conditions; therefore, the type of strain selected for inoculation in certain climatic conditions is crucial. Tate (2000) further states that when considering drought-sensitive strains, a number of attenuating actions such as increasing the organic matter content or increasing the clay content might be considered. Both of these actions will increase the water-holding capacity of the growth medium.

Soil and root temperature also plays a vital role in nodulation and nitrogen fixation; the optimum temperature for infection, nodulation and nitrogen fixation occurs between 20°C and 30°C (Gibson, 1971). Okon et al. (1976) stated that certain nitrogen-fixing bacteria grow best under temperatures ranging between 32°C and 38°C.

Soil salinity is one of the largest environmental threats on earth and influences almost 7% of all land areas (Salah et al. 2010; Li et al. 2010). Salah et al. (2010) further state that elevated levels of salinity in the soil decrease vegetation performance in terms of biomass production, which is caused by physiological factors like the inhibition of enzyme activities. When considering salinity as a rhizobial growth inhibitor, it can either have a toxic effect that is direct or that occurs through osmotic stress

towards the rhizobia (Tate, 2000; Zahran, 2001; Salah et al. 2010). High salinity values do not imply that no nodulation takes place, but rather that the fixed nitrogen levels are below the plant's minimum requirement (Tate, 2000). Zahran (2001) further states that when legume species are to be established in saline materials, the appropriate strain selection needs to be carried out for optimal infection and nodulation to occur. Salinity stress can also pose a threat to the germination of certain species and therefore, in some circumstances, the appropriate species need to be selected (Li et al. 2010).

Metal trace element concentrations within the growth medium can also place a constraint on the infection, nodulation and nitrogen fixation process (Holding and Lowe, 1971). Leyval et al. (1997) state that high trace metal concentrations in soil are toxic to bacteria and fungi, and inhibit their colonization and development.

Phosphate deficiencies can place a constraint on nitrogen fixation due to bacteria requiring a higher input of phosphate (Sprent, 1999). Sprent (1999) also states that deficient phosphate levels cause poor carbohydrate transport to the nodules by the host plant, and therefore nodule functioning is retarded.

Soil nutrients also play a fundamental role in vegetation establishment and growth; therefore, the parameters for nutrient content are given in Table 2.1. The nitrogen levels in this table provide an indication of sufficient levels to sustain vigorous plant growth.

Table 2.1: Sufficient range of nutrients necessary for efficient plant growth (Van Wyk, 2002)

Elements	Sufficient range
	%
Nitrogen (N)	2.0-5.0
Phosphate (P)	0.2-0.5
Potassium (K)	1.0-5.0
Calcium (Ca)	0.1-1.0
Magnesium (Mg)	0.1-0.4
Sulphate (S)	0.1-0.3
Sodium (Na)	1.0-10
Selenium (Si)	0.2-2.0
Chlorine (Cl)	0.2-2.0
	mg.kg⁻¹
Iron (Fe)	50-250
Zinc (Zn)	20-100
Manganese (Mn)	20-300
Copper (Cu)	5-20
Boron (B)	10-100
Molybdenum (Mo)	0.1-0.5
Cobalt (Co)	0.2-0.5
Vanadium (V)	0.2-0.5

Elevated nitrogen fixation can have indirect effects on the surrounding soil microbiota. These effects include increased microbial activity, stronger decomposition of organic matter, decrease in nitrogen fixation through free-living fixating bacteria, and an increase in microbial species with higher nitrogen demands (Marschner and Rengel, 2010). When legumious plant material decomposes, a large amount of nitrogen is released into the soil environment and can therefore drive the abovementioned effects even further (Palm and Sanchez, 1991).

Chapter 3: Materials and methods

3.1 Introduction

In order to establish a meaningful understanding of the development and production patterns of nitrogen-fixing bacteria in the different growth mediums on the different species, a variety of procedures were required. The procedures used are interrelated and comprise visual assessment procedures as well as analytical (chemical) procedures. Visual assessments were carried out to establish whether there were different growth patterns within the different growth media, and also to quantify the establishment potential. Analytical procedures were carried out in order to observe the differences, if any, on a chemical and physical scale, which in turn would lead to clarity on the micro scale level.

Adequate sampling material was required in terms of replicate amounts in order to ensure statistical accuracy, which in turn would also comply with budgetary constraints. Therefore, it was decided that there would be eight replicates of each plant species in each growth medium, totalling 560 plants. Due to budgetary constraints, three representative plants per species per growth medium were selected for sampling purposes after establishment. The three selected specimens were clearly marked and used during the entire sampling period.

3.2 Environmental experimental conditions

3.2.1 Locality

The study was conducted at the Potchefstroom Campus of the North-West University's Soil and Rehabilitation Research Facility, situated adjacent to the campus (S26°40'52.28" E27°05'50.36"). This facility comprises the appropriate experimental conditions with regard to irrigation and suitable placement areas.

The experimental bags were placed on a concrete floored area in order to prevent root growth to beyond the bag. All of the nitrogen produced by the plants had to be confined within the bag in order to achieve accurate quantification results. The experimental area was also covered with a 10% shade net roof during the establishment phase of the plants. The nets were removed after six months and were not replaced until the end of the study. The irrigation water and net roof were the two differences from natural field establishment.

No climatological control to create field conditions. The plants grew under natural climatic conditions (Figures 3.1 and 3.2), which would be the case under normal conditions of establishment during land re-vegetation practices.

Due to the study being conducted as pot trials with irrigation and shade cover during establishment the data generated during this study cannot be entirely be transferable to field conditions.

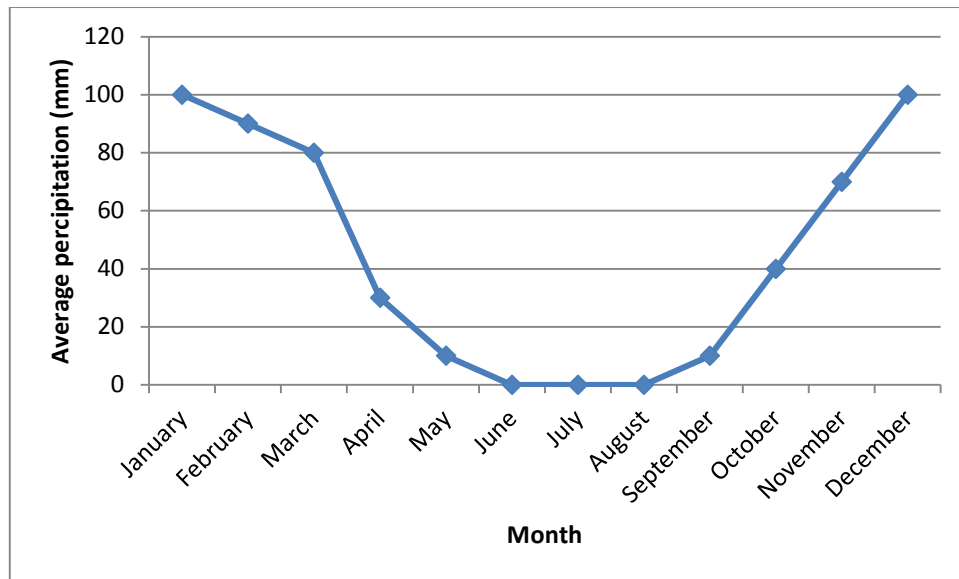


Figure 3.1: Illustration of the average rainfall for the Potchefstroom area over a period of 12 months for the past 20 years (<http://www.weatherbase.com>)

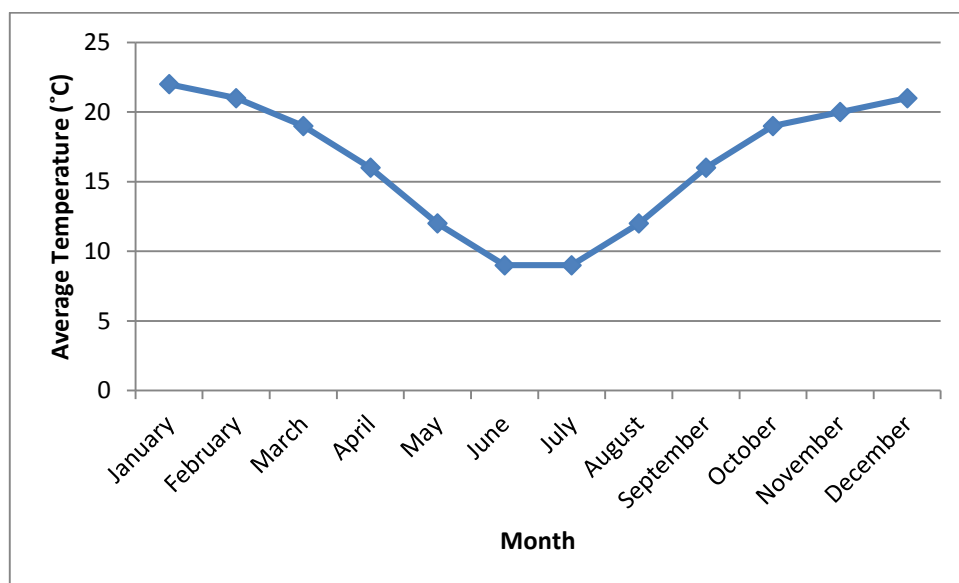


Figure 3.2: Illustration of the average temperatures measured in the Potchefstroom area over a period of 12 months for the past 20 years (<http://www.weatherbase.com>)

3.2.2 Irrigation

Due to the plants being established in bags that could only accommodate a certain amount of growth medium, the water-holding capacity per volume of growth medium was also limited. Therefore, the trial could not rely on rain water alone. Overhead irrigation was installed in order to simulate natural rainfall conditions. The irrigation system was manually used only when there was no or insufficient follow-up rainfall subsequent to the previous rainfall event.

This enabled optimal plant development during the growing season as well as optimal rhizobial growth and ultimate nitrogen fixation because, as stated by Sprent (1971), these bacteria are usually drought-sensitive. Good quality water from a municipal source was used (Electrical Conductivity value of ± 60 mS.m⁻¹). The later will most probably differ in an mining environment where process or re-cycled water is usually used for vegetation establishment.

In the event of the irrigation system being operated, watering was done manually for 10 minutes at a time (Figure 3.3); this ensured sufficient moisture replenishment for the growth medium, with an approximate precipitation of 20 mm/week during the summer months, and 5 mm/week during the winter months.

3.2.3 Growing conditions

As stated earlier, no climatic control was carried out, and only the solar intensity was controlled during the establishment phase of the trial by means of a 10% shade net coverage for the first 6 months. The plants were allowed to follow natural growth patterns and no pruning was carried out. The plants were established in black planting bags; two sizes were utilized (Figure 3.2). For the deep-rooted plants (lucerne and tree lucerne), 50 litre bags were used; for the remainder of the species, 20 litre bags were used (Figure 3.4).



Figure 3.3: Illustration of the overhead irrigation system used as a supplement to rainfall



Figure 3.4: Illustration of the 50 litre bags used for growth of the deep-rooted plants such as lucerne and tree lucerne

3.3 Experimental design

3.3.1 Species selection

The selection of adequate species formed an integral part of this study in order to add economic value to any applications thereof in the future, in the form of added benefits like increased nitrate levels in the growth medium. Due to the physical and chemical properties of the different tailings materials, species that were established in these mediums and, more importantly, those that were easy obtainable in the market or through hand-harvesting, were selected. Species selection plays a key role in re-vegetation success and was therefore was a critical consideration. Three uncommon rehabilitation species were also selected in order to expand the species selection list. The following legume species were selected for this study:

- Species 1: *Elephantorrhiza elephantina* (Elephant's root/'Olifantswortel') is a shrublet with erect, unbranched, annual stems that are usually 500 mm above the surface (Van Wyk and Malan, 1998). It also consists of a woody rootstock below ground; branchlets are hairless, bipinnate and fern-like (Van Wyk and Malan, 1998). The flowers emerge near to the ground during spring and are yellow in colour (Van Wyk and Malan, 1998). It is usually found in grasslands where dense stands are formed (Van Wyk and Malan, 1998).
- Species 2: *Vigna unguiculata* (Cowpea/'Akkerboon') is an important agricultural species and is known for high levels of nitrogen fixation; it plays an important role in rotational crop systems.
- Species 3: *Melilotus alba* (Bokhara clover/'Bokhaarklawer') is an annual herb that is erect up to 1.5 meters; the leaves are trifoliate and have a toothed margin (Van Wyk and Malan, 1998). The flowers are white in colour and are carried in long slender racemes. This species is regarded as a weed in South Africa and occurs along disturbed areas; it is native to Europe and Asia (Van Wyk and Malan, 1998).
- Species 4 and 5: Both *Sericea lespedeza* varieties are perennial, drought tolerant legumes which are tolerant to soil acidity and low fertility. *Sericea lespedeza* plants are able to establish and grow in almost all soils, but do very well on sandy and loam-type soils (www.lespedeza.co.za/).
 - Species 4: *Sericea lespedeza* var. *Au-louton* (Poor man's lucerne/'Armmans lusern') is the cultivar with lower tannin levels and is therefore found to be more palatable (www.lespedeza.co.za/).
 - Species 5: *Sericea lespedeza* var. *Au-grazer* (Poor man's lucerne/'Armmans lusern') is not as cold- and drought-tolerant as the *Au-louton* variety (www.lespedeza.co.za/).

- Species 6: *Medicago sativa* (Lucerne/'Lusern') is a very important grazing species due to its high protein content; it is native to Asia and Europe (Van Wyk and Malan, 1998). This is a herbaceous perennial shrublet with numerous erect branches arising from the crown of a woody taproot (Van Wyk and Malan, 1998). The leaf margins are smooth to finely-toothed towards the tip. The flowers are mainly purple and are carried in cylindrical clusters that can be up to 40 mm long (Van Wyk and Malan, 1998).
- Species 7: *Chamaecytisus palmensis* (Tree lucerne/'Boomlusern') is described as a perennial, evergreen, hardy tree. The 5-6 m high tree has drooping, leafy branches with bluish-green trifoliate leaves. This species originates from the Canary Islands in Spain, but is also extensively used in rehabilitation practices in New Zealand. Tree lucerne is very drought tolerant and has a wide soil pH adaptability, being able to thrive in a soil pH of 4.

3.3.2 Growth medium (tailings) selection

South Africa has an array of mining varieties with their own tailings materials and each presents its own constraints with regard to biological growth. The following tailings materials were selected for this study; these materials represent the vast majority of the different available tailings materials in South Africa. As for the chemical characteristics, each TSF is unique and therefore the results generated by this study are site- and material- specific.

- Control Material: Red sandy soil (Red apedel B-horizon from the Hutton soil form (Macvicar and De Villiers, 1991). This material was collected from the Potchefstroom area.
- Tailings 1: Gypsum. This tailings material was collected from the dormant OMV Kynoch gypsum TSF in Potchefstroom; it was a tail product of the fertilizer plant.
- Tailings 2: Gold with <1% pyrite. This tailings material was collected from Mine Waste Solutions number 4 TSF at Stilfontein in the North-West Province. The parent material of this material belongs to the Witwatersrand Supergroup and is characterized by quartzite, pyrite and conglomerate parent material.
- Tailings 3: Gold with >1% pyrite. This tailings material was collected from the Mine Waste Solutions number 5 TSF at Stilfontein in the North-West Province. The parent material is from the Witwatersrand Supergroup and is characterized by quartzite, pyrite and conglomerate parent material.
- Tailings 4: Platinum. This tailings material was collected from the Anglo Platinum Paardekraal TSF near Rustenburg in the North-West Province. The parent material belongs to the Bushveld Complex from the Merensky and UG2 Reef that consists of anorthosite and pyroxenite.

- Tailings 5: Kimberlite. This tailings material was collected from the Cullinan diamond mine in the Gauteng Province where diamonds were mined from the kimberlite pipe structure. This parent material is characterized by silicate minerals rich in magnesium, iron and alkali metals.
- Tailings 6: Coal Discard. This tailings material was collected from Witbank in the Mpumalanga Province at Anglo Coal.
- Tailings 7: Fluorspar. This tailings material was collected from Witkop Fluorspar Mine at Zeerust in the North-West Province. This parent material mainly consists of fluorite.
- Tailings 8: Andalusite. This tailings material was collected from a derelict mine at Groot Marico in the North-West Province that forms part of a contact metamorphic zone between the Bushveld Igneous Complex and shale from the Transvaal Supergroup.
- Tailings 9: Fine coal. This tailings material was collected from Witbank in the Mpumalanga Province at Anglo Coal.

3.3.3 Sampling design

Initial soil sampling of all of the material was conducted in order to derive a baseline for both chemical and physical characteristics. Due to chemical constraints and shortfalls in most of the tailings materials, and in order to support rigorous vegetation establishment, certain ameliorations were required and applied accordingly.

Due to specific growth patterns in the plants, soil microbes and bacteria, the sampling design was planned accordingly in order to derive the best readings of NO_3 production. The plants were established during September 2012 at the start of the growing season. The plants were left to establish and grow into more mature plants before sampling commenced. Three sampling intervals were decided upon in order to obtain a workable data series for NO_3 production. The first samples were collected during January 2013 in the peak growing period. The second set of samples was collected during autumn in late April 2013 when the selected species underwent a transition phase to dormancy for the winter period (all except for the *Sericea lespechea* species). Whilst the plants enter into a state of dormancy, they do not require nitrogen and therefore there may be an increase in soil available nitrogen. According to Barnard (2000), and Marschner and Rengel (2010), the peak in soil-available nitrogen was reached during the mid winter months when root nodules died off and started decaying. This served as justification to conduct the final sample series during the course of July 2013.

A soil auger comprising 30 cm long and 5 cm wide was used for sampling. In order to obtain material from a region where the plant's root development is most dense (Tate, 2000), the auger was used to sample up to a depth of 10 cm and as close as possible to the plant's main stem. The samples consisted

of approximately 100 grams per sample. The sampling left a cavity in the growth material and therefore each consecutive sampling series was carried out in a clockwise direction around the stem, as depicted in Figure 3.5.

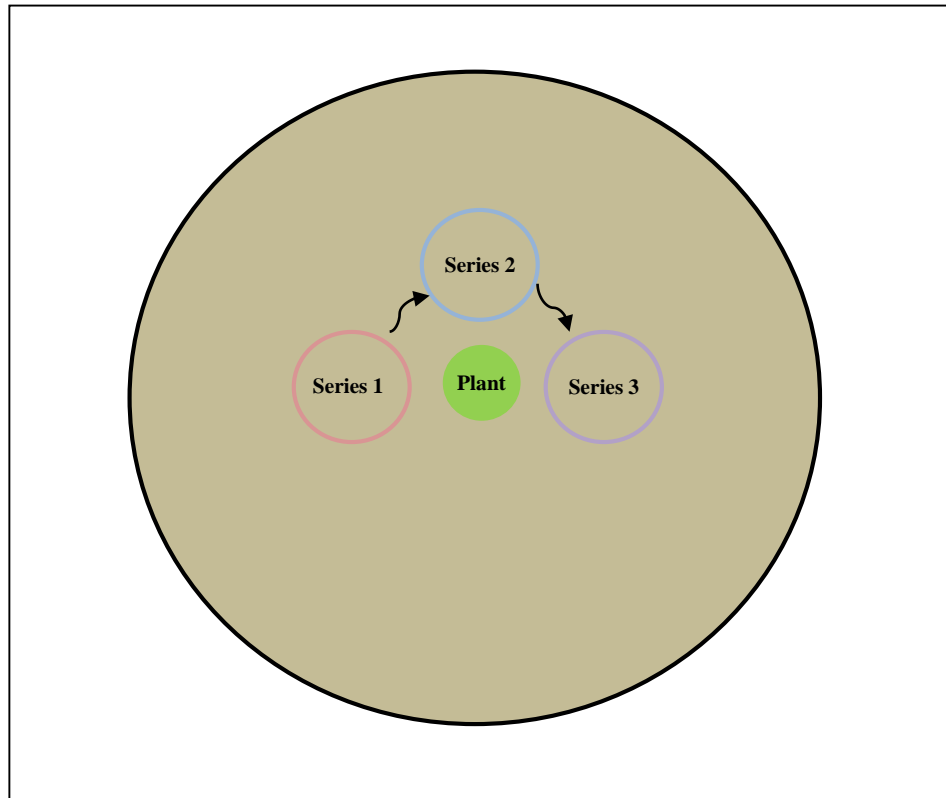


Figure 3.5: Schematic sampling series pattern

After each different tailings material was sampled, the soil auger was thoroughly washed in order to eliminate cross-contamination.

