Integrating ARD Prevention and Mine Waste Minimisation:

Soil Fabrication from Coal Waste

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

Rehabilitation post mining requires revegetation of lands and potentially of waste dumps. Both require availability of fertile soils, typically excavated and transported from surrounding areas, with associated cost and environmental impact. Stockpiled soils may contribute to the soils available for revegetation but are typically no longer fertile and not in sufficient amount to comply with the requirements of the Environmental Management and Rehabilitation Programme.

In this study, we focus on the potential for technosols, fabricated from fine coal waste and organic amendments, with or without bioaugmentation, to be constructed and applied as a soil substitute. This work is motivated threefold: by the need for increasing quantities of high quality, regenerative fertile soils for mine rehabilitation; by the desire to reduce excavation of natural soils and its associated environmental impact; and by the potential to re-purpose low-risk mine wastes, to both reduce the need for excavation of virgin materials and to reduce the waste disposal burden.

We, and others, have previously reported the proof of concept for production of technosols from fine coal waste through the addition of organic amendments and that these technosols support the growth of plant species typically used in mine site rehabilitation.

In this study, we expand our previous proof of concept through the following studies: the characterisation of fine coal waste fractions and stockpiled soils to determine key properties for soil fabrication; the upgrading of fine coal waste fractions by solid-solid separation for coal recovery and technosol manufacture were needed; the characterisation of organic material fractions as amendments; the volume of organic amendment required for optimal soil properties; the benefit of biostimulation through microbial inoculation; and the determination of soil properties and associated plant growth in response to varying the above factors, all through experimental studies. In addition, through desktop study we address the sourcing and availability of varied amendments and the consideration of the economics of technosols production relative to excavation and burrowing or the use of commercial fertile topsoils.

Three coal fine waste streams were characterised in terms of particle size distribution, sulphur grade, mineral content, organic C and ARD potential. Of these, one ultrafine stream was considered not suitable for soil manufacture owing to the large amendment requirements. The further two streams were processed further.

In considering the upgrading of the fine coal waste materials, we used a two-stage flotation approach to recover saleable coal and to reduce sulphur loading in the resultant tailings for soil manufacture. Here it is necessary to minimise flotation chemicals and we demonstrated this for a thickener underflow stream and a flotation tailings stream. Coal of sufficient quality was recovered from both streams. Based on S characterisation, only the thickener underflow slurry was exposed to 2nd stage flotation for S reduction which was satisfactorily demonstrated.

Owing to ensuring technosols, manufacture is a simple and low cost process and based on the findings of the two stage flotation process, it was decided to explore technosols using the ‘as is’ coal waste. In the first experimental stage, a wide range of technosols were manufactured from both CW streams using a range of amendments. These were characterised in terms of the resultant soil properties as the soils matured over a 60 day period, prior to planting.

In the second experimental study, technosols were fabricated from the coal waste tailings by combination with malt residue as amendment at 0, 2.5, 5.0 and 7.5% loading by volume. Controls included fertile topsoil, compost and stockpiled soil. Further bioaugmentation was achieved by inoculation using a commercial microbial inoculum EM ProSoil. *E. tef* was used as a model plant species in these studies as it is an ‘early coloniser’ in rehabilitation and use of a single species was preferred

for accuracy of pot studies. Results were collected in terms of soil characteristics pre- and post-planting, microbial consortia within the soil, plant growth and quality of plants resulting.

While pure coal waste supported plant growth, growth was poor owing to the poor compact soil structure, the low P and K concentrations and the tendency for water run-off. On applying 2.5, 5.0 and 7.5% malt residue, best soil structure and growth was attained with 2.5 to 5.0% malt residue. MR was both a structural and a nutritional amendment. The water holding capacity of the technosols was improved by MR addition as was the nutrient availability. The plant growth at 2.5 and 5.0% MR was similar and reflected that previously reported in our studies (Amaral Filho et al., 2020). Specifically, amendment at this ratio stimulated roots and shoots. Bioaugmentation through the inoculation of the soils with a microbial cocktail resulted in improved performance owing to the role of the microorganisms in decomposition of organic matter and cycling of nutrients. Potting soil formed a valuable positive control, demonstrating the best growth while compost proved an insufficient matrix for growth of plants owing to its low water holding capacity and nutrient content. The *E. tef* plant and seeds harvested was shown to be usable as animal feed. While Na, Mg, Ca, Zn and Fe were increased within the desired concentration range, no metal accumulation with risk of ecotoxicity was found.

In the production of technosols, expansion of the range of amendments to those readily available in the mining region is considered with manure, compost, the organic fraction of domestic waste, sewage sludge, malt residue from the brewery and solid fraction from pulp and paper mills wastewater processing being considered for potential use. Their potential volumes within a 160 km radius of the coal fields is considered to assess applicability and transport costs. Based on these findings, it is recommended that further amendments be explored experimentally for technosols manufacture. It is estimated that through re-purposing malt residue to soil fabrication some 1000 to 1800 ha can be rehabilitated per year. Additional amendments are required to extend this.

The economic and environmental aspects of technosols manufacture were considered, focusing primarily on the raw material requirements and transport. The use of a high quality fertile soil was compared with excavation and stockpiled soils and with technosols manufacture. The economic feasibility of technosols manufacture is good where MR is obtained as a free good and transport from the two Gauteng breweries accounted for. While the use of microbial inocula is beneficial, the commercially available inocula are cost-prohibitive. Hence, the production of inocula for bio-augmentation and bio-stimulation to reduce the costs of commercially available cultures should be assessed, as there is potential for onsite production, reducing the need for formulation costs for transport and storge. This is routinely used in organic farming applications, hence is practical.

Further, through soil design, it is demonstrated that the water requirements for revegetation can be tailored, a feature of importance in the water-scarce regions.

On considering the performance of the manufactured technosols and economic and environmental considerations, this approach shows promise for the effective rehabilitation of mine lands and the reduction of coal waste for disposal.

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ACRONYMS & ABBREVIATIONS

ABA	Acid Base Accounting
AMF	arbuscular mycorrhizal fungi
ANC	Acid Neutralisation Capacity
ARD	Acid Rock Drainage
CW	Coal waste
D_{50}	PSD where 50% of the material is less than the stated size
D_{80}	PSD where 80% of the material is less than the stated size
DNA	Deoxyribonucleic acid
EcM	actomycorhizal fungi
EIA	Environmental Impact Assessment
EMP	Environmental Management Programme
FAO (UN)	Food and Agriculture Organization of the United Nations
FC	Field Capacity
FDA	Fluorescein Diacetate
MPA	Maximum Potential Acidity
NAF	Non-Acid Forming
NAG	Net Acid Generation
NAPP	Net Acid Production Potential
PAF	Potentially Acid Forming
PGPR	Plant Growth Promoting Rhizosphere Bacteria
PSD	Particle Size Distribution
qPCR	quantitative Polymerase Chain Reaction
ROM	Run-Of-Mine
SIR	Substrate Induced Respiration
SOM	Soil Organic Matter
TOC	Total Organic Carbon
UCT	University of Cape Town
WHC	Water Holding Capacity
WRB	World Reference Base for Soil Resources
WRC	Water Research Commission, South Africa

GLOSSARY OF TERMS

Circular Economy	In contrast to the linear economy, a circular economy aims to close the gap between the production and the natural ecosystems' cycles by reducing the waste generation and maximizing natural resource efficiency through re-use, recycling and re-purposing
Field Capacity	"Field capacity" is used interchangeably with the terms "water holding capacity" and "water retention capacity" and defined as the soil moisture or water content held in soil after drainage of excess water and material decrease of the rate of downward movement
Flotation Tailings	A ore slurry generated after mineral concentration in flotation units, usually with no market value
Geomembrane	Very low permeability synthetic membrane liner or barrier used with any geotechnical engineering related material so as to control fluid (liquid or gas) migration in a human-made project, structure, or system. Geomembranes are made from relatively thin continuous polymeric sheets, but they can also be made from the impregnation of geotextiles with asphalt, elastomer or polymer sprays, or as multilayered bitumen geocomposites. Continuous polymer sheet geomembranes are, by far, the most common.
Pedogenesis	Process of evolution of soil under the influence of various physical, biological, climatic, and geological factors. Pedogenesis occurs via a series of changes to the parent material, all of which lead to the formation of layers of soil, also called soil horizons. These layers can then be separated on the basis of the composition and other physical properties.
Slurry	Mixture of water and ultrafine mineral particles with flow properties allowing its transport by flow
Soil Ameliorant	A material or substance that, when incorporated in the soil, enhances its quality and productivity by improving the physical and bio-chemical conditions
Technosols	Fabricated soils with properties and pedogenesis dominated by their technical origin. They contain significant amounts of artefacts (made or extracted from the earth by humans), some sort of geotechnical liner, or are sealed by technic hard rock (hard material created by humans, having properties unlike natural rock). They include soils constructed from wastes, pavements with their underlying unconsolidated materials, soils with geomembranes and constructed soils in human-made materials.
Topsoil	Upper and outermost layer of soil. This soil is fertile and has the highest concentration of organic matter and microorganisms
Ultrafines	Particles generated during the mineral beneficiation processes, usually with average diameter lower than 0.25 mm