3.4 Growth medium analysis

The soil physical and chemical analyses of all of the materials and samples were assigned to GeoLab (an Agrilasa-accredited laboratory).

3.4.1 Physical analysis

In order to determine the physical characteristics of the different materials, a particle size distribution analysis was conducted. This entails the use of different sieve sizes stacked on top of each other. The sieve stack is stacked vertically with the largest sieve size at the top and the smallest at the bottom. Underneath the last sieve there is a catch pan to retain all of the particles that passed through all of the sieve sizes. Before the soil is inserted at the top, it is oven-dried and weighed. The sieve stack is then placed onto a shaker that is operated until no particle movement through the sieves is observed.

The material trapped by each sieve is weighed and the cumulative weight percentage of each sieve size is then plotted on the vertical axis of a graph (depicted in Figures 4.2– 4.11); the sieve opening sizes are plotted on the horizontal axis (Winegardner, 1995).

3.4.2 Chemical analysis

Chemical analyses were conducted on each tailings type and on the control growth medium in order to derive certain benchmark values and to finally compare them to the experimental result values. The most important benchmark value derived from the initial chemical analyses was the NO_3 value which served as a background value. All of the different conducted analyses are outlined and discussed in detail below.

1. The exchangeable cations adsorbed onto the reactive colloidal surfaces of the soil/tailings were measured using an ammonium acetate (NH_4OAc) extract. In this method, the exchange sites are saturated with NH_4 as an index cation, after which the soil is then filtered to remove all of the excess salts. The cations (Mg, Ca, K, Cl, Na) that are adsorbed by the soil colloids are then displaced into solution and measured to give the exchangeable concentrations for each cation (United States Department of Agriculture, 2004).
2. pH was measured by the H_2O method with a 1:2.5 soil:water ratio suspension on a mass basis. The pH of the medium can be expressed as the negative logarithm to base 10 of the H^+ ion activity. After the 1:2.5 soil/water ratio is prepared, the pH is measured by using a pH meter after one hour (Soil Science Society of SA, 1990).
3. Electrical Conductivity (EC) provides an indication of the total dissolved salts in the extract and therefore gives the concentration of the soluble salts in the soil. In turn, the EC value provides a salt hazard indication that the soil can pose towards vegetation establishment (Soil Science Society of SA, 1990). A saturated paste was prepared with the material and de-ionised water, and left to stand overnight. The soil paste was then filtered by suction through Whatman number 50 paper. The EC was then measured from the saturation extract derived from the latter process, and expressed as mS.m^{-1} (Soil Science Society of SA, 1990).
4. Cation Exchange Capacity (CEC) was measured in order to determine the exchange ability of the soil's reactive surfaces. This method entails the use of an NH_4OAc solution (1 mol.dm^{-3}) and is buffered by a neutral pH of 7 (Soil Science Society of SA, 1990). The method is to 1) saturate the exchange site with Na, 2) leach out excess Na with water and alcohol, and 3) displace adsorbed Na with ammonium acetate, and determine Na.
5. Phosphorus (P) was measured by means of the Bray 1 method which entails the use of a Bray 1 solution consisting out of ammonium fluoride and hydrochloric acid. After the Bray solution

is added to the soil it is then filtered through Whatman number 2V filter paper into a suitable bottle. A continuous flow analyser is then used to determine the P concentration of the extract (Soil Science Society of SA, 1990).

6. Nitrate (NO_3) concentrations were measured according to the KCl (1 mol.dm^{-1}) method. After the samples were sifted with a 2mm sieve and dried at room temperature, the NO_3 content was determined after extraction with 1N KCl (Soil Science Society of SA, 1990).

3.5 Visual and establishment potential assessment

As part of the research question, the aim is to determine whether the selected species will germinate and develop into mature plants within the different growth mediums; therefore, it was decided to conduct a visual assessment to determine this. After sowing and planting, a six-month period was granted before this assessment was conducted. The process entailed quantifying the amount of bags that had successful plant establishment; this was done per species and per tailings material. The data were then expressed as a percentage of the total amount of bags per species per tailings material.

3.6 Amelioration

It is evident from the discussions above that certain tailings materials are unable to support vegetation and biological growth. Therefore, as a standard practice with regard to vegetation establishment on these materials, soil amelioration is of utmost importance in order to achieve rehabilitation success. The chemical analysis conducted and presented in Tables 4.1 and 4.2 were utilized during the formulation of the amelioration plan for each material. The amelioration for each different material, as recommended by GeoLab, is provided in Table 3.1 below.

Note that these amelioration recommendations were done according to the specific material and therefore cannot be applied to just any rehabilitation project or study.

Table 3.1: Amelioration specifications according to GeoLab

T1	Broadcast 5 ton/ha dolomite lime six weeks before planting and work in 15-20 cm.
	Broadcast 80 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 250 kg/ha 4:3:4(33) immediately before planting and work in 5 cm.
	Top-dress 200 kg/ha LAN six weeks after planting.
T2	Broadcast 15 ton/ha dolomite lime six weeks before planting and work in 15-20 cm.
	Broadcast 35 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 350 kg/ha 4:3:4(33) immediately before planting and work in 5 cm.
	Broadcast 150 kg/ha Superphosphate with seeds.
	Top-dress 150 kg/ha LAN six weeks after planting.
T3	Broadcast 116 ton/ha dolomite lime six weeks before planting and work in 15-20 cm.
	Broadcast 55 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 350 kg/ha 4:3:4(33) immediately before planting and work in 5 cm.
	Broadcast 150 kg/ha Superphosphate with seeds.
	Top-dress 150 kg/ha LAN six weeks after planting.

T4	Broadcast 35 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 350 kg/ha 4:3:4(33) immediately before planting and work in 5 cm.
	Broadcast 150 kg/ha Superphosphate with seeds.
	Top-dress 150 kg/ha LAN six weeks after planting.
T5	Broadcast 35 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 250 kg/ha 3:2:0(25) immediately before planting and work in 5 cm.
	Top-dress 150 kg/ha LAN six weeks after planting.
T6	Broadcast 168 ton/ha dolomite lime six weeks before planting and work in 15-20 cm.
	Broadcast 65 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 350 kg/ha 4:3:4(33) immediately before planting and work in 5 cm.
	Broadcast 150 kg/ha Superphosphate with seeds.
	Top-dress 150 kg/ha LAN six weeks after planting.
T7	Broadcast 45 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 350 kg/ha 4:3:4(33) immediately before planting and work in 5 cm.
	Broadcast 150 kg/ha Superphosphate with seeds.
	Top-dress 150 kg/ha LAN six weeks after planting.
T8	Broadcast 3 ton/ha calcite lime six weeks before planting and work in 15-20 cm.
	Broadcast 35 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 350 kg/ha 3:2:1(25) immediately before planting and work in 5 cm.
	Broadcast 100 kg/ha Superphosphate with seeds.
	Top-dress 150 kg/ha LAN six weeks after planting.
T9	Broadcast 255 ton/ha dolomite lime six weeks before planting and work in 15-20 cm.
	Broadcast 55 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 350 kg/ha 4:3:4(33) immediately before planting and work in 5 cm.
	Broadcast 150 kg/ha Superphosphate with seeds.
	Top-dress 150 kg/ha LAN six weeks after planting.
TC	Broadcast 6 ton/ha dolomite lime six weeks before planting and work in 15-20 cm.
	Broadcast 30 ton/ha compost four weeks before planting and work in 5-10 cm.
	Broadcast 350 kg/ha 3:2:1(25) immediately before planting and work in 5 cm.
	Broadcast 100 kg/ha Superphosphate with seeds.
	Top-dress 150 kg/ha LAN six weeks after planting.

**As an exception, the compost application was limited to 5% throughout all of the types of material.

Soil amelioration was done after the analysis was completed and the final recommendations were received. GeoLab compiled the amelioration plan according to normal grassing specifications, and not specifically for legumes, since this is the standard recommendation protocol during rehabilitation projects. The material was tipped in 1 ton heaps; the appropriate amounts of ameliorants were applied and thoroughly worked through. The materials were then placed into the bags after this process was completed and left for one week before seeding. Due to the amelioration and alleviation of acidic pH, the effect of metal toxicity is minimised; as the alkaline conditions promote the precipitation of metals. The sorption of metals to the negatively-charged colloids is also achieved, and therefore availability for uptake by plants is lessened (Titshall et al., 2013).

3.7 Data analysis

Statistical data analysis was done with the aid of Statistica version 11 software, where comparative graphs of the different growth mediums and plant species establishment percentage were generated (Statsoft, 2013). Comparative graphs were also generated in order to depict the NO₃ assimilation of the different species within each growth medium. The NO₃ assimilated within each treatment was depicted in mg.kg⁻¹.

The non-parametric Kruskal-Wallis ANOVA for comparing multiple independent samples was used to determine differences between the various tailings materials, control medium and the different species ($P < 0.05$).

In order to determine whether there was any correlation between substrate physical and chemical characteristics, vegetation establishment and NO₃ assimilation, a correlation matrix was created by using the Spearman Rank Order Correlation method in Statistica version 11 (Statsoft, 2013).

Principal Component Analysis (PCA) analysis was also conducted on the data in order to establish whether there were any associations between NO₃ assimilation, substrate pH, EC and specific particle fractions. The data were normalized before the PCA analysis was conducted.

Chapter 4: Results and discussion

4.1 Introduction

Due to the nature of this study, the research question was addressed through a basic NO_3 analysis of the soil and a visual vegetation assessment. The results are depicted below in a chronological sequence from the control growing material to the last tailings material.

4.2 Physical and chemical analyses of the growth mediums

Initial chemical analyses were conducted to determine the amelioration requirement for rehabilitation specifications. These analyses are presented in Table 4.1. The amelioration requirement for each material, as provided by the laboratory, is presented in Table 4.2. The data are discussed under each material's section.

The sand grade and texture class diagrams were used as classification method for physical classification of the different tailings materials (see Figure 4.1).

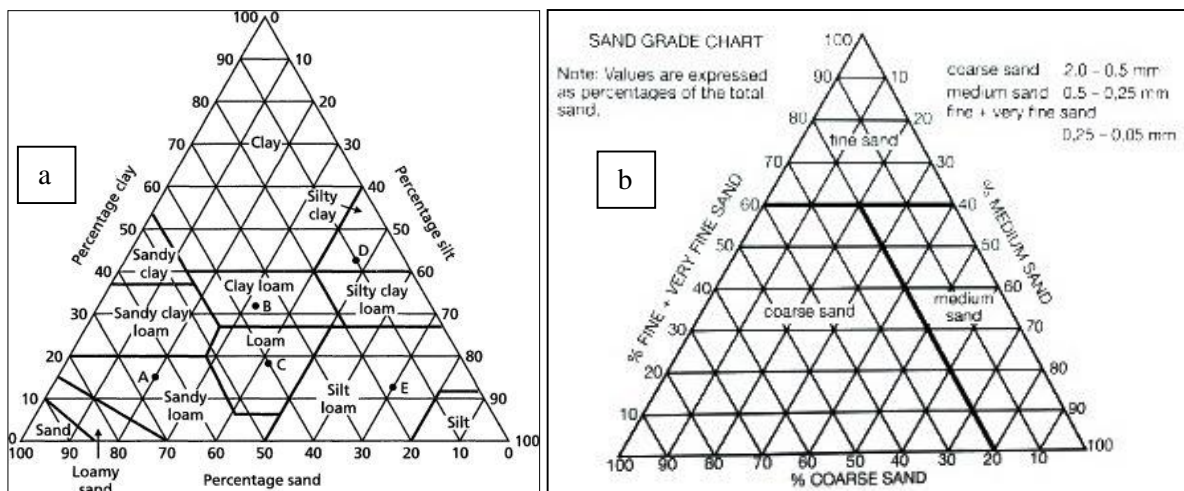


Figure 4.1: a) Sand texture classification diagram, and b) Sand grade classification diagram

Table 4.1: Initial chemical analysis for each growth medium (GeoLab)

Tailings	pH(KCl)	pH(H ₂ O)	EC	SO ₄ -S	P(Bray 1)	K		Ca		Mg		Na		CEC	Al	ESP	Al
			mS.m ⁻¹	mg.kg ⁻¹	mg.kg ⁻¹	cmol.kg ⁻¹	mg.kg ⁻¹	cmol.kg ⁻¹	mg.kg ⁻¹	cmol.kg ⁻¹	mg.kg ⁻¹	cmol.kg ⁻¹	mg.kg ⁻¹	cmol.kg ⁻¹	cmol.kg ⁻¹	%	%
Gypsum	4.1	3.9	199	159824	252	0.051	20	27.976	5595	0.142	17	0.050	12	0.2	0.62	22.19	275.5
Gold <1% pyrite	5.3	5.4	193	1674	1	0.076	30	8.965	1793	0.779	94	0.039	9	1.3	0.04	3.11	3.2
Gold >1% pyrite	6.4	6.1	422	447	0	0.115	45	10.213	2043	2.158	261	0.510	117	0.8	0.00	61.05	0.1
Platinum	8.0	6.6	205	143	0	0.079	31	1.964	393	0.407	49	0.297	68	1.2	0.00	24.94	0.0
Kimberlite	7.6	9.7	121	32	5	1.846	720	9.753	1951	1.934	234	3.746	862	9.5	0.00	39.35	0.0
Coal discard	2.3	2.4	1090	6958	0	0.065	25	6.620	1324	1.292	156	0.095	22	0.6	9.15	15.33	1482.8
Fluorspar	7.9	5.7	146	122	1	0.061	24	10.114	2023	1.325	160	0.199	46	1.1	0.00	18.86	0.0
Andalusite	5.5	6.0	11	20	3	0.222	87	1.958	392	3.352	406	0.086	20	3.6	0.00	2.40	0.0
Fine Coal	3.8	2.5	390	709	1	0.064	25	11.186	2237	1.747	211	0.012	3	0.8	21.28	1.49	2718.3
Control medium	4.1	4.1	13	12	4	0.160	62	0.441	88	0.406	49	0.004	1	1.4	0.31	0.32	22.5

Table 4.2: Initial chemical analysis for each growth medium (GeoLab)

Sample no.	Anions in saturated paste extract											
	Neutr pot	Titr acid	Acid pot 1	Nett acid pot	Total S	Organic C	Total N	Cl	SO ₄	NO ₃	F	HCO ₃
	ton/ha	ton/ha lime	ton/ha lime	ton/ha lime	%	%	%	mg/l	mg/l	mg/l	mg/l	mg/l
Gypsum	0	5	0	5	13.43	0.00	0.05	6.6	1813.3	18	27.5	7.3
Gold <1% pyrite	0	4	11	15	0.26	0.00	0.04	14.3	2186.9	13.8	0.1	65.9
Gold >1% pyrite	0	1	115	116	1.03	0.12	0.04	100.7	3808.2	168.6	0.1	80.5
Platinum	34	0	2	-32	0.03	0.00	0.02	549.4	1109.9	48.5	0.0	124.4
Kimberlite	121	0	0	-121	0.00	0.09	0.04	10.6	448.3	7.4	1.2	285.5
Coal discard	0	76	92	168	1.48	26.60	0.63	7	114585	12.4	1.4	0
Fluorspar	179	0	40	-139	0.38	12.05	0.04	135	1574.9	1.7	14.9	153.7
Andalusite	0	3	0	3	0.00	0.28	0.06	11	96.7	16	0.1	131.8
Fine coal	0	103	152	255	1.37	54.34	1.28	3	30340	13.3	0	0
Control medium	0	6	0	6	0.00	0.17	0.05	3.4	31.10	48.8	0	95.2

4.2.1 Red sandy loam

Physical properties

As a control growth medium, a red sandy soil with less than 5% clay was selected; it is referred to as TC. This is a naturally-occurring soil with particle sizes ranging between 0.85 mm and 0.002 mm (see Figure 4.2 below). As for the specific particle size distribution of TC, it consists of 0.5% very coarse sand, 4.1% coarse sand, 25.3% medium sand, 38.2% fine sand, 24.5% very fine sand, 2.9% silt and 4.3% clay. Therefore, this material is associated with the pure sand–fine sand class according to Figure 4.1.

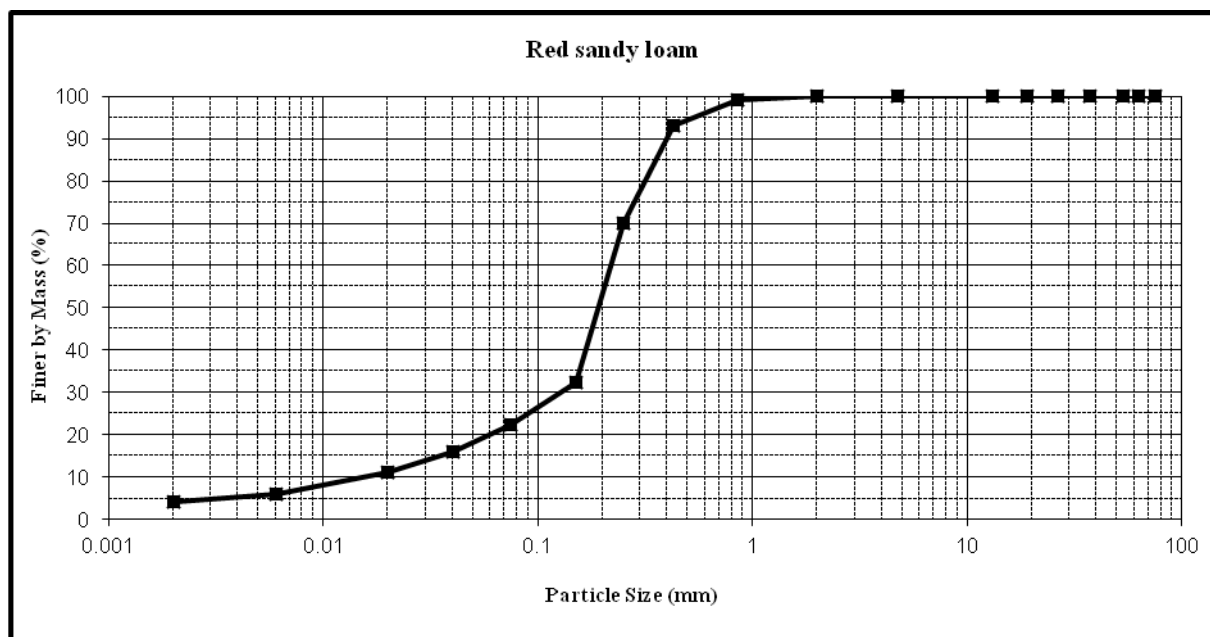


Figure 4.2: Particle size distribution of red sandy loam

Chemical properties

As depicted by the data in Tables 4.1 and 4.2, it is evident that TC has an acidic pH of 4.1; whereas, a pH of between 6.5 and 7.5 is considered neutral. From a chemical perspective, this material does not place any other constraints on vegetation establishment.

4.2.2 Gypsum tailings

Physical properties

Gypsum is the first tailings type and is referred to as T1. This tailings material is an end-product of various manufacturing processes, e.g. the production of fertilizers. It is usually snow white in colour and consists of a fine powder. Particle sizes for T1 range between 0.15 mm and 0.002 mm (Figure 4.3). The particle size distribution consists of 0.3% coarse sand, 0.5% medium sand, 0.9% fine sand, 76.6

very fine sand, 14.7% silt and 6.9% clay particles. As depicted in Figure 4.1, T1 is characterized as sand–fine sand class.

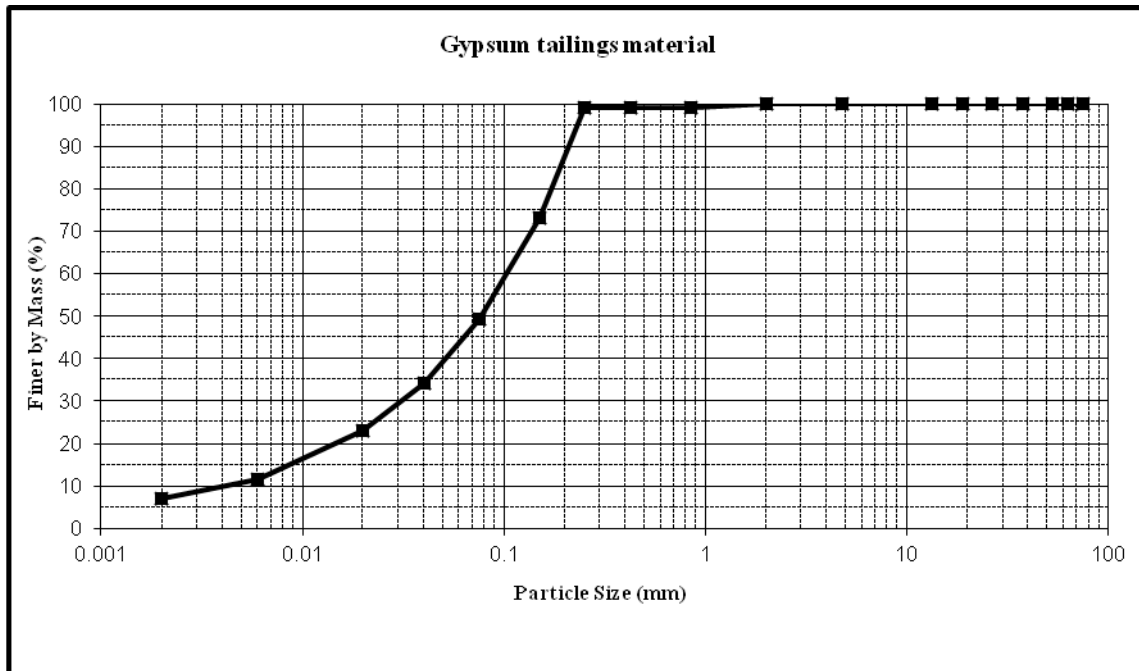


Figure 4.3: Particle size distribution of gypsum tailings material (T1)

Chemical properties

See Tables 4.1 and 4.2 for the chemical properties of T1. As depicted by the data, T1 also has an acidic pH, high sulphate levels, high Al content, high P and ESP. As mentioned in Chapter 2, the high Al content in conjunction with low pH values can place certain constraints on nitrogen fixation due to Al toxicity. Furthermore, the high ESP refers to elevated sodium levels within the medium and therefore, as referred to earlier, can cause osmotic constraints and ultimately hamper nitrogen fixation as a result of physiological stress.

4.2.3 Gold tailings with <1% pyrite

Physical properties

The second tailings material used in this study is gold tailings, further referred to as T2. Tailings 2 is the end-product of gold extraction practices and processes. The gold-bearing material usually consists of pyrite, which causes severe acidification of the surrounding material when oxidized (Rossouw, 2010). The particle size distribution of this material consists out of 0.2% very coarse sand, 0.6% coarse sand, 2.9% medium sand, 33.9% fine sand, 33.5% very fine sand, 24.2% silt and 4.6% clay. Although a clay class is present within this material, it is important to know that materials in this class size do not function as clay in natural soils, but are rather a by-product due to mechanical and metallurgical processes. As depicted in Figures 4.1 and 4.4, T2 falls within the sandy loam–fine sand class.

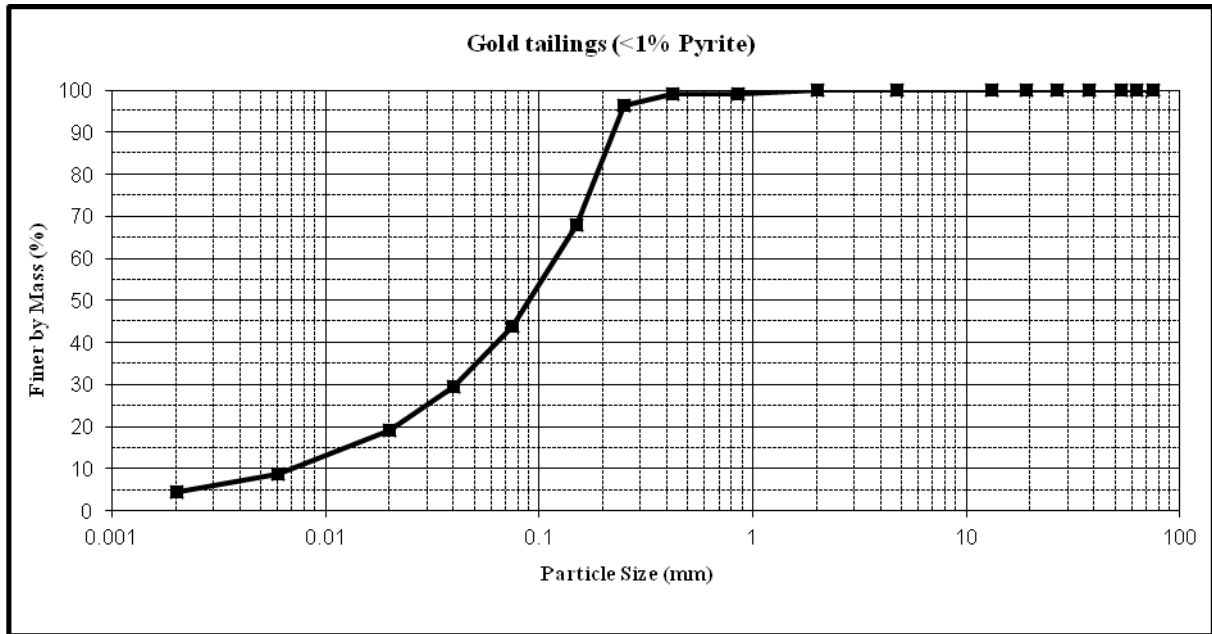


Figure 4.4: Particle size distribution of gold tailings material with less than 1% pyrite (T2)

Chemical properties

Tables 4.1 and 4.2 present the chemical data for T2. From this data, it is evident that the medium has high sulphate levels and a moderately acidic pH. The moderate pH is an indication that the oxidation process of the pyrite has not yet been completed.

4.2.4 Gold tailings with >1% pyrite

Physical properties

The third tailings type is gold tailings with more than 1% pyrite, further referred to as T3. As discussed earlier, the pyrite content together with oxygen and moisture determines the acidification potential of the material. Therefore, a material with high levels of pyrite is anticipated to cause more intense acidification, which in turn will lead to greater biological stress. The particle sizes range between 0.85 mm and 0.002 mm (see Figure 4.5 below).

The particle size distribution consists of 0.1% very coarse sand, 0.6% coarse sand, 6.5% medium sand, 45.7% fine sand, 33.2% very fine sand, 11.8% silt and 2.1% clay particles. Therefore, with the major part of the texture ranging between fine sand and very fine sand, this material falls within the loamy sand–fine sand class (according to Figure 4.1).

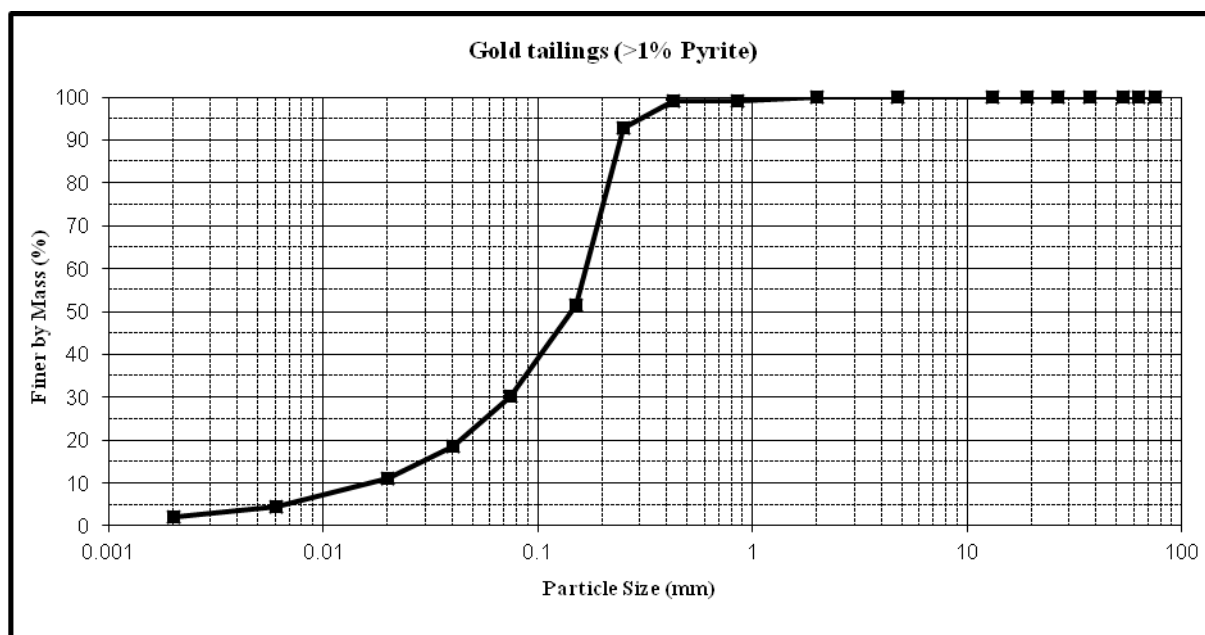


Figure 4.5: Particle size distribution of gold tailings material with more than 1% pyrite (T3)

Chemical properties

Tables 4.1 and 4.2 depict the chemical data for T3; from the data it is evident that this material has a very high salinity due to the high EC value, which may have a negative influence on biological growth due to osmotic constraints. T2 also has a slightly lower pH than found in natural conditions and can also be ascribed to the on-going oxidation of the pyrite in the material. This material is also characterized by a high ESP value which in turn causes soil aggregate destabilisation, and this leads to high levels of soil erosion. In this material, the phosphorus level is zero, which can lead to poor initial root development.

4.2.5 Platinum tailings

Physical properties

The fourth tailings material is platinum, further referred to as T4. This is also regarded as a very sandy material that is dark in colour due to the dark colour of the pyroxenite in the host rock. This material is also characterised as a material with high infiltration rates and low water retention; it is therefore prone to exhibit moisture stress on the biota living there. A further drawback of this material is natural compaction whereby vertical water movement through the material causes finer particles to illuviate through the profile; this causes compaction at a deeper level that can pose possible root development constraints. The particle sizes for T4 range between 0.85 mm and 0.002 mm (see Figure 4.6). The particle size distribution consists of 0.3% coarse sand, 8.7% medium sand, 48% fine sand, 33.4% very fine sand, 7.4% silt and 2.1% clay particles. As discussed earlier, the data confirm that this is a very sandy material with 90.4% of its texture within the fine sand–sand class (according to Figure 4.1).

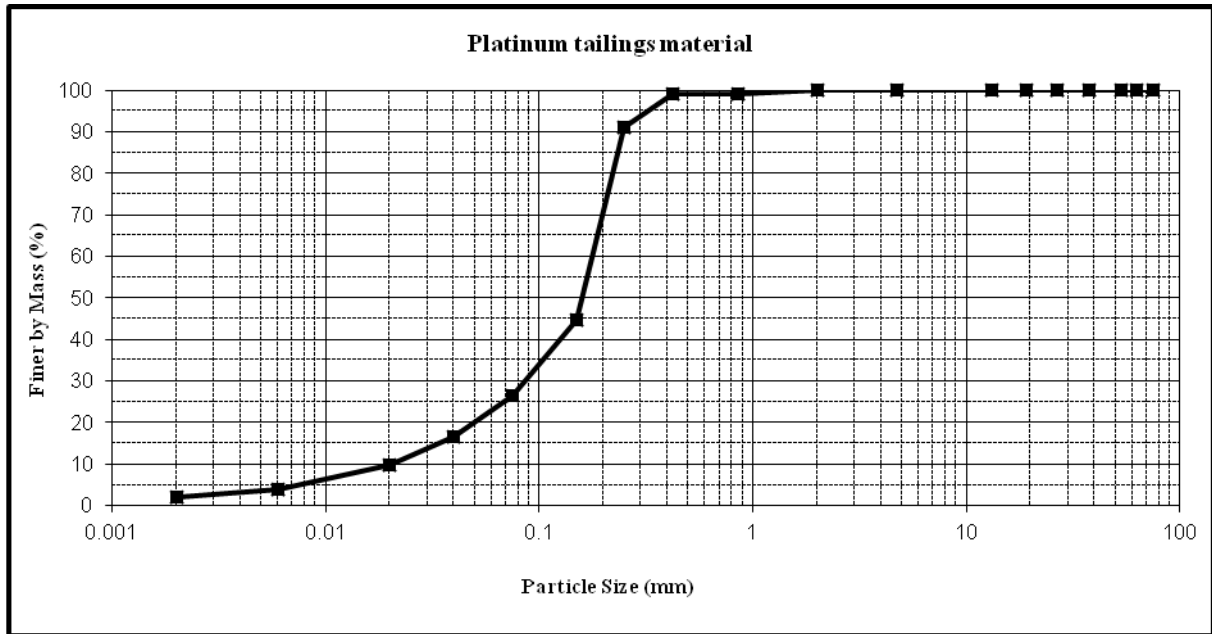


Figure 4.6: Particle size distribution of platinum tailings material (T4)

Chemical properties

In Tables 4.1 and 4.2, the chemical properties of T4 are presented. From this data, it is evident that this material has a high ESP value and no P content, which can lead to poor root development and ultimately no establishment.

4.2.6 Kimberlitic tailings

Physical properties

The fifth tailings material is kimberlite, further referred to as T5. This is regarded as gravelly tailings material that is dominated by gravel-sized stones. This material is also characterised as a material with high infiltration rates and low water retention; it is therefore prone to exhibit moisture stress on the biota. The particle sizes for T5 range between 10 mm and 0.002 mm (see Figure 4.7). Particle size distribution consists of 34% very coarse sand, 33.2% coarse sand, 13.2% medium sand, 7.7% fine sand, 3.6% very fine sand, 2.9% silt and 4.3% clay particles. As discussed earlier, the data confirm that this is a very gravelly to sandy material with $\pm 16\%$ of the particles in the >2 mm class (Figure 4.1).

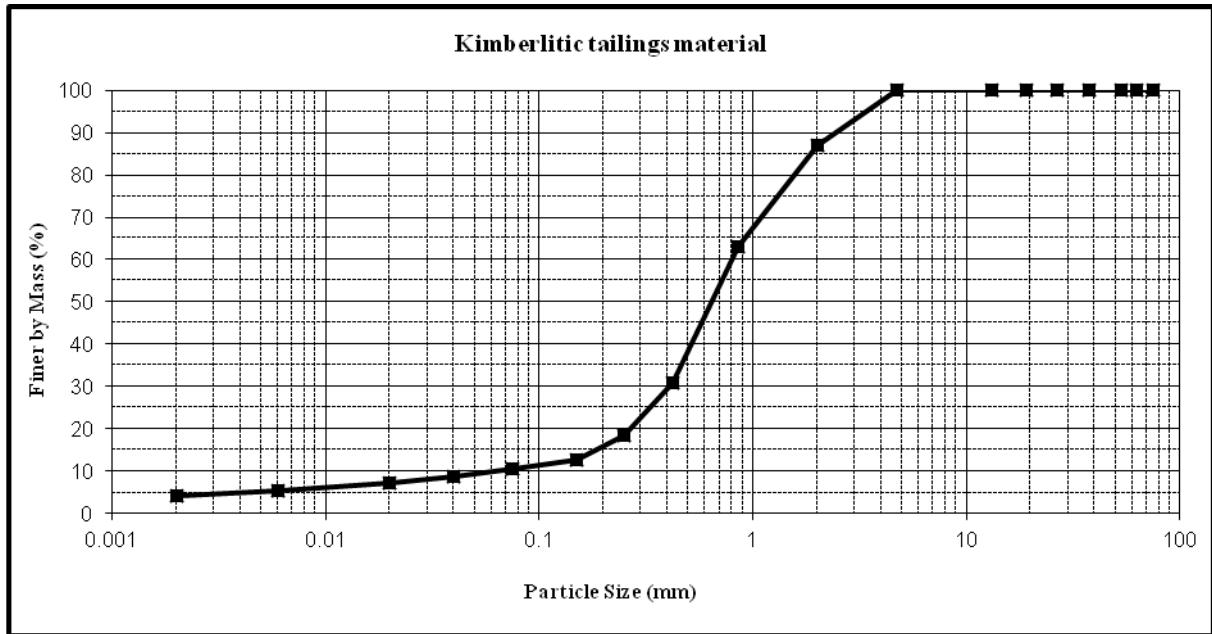


Figure 4.7: Particle size distribution of kimberlitic tailings material (T5)

Chemical properties

In Tables 4.1 and 4.2, the chemical properties of T5 are presented. From this data, it is evident that this material has a high pH of 9.7 which can pose a problem relating to the availability of certain nutrients. The ESP value is also high and can cause aggregate destabilization.

4.2.7 Coal discard tailings

Physical properties

The sixth material is coal discard, further referred to as T6. This is also regarded as a stony-gravelly type of material that is dominated by gravel-sized stones and sand. This material is characterised as a material with high infiltration rates and low water retention; it is therefore prone to exhibit moisture stress on the soil biota. The particle sizes for T6 range between 25 mm and 0.002 mm (Figure 4.8). Particle size distribution consists of 22.3% very coarse sand, 30.2% coarse sand, 26.3% medium sand, 10.9% fine sand, 2.9% very fine sand, 5.2% silt and 2.1% clay particles. As discussed earlier, the data confirm that this is a very gravelly to sandy material with $\pm 14\%$ of the particles falling in the >2 mm class.

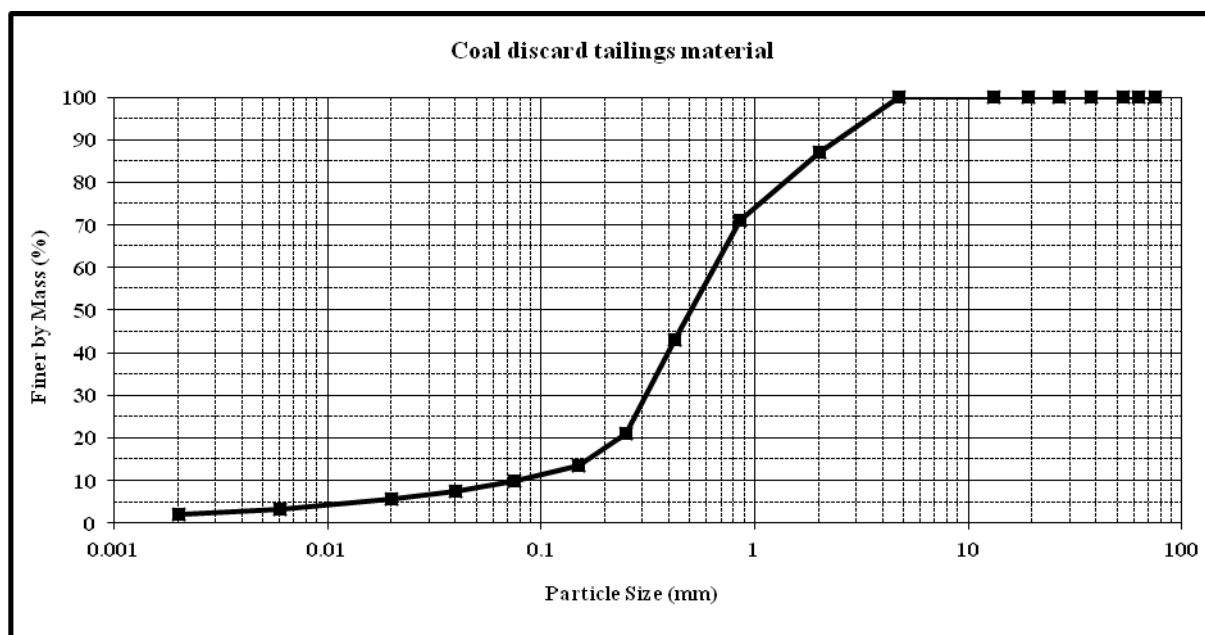


Figure 4.8: Particle size distribution of coal discard tailings material (T6)

Chemical properties

In Tables 4.1 and 4.2, the chemical properties of T6 are presented. From this data, it is evident that this material places great constraints on biological growth (from a chemical perspective) due to the extremely low pH of 2.4 which, as discussed earlier, causes aluminium toxicity. Furthermore, the EC value of 1090 mS.m⁻¹ causes significant osmotic constraints. The SO₄ value is also exceptionally high which will, in turn, lead to major physiological constraints within the plants. The P value is zero and can cause poor vegetation establishment. The CEC is also low; this is an indication of a soil's ability to retain nutrients and other chemical substances. This low value indicates that the medium loses a lot of nutrients through the process of leaching. Unstable aggregates can be expected due to the higher ESP value.