1 BACKGROUND TO COAL WASTES AND FABRICATED SOILS

1.1 Introduction to Coal Wastes, their Legacy and Associated Opportunity

Despite the global trend towards a low-carbon economy, coal still accounts for 40% of electricity generation worldwide, and approximately 83% of electricity production in South Africa (Minerals Council South Africa, 2022). In the South African context, run-of-mine (ROM) coal is washed to reach market standards this leads to considerable quantities of waste material with increased sulphur and ash content and concomitant economic, environmental and social impacts (Falcon and Ham, 1988). Land disposal of coal processing wastes, including discards and ultra-fine slurry, blemishes the landscape and poses a risk to local communities, through its impact on air quality, soils and water sources as a result of dust and acid rock drainage (ARD) emissions (Bell et al., 2001). With over 60 million tonnes of material added to coal waste dumps in South Africa annually (Eberhard, 2015), it becomes increasingly important to manage the legacy waste deposits and to adopt new approaches to the handling of the streams of new waste to alleviate the environmental and social burden created by this industry (Harrison et al., 2013, 2020; Edraki et al., 2014).

Further to alleviation of the negative impact, the management of coal waste offers potential for retrieval of coal values and repurposing of associated “waste” materials to uses consistent with the industrial ecology and circular economy approaches. In regions such as the eMalahleni (Witbank) coal fields in South Africa, several local collieries are approaching the end of productive life. This requires the locus of production to shift away from coal processing to alternative economic activities. Further, in many areas there is pressure for coal mining to be phased out in favour of renewable energy, requiring both the shift in focus of economic activities and the framework in which to achieve responsible mine closure with long-term mitigation of environmental burden and with responsible use of resources. The coal industry in South Africa employed over 90,000 people in 2021 (Minerals Council South Africa, 2022), hence the combination of the depletion of coal fields and the move away from coal for energy as part of the low carbon economy opens up a need for options for a post-mining economy for the surrounding communities. Despite of all the efforts to move toward a mixed matrix, the energy sector in South Africa will rely on coal for at least more 2 or 3 decades, making the coal sector a crucial consideration in planning for and delivering just transitions towards a greener economy.

Both in ongoing active coal mining areas and where end-of-mine frameworks are sought, coal waste is best viewed as a multi-product resource rather than a waste product; this enables us to maximise a responsible mining approach. Through viewing the waste material as a resource with potential to provide economic, social and environmental benefit, resource productivity can be enhanced and environmental and social burden relieved. Re-processing of fine coal waste for thermal coal recovery has been explored in some places in South Africa; however, repurposing of the non-combustible fraction is not practised. Further up-grading of coal waste fractions, such as discards and fines, can augment the processing of the fractions of suitable calorific value, thus increasing the core coal product.

Increasingly, studies are being conducted into the reduction of the impact of coal processing waste through not only appropriate disposal but also through its valorisation and repurposing (Harrison et al., 2020; Harrison et al., 2013). The current waste disposal management, in a manner to constrain seepage and leachate, thereby complying with legal requirements, is both costly and leaves an ongoing liability into the future. Facilities for ARD treatment and treatment of neutral mine drainage to address metal- and salt-rich polluted waters are also costly and need to be run over excessive time frames of 10s to 100s of years owing to the persistent pollution. In preference, preventive approaches applied from the early stages of a mine project and throughout the project have potential for removal or reduction of legacy, enhanced resource efficiency and improved efficacy from a sustainable development perspective. This requires either risk mitigation through new approaches to use, re-purposing and valorisation of these mining wastes. Where the mining wastes are sulphidic, risk of oxidation of sulphidic minerals past life of mine can be prevented by removal of sulphide prior to re-purposing or disposal using mineral processing and metallurgical techniques (Amaral Filho et al., 2022; Harrison et al., 2020; Harrison et al., 2013; Machado and Schneider, 2008; Marcello et al., 2008; Soares et al., 2009; Tambwe et al., 2020). “Towards zero waste” strategies include both recovering lower volume products of added value and re-purposing the major coal ash fraction, examples of the latter being to fabricated soils (Amaral Filho et al., 2016, 2020; Firpo et al., 2015; Harrison et al. 2020b; Weiler et al., 2018) , road materials or construction materials (Argane et al., 2016; Santos et al., 2013).

By law, in South Africa, mining companies are required to rehabilitate the mine sites and mine land at the termination of the mining process to receive the mine closure certificate. The Environmental Impact Assessments (EIAs) developed to examine the environmental risks associated with mining developments, such as coal mine development, and the legislated Environmental Management Plans (EMPs) developed for mines to ensure delivery of risk mitigation, provide guidance on and bring attention to the economic, social and environmental value of waste management systems. Waste management schemes developed from these EIAs and EMPs are set up to prioritise sustainable development to minimise detrimental environmental impacts. They require the mine site to be returned to a state approaching the pre-mine state, thus requiring re-vegetation. However, the shortfall of topsoils to complete the rehabilitation of the mine site post mining according to the approved EMPs is one of the major concerns for compliance with the legal requirements.

It is proposed that a cornerstone of a well-considered sustainable development approach to mining centres on waste reduction through its re-purposing to other uses, following its processing, where necessary, to a largely benign state. This approach is accompanied by improved resource efficiency. One such approach is the fabricating of soil-like materials, otherwise known as fabricated soils or technosols. This simultaneously reduces the mass of mine waste abandoned in waste disposal facilities, overcomes the topsoil shortage and avoids the transportation of soils from borrow pits (Schad, 2018). These fabricated topsoils address the challenges related to mine waste disposal both by repurposing the waste into a useful growth substrate for land rehabilitation and by defining the topsoil mixture of substances containing carbon, nitrogen, phosphorus and mineral elements balanced to promote sustainable plant growth according to the local needs and conditions.

The use of a strategy for industrial and urban waste recycling to preserve natural soil resources through fabricating soils has been demonstrated. Rokia et al. (2014) demonstrated the feasibility of producing fabricated topsoils (technosols) using urban wastes, including earth materials and organic wastes. In terms of using mine waste for topsoil fabrication, Firpo (2015), Ginocchio et al. (2016), Weiler et al. (2018), Harrison et al. (2020b) and Amaral Filho et al. (2020) have shown that several wastes, including biosolids, algal biomass, wastes from steel industries, and food and beverage processing waste, can be applied to mine wastes to speed-up soil formation and are critical for the establishment of a self-sustainable plant cover and for root development.

In summary, this project addresses specific issues related to coal mine rehabilitation, associated environmental (and accompanying social) legacies and resource efficiency following mine activities:

- The excess of spoils and processing wastes requires containment facilities with inherent long-term liabilities. Their appropriate treatment and re-purposing can reduce or avoid this.
- The current use of very fine coal for power generation is limited by market and technology specifications.
- The shortage of topsoils to comply with rehabilitation plans at the mine site results in extra costs, carbon emissions from transport, and secondary environmental disturbance at borrow pits. Generation of such soil matrices on site has potential to mitigate these.
- The quality of soil used in the current rehabilitation strategies requires the use of fertilisers for sustaining long-term plant growth. Healthy soils can mitigate the extent of fertiliser use.

- The ARD treatment and mitigation strategies must comply with minimum standards, and are costly and energy consuming.
- Long-term environmental legacies are best avoided to ensure long-term efficacy of mine closure strategies.

In addressing these, this project is focussed on the purposing of the ash or mineral component of coal waste, drawn from coal fines or coal discards or, where appropriate and non-polluting, the ‘as is’ fine coal waste stream, to reduce mine waste disposal and to produce fertile soils, devoid of environmental legacy, for the rehabilitation of mine sites and associated mine lands in order to contribute positively to long-term rehabilitation of mine sites through application of nature-based sustainable environments.

1.2 Introduction to Soil Quality and Fabricated Soils

Soils play a key role in the environmental quality of the earth’s biosphere. When looking at soils, consideration must be given to multiple components, including mineral matter, organic matter, water, availability and transfer of gases, and living organisms such as earthworms, insects, bacteria, fungi, algae, and nematodes. Soils serve as an essential reservoir of water for plants and microorganisms and as a filtering and purifying medium through which water passes before collecting in underground and superficial water bodies. The soil quality is affected by a matrix of factors and is usually assessed using indicators that represent a selection of constituents, processes or conditions. Soil characteristics and quality depend highly on the parent materials to define geomorphology, hydrology and the kind of vegetation which should be introduced.

According to Doran et al. (2013), soil quality can be defined as the capacity of the soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health. However, soil quality indicators are difficult to define since they are also impacted by external factors such as land use and soil management, ecosystem and environmental interactions, socioeconomic and political priorities. Managing and maintaining the soil in an acceptable state as part of our natural capital for the next generations is a prerequisite and requires particular attention in regions where we disturb the bio- and lithosphere for the extraction of natural resources, including minerals.