4.2.8 Fluorspar tailings

Physical properties

The seventh material that was used is fluorspar, further referred to as T7. According to the texture analysis, this material consists of very fine particles and could therefore place a constraint on infiltration. The following particle sizes occur: 0.1% very coarse sand, 2.3% coarse sand, 16% medium sand, 36% fine sand, 31.3% very fine sand, 12.2% silt and 2.2% clay particles (Figure 4.9). The data confirm that this is a very fine material with 81.6% of its texture within the fine sand–sandy loam class.

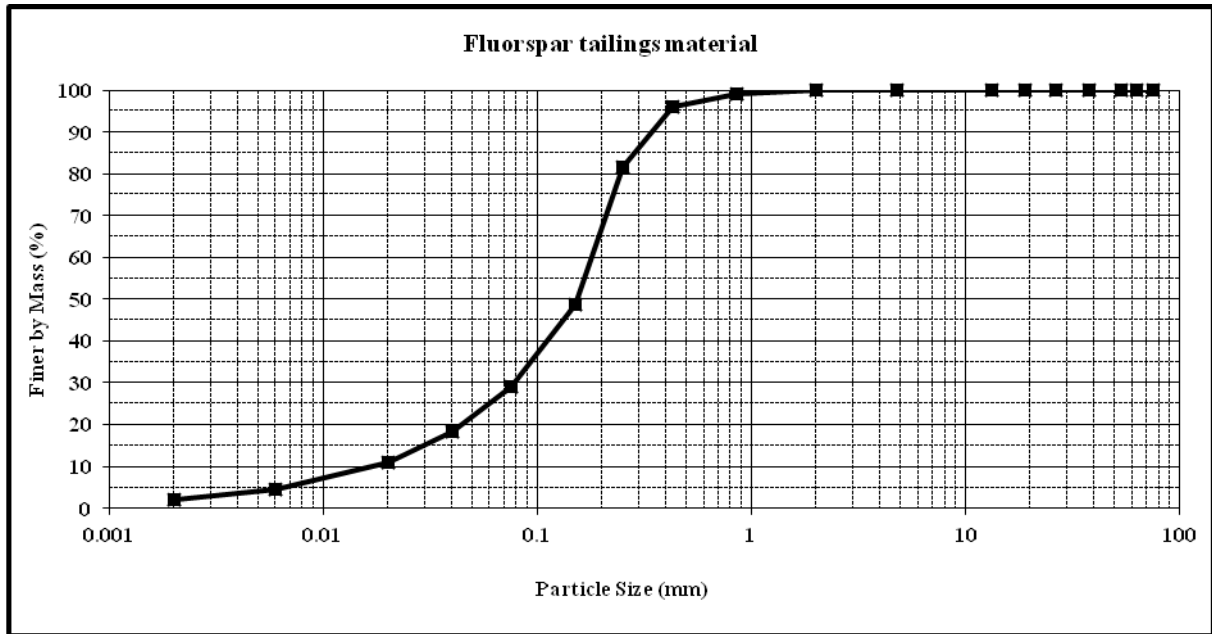


Figure 4.9: Particle size distribution of fluor spar tailings material (T7)

Chemical properties

In Tables 4.1 and 4.2, the chemical properties of T7 are presented. From the data, it is evident that poor root development can be expected due to the low P value. Furthermore, the ESP value is high and can cause aggregate instability. The pH of this material is also low at a pH(KCl) of 7.9 and pH(H₂O) of 5.7; this can cause solubility of certain heavy metals that can hamper vegetation growth and establishment.

4.2.9 Andalusite tailings

Physical properties

Andalusite is the eighth material used in this study and is further referred to as T8. This material has a very fine texture and therefore causes low water infiltration rates, as well as the consequent waterlogging conditions, which can lead to poor biological growth in certain species. The particle size distribution (Figure 4.10) of T8 consists of 2.2% very coarse sand, 4% coarse sand, 3.4% medium sand, 2.4% fine sand, 4.1% very fine sand, 68.6% silt and 15.2% clay particles. The data confirm that this is a very fine material with 90.4% of its texture within the fine sand to clay class and therefore falls within the silty-loam class (according to Figure 4.1).

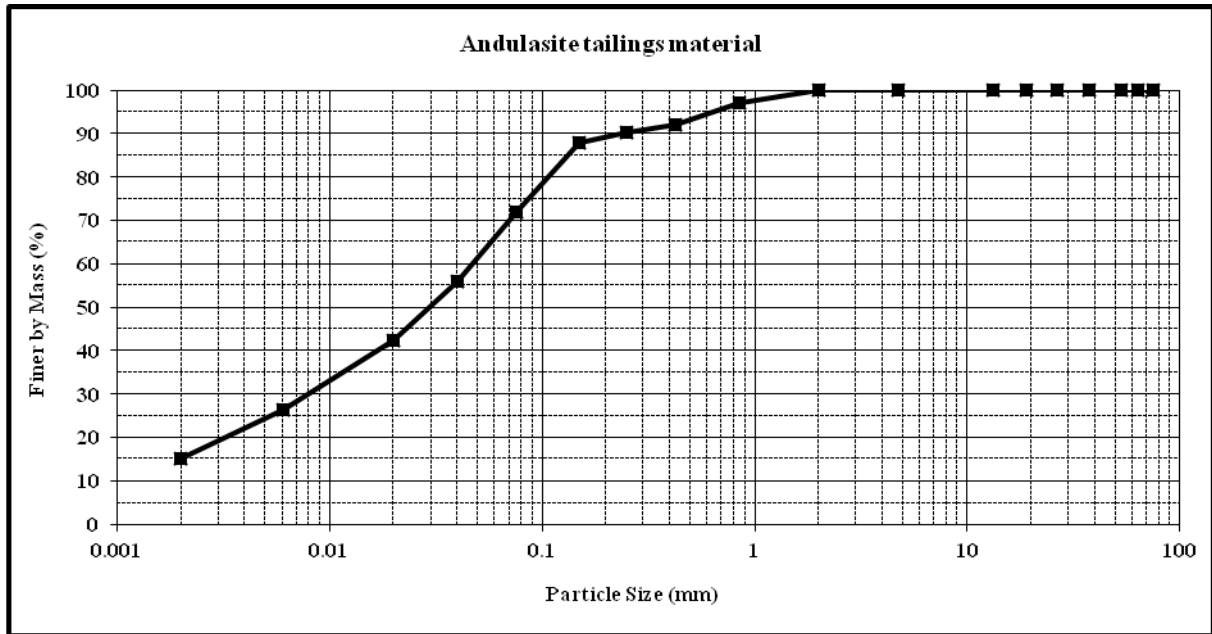


Figure 4.10: Particle size distribution of andalusite tailings material (T8)

Chemical properties

In Tables 4.1 and 4.2, the chemical properties of T8 are presented. From this data, it is evident that this material does not place any constraint on biological growth from a chemical perspective.

4.2.10 Fine coal tailings

Physical properties

The ninth and final material is fine coal tailings, further referred to as T9. The coal discard, T9, is black in colour but is dominated by a very fine texture which leads to varying infiltration rates and water movement characteristics. Due to the domination of fine texture classes, this material can cause waterlogged conditions as well as low infiltration rates. These factors can cause biological growth constraints among certain species. The particle size distribution (Figure 4.11) of T9 consists of 1.1% very coarse sand, 6% coarse sand, 13.1% medium sand, 21.2% fine sand, 18% very fine sand, 20.9% silt and 19.7% clay particles. The data confirm that this is a very fine material with 79.9% of its texture within the sandy–clay loam class.

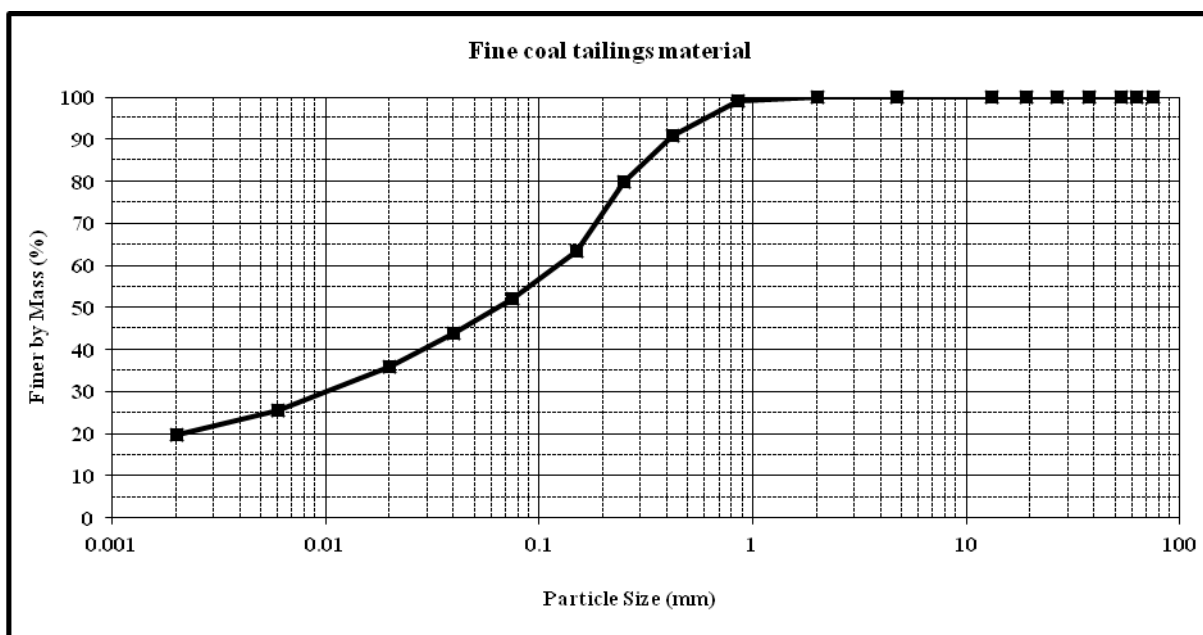


Figure 4.11: Particle size distribution of fine coal tailings material (T9)

Chemical properties

In Tables 4.1 and 4.2, the chemical properties of T9 are presented. It is evident that this material places great constraints on biological growth from a chemical perspective. The pH of this material, as for T6, is extremely low; it will inhibit seed germination and, ultimately, vegetation establishment. Furthermore, the low pH will cause heavy metal solubility and toxicity; in this case, the AI value is also high and will therefore be soluble - available for biological uptake. Electrical conductivity (EC) is also high and, as previously mentioned, can have osmotic effects on the plants. The P contents within the soil are low and will not be able to support sufficient root growth. The CEC value is also low and may cause poor nutrient retention.

4.3 Vegetation Establishment Potential Data

Establishment potential of a species within a certain growth medium is species-specific and depends on the specific reaction of that species towards the chemical and physical characteristics of the growth medium. This study aims to determine the establishment potential of different species within different growth mediums which will serve as an indication of, and guideline for, seeding quantities.

The establishment potential of the seven selected legume species in nine different tailings materials was measured in terms of a visual assessment of the species as well as their occurrence in the different tailings mediums. The assessment was conducted six months after initial seeding was completed. In order to present these findings, a general discussion of each species and its establishment success within the different tailings mediums will be provided.

As a general note regarding establishment, there was no germination of Species 3 (Clover) in any of the tailings materials and therefore this species was discarded from the study. The reason for the latter phenomenon can be ascribed to possible infertile seed or the lack of a certain required environmental trigger that stimulates germination.

Furthermore, for tailings 2 (gold tailings material with <1% pyrite), there was no establishment of any of the species and therefore this tailings type was discarded from the sampling and data analysis process.

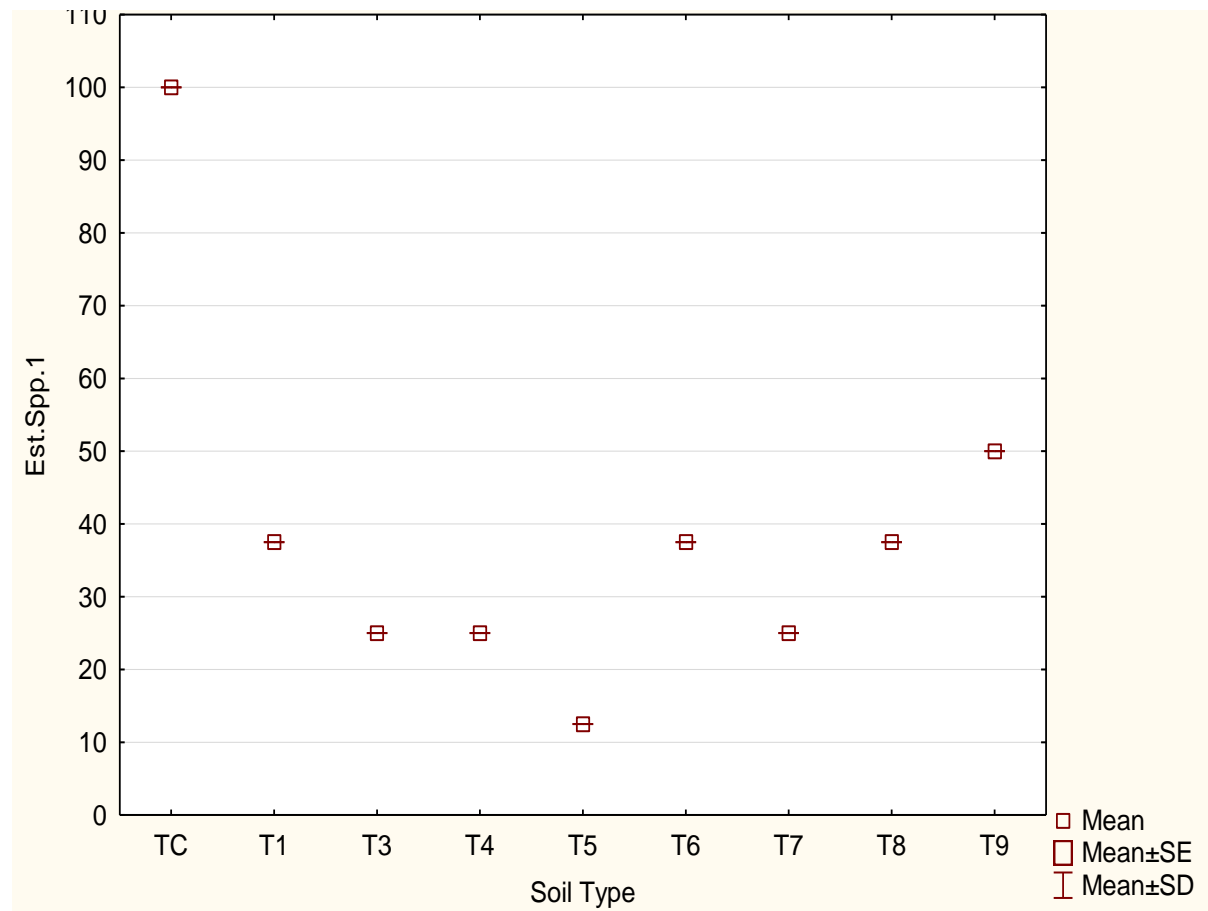


Figure 4.12: Percentage establishment of Species 1 (Elephant's root/*Elephantorrhiza elephantina*)

Significant differences ($P < 0.05$) were calculated between the establishment potential of Species 1 and the different tailings materials. From this data, it is evident that establishment was the greatest for the control soil and tailings 9 (fine coal), which had the second best establishment (Figure 4.12). As for overall establishment a minimum of 12.5% and a maximum of 100% with an average of 39.8% were achieved.

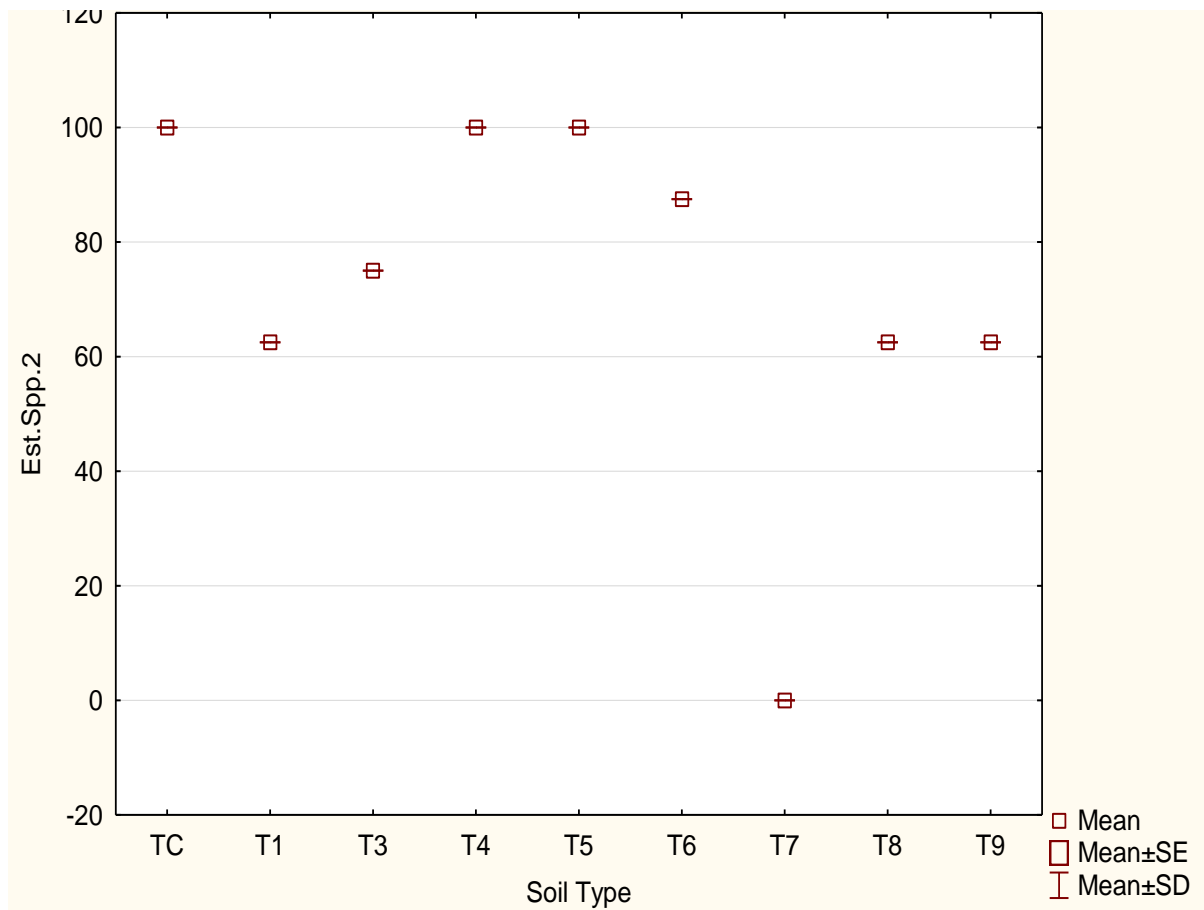


Figure 4.13: Percentage establishment of Species 2 (*Cowpea/Vigna unguiculata*)

According to Figure 4.13, the overall establishment of Species 2 was significant within all of the growth mediums, except for tailings 7 (fluorspar). This is further emphasised by tailings 7 differing the most ($P < 0.05$) from all of the other growth mediums. This can possibly be ascribed to the very fine texture of the fluorspar tailings. As for overall establishment a minimum of 0% and a maximum of 100% with an average of 71.6% were achieved.

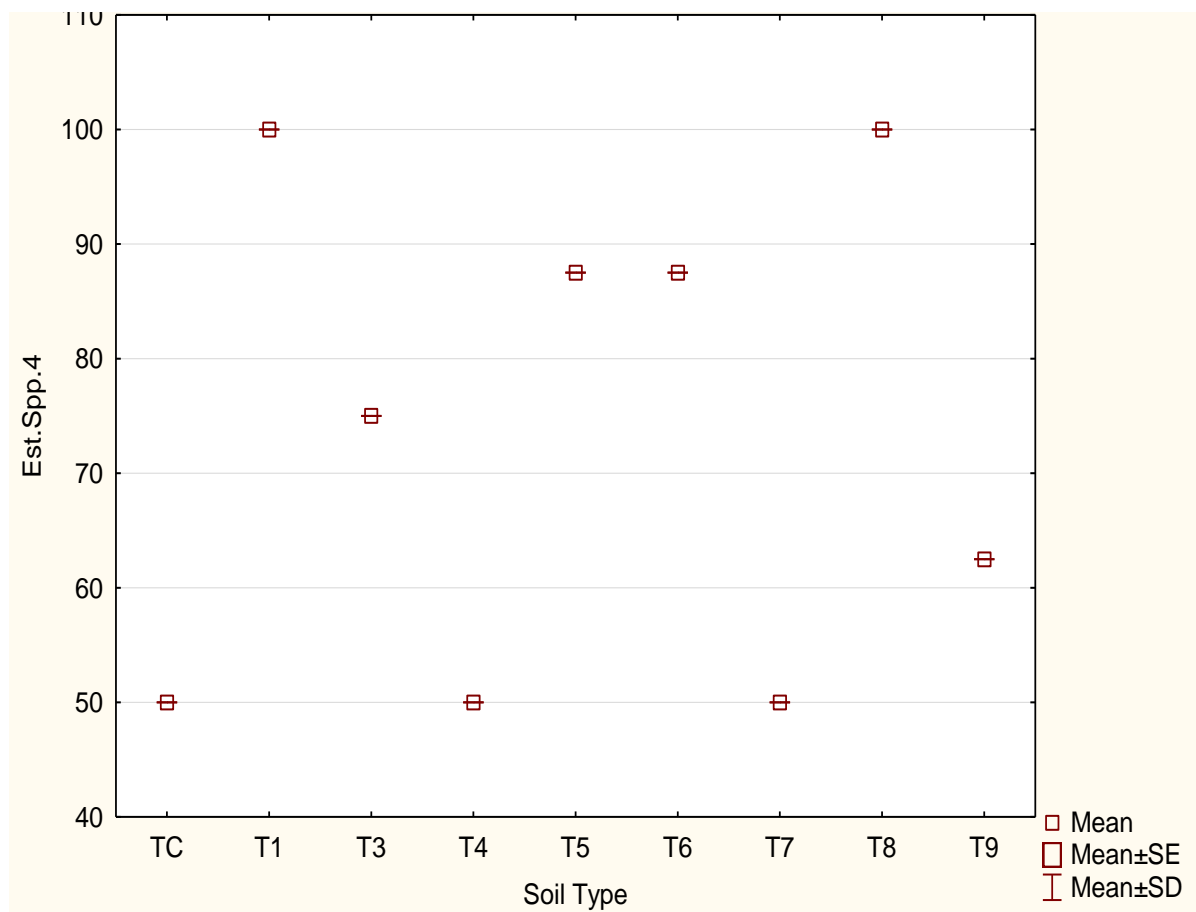


Figure 4.14: Percentage establishment of Species 4 (Poor man's Lucerne/*Sericea lespedeza* var. Au-louton)

Species 4 (Poor man's Lucerne, Au-louton) showed significant differences ($P < 0.05$) in establishment. The data indicate that Species 4 established the best within tailings 1 (gypsum) and tailings 8 (andalusite). As for the other tailings types and the control medium, establishment was 50% and above (Figure 4.14). As for overall establishment a minimum of 50% and a maximum of 100% with an average of 73.3% were achieved.

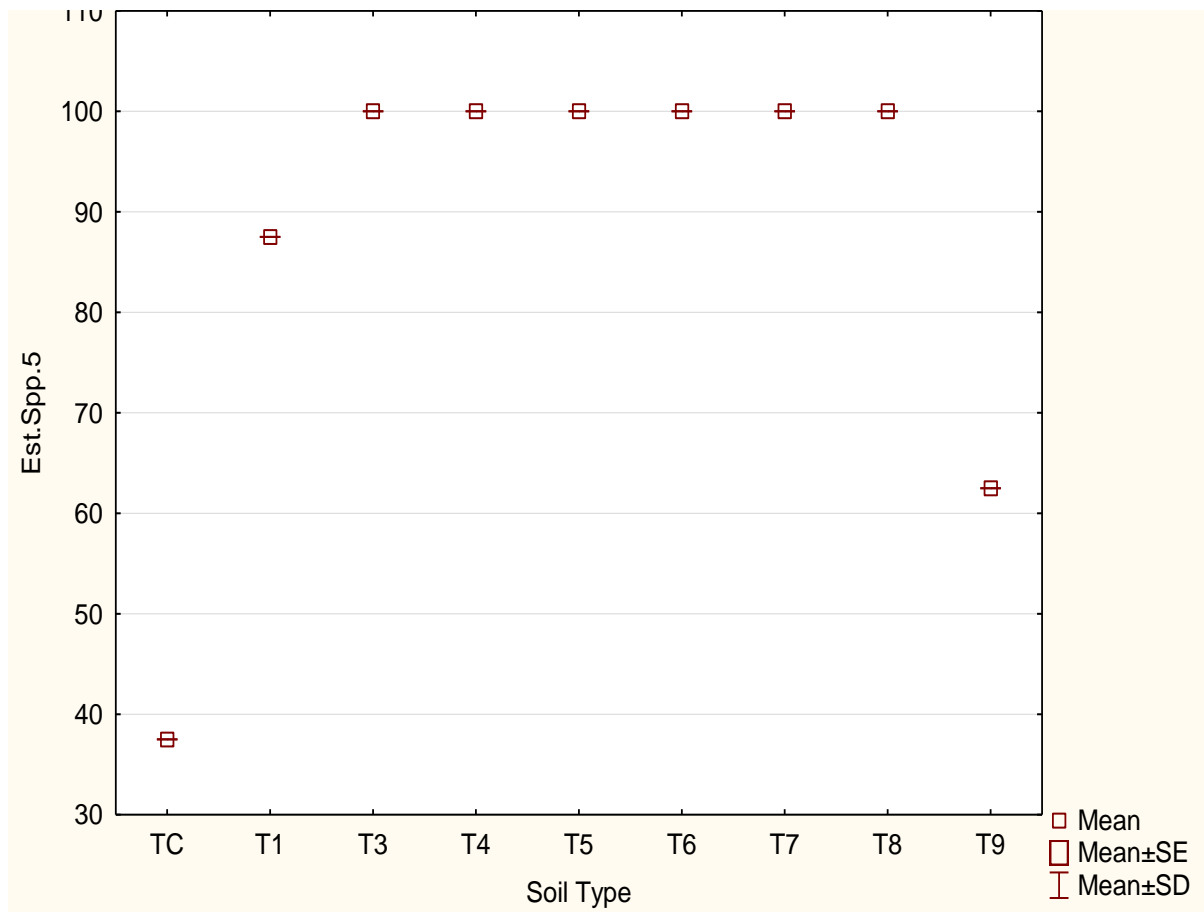


Figure 4.15: Percentage establishment of Species 5 (Poor man's Lucerne/ *Sericea lespedeza* var. Au-grazer)

As depicted in Figure 4.15, Species 5 (Poor man's Lucerne, Au-grazer) showed significant establishment in all of the tailings materials, except in the control medium and tailings 9 (fine coal). Significant differences ($P < 0.05$) were calculated for the latter two mediums. As for overall establishment a minimum of 37.5% and a maximum of 100% with an average of 86.9% were achieved.

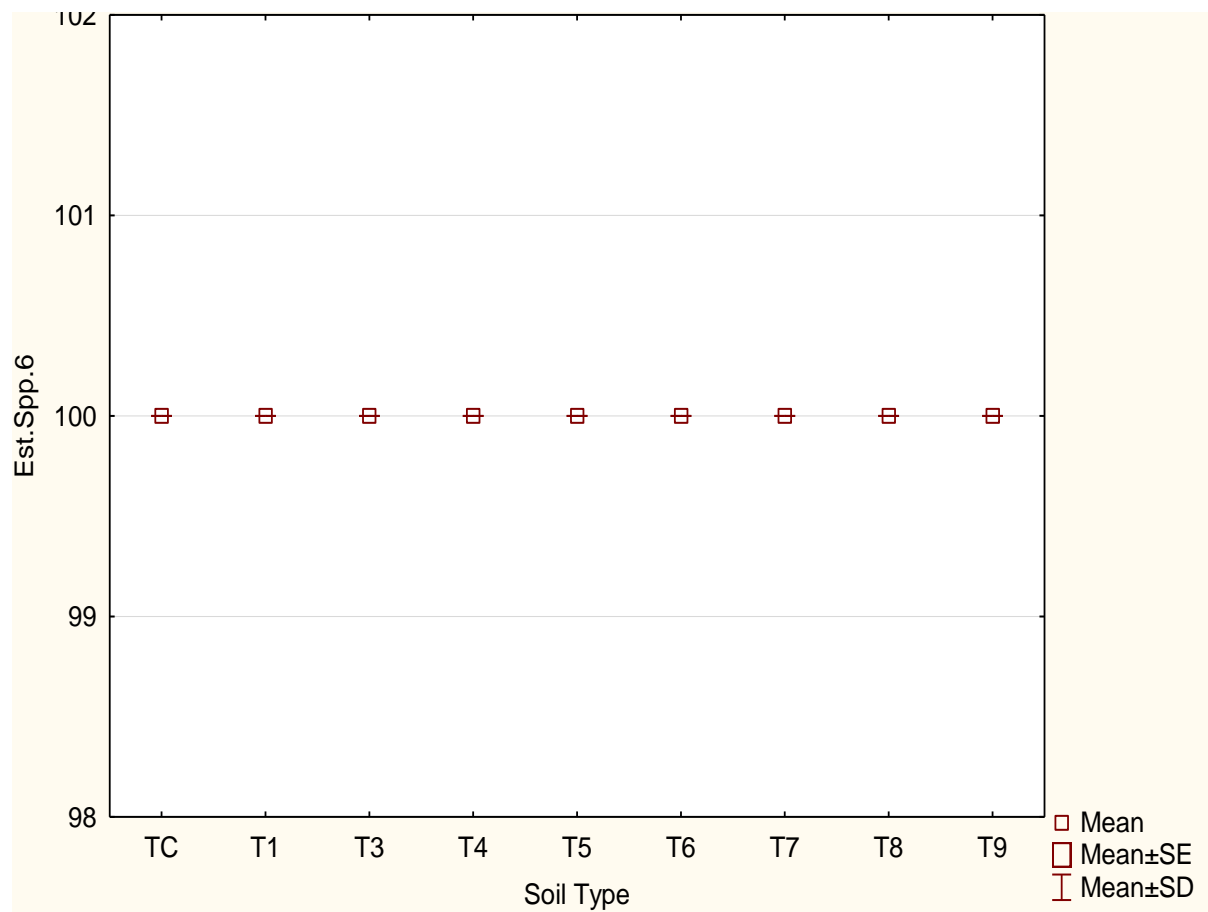


Figure 4.16: Percentage establishment of Species 6 (*Lucerne/Medicago sativa*)

No statistical differences were calculated for Species 6 and, as depicted in Figure 4.16, it is evident that Species 6 showed 100% establishment within all of the tailings materials and the control medium. As for overall establishment a minimum of 100% and a maximum of 100% with an average of 100% were achieved.

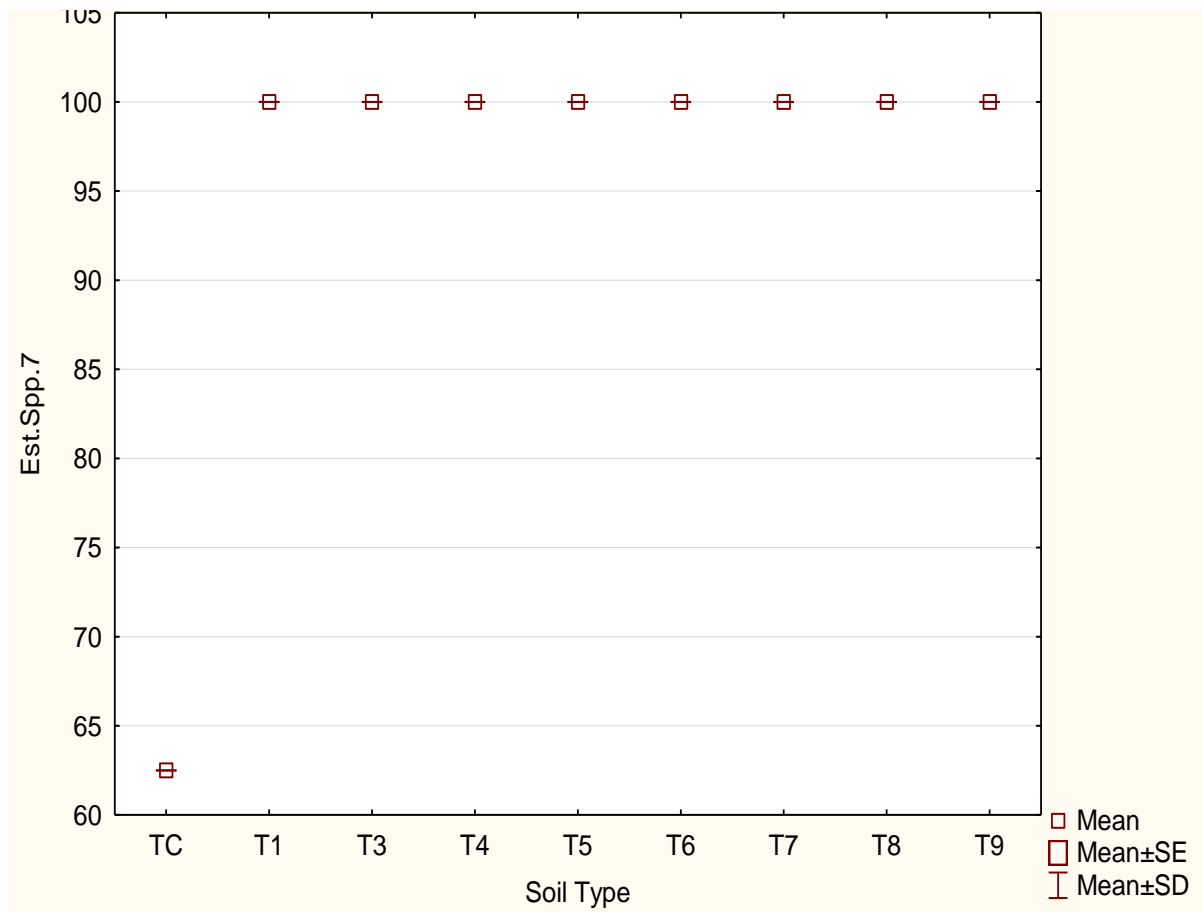


Figure 4.17: Percentage establishment of Species 7 (Tree Lucerne/*Chamaecytisus palmensis*)

Establishment of Species 7 was significant in all of the tailings materials but poor in the control material. The most differences ($P < 0.05$) were calculated for the control material. The establishment of Species 7 in the control material differed significantly from the tailings material ($P < 0.05$). As for overall establishment a minimum of 62.5% and a maximum of 100% with an average of 95.6% were achieved.

Table 4.3 provides a summary of the data from the establishment graphs for each tailings type and each species established therein.

Table 4.3: Percentage establishment for every species in different tailings materials

Tailings material	Species	Percentage Establishment (%)
Hutton Soil	1 (<i>E. elephantina</i>)	50
Hutton Soil	2 (<i>V. unguiculata</i>)	25
Hutton Soil	4 (<i>S. lespedeza</i> var. Au-louton)	50
Hutton Soil	5 (<i>S. lespedeza</i> var. Au-grazer)	37.5
Hutton Soil	6 (<i>M. Sativa</i>)	100
Hutton Soil	7 (<i>C. Palmensis</i>)	62.5
Gypsum	1 (<i>E. elephantina</i>)	37.5
Gypsum	2 (<i>V. unguiculata</i>)	62.5
Gypsum	4 (<i>S. lespedeza</i> var. Au-louton)	100
Gypsum	5 (<i>S. lespedeza</i> var. Au-grazer)	87.5
Gypsum	6 (<i>M. Sativa</i>)	100
Gypsum	7 (<i>C. Palmensis</i>)	100
Gold >1% Pyrite	1 (<i>E. elephantina</i>)	25
Gold >1% Pyrite	2 (<i>V. unguiculata</i>)	75
Gold >1% Pyrite	4 (<i>S. lespedeza</i> var. Au-louton)	75
Gold >1% Pyrite	5 (<i>S. lespedeza</i> var. Au-grazer)	100
Gold >1% Pyrite	6 (<i>M. Sativa</i>)	100
Gold >1% Pyrite	7 (<i>C. Palmensis</i>)	100
Platinum	1 (<i>E. elephantina</i>)	25
Platinum	2 (<i>V. unguiculata</i>)	100
Platinum	4 (<i>S. lespedeza</i> var. Au-louton)	50
Platinum	5 (<i>S. lespedeza</i> var. Au-grazer)	100
Platinum	6 (<i>M. Sativa</i>)	100
Platinum	7 (<i>C. Palmensis</i>)	100
Kimberlite	1 (<i>E. elephantina</i>)	12.5
Kimberlite	2 (<i>V. unguiculata</i>)	100
Kimberlite	4 (<i>S. lespedeza</i> var. Au-louton)	87.5
Kimberlite	5 (<i>S. lespedeza</i> var. Au-grazer)	100
Kimberlite	6 (<i>M. Sativa</i>)	100
Kimberlite	7 (<i>C. Palmensis</i>)	100

Coal discard	1 (<i>E. elephantina</i>)	37.5
Coal discard	2 (<i>V. unguiculata</i>)	87.5
Coal discard	4 (<i>S. lespedeza</i> var. Au-louton)	87.5
Coal discard	5 (<i>S. lespedeza</i> var. Au-grazer)	100
Coal discard	6 (<i>M. Sativa</i>)	100
Coal discard	7 (<i>C. Palmensis</i>)	100
Fluorspar	1 (<i>E. elephantina</i>)	25
Fluorspar	4 (<i>S. lespedeza</i> var. Au-louton)	50
Fluorspar	5 (<i>S. lespedeza</i> var. Au-grazer)	100
Fluorspar	6 (<i>M. Sativa</i>)	100
Fluorspar	7 (<i>C. Palmensis</i>)	100
Andalusite	1 (<i>E. elephantina</i>)	37.5
Andalusite	2 (<i>V. unguiculata</i>)	62.5
Andalusite	4 (<i>S. lespedeza</i> var. Au-louton)	100
Andalusite	5 (<i>S. lespedeza</i> var. Au-grazer)	100
Andalusite	6 (<i>M. Sativa</i>)	100
Andalusite	7 (<i>C. Palmensis</i>)	100
Fine coal	1 (<i>E. elephantina</i>)	50
Fine coal	2 (<i>V. unguiculata</i>)	62.5
Fine coal	4 (<i>S. lespedeza</i> var. Au-louton)	62.5
Fine coal	5 (<i>S. lespedeza</i> var. Au-grazer)	62.5
Fine coal	6 (<i>M. Sativa</i>)	100
Fine coal	7 (<i>C. Palmensis</i>)	100

4.4 Chemical data analysis

CANOCO Principal Component Analysis (PCA) (Figure 4.18) was done on the NO₃ production of both sampling series, as well as on chemical and physical parameters in order to determine whether there is an association between these factors. The first ordination axis had an Eigenvalue of 38.63% which indicated the amount of variance in the dataset. Clay content and electrical conductivity (EC) had positive correlations with the first ordination axis and therefor explained most data variance. The positive correlations of the clay content and EC with the first ordination axis are coupled together with their strongly positive correlation with one another. The second ordination axis had an Eigenvalue of

26.63%, indicating the amount of variance in the chemical dataset of both the NO₃ sampling series. Both the NO₃ sampling series and pH(KCl) correlated positively with the second ordination axis. NO₃ production correlates negatively to pH(KCl) which states that there are little influence between these two factors. According to Figure 4.18, it is evident that from all of the abovementioned factors, clay content had the strongest association with NO₃ production; whereas, the chemical parameters after amelioration (EC and pH) had a weak association with NO₃ production.