Soil characteristics and quality depend on the constituent parent materials to define geomorphology, hydrology and the kind of vegetation which should be introduced. These are also influenced by the organic components, the microbial components and the soil (bio)chemistry.

According to Husson (2013), an 'ideal' soil would also provide favourable conditions for development of 'useful' soil microorganisms and unfavourable conditions for pathogens. In such an 'ideal' soil, energy use efficiency is at a maximum to ensure cell homeostasis. Most photosynthetic products can thus be used for the metabolism and growth of plants and associated microorganisms. Ideally, plant production is optimized; the resulting high biomass production generates carbon fluxes in the soil through exudate production, as well as more humus formation and sustenance of functional microbial consortia to maintain functional soils. These fluxes and the microbial activity in the soils contribute to the buffering of the soil's Eh and pH at favourable levels. Further, the elemental cycles are maintained within the soils, particularly the nitrogen, sulphur and phosphorus cycles, maintaining nutrient availability. The soil-plant-microorganism system is thus efficient and stable.

When addressing rehabilitation, the desired characteristics of the soil to be delivered also need to take into account the geographical area and final use of the soil. For example, in agricultural areas, soils have to supply nutrients and water for the chosen crop (not native vegetation) while maintaining its chemical, physical and biological quality (Wick et al., 2013), thus requiring ability to retain sufficient moisture and to ensure bioavailable nutrients. Conversely, in a natural environment, the supply of nutrients may cause a shift in the consortium of plants co-existing and so disrupt the natural, diverse and robust ecosystem. From a socio-economic and environmental perspective, a fabricated soil should mimic the conditions of surrounding healthy and desirable soils, particularly where it is desirable to reestablish the indigenous vegetation, post-mining. Reproducing original (pre-mining) land capability conditions is congruent with mine closure objectives.

The concept of returning the area to its pre-mining state forms the basis of the South African approach to mine closure, and so is a central consideration when developing soils for mine-site rehabilitation. In this approach, development of a healthy vegetation cover prevents soil erosion and contaminant mobilization, improves plant succession and creates a beneficial habitat for wildlife (Kabas et al., 2012; Mendez and Maier, 2008; Tordoff et al., 2000). However, alternative approaches may be desirable when factoring in the post-mining economic activities desired; hence cognisance must be given to the geographical region, the political and cultural context and the planned future land uses for the post-mining environment in order to define the required soil quality and characteristics to be delivered to the post-mine environment. For example, if prior grasslands are to be converted to agricultural lands for active energy cropping, the ideal soil requirements are expected to differ.

In constructing fabricated soils from waste materials for rehabilitation, the addition of amendments to a bulk particulate material, used as the main soil substrate, is required to manipulate the chemical, physical and biological aspects of the material, in order to produce a good soil. As with natural soils, the geomorphology and hydrology of fabricated soils depend greatly on their parent material constituents and define the kind of vegetation which can be introduced; these are related to biochemical and physical proprieties of the soil (Tordoff et al., 2000; Van Ham et al., 2007). When fabricating soils using mine waste key considerations for soils derived from mine wastes include the acidity and neutralization reactions associated with the mine waste and thereby the resultant soil, macro and micronutrient availability, organic matter content, microbial community, metals and phytotoxic compounds, and physical structure.

Degraded mine soils and poorly constructed fabricated soils may lack soil structure, due to either their base or parental material or their depleted organic fractions. On fabricating soils as an artificial mixture of mine waste materials and other amendments, choosing a particle size distribution to promote a balance between macro and micropores and choosing appropriate organic matter content is critical for its establishment as a functional soil (Daniels, 1996; Ussiri and Lal, 2005). Many different materials can be combined to improve a degraded soil or to create a functional soil provided the aspects mentioned above are taken into account. In this context, the use of coal mine waste appears as an opportunity to amend and fabricate soils, especially for use in environmental remediation activities (Tordoff et al., 2000; Van Ham and Teshima, 2005; Van Ham et al., 2007). When combined with alternative materials, this prevents the need for the use of natural top soils, and hence avoids their burrowing, and associated environmental burden. Further, it offers the benefit of recycling municipal and industrial wastes (U.S. EPA, 2007). The technosols developed in this investigation are fabricated from fine coal processing waste and amended with malt residue and compost. Virgin and stockpiled soils are used as auxiliary parental material and controls.

1.3 Introduction to the Scope of the Project

In our earlier work, reported in WRC K5/2231 (Harrison et al., 2020a,b), we demonstrated the proof of concept of using sulphur-lean and coal-lean fine coal waste as the main parental material for soil fabrication. In this study, the feasibility of using local ultrafine coal processing wastes with no market value, in combination with organic supplements and nutrient sources, is further explored as an option for providing topsoil during the rehabilitation of mine sites in South African coalfields. In particular, the preparation and characterisation of appropriate fine coal waste sub-fractions is considered. We address the feasibility of using a prior separation step in the preparation of the fine coal waste. We also consider the potential to use coal wastes with appropriate characteristics directly for soil fabrication, without pre-processing. The requirements of the fabrication process to produce a technosol that complies with the specifications of a topsoil for rehabilitation are further explored with particular consideration being given to the physicochemical soil structure and its ability to support plant growth. We also consider the establishment of soil microbiology to ensure nutrient cycling and availability (Amaral Filho et al., 2020).

Through the development of protocols for soil fabrication from fine coal waste and the development of analytical tools for the characterisation of these fabricated soils, we will build understanding of the key factors influencing their quality and productivity. Enhanced plant growth using these matrices is sought and the economic and environmental feasibility of fabricating soils from coal waste for mine site rehabilitation and extended uses is addressed.

2 LITERATURE REVIEW

2.1 Coal Mining in South Africa

2.1.1 South African coal mine waste

In South Africa, run-of-mine (ROM) coal contains gangue minerals, impurities and unrecovered minerals in addition to the desired combustible coal. In order to meet the market specifications (Table 2-1) ROM coal is beneficiated, resulting in separation of saleable coal from waste material.

Table 2-1 South African thermal coal standards (Steyn & Minnitt, 2010)

	Domestic thermal coal	Export coal
Sulphur (%)	0.7-1	<1.0
Calorific Value (MJ/kg)	24-27	27
Ash (%)	15-21	<15

In Figure 2-1 a schematic diagram of South Africa's coal production in 2016 is presented. It observed that over 40% of the ROM coal was used for electricity generation and roughly 25% of the coal is suitable to export. In terms of coal waste production, in 2016 almost 75 Mt of coal waste was generated, over 20% of the total mass of the produced ROM coal. Typically, South African coal wastes are dumped as coarse discards and slurry in waste disposal facilities. In some cases, in order to reduce the formation of ARD, co-disposal is practised. Usually, the coal coarse discards are generated from the dense medium separators. In contrast, coal slurry is typically a mixture of fine (-1000+150 micron) to ultrafine ROM coal (-150 micron) gangue minerals and process water leaving the beneficiation plant.

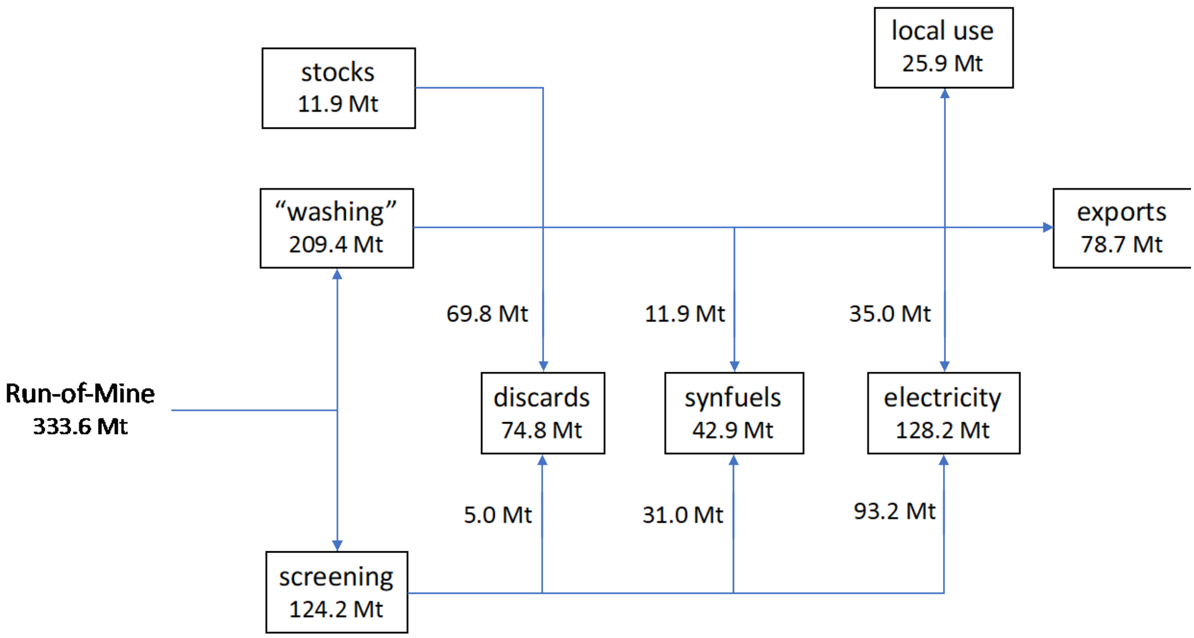

Figure 2-1 South Africa's coal chain figures from 2016. Courtesy Xavier Prevost (external communication)

Analysis of the particle size distribution of examples of South African coal slurries wastes (Figure 2-2) showed that the samples presented a D_{50} lower than 250 µm (Iroala, 2014; Kazadi Mbamba, 2011), indicating suitability to be further processed by flotation (Horsfallt et al., 1986).