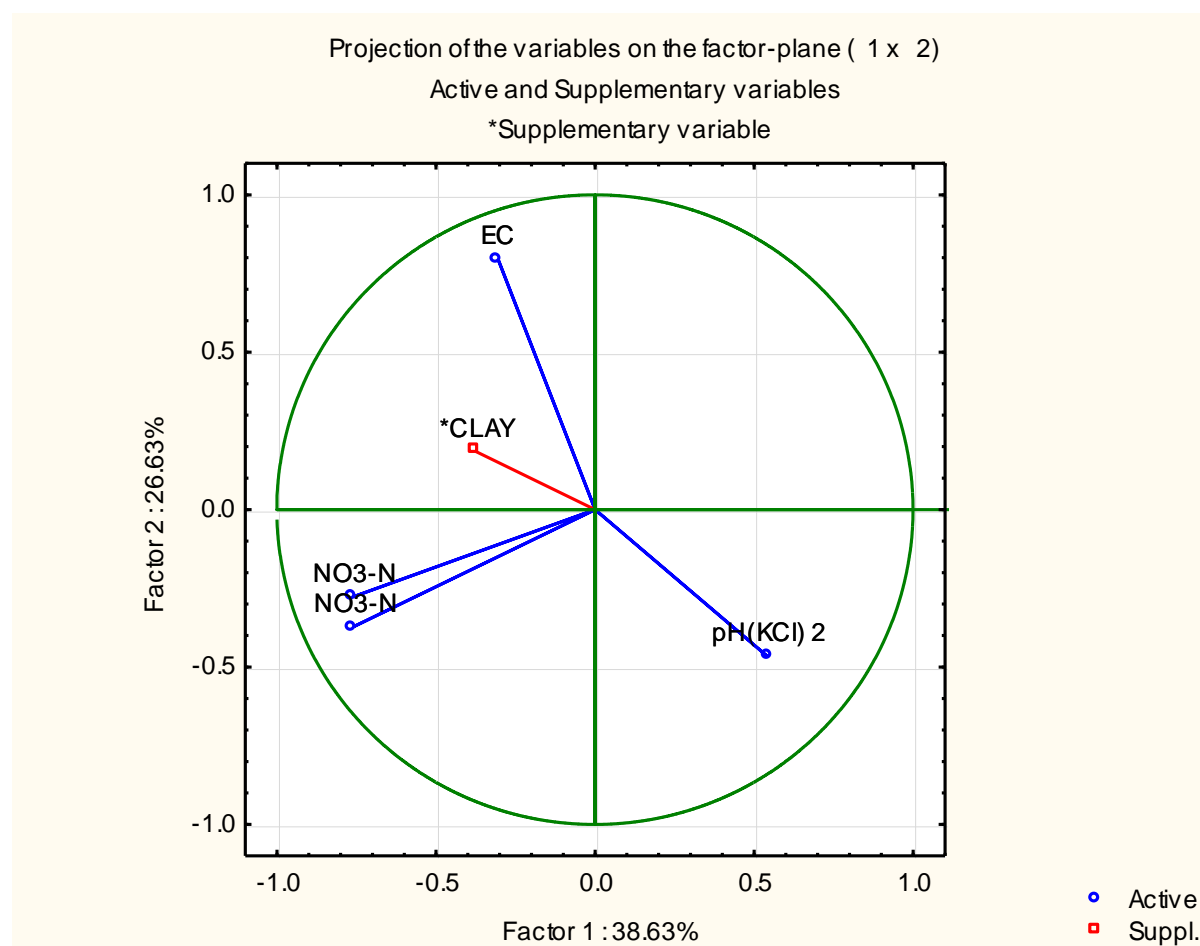


Figure 4.18: Principal Component Analysis (PCA) for the NO₃ production of both sampling series, as well as physical and chemical parameters

One of the main focus areas of this study was to determine whether the selected legume species, in conjunction with their associated symbionts, still had the ability to fixate atmospheric nitrogen into forms that were more readily available to plants. As mentioned in Chapter 2, one of these forms is NO₃ released into the surrounding soil environment (Mulder et al., 2002).

The following graphs depict the NO_3 release into the substrate across the three sampling series. According to Salvagiotti et al. (2008), 50-60% of soybean nitrogen demand is produced by the nodules on the roots.

As depicted in Figure 4.19 for the control growth medium, it is evident that all of the species contributed to NO_3 enrichment. In some of the species, NO_3 enrichment amounts did not surpass the initial NO_3 value and this can be attributed to the fact that the legume used more NO_3 than it released.

Species 2 (*V. unguiculata*), 4 (*S. lespedeza* var. Au-louton) and 5 (*S. lespedeza* var. Au-grazer) had the highest fixating ability within the control medium. As stated in Chapter 3, higher NO_3 levels can be expected during the dormant phase (Series 2) due to release of NO_3 during this phase, but the finding was that this is species-specific and is not indicated by all of the species as a norm. For Species 6 (*M. sativa*), the latter is evident and although not surpassing the initial NO_3 value, the higher NO_3 value during sample Series 2 is an indication of active NO_3 release. The same is applicable for Species 7 (*C. palmensis*) and especially Species 5 (*S. lespedeza* var. Au-grazer).

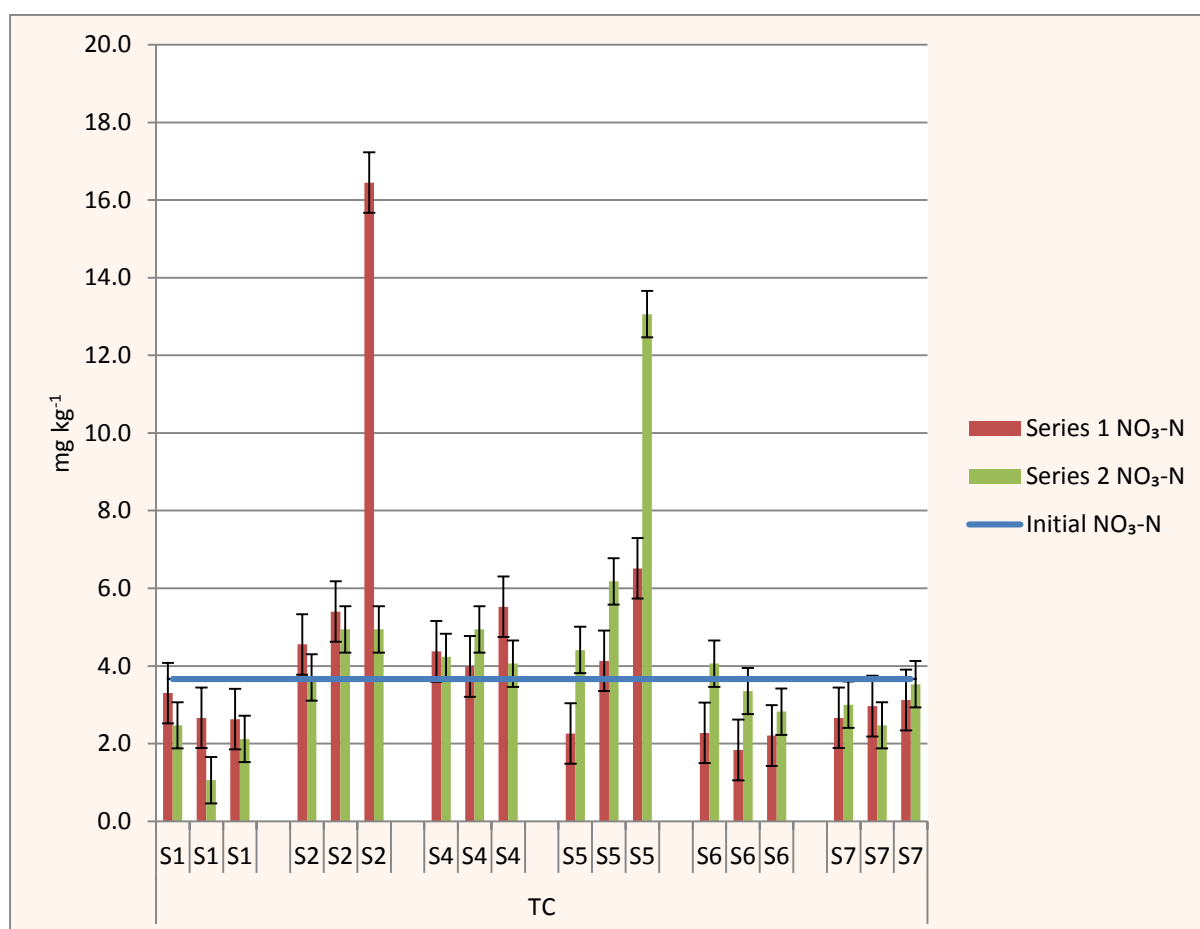


Figure 4.19: Chemical analysis depicting the initial NO_3 concentration, NO_3 during the peak growing period (Series 1), and NO_3 during the dormant state (Series 2) for the red sandy loam soil (TC)

From Figure 4.20, it is evident that all species surpassed the initial NO_3 levels through enrichment. As mentioned with TC, the statement that available NO_3 is higher during dormant phases is only valid for certain species. Within the gypsum tailings, this is only valid for Species 5 (*S. lespedeza* var. Au-grazer), which had a higher NO_3 value during the second sampling series. It is also evident that Species 2 (*V. unguiculata*), 6 (*M. sativa*) and 7 (*C. palmensis*) performed better within Tailings 1 (gypsum). Therefore, for gypsum tailings, the latter species used in this study can be recommended as the most suitable species for NO_3 enrichment in this tailings material.

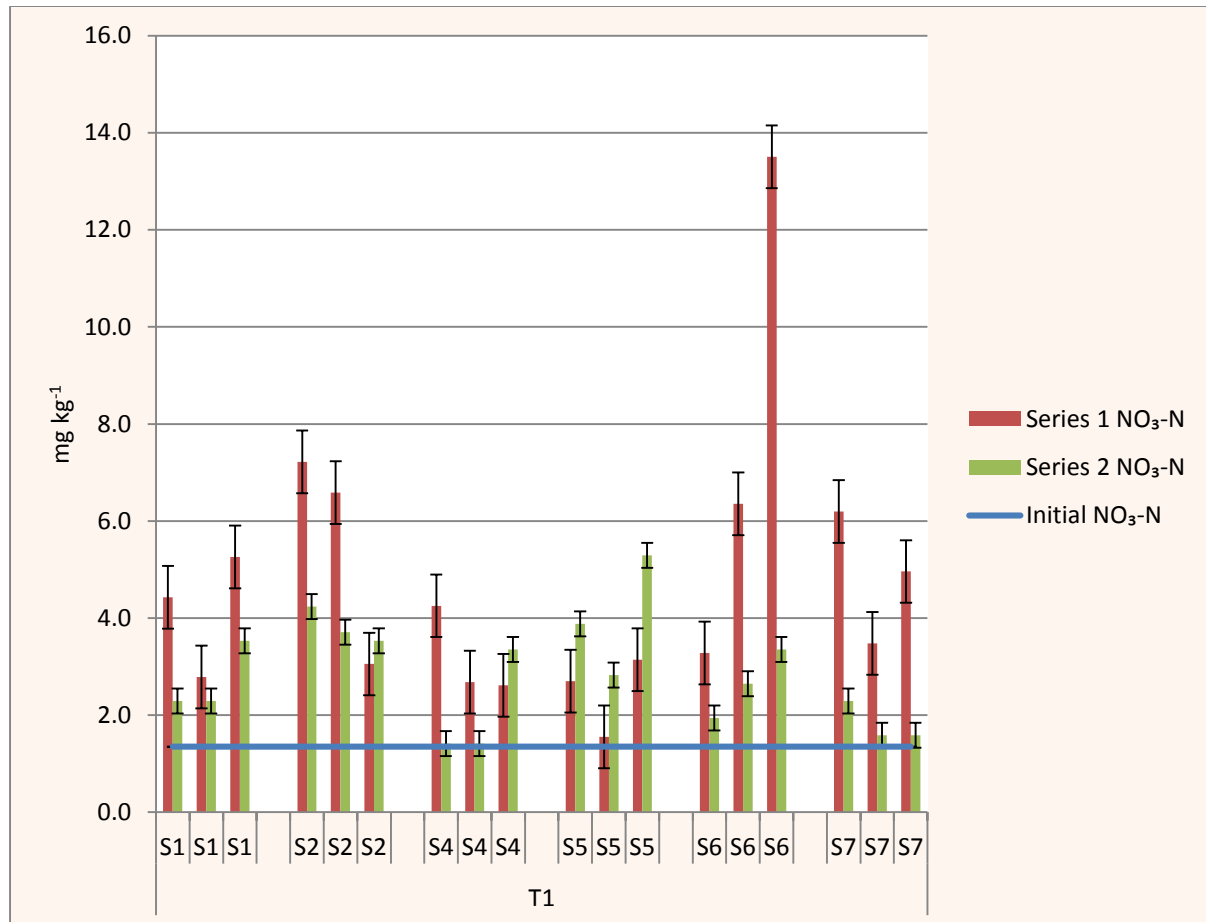


Figure 4.20: Chemical analysis depicting the initial NO_3 concentration, NO_3 during the peak growing period (Series 1) and NO_3 during the dormant state (Series 2) for the gypsum tailings material (T1)

The data presented by Figure 4.21 indicate that for Tailings 3, the initial NO_3 content was much higher than that of the first experimental sampling values. This can possibly be ascribed to the fact that there was a source of nitrogen that elevated the NO_3 content of the soil at the source area. After the soil was left to settle, it most likely leached out or was used by the plants during establishment. The persistence of NO_3 through all of the sampling series is an indication that fixation contributes to the available NO_3

in the tailings. This is further emphasised by Species 5 (*S. lespedeza* var. Au-grazer) and 6 (*M. sativa*) which had a higher NO₃ value within one replicate during the second sample series.

Therefore, for the gold tailings material, the following species used during this study can be recommended for NO₃ enrichment: *E. elephantina*, *S. lespedeza*, *M. sativa* and *C. palmensis*.

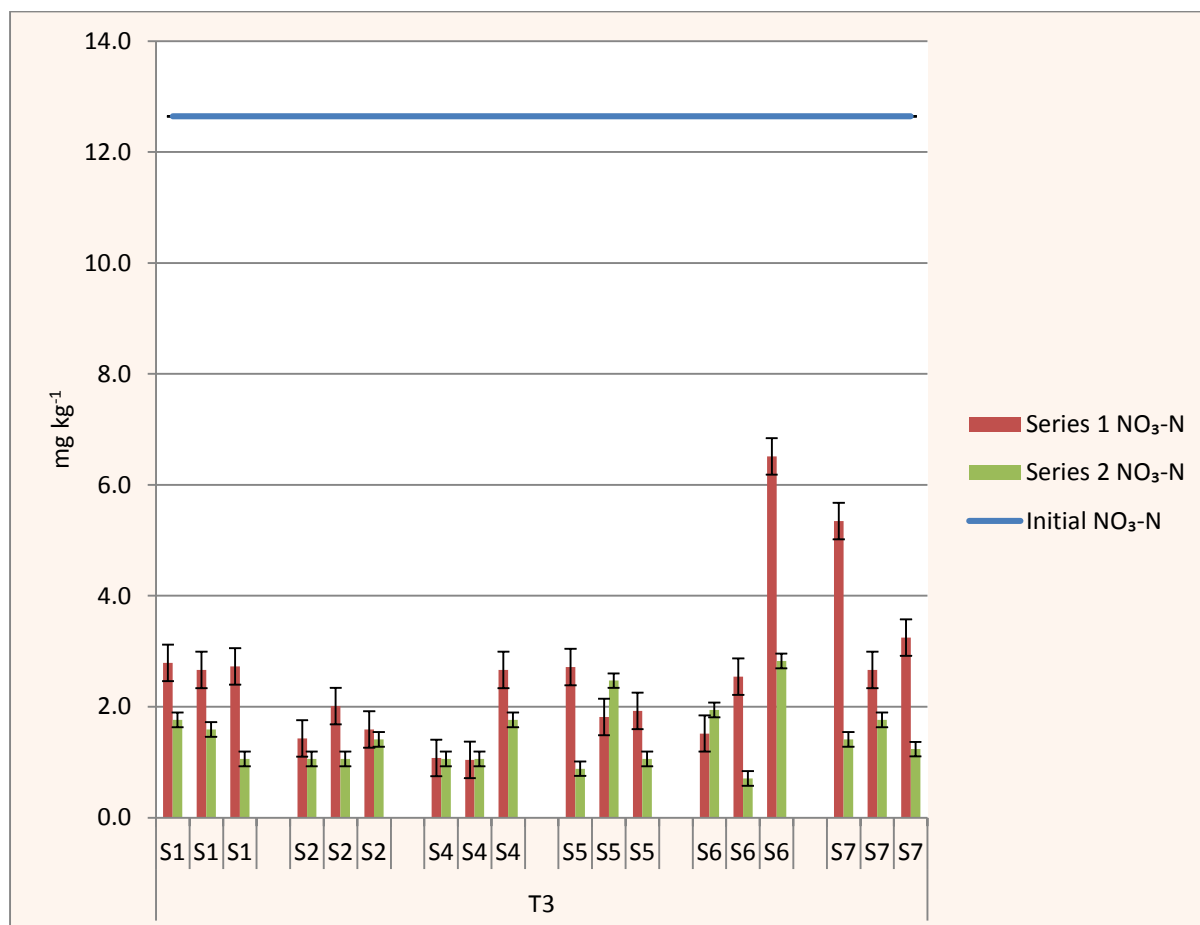


Figure 4.21: Chemical analysis depicting the initial NO₃ concentration, NO₃ during the peak growing period (Series 1), and NO₃ during the dormant state (Series 2) for the gold tailings material with more than 1% pyrite (T3)

As depicted in Figure 4.22, it is evident that the initial NO₃ value is higher than that of the experimental sampling values in most samples.

This material falls within the sandy class and therefore this material is characterized by high leaching abilities. This will in turn cause nutrients like NO₃ to be leached out easily and this can have a definite effect on NO₃ sampling results.

The substantial occurrence of NO₃ throughout all of the sampling series is an indication that NO₃ is present at the plants growing in the material. This is further supported by the fact that Species 1 (*E. elephantina*), 2 (*V. unguiculata*), 4 (*S. lespedeza* var. Au-louton), 5 (*S. lespedeza* var. Au-grazer) and 7 (*C. palmensis*) indicate higher NO₃ levels during the second sampling series.

As for the platinum tailings, all the species used in this study can be planted in order to produce NO₃.

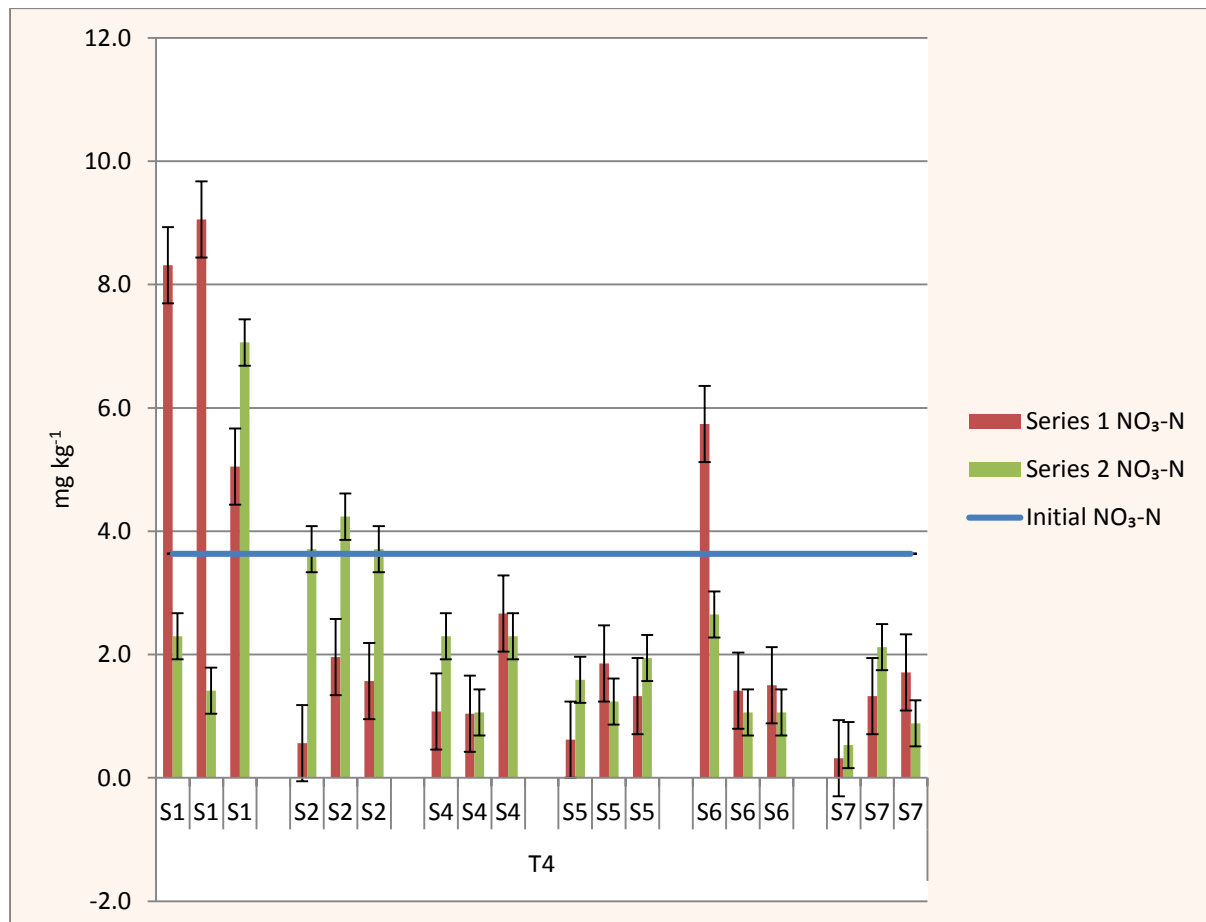


Figure 4.22: Chemical analysis depicting the initial NO₃ concentration, NO₃ during the peak growing period (Series 1), and NO₃ during the dormant state (Series 2) for the platinum tailings material (T4)

The data presented in Figure 4.23 indicate that for Tailings five (Kimberlitic tailings), the initial NO₃ content was surpassed by all of the species' sample series values. This is a positive indication that all of the species have the ability to enrich the medium with NO₃.

The data further emphasised that Species 1 (*E. elephantina*), 2 (*V. unguiculata*), 4 (*S. lespedeza* var. Au-louton) and 5 (*S. lespedeza* var. Au-grazer) contributed to higher NO₃ values during the second sampling series, which was during the plants' dormant phase. Therefore, the latter supports the

statement in Chapter 3 regarding NO₃ release during the dormant phase. All of the species performed well in this tailings material.

Although Species 1 (*E. elephantina*) had significant NO₃ enrichment potential, it only had a 12% establishment success within this medium and would not be prescribed for kimberlitic tailings. Therefore, for the kimberlitic tailings material, the following species can be recommended for NO₃ fixation: *E. elephantina*, *V. unguiculata*, *S. lespedeza*, *M. sativa* and *C. palmensis*.

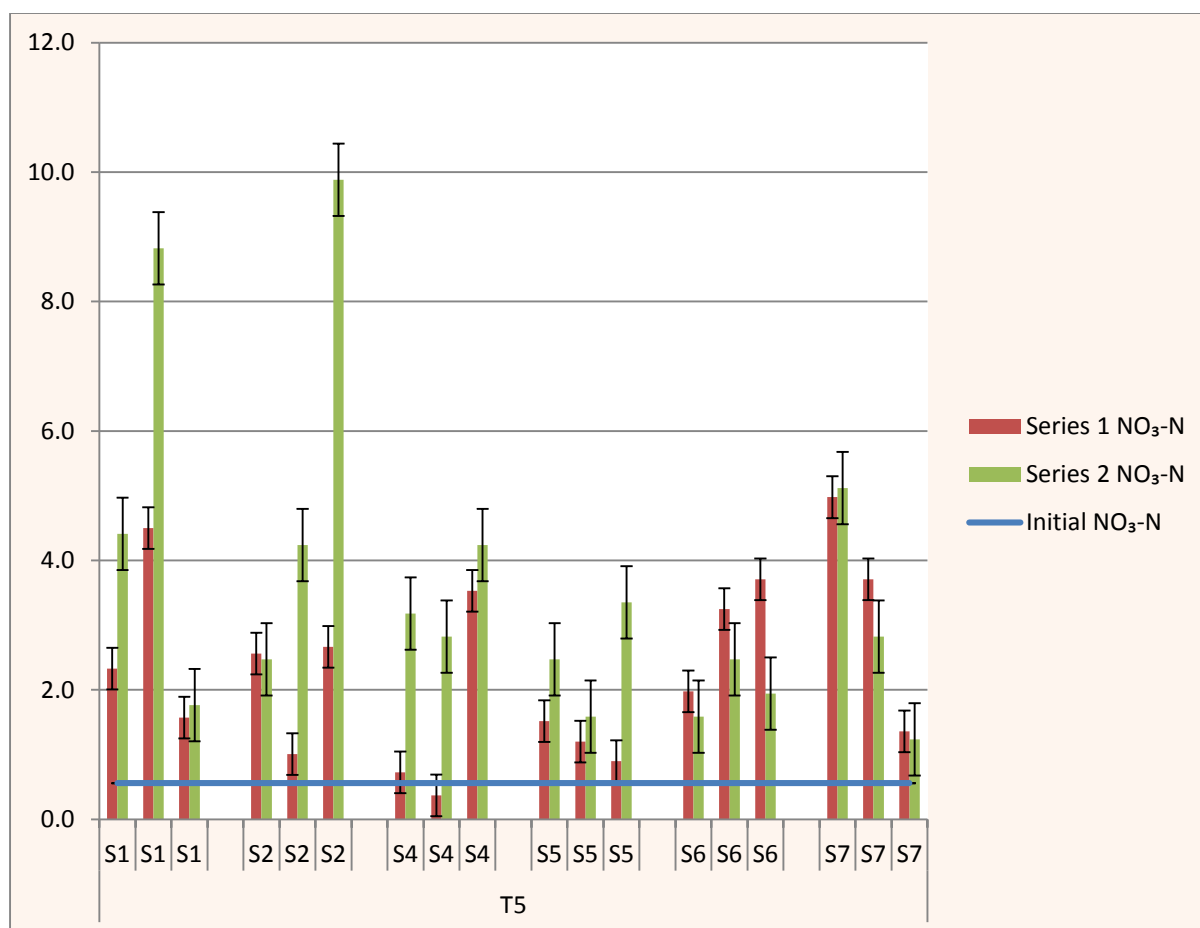


Figure 4.23: Chemical analysis depicting the initial NO₃ concentration, NO₃ during the peak growing period (Series 1), and NO₃ during the dormant state (Series 2) for the kimberlitic tailings material (T5)

As depicted in the data (Figure 4.24), it is evident that the initial NO₃ value for Tailings six (coal discard) was surpassed by both of the sampling series' NO₃ values. This emphasises the fact that all of the species depicted in Figure 4.24 had the ability to successfully enrich the coal discard material with NO₃.

It is also evident that Species 4 (*S. lespedeza* var. *Au-louton*), 6 (*M. sativa*) and 7 (*C. palmensis*) yielded higher NO₃ values during the dormant phase, indicating NO₃ release into the surrounding material.

Therefore, all of the species in this study can be recommended as positive nitrogen contributors within coal discard tailings.

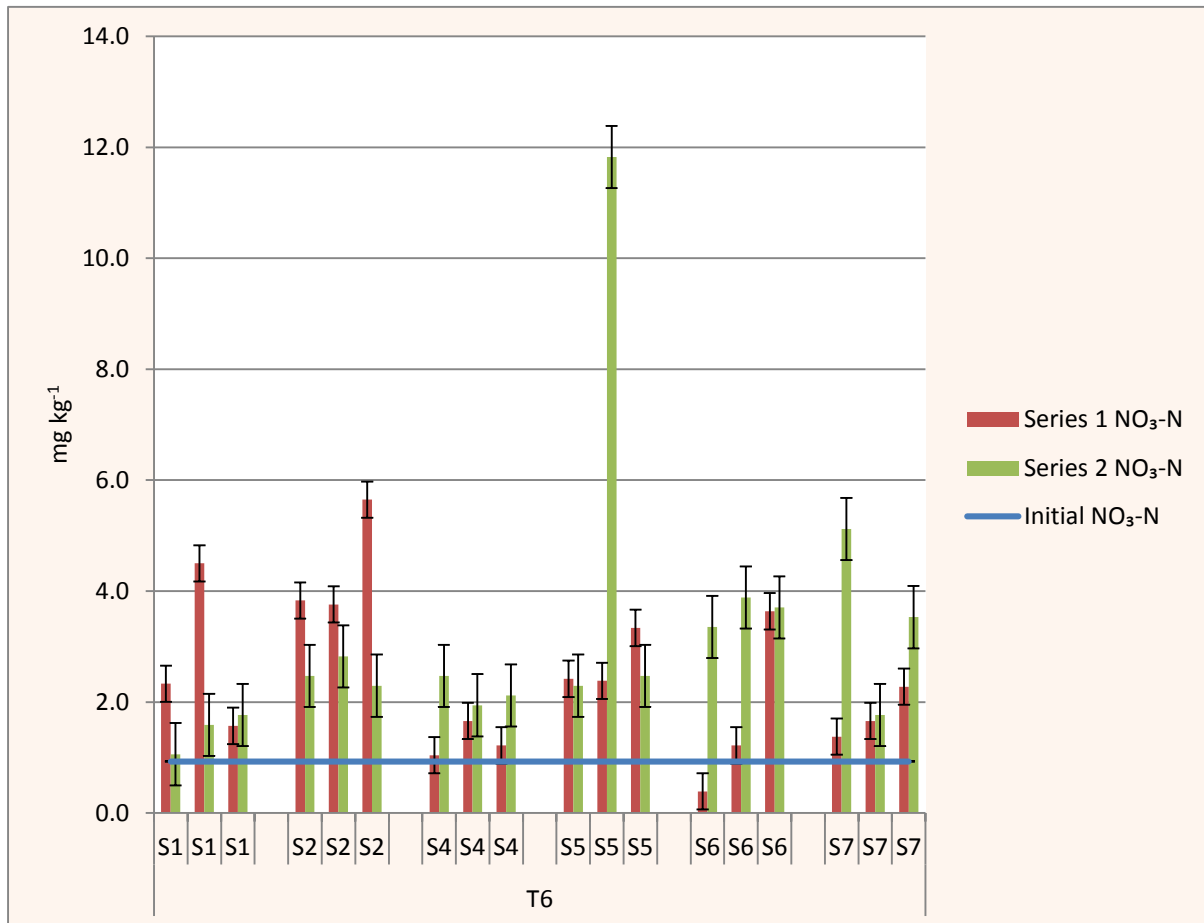


Figure 4.24: Chemical analysis depicting the initial NO₃ concentration, NO₃ during the peak growing period (Series 1), and NO₃ during the dormant state (Series 2) for the coal discard tailings material (T6)

The data presented in Figure 4.25 for Tailings seven (fluorspar tailings) indicate that the initial NO₃ content of Tailings seven was greatly surpassed by the NO₃ values. As stated earlier, this is a positive indication that the species were contributing to higher nitrogen levels within the growth medium. As for Species 4 (*S. lespedeza* var. Au-grazer) and 6 (*M. sativa*), the greatest NO₃ release was during the dormant phase.

Therefore, all of the species that established within the fluorspar tailings can be recommended to increase soil-available NO₃.

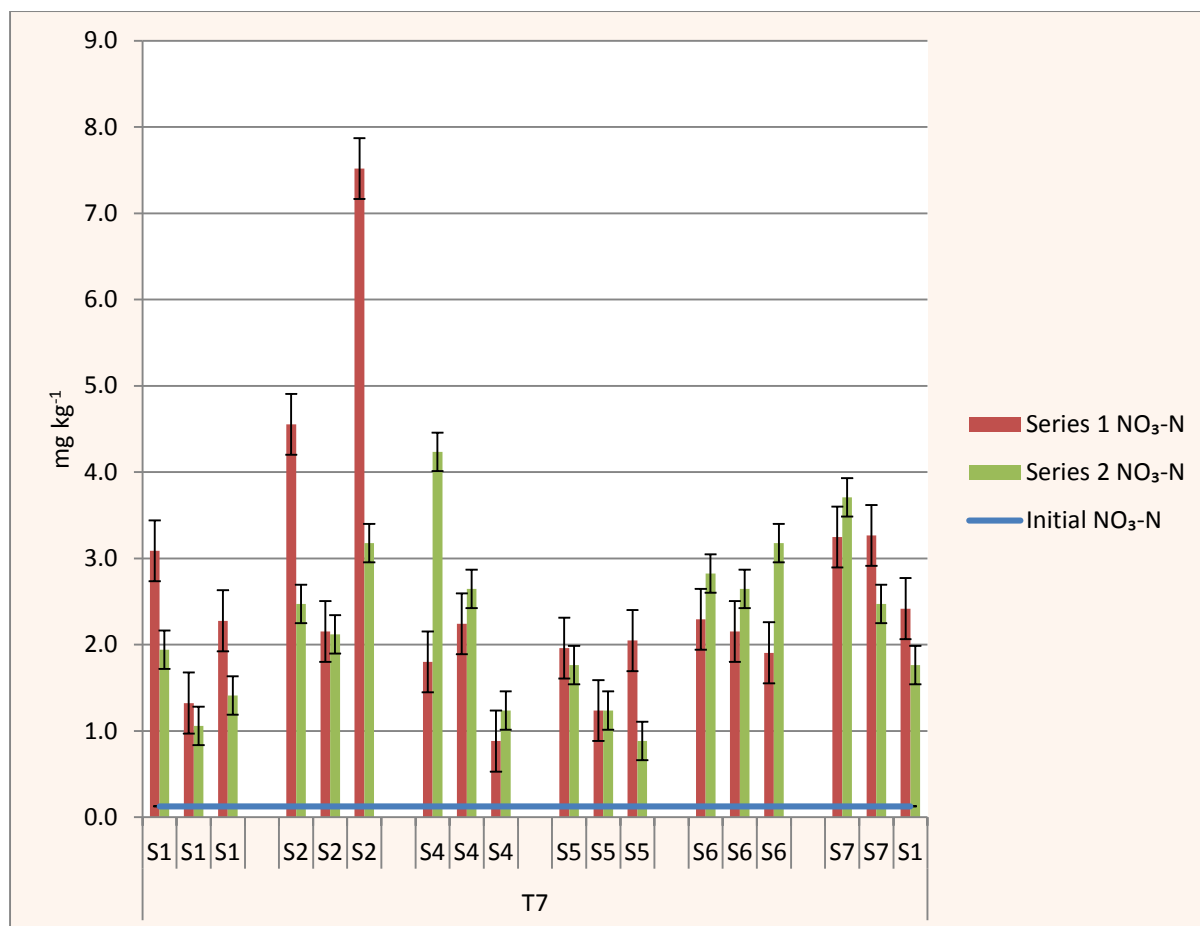


Figure 4.25: Chemical analysis depicting the initial NO₃ concentration, NO₃ during the peak growing period (Series 1), and NO₃ during the dormant state (Series 2) for the fluorspar tailings material (T7)

As depicted in Figure 4.26, it is evident that all of the experimental NO₃ values surpassed those of the initial value for Tailings 8 (andalusite). Species 2 (*V. unguiculata*), 4 (*S. lespedeza* var. Au-louton) and 5 (*S. lespedeza* var. Au-grazer) had higher NO₃ levels during the dormant phase than in the peak growing season, indicating NO₃ release during this period.

Therefore, Species 1 (*E. elephantina*), 2 (*V. unguiculata*), 4 (*S. lespedeza* var. Au-louton) and 5 (*S. lespedeza* var. Au-grazer) can be recommended to enrich NO₃ in andalusite tailings.

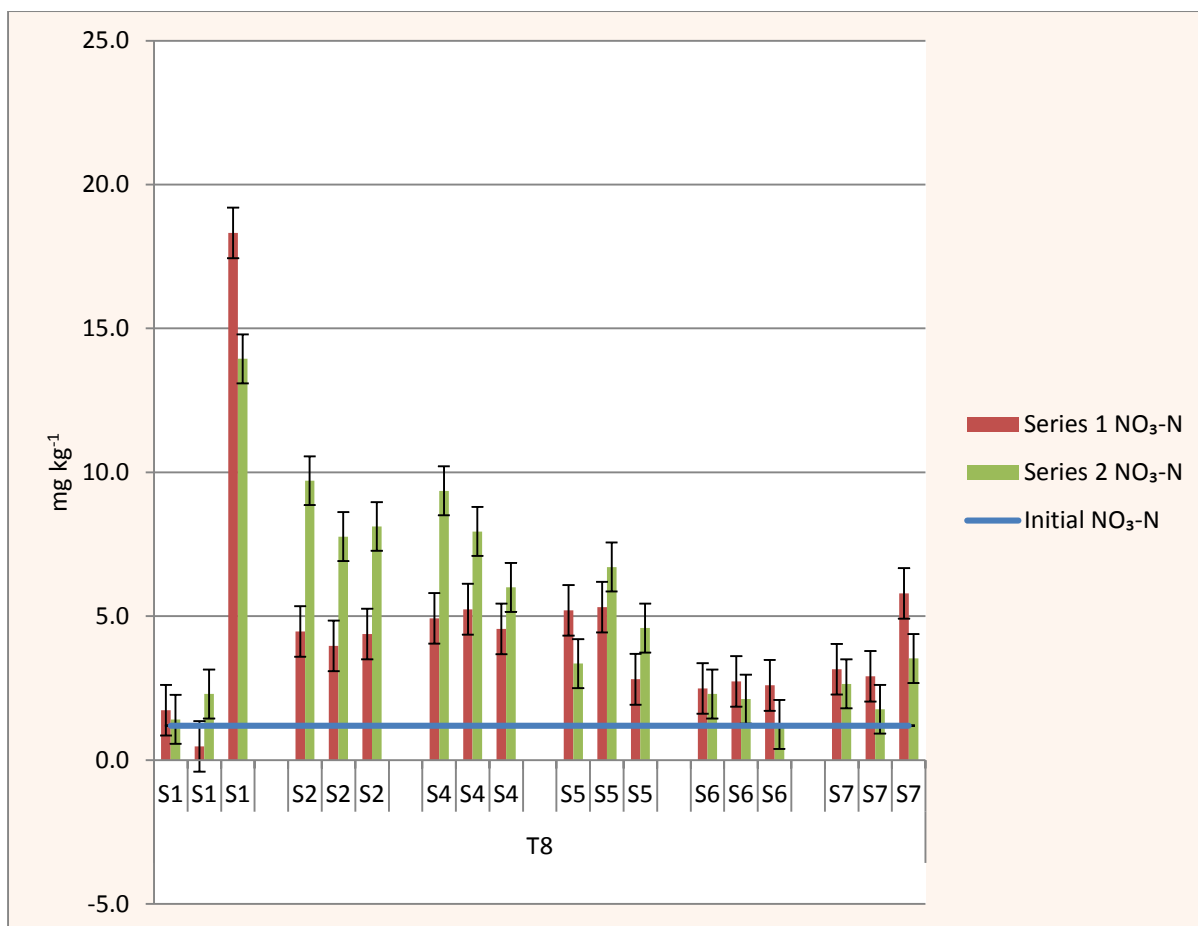


Figure 4.26: Chemical analysis depicting the initial NO₃ concentration, NO₃ during the peak growing period (Series 1), and NO₃ during the dormant state (Series 2) for the andalusite tailings material (T8)

The data presented for Tailings 9 in Figure 4.27 indicate that all of the experimental sampling values were greater than those of the initial NO₃ value, therefore indicating a positive contribution to soil available NO₃. As for Species 2 (*V. unguiculata*), 4 (*S. lespedeza* var. Au-louton) and 5 (*S. lespedeza* var. Au-grazer), the NO₃ values were higher during the dormant phase.