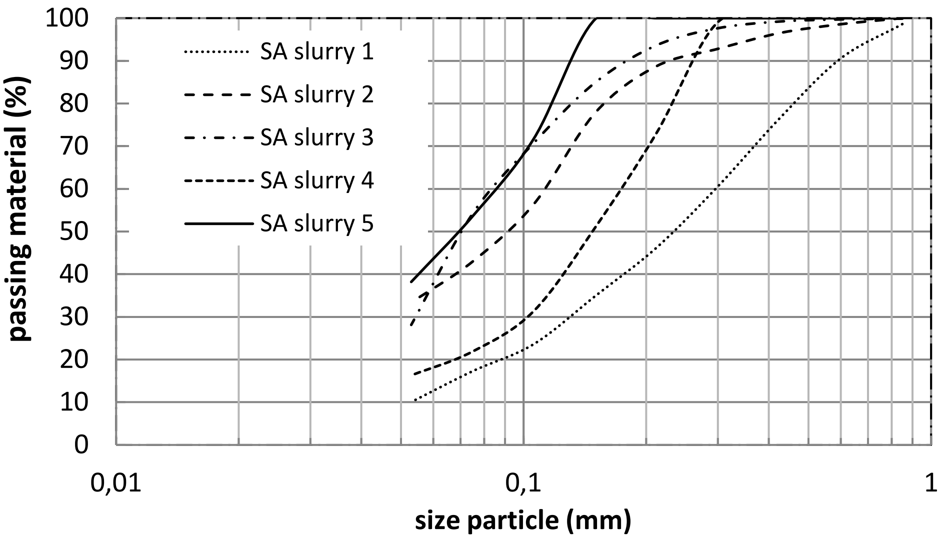

Figure 2-2 Particle size distribution of the discarded fine coal slurry from South African mines (Iroala 2014, Kazadi Mbamba 2011)

Kotelo (2013) studied the distribution by size of the mineral constituents in a South African coal tailings sample collected from a thickener underflow in the Witbank area (SA Slurry 4 – Figure 2-2). The relatively inert minerals, quartz (34-38%) and kaolinite (52-58%), contributed the highest proportion of ash-forming mineral constituents in all the size fractions investigated. Other ash forming minerals included acid-forming pyrite (2-3%), gypsum (3-8%) and jarosite (<1-3%).

Usually ultrafine coal waste has a high calorific value and potential for coal recovery, if further processed. Reddick (2006) estimated that between 14 Mt and 18 Mt of coal slurry was discarded in 2006. These figures are likely to have increased since then, due to increased coal production (Chamber of Mines of South Africa, 2013) and declining ore grades. Apart from representing a loss of revenue from coal, provision for slurry deposits has been estimated to contribute 3% to the capital cost of establishing an open pit coal mine, equivalent to 10% of the capital cost of the processing plant (Mohutsiwa and Musingwini, 2015).

As indicated above, coal slurry waste contains sulphide sulphur in the form of pyrite, creating an ARD risk. As demonstrated by Kotelo (2013), despite the relatively low sulphur content (1.1%) of the sample, the studied coal slurry was found to be acid generating. ARD generation occurs when the sulphidic minerals in the fine coal waste or coal discards react with oxygen and water, being oxidised to release iron compounds and sulphuric acid into solution (McCarthy, 2011; Rohwerder et al., 2003). Microbial oxidation of the ferrous iron and the sulphur compounds present results in the generation of ferric iron and acidity which accelerate the mineral oxidation such that it occurs much faster than inorganic chemistry predicts (Rohwerder et al., 2003). This results in potentially acidic, sulphate-rich mine waters known as ARD, although neutral mine drainage is also found in the presence of high acid-neutralising capacity. ARD drives heavy metal mobility and subsequent degradation of water resources and agricultural land due to both the acidity and salinity inherent in these mine waters (McCarthy and Pretorius, 2009). In the South African coal fields, ARD, or neutral mine drainage, is commonplace in areas in Mpumalanga and presents a significant problem (McCarthy and Pretorius, 2009). Bell et al. (2001), in a work carried out in the Witbank coalfield, stated that spoil heaps not only generate ARD but also form blemishes on the landscape and result in air pollution as a result of spontaneous combustion and dust.

2.1.2 Value recovery and risk reduction for coal mine waste slurries

In order to recover value and reduce the ARD risk associated with sulphidic mineral tailings, researchers at the University of Cape Town have developed a two-stage separation process (Harrison et al., 2013; Hesketh et al., 2010; Iroala, 2014; Kazadi Mbamba et al., 2012). This process (Figure 2-3), using separation techniques such as flotation or reflux classification, concentrates a fine sulphidic waste stream into a recovered valuable fine coal product stream, a non-acid generating sulphide-lean stream which is relatively benign, and a sulphide-rich stream which is typically acid generating. This process has been shown to be effective with coal waste and base mineral tailings. The sulphide-rich stream has a reduced volume and a concentrated sulphide content (typically 10 to 25% of overall fine waste stream), while the majority of the waste volume resides in the sulphide-lean stream presenting reduced or negligible environmental risk. Each of the three resultant streams has potential for re-purposing for enhanced value recovery and reduced environmental burden.

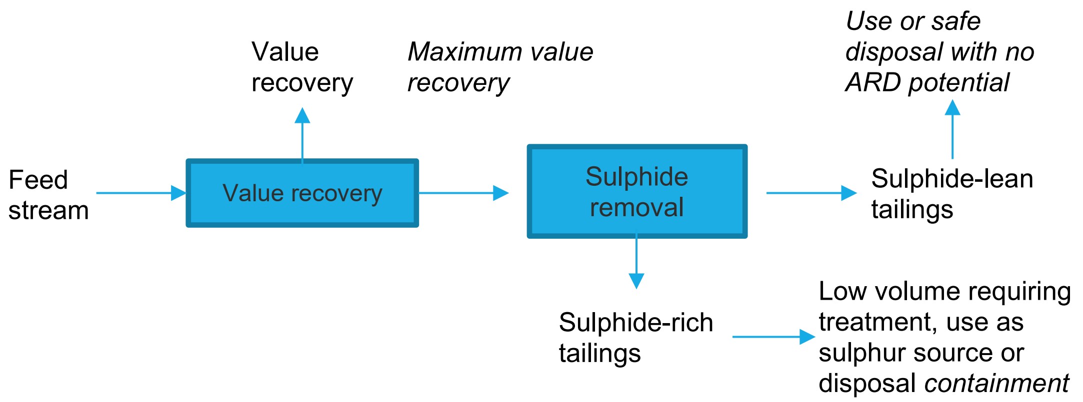

Figure 2-3 The two-stage separation process towards risk removal in disposal of mining tailings and fine coal waste developed at the University of Cape Town's Chemical Engineering department.

Previous studies of this process were carried out with laboratory-scale tests on fine coal slurry waste from different collieries in the Witbank and Waterberg coalfields in South Africa. Together the studies showed that results vary significantly for different coal wastes in terms of deportment of key components (coal, ash and sulphur) to the three final streams. The process results in a coal product, comprising between 25% and 80% of the incoming stream, with reduced ash and sulphur. The sulphide-rich stream comprises a very much reduced volume stream and, where sufficiently upgraded, valorisation options can be considered as presented in the original study (Harrison et al., 2020); alternatively, disposal with containment is required. The ARD potential was assessed using several acid rock characterisation tests (Broadhurst et al., 2013; Iroala, 2014; Kazadi Mbamba et al., 2012). In terms of the product streams obtained by the two-stage flotation, the sulphide-lean tailings were consistently net acid neutralising. This sulphide-lean material has been demonstrated to have potential for soil fabrication (Amaral Filho et al., 2020; Firpo et al., 2015; Harrison et al., 2020) and is the object of this study.

2.2 Sustainability in Mining

2.2.1 Mine rehabilitation

The impacts of coal mining processes on the mined lands have to be mitigated and minimised through the successful implementation of sustainable mine rehabilitation schemes. The development of mine rehabilitation strategies involves political, economic, social and environmental factors. The implementation of sustainable mine restoration is dependent on the interplay of these dynamic drivers and is further complicated by conflicting ideas from stakeholders. This often results in minimum regulatory compliance. However, the incorporation of sustainability principles into policies and legislation has increased local and national awareness.

Sustainable development has become an imperative of mining processes and is being incorporated into mine rehabilitation and closure procedures (Coaltech, 2019). For example, rehabilitation has been shown to effectively reduce the amount of greenhouse gases emitted by coal waste dumps. According to Cook and Lloyd (2012), carbon dioxide emissions was reduced to 1 kg/m^2/a instead of 100 kg/m^2/a in the absence of rehabilitation schemes and 7000 kg/m^2/a in the presence of spontaneous combustion.

Reclamation is defined as the process through which degraded land is restored to productive land through anthropogenic and natural solutions, returning the mined site to its previous capability. It is achieved through land rehabilitation and regeneration, as well as remediation in the case of contaminated areas. Restoration of degraded post-mine sites is dependent on the landscape needs (Van Deventer et al., 2008). Soil stockpiling, soil replacement, soil amelioration, revegetation and the removal or redesign of available infrastructure are common mine rehabilitation strategies that aim to transform the derelict land. A site-specific rehabilitation framework was proposed by the Land Rehabilitation Guidelines for Surface Coal Mines (Coaltech, 2019) to facilitate the development of a detailed plan for rehabilitation. It outlines an iterative process consisting of four stages: (1) planning, (2) implementing, (3) monitoring, and (4) refining, correcting and re-planning.

Mine closure is the final stage in the lifecycle of a mine. It can only be reached once rehabilitation of the land site during the decommissioning and post-mining stages is classified as successful and complete (Coaltech et al., 2019). At this point, the land can be commissioned to a new owner. When achieving this final goal, the land complies with the criteria set in all three pillars of sustainable development (environmental, social and economic). The overarching aim of rehabilitation strategies is thus to restore the mine site to provide a land that is financially viable for the new owner to invest in, whilst simultaneously having the capacity to be prosperous for environmental and social development. However, site relinquishment is rarely achieved due to inadequate or failure of administered remediation, restoration or regeneration schemes of the mine land and mining waste. The ineffectiveness of current mine remediation strategies is evident when considering statistics concerning greenhouse gas emissions from mining waste dumps, such as those presented by (Cook and Lloyd, 2012). The sand covered dumps continue to burn at rates similar to those of unrehabilitated waste dumps. Therefore, alternative mine rehabilitation methods to be implemented from initial land disturbance until the decommission of the mine site are required.

Waste management rarely has a one-glove-fits-all solution. Feasible coal mine waste management is a complex and dynamic problem to which the literature, research and case studies are a testament. It is unlikely that an insular, rigid and ‘added-on’ technology will be able to solve this problem; however, using innovative, proactive and flexible technologies in conjunction with one another may hold multiple benefits. This encompassing approach is a characteristic of the circular economy principle for sustainable mine waste management systems.

2.2.2 The Circular Economy Principles

The increased awareness of sustainability in mine development has shifted the focus from linear economy principled waste treatment and waste disposal schemes such as ARD treatment facilities (Taha, et al., 2017), to preventative methods for controlled resource management and effective waste management with mitigated impacts on the environment (Kinnunen and Kaksonen, 2019; Kotsiopoulos and Harrison, 2017). These methods are based on zero waste strategies and waste valorisation which forms part of the circular economy principle.

The circular economy principle is based on an economic framework where waste and additional by-products are placed back into a process for the cyclic use of materials in a system (Lèbre et al., 2017). Reusing and repurposing the wastes are cost-effective approaches to waste management and it simultaneously encourages sustainability principles. When successfully implemented, the volume of primary resources used and overall amount of waste generated in the processes are significantly reduced (Amaral Filho et al., 2017, 2013; Broadhurst et al., 2013b; Lèbre et al., 2017). The cyclical use of materials prolongs the lifetime of resources to improve the process economy. It can be incorporated into industrial processes through the industrial ecology integration tool which provides guidance on the cradle-to-grave use of materials (Jelinski et al., 1992).

A circular economy approach mainly focuses on primary resource management when applied in the early phases of a mining project; however, it can also be integrated into the mining sector as a strategy to manage discarded waste. In industries, such as the food and beverage industry and wastewater industry, sustainable development uses the circular economy principle to reduce the amount of initial resources brought into a specific system. In the mining industry, the focus is rather on minimizing the volume of waste produced since current mining by-products cannot yet replace the value of the primary excavated resources. Mining waste is often disregarded in the financial system of a mine since the value of the material contained in the final product overshadows the material losses in the product value supply chain. However, the circular economy demands a new value chain and thereby a different perspective on resources in a process. When implemented at mines, minerals would be excavated without exceeding environmental limits and resources would be utilized to their full potential (Lèbre et al., 2017).

Repurposing the mining waste aligns with the United Nations' Sustainable Development Goals (SDGs), and has potential solution to contribute to the environmental SGD 11 ('Make cities and human settlements inclusive, safe, resilient and sustainable') and economic SDG 12 ('Ensure sustainable consumption and production patterns') as well as to environmental SDG 15 ('Protect, restore and promote sustainable use of terrestrial ecosystem, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss') (Department of Statistics South Africa, 2019). Multiple investigations on valorising mining waste from open-pit and underground coal mines have been performed, many of which demonstrated its feasibility. The publications by Amaral Filho et al. (2020) and Weiler et al. (2020) combined coal waste and organic material amendments to fabricate technosols that can be used as a sustainable mine rehabilitation strategy and as a cattle feed source respectively. Kinnunen and Kaksonen (2019) reported that research on methods for integrating the circular economy principle in the mining sector globally through waste valorisation, is lacking. However, the authors corroborate the research by Weiler et al. (2020) and Amaral Filho et al. (2020) by suggesting additional applications for the reuse of tailings in 3D printing and as a mineral matrix in geopolymers. Taha et al. (2017) demonstrated this by developing a feasible process for producing eco-friendly fired bricks from coal tailings and the residual coal from the mineralized waste. Thus, fabricating technosols from mining and other waste sources is an example of using the circular economy principle to produce a value-added product (Macia, et al., 2014).

2.3 Pedology

Pedology is the scientific field of soil research that can be traced back to the 18th century. The research has expanded significantly over the last century to include the influence of the Anthropocene age on pedogenesis (Richter, 2007; Vittori Antisari et al., 2014) describes the importance of pedology by inferring that the answers to modern scientific questions regarding the natural environment are rooted in the future of soil. For the last decade, the quality of soils has been a global concern. In South Africa, less than 15% of the soil is arable. Soil quality have mostly been defined by its agricultural potential or land use (Mills and Fey, 2004a); however, soil fertility is strongly influenced by both biotic (plant and microbial productive capacity) and abiotic factors (e.g. environmental conditions, human and animal health) (Valarini et al., 2003). The exponential increase in the population leads to accelerated rates of urbanization and pollution, and the expansion and continuous development of technologies, agricultural practices, industrial production systems and mining processes which consequently alter the affected soil's quality, fertility and structure (Richter, 2007) .

2.3.1 Technosols

The World Reference Base (WRB) for Soil Resources classifies technosols as engineered soils with parental materials (primary waste sources) derived from anthropogenic activities and pedogenesis influenced by their technical origin. It I s often constructed from either urban or mine waste (Food and Agriculture Organization of the United Nations, 2015). Technosols are designed on the principle of waste valorisation to reduce the volume of waste accumulated and to enhance the rehabilitation of degraded land through the accelerated formation of a fertile soil (Weiler et al., 2020; Zornoza et al., 2017). Characteristics of the parental materials and of the associated amendments influence the design goal of the fabricated soil. The amendment materials are specifically chosen to ameliorate the parental material(s) to ensure a self-sustaining soil.

The large volumes of mining and urban waste that are constantly generated globally, are the ideal primary waste sources for parent materials for technosols. Other common waste sources for parent materials include fly ash, biosolids (sewage sludge), ashes, and landfillClick or tap here to enter text.. Research by Deeb et al. (2017), Macía et al. (2014), Jordán et al. (2017), Herran Fernandez et al. (2016), and Neina et al. (2016) show that technosols are not only constructed from coal mining waste but also from degraded soils originating from mining activities and the excavation of rock.

The research also suggest that Technosols can be implemented in various geographical and geological settings. The paper by (Deeb et al., 2017) focused on weathered limestone from the Parisian basin. Macía et al. (2014) investigated marine dredged material, whereas Jordán et al. (2017) focused on limestone quarries. Herran Fernandez et al. (2016) formulated a technosols primarily from demolition waste from a construction treatment plant in Gardelegui (Spain), and Neina et al. (2016) focused on tantalite mining in Rwanda. The various parental materials used in the studies are in accord with the WRB definition of Technosols (Food and Agriculture Organization of the United Nations, 2015). None of these studies provide justification for the technosols formulation. However, all of the investigations presented auspicious results for plant growth and phytodegradation. It is an indication of the infancy of the research in this field and that the research on Technosols are performed to identify which technosols make-up (ratios of waste, native soil, and amendment materials) will result in a fertile but also economically-viable soil.