From the data, it is evident that Species 2 (*V. unguiculata*), 4 (*S. lespedeza* var. Au-louton) and 5 (*S. lespedeza* var. Au-grazer) can be recommended for fine coal tailings as positive NO₃ contributors.

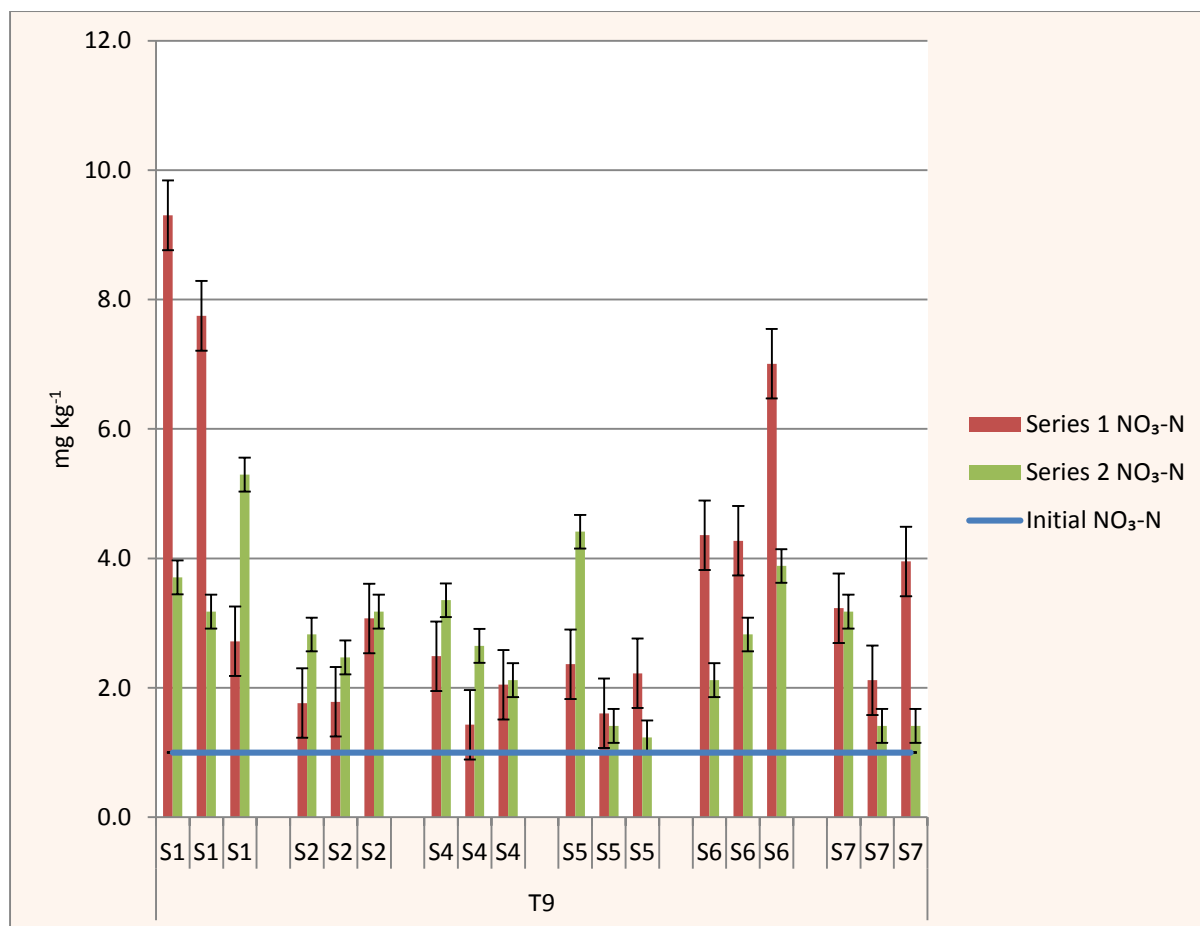


Figure 4.27: Chemical analysis depicting the initial NO₃ concentration, NO₃ during the peak growing period (Series 1), and NO₃ during the dormant state (Series 2) for the fine coal tailings material (T9)

According to the Spearman Rank Order Correlation Matrix it is evident that there are correlations between certain substrate chemical factors and NO₃ production (Table 4.4); these correlations will be discussed accordingly. Establishment potential of species 1 (*E. elephantina*) over all the treatments negatively correlated with pH treatment, correlation > -0.8 and as $P < 0.05$, the finding is statistical proven. As for species 1 it also correlated positively with the [Al] content, therefore the establishment potential of this species will increase with a decrease in [Al]; the finding was statistically accurate $P < 0.05$. Species 5 (*S. lespedeza* var. Au-grazer) over all the treatments positively correlated with the pH treatment with a correlation > 0.8 and as $P < 0.05$, the finding is statistically proven. As the pH is corrected, this species establishment will increase. In the same way establishment will decrease with an increase in [Al] as it negatively correlated with [Al] and the finding was statistically accurate $P < 0.05$.

NO₃ production in both sampling series had a correlation with pH, EC (initial) and phosphate levels ($P < 0.05$). Porter and Sheridan (1980) found that pH values below 4 had detrimental effects on the nitrogen-fixing process in root nodules. With this implication in mind, these factors can possibly influence NO₃ production. According to Zahran (2001), nodule activity is strongly influenced by the

EC value of the substrate. According to Sprent (1999) and Good (2000), these plants have a high P requirement. As for the correlation between pH and NO₃ release, Good (2000) states that legume species are directly affected by pH values under 5.5 and above 8. Zahran (2001) also found that saline mediums decrease the growth of legumes; therefore, the correlation between the EC values and the establishment potential of certain species is relevant.

Table 4.4: Spearman Rank Order Correlation of NO₃ production, species and chemical characteristics

All Groups Spearman Rank Order Correlations (Spreadsheet1) MD pairwise deleted Marked correlations are significant at p <.05000													
	NO ₃ -N	NO ₃ -N	Est.Spp.1	Est.Spp.2	Est.Spp.4	Est.Spp.5	Est.Spp.7	pH(KCl)Initial	pH(KCl)After	EC (Initial)	EC (After)	P(Bray 1)	Al
NO ₃ -N	1.000000	0.377645	0.318545	-0.153336	0.204429	-0.288488	-0.166976	-0.209843	-0.353050	-0.204701	0.085913	0.306304	0.090287
NO ₃ -N	0.377645	1.000000	0.305326	0.044590	0.134781	-0.237757	-0.250603	-0.220960	-0.310740	-0.315968	-0.133667	0.249980	0.091046
Est.Spp.1	0.318545	0.305326	1.000000	-0.077388	0.011387	-0.807358	-0.578503	-0.761750	-0.838472	-0.072671	0.139073	0.141621	0.600425
Est.Spp.2	-0.153336	0.044590	-0.077388	1.000000	-0.229737	-0.036667	-0.439606	0.089936	-0.036144	0.021027	-0.579098	-0.073577	0.182101
Est.Spp.4	0.204429	0.134781	0.011387	-0.229737	1.000000	0.170433	0.423478	-0.380191	-0.251670	-0.061035	0.482702	0.430256	0.149208
Est.Spp.5	-0.288488	-0.237757	-0.807358	-0.036667	0.170433	1.000000	0.654596	0.522810	0.856870	0.135257	-0.003632	-0.434742	-0.601629
Est.Spp.7	-0.166976	-0.250603	-0.578503	-0.439606	0.423478	0.654596	1.000000	0.192501	0.493664	0.411700	0.558445	-0.295318	-0.119716
pH(KCl)Initial	-0.209843	-0.220960	-0.761750	0.089936	-0.380191	0.522810	0.192501	1.000000	0.695899	-0.317787	-0.518919	-0.069847	-0.817460
pH(KCl)After	-0.353050	-0.310740	-0.838472	-0.036144	-0.251670	0.856870	0.493664	0.695899	1.000000	0.179564	-0.073476	-0.504359	-0.634328
EC (Initial)	-0.204701	-0.315968	-0.072671	0.021027	-0.061035	0.135257	0.411700	-0.317787	0.179564	1.000000	0.635578	-0.635059	0.555869
EC (After)	0.085913	-0.133667	0.139073	-0.579098	0.482702	-0.003632	0.558445	-0.518919	-0.073476	0.635578	1.000000	-0.116760	0.478737
P(Bray 1)	0.306304	0.249980	0.141621	-0.073577	0.430256	-0.434742	-0.295318	-0.069847	-0.504359	-0.635059	-0.116760	1.000000	-0.037972
Al	0.090287	0.091046	0.600425	0.182101	0.149208	-0.601629	-0.119716	-0.817460	-0.634328	0.555869	0.478737	-0.037972	1.000000

4.5 Root nodulation

This section was included in the study in order to provide a visual reference for whether nodulation occurred in the different tailings materials. This section by no means contributed scientifically to the study. Due to the amount of species and tailings materials in this study, it was decided that the species that established the best in all of the mediums would be examined. Species 6 (*Medicago sativa*), with a 100% establishment within all of the tailings types and control medium, was identified for the root nodulation examination.

The scale that was used is a South African five rand coin with a diameter of 26 mm. It was evident throughout all of the examined roots that nodules were present, with variation found only in the abundance thereof.

In Figure 4.28, it is evident that large clumps of nodules as well as single bodies occurred along the root hairs of Species 6 in the gypsum tailings.



Figure 4.28: Nodule formation on Lucerne (*M. sativa*) in gypsum tailings material (T1)

As for Species 6 in the gold tailings with more than 1% pyrite, it was evident that nodulation occurred in clumps near the plant base (Figure 4.29).



Figure 4.29: Nodule formation on Lucerne (*M. sativa*) in gold tailings material with more than 1% pyrite (T3)

As depicted in Figure 4.30, it is evident that single-bodied nodules occurred more at the plant base just beneath the soil surface and that they were more elongated in Tailings 4 (platinum tailings).



Figure 4.30: Nodule formation on Lucerne (*M. sativa*) in platinum tailings material (T4)

In the kimberlite tailings, it was evident that sparse single-bodied nodules occurred among the finer roots at the base of the plant (Figure 4.31).



Figure 4.31: Nodule formation on Lucerne (*M. sativa*) in kimberlitic tailings material (T5)

In Figure 4.32, it is evident that elongated nodules occurred among the fine roots, with small clumps starting to develop. The same can be said for Tailings 7 (fluorspar tailings) which had a similar nodulation pattern.



Figure 4.32: Nodule formation on Lucerne (*M. sativa*) in coal discard tailings material (T6)



Figure 4.33: Nodule formation on Lucerne (*M. sativa*) in fluorspar tailings material (T7)

As depicted in Figure 4.34, it is evident that the fine root development within the andalusite tailings was much less than in the other tailings materials. The few fine roots that were present had development of rather large nodule clumps on them.



Figure 4.34: Nodule formation on Lucerne (*M. sativa*) in andalusite tailings material (T8)

As for Tailings 9 (fine coal tailings), it was observed that nodulation occurred the least compared to the other tailings materials, with only a few nodules present on the fine root system.



Figure 4.35: Sparse nodule formation on Lucerne (*M. sativa*) in fine coal tailings material (T9)

Chapter 5: Conclusion and recommendations

5.1 Introduction

The mining industry as a whole has an array of challenges regarding its “tail end” of production (or better known as the tailings material that it produces). These constraints include, firstly, the stockpiling or storage of tailings in a stable and safe manner; secondly, the stabilization thereof in order to maintain its intended geomorphological structure; and lastly, obtaining a closure certificate.

One of the most important outcomes from this study is the improvement of the stabilization of these structures and the further development of standard practice techniques. Numerous studies and development strategies have been trialled and tested over the years by both universities and institutional companies in order to get the grassing technique to what it is today. Due to the complexity of this field, continual studies are of utmost importance to achieve continual improvement in the standard practice in the industry.

The following sections will briefly discuss the outcomes of this study and highlight how the use of legumes can possibly contribute to a more sustainable herbaceous layer on the surface areas of TSFs.

5.2 Vegetation establishment potential

The main objective of this section was to establish whether the selected plant species would be able to germinate in the different materials, and secondly, whether they would reach maturity. As discussed in Chapter 3, no germination was observed within Tailings 2, which was the gold tailings with <1% pyrite. There is no immediate explanation for this phenomenon and due to the timeline of this study; Tailings 2 were discarded for this experiment. As for the other tailings types and the control medium, varying establishment successes were observed. Species 3 did not show any germination throughout any of the tailings materials and therefore this species was also discarded from the study; as such, it cannot be recommended for establishment on any of the tested tailings. Establishment for each material will be discussed in chronological sequence.

As for vegetation establishment on the gypsum tailings or Tailings 1, Species 4 (Poor man's Lucerne, Au-louton), 5 (Poor man's Lucerne, Au-grazer), 6 (Lucerne) and 7 (Tree Lucerne) had the most successful establishment of all of the species ($P < 0.05$), and can therefore be recommended for legume establishment in this tailings type.

Tailings 3, or the gold tailings with >1% pyrite, had an average vegetation establishment of 79%, where all of the species except the Elephant's root showed satisfactory establishment ($P > 0.05$); therefore, Species 2, 4, 5, 6 and 7 can be recommended for establishment due to ($P < 0.05$). Although Species 1 (Elephant's root) had lower establishment rates, it does not mean that it needs to be excluded from seed

mixtures; rather, it is an indication that seeding density needs to be elevated if a specific germination rate is required.

When looking at the vegetation establishment success in Tailings 4, or the platinum tailings, it had an overall establishment of 79%. Although all species germinated and grew, Species 2 (Cowpea), 5 (Poor man's Lucerne, Au-grazer), 6 (Lucerne) and 7 (Tree Lucerne) showed exceptional establishment rates ($P < 0.05$).

The overall establishment success in Tailings 5, or kimberlitic tailings, was 83%. Although establishment was evident throughout all of the species, Species 2 (Cowpea), 5 (Poor man's Lucerne, Au-grazer) and 7 (Tree Lucerne) showed 100% establishment, having the least differences in establishment success ($P < 0.05$). Therefore, these legume species can be recommended for this type of tailings material.

As for Tailings 6, or coal discard tailings, an overall establishment of 85% was achieved throughout all of the species. The species that showed the best establishment, the least differences ($P < 0.05$), were Species 2 (Cowpea), 4 (Poor man's Lucerne, Au-louton), 5 (Poor man's Lucerne, Au-grazer) and 7 (Tree Lucerne). It can therefore be anticipated that these species will have satisfactory establishment in this material in future rehabilitation projects.

An overall vegetation establishment of 75% was achieved in Tailings 7, or fluorspar tailings, with Species 4 (Poor man's Lucerne, Au-louton), 5 (Poor man's Lucerne, Au-grazer) and 7 (Tree Lucerne) having the highest establishment potential - with 100% establishment each and ($P < 0.05$).

For Tailings 8, or andalusite tailings, there was an average establishment of 79% with Species 4 (Poor man's Lucerne, Au-louton), 5 (Poor man's Lucerne, Au-grazer) and 7 (Tree Lucerne) that had the highest establishment rates and the least differences in establishment ($P < 0.05$).

Lastly, Tailings 9, or fine coal tailings, had an average establishment of 73% throughout all of the species, with Species 2 (Cowpea), 4 (Poor man's Lucerne, Au-louton), 5 (poor man's Lucerne, Au-grazer) and 7 (Tree Lucerne) that had the best establishment ($P < 0.05$). As for Species 1, (Elephant's root), the establishment was on average lower ($P > 0.05$) than all of the other species in all of the materials; this can possibly be an indication of lower germination potential of the seeds.

The main finding of this section was that all of the species did establish, albeit at differing percentages and that all of them can be included in rehabilitation projects as legume species.

5.3 Ability to enrich soil with nitrate

The aim of this section is to provide a brief discussion of the NO₃-enriching abilities of the different species in the different growth mediums. This will then serve as further motivation to establish these species in the different tailings materials.

As for Tailings 1 (gypsum), Cowpea, Lucerne and Tree lucerne can be recommended. For Tailings 2 (gold with <1% pyrite), Elephant's root, both Poor man's Lucerne varieties, Lucerne and Tree Lucerne can be recommended. As for Tailings 4 (platinum), Elephant's root, Cowpea, both Poor man's Lucerne varieties and Tree Lucerne contributed significantly to soil-available NO₃ levels. In Tailings 5 (kimberlite), Elephant's root, Cowpea and both Poor man's Lucerne varieties can be recommended. As for Tailings 6 (coal discard), Poor man's Lucerne, Lucerne and Tree Lucerne can be recommended. For Tailings 7 (fluorspar), Poor man's Lucerne, Lucerne and Tree Lucerne can be recommended. For Tailings 8 (andalusite), Elephant's root, Cowpea and both Poor man's Lucerne varieties can be recommended. As for Tailings 9 (fine coal), Cowpea and both Poor man's Lucerne varieties can be recommended.

As for NO₃ enrichment by the legumes in the different tailings materials and the control material, it was evident in all experimental samples that this biological process did take place. Therefore, this serves as an indication that the selected legume species for this study fulfilled their ability to supply NO₃ that can be used by the surrounding plants. As such, these plants can be recommended as legume species for future rehabilitation projects.

5.4 Limitations of the study

Due to the fact that this study was conducted under semi-controlled conditions and in bags, a cautionary note is necessary on the extrapolation of the findings to field conditions; this is due to the fact that field conditions vary greatly from those of the study, and therefore restrict the value of the results (Titshall et al. 2013). Titshall et al. (2013) further states that these types of experiments increase our understanding of certain behaviours and selected conditions. In order to get the full understanding of this study, it needs to be carried out under field conditions and then compared to the current study.

Due to certain constraints regarding the management of, and environmental factors relating to, these unnatural structures, Gibson (1971) suggests that the most appropriate strains need to be selected and that management practices need to be adapted in order to minimize the effects of these factors. Gibson (1971) also states that the ideal temperature for the optimal functioning of fixation is between 20 and 30°C; therefore, this is an important aspect to keep cognisance of in management practices.

5.5 Recommendations

As derived from the NO₃ fixation results, certain species were identified that had a better ability to enrich the surrounding growth medium with elevated levels of NO₃. For each tailings material, the identified species differed, and these species are listed in Table 5.1. This table must be read and interpreted in conjunction with Table 4.3, which depicts the establishment success rate.

Table 5.1: Recommended species for NO₃ enrichment within the different tailings materials

Tailings	Species					
	1 <i>E. elephantina</i>	2 <i>V. unguiculata</i>	4 <i>S. lespedeza var. Au- louton</i>	5 <i>S. lespedeza var. Au- garzer</i>	6 <i>M. sativa</i>	7 <i>C. palmensis</i>
1 (Gypsum)		x			x	x
2 (Gold < 1% Pyrite)	x		x	x	x	x
4 (Platinum)	x	x	x	x		x
5 (Kimberlite)	x	x	x	x		
6 (Coal Discard)			x		x	x
7 (Fluorspar)			x		x	x
8 (Andalusite)	x	x	x	x		
9 (Fine Coal)		x	x	x		

5.6 Recommendations for further research

- In order to determine whether these species will grow under field conditions on a TSF, an extended study needs to follow to determine establishment success.
- There are many different legume species in South Africa and only a very small amount was used during this study. Further studies can identify more species that are suitable for growth on these tailings materials.
- A more chemically-orientated study that focuses on nodule development success based on the chemical constraints posed by the tailings materials, and also to determine establishment constraints.

Chapter 6: References

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