The properties of a soil are strongly influenced by the origin and the physical and chemical properties of the parental materials. Therefore, when developing innovative and sustainable solutions for waste management initial feasibility studies must be performed to evaluate the feasibility of using a specific waste source to construct a value-added product. Coal deposits are often associated with silicates and sulphur. According to the ARD potential, to minimise the possible negative impact of these fractions on the endemic environment, the CW can be desulphurised prior to fabricating the technosols. In South Africa, ultrafine coal slurry from the eMalahleni colliery was processed in a two-stage desulphurisation system to produce a low sulphur CW that was used to fabricate the technosols by Amaral Filho et al. (2020) . Weiler et al. (2018), collected coal mine discard from collieries in the Barro Branco seam in Brazil. It was subjected to dense medium separation to split the sulphide-rich and carbon-rich fractions of the waste. Comminution of the parental waste material, form part of the preparation for the process of technosols construction. The coarse solid CW particles are often grounded (in a jaw or mill crusher) and sieved to obtain the desired particle diameter that resembles native soil (usually to an average particle size diameter below 2 mm) (Deeb et al., 2017; Firpo et al., 2015; Weiler et al., 2018).

The primary waste materials cannot be used by itself as a soil due to poor water permeability (Macía et al., 2014), imbalanced pH (Firpo et al., 2015), and a lack of nutrients (Amaral Filho et al., 2020; Weiler et al., 2020). Hence, the addition of amendments to parental materials in technosols play a significant role in modifying the quality, structure and fertility of the fabricated soil (Herran Fernandez et al., 2016). Properties such as acidity adjustment, physical structure (water holding capacity or porosity) improvement, alkalescent source, microbial augmentation, organic matter and nutrients provision are important when choosing which parental material amendments to incorporate into Technosols (Firpo et al., 2015; Jordán et al., 2017; Novo et al., 2013).The availability and proximity of the amendment materials to the construction and planned implementation site also play a role in the selection process of the appropriate sources and amounts of amendments. (Weiler et al., 2018), amended a spolic technosols with rice husk ash (a physical structure ameliorant), sewage sludge (source of organic matter) and steel slag (neutralisation agent and source of micronutrients) that were all near the mine site. Various waste sources can be used as amendments. The research by Weiler et al. (2020) and Amaral Filho et al. (2020), incorporated various ratios of either native topsoil, compost, anaerobic digester sludge, or malt residue to amend the CW-derived technosols. Additionally, Sekhohola and Cowan (2017) inoculated the Technosols with a fungal culture. The proportions in which these amendments were added, ranged between 2.5% and 5% w/w (combined). The research all reported positive results for technosols fertility (metal content was remediated to below toxic levels, and the Ca, Mg and S nutrient levels were sufficient to support plant growth). Santos et al. (2019) demonstrated that the addition of 3% of organic matter to a technosols formulated from sulphide-rich mining waste produces alkaline soil conditions, which are advantageous for plant growth (*Lavendula pedunculata* and *Cistus ladanifer*) at long-term (three years) and controlled conditions. This supports the theoretical basis on which amendments are incorporated into technosols.

Amaral Filho et al. (2020) suggests that the high ash content, low phosphate content, and low organic carbon content of the compost used resulted in it functioning as a secondary soil source not contributing for the quality and production aspects, whereas the anaerobic digester sludge and malt residue had a greater contribution to organic amendment in the technosols. Therefore, it is possible to hypothesize that when amendments to the fabricated soils are used in conjunction with one another it would lead to a more fertile soil (high plant biomass) and even a self-sufficient soil since certain amendment types have a higher content of accessible organic matter.

Amendments such as leaf litter and animal manure have been incorporated into arable soils for centuries. In recent years, compost has become a popular amendment to industrial and agricultural soils, and to Technosols. It is generally added in a specific ratio to the soil, to maintain or produce a soil with a circumneutral pH and an organic matter content of 2%. Weiler et al. (2018), Herran Fernandez et al. (2016), suggests that compost is a better suited amendment for Technosols compared to sewage sludge which is often associated with a high heavy metal content. Based on the net neutralisation potential (NNP), the ratio of compost additions can be determined. NNP is the difference between the neutralisation potential (NP) and acid potential (AP) of a substrate. Negative NNP values are an indication of potential acid drainage. In the previously mentioned papers, none of the authors justify the ratios in which the amendment materials were added, except for (Weiler et al., 2018). The variations in ratios of added amendments in each of the investigations are another indication of the infancy of the research in this field but also to effectively determine the amendment type(s) and dosage(s) that will lead to a sustainable product (technosols) in terms of economic, social and environmental factors.

Research by Amaral Filho et al. (2020), Deeb et al. (2017), and Weiler et al. (2018, 2020) reports on the beneficial effects of various amendments to Technosols. The results showed increased microbial community activities, improved soil structure and aggregations, augmented nutrient cycles (through sulphur speciation), and sustained plant growth. The research is corroborated by multiple other studies (Firpo et al., 2015; Neina et al., 2016) and provides justification for the use of amendments in engineered soils. However, the period for which these amendments have beneficial effects on the Technosols are unclear. For this, the application rates and dosages of amendments must be investigated for several plant growth cycles.

The incorporation of microorganisms in waste management schemes have shown to be advantageous in many applications, especially in the wastewater industry. Bioremediation is a technology developed to degrade, minimize or transform pollutants in natural environments through the biological activity in microorganisms. It is commonly used in the wastewater industry and has only recently been applied in hazardous waste management systems. The process of bioremediation is dependent on the ability of the selected microorganisms to rapidly acclimate and perform specific metabolic activities that target the contaminants. The success thereof is determined by the pollutant concentration and bioavailability, nutrient availability and environmental conditions (such as moisture content, pH, temperature and oxygen levels) for promoting the metabolic functions, growth and proliferation of the microbial cultures. Biostimulation is an extension of bioremediation through optimising the environmental conditions by applying limiting nutrients such as carbon, oxygen, nitrogen and phosphorus (Adams et al., 2015). Recent research has shown the feasibility of treating mining waste through microbial activity (Sekhohola and Cowan, 2017; Zornoza et al., 2017) . Bioremediation and biostimulation have been tested in several applications and although the biological approach to remediation in the mining sector seems promising since they are directly related to soil quality, it is yet to be established.

Microorganisms are essential to soil health and plant growth and remediation strategies are often unsuccessful when using only applied nutrients or an inoculum. Hence, for sustained remediation, a simultaneous approach consisting of biostimulation and bioaugmentation is required (Adams et al., 2015). However, the diverse native microbial communities associated with self-sustaining soils are initially absent in these Technosols. Therefore, by using the proposed combined approach of a biostimulated and bioaugmented-technosols (combined use of parental material amendments with exogenous microbial communities), it will stimulate the indigenous microbiome to establish a stable microbial population that is able to sustain itself (Zornoza et al., 2017).

Bioaugmentation is defined as the process through which a specific environment is supplied with an external source of specialized microorganisms to augment the biodegradation of pollutants or replace the indigenous microbial. In agriculture it has been used as an effective bioremediation tool (da Silva and Alvarez, 2010). Valarini et al. (2003) showed that degradative processes, especially the humification of fresh organic material (such as plant leaf litter), are improved when integrating exogeneous microorganisms into soils. The microorganisms are selected for their capability to perform a specific metabolic function in an environment. Another form of bioaugmentation exists that is not based on the establishment of a microbial community. The aim of the inoculum is only to catalyse biodegradation in an environment where external conditions inhibit the ability of the microbial community to be established. Examples of such external conditions include abiotic properties such as acidic conditions, toxic pollutants, low nutrient levels and inability to sufficiently retain water, as well as biotic properties such as competitive native microorganisms. For this reason, additional sources of nutrients and physical ameliorants are also applied to the specific environment (e.g. soil) to ensure successful bioaugmentation (da Silva and Alvarez, 2010).

The benefits of bioaugmentation in Technosols include rapid acclimation of microorganisms and enhanced degradation of the priority pollutants (da Silva and Alvarez, 2010). Zornoza et al. (2017) reported that the addition of extrinsic microorganisms to Technosols resulted in soil carbon sequestration. The metabolic activity of the microorganisms resulted in calcite precipitation, and thus the degradation of inorganic carbon. This study, along with several other such as Valarani et al. (2003), motivates the application of mixed cultures au lieu of single bacterial species. Single strains, or pure cultures, are primarily used when targeting a specific contaminant. Another technique that has shown significant potential is the use of genetic elements as an inoculum to transfer genes with the desired catabolic potential to the native bacteria. This minimizes the risk of external conditions affecting the exogenous microorganisms' ability to adapt to the environment and to perform necessary microbial activities (da Silva and Alvarez, 2010). Another well-known inoculant in bioaugmentation technology is genetically modified organisms (GMOs). The widespread use of GMOs is a testament to their success; however, the extended negative impacts remain unclear. In agriculture, it could cause the loss of biodiversity from modifications to the microbiome structure through extrinsic gene transfer to the local microorganisms.

2.3.2 Advantages and Disadvantages

The advantages of Technosols extend past the mining industry into metropolitan and industrial areas since various sources of waste materials can be used for the fabrication of Technosols. Research on Technosols also suggest multiple applications thereof. A study conducted by Weiler et al. (2020) showed that when plants such as alfalfa (*Medicago sativa*) are grown in Technosols constructed from CW and amended with compost, it is a feasible cattle feed. Amaral Filho et al. (2020) successfully investigated the feasibility of integrating Technosols in collieries for the restoration of degraded mine land. According to Amaral Filho (2020), the waste is valorised, and the volume of borrowed natural soil required for mining rehabilitation is significantly reduced. Herran Fernandez et al. (2016) suggest that phytoremediation through Technosols formulated from demolition waste, steel slag and compost is a suitable solution for the lack of natural soil in vacant municipal areas. The research is corroborated by Sekhohola and Cowan (2017), Weiler et al. (2020), and Zornoza et al. (2017).

Indirect advantages of mine land reclamation through this technology include carbon and sulphur sequestration (Weiler et al., 2018). The use of various waste sources as parental materials and amendments are beneficial because it is available in large quantities and at low cost often near the mines. It is safe for land application and ensures the reuse of organic matter that would otherwise be wasted (Santos et al., 2019). Socio-economic and environmental impacts of coal mine related processes are also minimized by:

- Reducing the amount of natural topsoil required for coal mine rehabilitation (Amaral Filho et al., 2020).
- Reducing the volume of coal waste disposed in dumps deposits (Weiler, et al., 2020).
- Reducing surface and groundwater contamination (Jordán et al., 2017).

Technosols are developed based on the sustainable development framework that calls for a circular economy. The challenges of implementing this in the mining industry are linked to the technological, environmental, social and economic factors inhibiting the development of new value chains. Kinnunen and Kaksonen (2019), suggest that the valorisation of mining waste must be a primary development aim before the stage of site relinquishment is reached. This aligns with the recommendation made in the Land Rehabilitation Guidelines for Surface Coal Mines (Coaltech et al., 2019) which advocates for the concurrent implementation of mine rehabilitation strategies and excavation processes. Therefore, mine restoration and site relinquishment plans must be developed in the feasibility study of the mining project.

Different methods for coal beneficiation lead to variation in the waste streams between mines. Thus, the opportunities for waste valorisation differs between each mine. Since the formulation of Technosols are dependent on the characterisation of the specific primary waste source, the process becomes site-specific thereby limiting quick and simple implementation schemes (Kinnunen and Kaksonen, 2019).

The infancy of waste valorisation in the mining sector means that the value of implementing waste valorisation systems into existing mining processes are unknown to stakeholders (Kinnunen & Kaksonen, 2019). The functionality and the contribution of Technosols to the mining economy, to the surrounding environment, to communities and to future potential investors must first be assessed and then justified. (Firpo et al., 2015; Novo et al., 2013), both conclude that the long-term behaviour of Technosols need to be investigated prior to the implementation thereof. Such studies must include assessments of appropriate plant species based on the specific types of parental materials in the technosols, since the success of rehabilitation strategies is reduced by slow vegetational growth (Santos et al., 2019). Investigations on the long-term effects of initial and periodic applications of amendments have not yet been performed. Some amendments may require regular applications which could increase the cost of sustaining Technosols as a mine rehabilitation scheme. However, when plant species with an economic value (such as *Lavandula pedunculata*) are cultivated in Technosols, the income generated from the plant biomass production could offset the implementation cost (Santos et al., 2019).

For a technology or a process to be classified as feasible, it must comply with certain criteria. It must be simple, efficient, economically viable and environmentally friendly (when valuing the principle of sustainability) with positive impacts on the community affected by the operations. The operating, maintenance and capital costs of the implemented Technosols are trivial when determining the feasibility of the strategy to rehabilitate degraded mine sites. Implementation costs for Technosols are dominated by capital costs. The primary costs associated with these Technosols are the cost of parental material amendments, exogenous microorganisms, plant species, logistical costs. However, the ability to be self-sustaining is largely determined from the monitoring costs.

Multiple studies have been performed on the feasibility of Technosols as a tool for waste management. Firpo et al. (2015), established a framework for constructing fabricated soils from coal mining waste amended with biosolids and steel slag. Whereas, papers by Amaral Filho et al. (2020), Herran Fernandez et al. (2016), Novo et al. (2013) and Sekhohola and Cowan (2017), directly investigated the feasibility of using mining or urban waste amended Technosols to sustain plant growth without negative socio-environmental impacts. In most studies, plant growth experiments were performed since the Technosol's potential to sustain plant growth is a measure of its feasibility. These experiments were performed under controlled conditions. The variations in the parent material amendments, in the plant growth experiments, and the different characterisation methods that were used for analyses, all led to auspicious results that support the feasibility of using a technosols as a waste management tool. However, not enough research has been done on the soil microbial community and activity and on plant growth in Technosols at ambient (subject to seasonal variation) conditions. This study aims to answer key questions regarding the function and dynamic structure of a technosols microbiome through conducting several cycles of plant growth experiments in a bioaugmented-technosols and a non-inoculated technosols at endemic site conditions.

2.3.3 Pedogenesis

The fertility, quality and physical structure of a soil are multifaceted and interlinked aspects. Therefore, various approaches have been developed and can be followed to assess soil quality. Assessing and maintaining soil fertility requires understanding of the soil pedogenesis, soil characteristics and requirements, of the relationship between pedology and hydrology, and of the functioning and origin of erosional cycles (Richter, 2007; Valarini et al., 2003). In literature, a synonymity between soil quality, soil fertility and soil structure exist. It prompts the equal consideration of all soil properties (chemical, physical and biological) for more effective and thorough indications of soil ecosystem functions (Mills and Fey, 2004; Valarini et al., 2003) . This interpretation of soil characterisation is corroborated by Deeb et al. (2017) who examined soil aggregation through the interactive effects of compost, plants and earthworms. The authors highlighted the significance of evaluating soil quality through both organic matter content and soil biota.

Pedogenesis is the mechanisms (chemical, physical and biological processes) that play a role during the formation of a soil from a parental material (minerals, rocks, organic materials or artefacts). A soil can be defined as an active process and response structure (Sauer, 2015), that operates as an open thermodynamic system with dynamic energy and material inputs and outputs, biophysical translocations (such as dispersion, diffusion, nutrient absorption and decomposition), biogeochemical cycles, and biomass production. This system interacts with multiple interfaces such as the atmosphere, hydrosphere, lithosphere and biosphere. The pedogenesis (soil formation) of any soil is dependent on five interlinked factors: climate, time, relief (topography), parental material and the indigenous microbial activity (Osmond, 1961). The following equation was developed by Jenny (1941),to describe a soil (s) as a function of the five soil-formation factors:

$$s = f(cl, t, r, p, o \ldots)$$

Where, *cl* represents the climate, *t* the time, *r* the relief, *p* the parental material, *o* the organisms and […] is the unidentified factors that may need to be considered.

The formation factors determine the chemical, physical, mineralogical and morphological characteristics of a soil (da Silva and Alvarez, 2010; Sauer, 2015) ;thus, the quality, structure and fertility or agricultural potential of the soil. Soil distribution is influenced by the topography and lithography of the specific area. Consequently, understanding the pedogenesis and characteristics of a soil is key to accurately determine its agricultural potential. The rate of change of soil properties are influenced by these interlinked factors that concurrently change due to anthropogenesis. Soil chrono-sequence studies are used to evaluate the rates of processes that play a role in pedogenesis(Sauer, 2015).

The challenge is to accurately quantify the effect or contribution of each of these formation factors, especially the biological activities, on pedogenesis. The assessment of microbial community activity and diversity is complicated by the complex microbiome function, diverse dynamic structure, seasonal influences and spatial variation. The characterisation of anthropogenic soils is useful for investigating the influence of any one of the five formation factors, especially that of the parental materials. Therefore, these soils are often used in research to investigate the role of microbial activities and parental materials on soil constitution and purpose (Deeb et al., 2017). Good soil health implies effective and undisturbed soil functions. Important soil functions are biomass production, water and nutrient filtration, nutrient accumulation and stabilization (Bonfante et al., 2019), soil aggregation, pollutant degradation and energy transformations (Suzuki et al., 2005). Therefore, good soil health is an indication of a biologically active and self-sustaining soil.

How well a soil functions, is an indication of its ability to sustain itself. For Technosols to be self-sustaining, the engineered soils must fulfil the primary soil functions as defined by (Nortcliff, 2007) whilst simultaneously present a low ecological potential risk. Additionally, the concentration levels of major elements (carbon, nitrogen, phosphorus) and minerals, as well as of trace elements (e.g. boron and molybdenum) must be balanced and below hazardous levels (Firpo et al., 2015). When the characteristics of the design goal for a specific technosols are accurately defined through technological application and scientific knowledge, the technosols should be self-sustaining by functioning as a circular economy tool. The pedoderm is the top nutrient rich surface layer of a soil. Abiotic and biotic factors that negatively influence soil quality, first alter the nutrient, humus and moisture content of the pedoderm (Mills and Fey, 2004a) . Thus, any attempts in maintaining soil quality, should be aimed at conserving the structure and function of the pedoderm.

Soil disruption as a result of mining or agricultural activities, reduces the plant leaf and root biomass in the soil and exposes the soil organic material to biotic and abiotic factors. The exposed organic material reacts with the atmospheric oxygen. This causes decreased microbial activities and significant reductions in nitrogen levels. The abiotic factors that modify the soil biota, vegetation, electrolyte concentration and nutrient content inherently effect the soil's structural stability. This consequently influences the soil's ability to effectively retain moisture and nutrients, and the soil's erodibility. In some instances, it results in crusting; a phenomenon described by a reduced or collapsed soil porosity when soil aggregates are continuously exposed (Mills and Fey, 2004a).

The parental materials and amendment materials influence the characteristics of a technosols. Materials are selected based on specific characteristics (for example an alkaline pH) and their contributions to improving soil fertility. Zornoza et al. (2017) reported that an alkaline technosols pH minimizes heavy metal (such as aluminium) toxicity through the absorption or coprecipitation of insoluble ferric oxyhydroxides with the free metal ions. The results in the study by Amaral Filho et al. (2020), reported a pH above 7 for all technosols substrates, excluding the control (native soil) suggesting that the higher pH supports plant growth and reduces the possibility of aluminium toxicity in the soil. This is corroborated by Botta (2015), who identified aluminium solubility in acidic soils. Here, the effect of pH on iron toxicity was not investigated, nevertheless it reported the beneficial effects of a slight alkaline soil on plant growth as a result of improved nutrient availability and the degradation of pollutants through augmented microbial activity. The research highlights the effect of soil pH on the quality and fertility of Technosols, thus, the significance of selecting the appropriate amendment type(s) and dosage(s).

The influence of earthworms to soil formation and soil quality is widely published. Earthworms promote the aggregation of soil particles(Mills and Fey, 2004). Satchell (1955) described the value of earthworm activities in soil through the increased macro-porosity, enhanced soil aeration and drainage, and improved soil moisture infiltration. Deeb et al. (2017), showed that earthworms can significantly improve the stability of soil aggregates, especially in technosols, and that the proclaimed benefits of compost are only manifested in the presence of plants or earthworms.

Pollution indexes are practical tools for defining the suitability of technosols for specific applications, and for evaluating the pedogenesis of a technosols. To assess the environmental soundness of a technosols, ecological risk parameters such as the Risk Index (RI) are employed. A RI value below 150 is an indication of low risk for pollution through heavy metals (Herran Fernandez et al., 2016) .

2.3.4 Soil Properties

Soil health and the ability of a soil to sustain itself is determined through assessment of the soil chemical, physical and biological properties (Botta, 2015) .

The numerous factors influencing soil fertility have resulted in multiple indicators for soil health. A set of soil parameters was developed for Southern Africa soil by employing a Soil and Terrain Database (SOTERSAF version 1.0) and auxiliary soil profiles that are held in the International Soil Reference and Information Centre – World Inventory of Soil Emission Potentials (ISRIC-WISE) database. These soil parameter estimates provide information for when conducting biophysical assessments, agricultural process modelling and simulation, ecological zoning and environmental change evaluation Click or tap here to enter text.(Batjes, 2004). The parameters are listed in Table 2-2.

Table 2-2: Soil Parameters or key attributes commonly required in soil studies according to ISRIC Report 2004/04 (Batjes, 2004).

Parameter	Unit
Organic Carbon	mg/kg (ppm)
Total Nitrogen	mg/kg (ppm)
pH	
CEC_{soil}	cmol(+)/kg
CEC_{clay}	cmol(+)/kg
Base saturation	% CEC_{soil}
Effective Cation Exchange capacity (ECEC)	Defined in terms of Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and exchangeable H^{+} and Al^{3+}
Aluminium saturation	% ECEC
Calcium carbonate content	%
Gypsum content	%
Exchangeable sodium percentage (ESP)	%
Electrical conductivity of saturated paste (ECe)	dS/m
Bulk density	Dry weight per unit volume soil
Coarse fragments (> 2 mm)	Volume %
Sand	Mass %
Silt	Mass %
Clay	Mass %
Available water capacity (AWC)	mm/m, from -33 to -1500 kPa; % w/v

The above-listed parameters must be within the limits set by legislation depending on the land use (Botta, 2015). Prior to experimentation and technosols fabrication procedures, the amendments and parental materials are characterised based on these and additional parameters. The level of complexity of the analyses is directly dependent on the objectives of the research. Characterisation of the substrates (soil-like mixtures) are also conducted. The results (such as the heavy metals concentrations) are often more attenuated compared to the individual substrate ingredients (Herran Fernandez et al., 2016). This is a direct consequence of the ameliorating effects of amendments to parental materials in Technosols.

Weiler et al. (2018), conducted plant growth experiments with CW amended-Technosols. Results for fertility parameters showed that a high cation exchange capacity (CEC) is favourable in a circumneutral soil pH since it improves the ability of the soil to support nutrients (facilitate the cycling of cations in the soil water for uptake by plants) (Botta, 2015). The high CEC thereby minimizes the adverse losses associated with leaching. When considering the plant nutrients and the ions in which they are present in soil, a low CEC is an indication of a soil with a low organic matter content. Therefore, soil fertility is sound when the higher CEC indexes are mainly due to basic cations (Botta, 2015; Weiler et al., 2020).

The above-listed soil parameters are used for standard soil characterisation analysis, but additional soil fertility tests are conducted to investigate all aspects of soil quality, structure and fertility. The organic matter (OM) content (%), electrical conductivity (EC; dS/m), and phosphorus, potassium, sulphur concentrations (ppm) are useful to evaluate the chemical soil properties. However, many authors rely on microbial activity as the primary indicator of soil fertility and quality. A study by Valarini et al. (2003) investigated the initial and integrated health of a clay loam soil through the biological activities of exopolysaccharides and from the phosphatase and esterase enzymes. The physical and chemical characteristics of the soil samples were also measured and were used as supporting evidence for the microbiological fertility parameters. The chemical properties define the nutrient contents of a soil that are dependent on the climate conditions, land use and plant growth. The Soil Organic Matter (SOM) is frequently used as an indicator of soil quality (Mills and Fey, 2004; Valarini et al., 2003) and is pertinent for developing Technosols (Herran Fernandez et al., 2016; Novo et al., 2013). It plays a key role in the soil's ability to perform geochemical cycles and to percolate water (Botta, 2015; Weiler et al., 2018) . The OM in a soil is an indication of the biologically active components (such as bacteria, earthworms and fungi) and a representation of the concentration of organic nutrients available in the soil . It is mainly characterised based on the concentrations of carbon (C), nitrogen (N), sulphur (S) and phosphorus (P) in the soil (Weiler, et al., 2018). These nutrients are used during vegetational growth; thus, are depleted first in agricultural soils. Soils are commonly characterised by the macronutrients content for calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K). The important micronutrients, or trace elements, are copper (Cu), boron (B), zinc (Zn) and manganese (Mn) that are usually present in lower concentrations (Botta, 2015). The sulphur (S), iron (Fe) and chromium (Cr) levels are also of significance. The macro- and micronutrients levels are measured to determine if the concentrations are above hazardous levels as established by local authorities. Table 2-3: lists the standard plant nutrients that are present as cations or anions in most soils.

Table 2-3: Chemical names, symbols and their ions of nutrients present in soil (Botta, 2015)

Chemical Name	**Chemical Symbol**	**Ion form necessary for plant growth**
Aluminium	Al	Al^{3+} (also present in other forms)
Boron	B	$H2BO^{3}$-, HBO_3^{2-} (also present in other forms)
Calcium	Ca	Ca^{2+}
Chlorine	Cl	Cl^-
Copper	Cu	Cu^{2+}
Hydrogen	H	H^+
Hydroxyl	OH	OH^-
Iron	Fe	Fe^{2+} (also present in other forms)
Magnesium	Mg	Mg^{2+}
Manganese	Mn	Mn^{2+}
Molybdenum	Mo	MoO_4^{2-}
Nitrogen	N	NO^{3-}, NH^{4+} (also present in other forms)
Potassium	K	K^+
Phosphorus	P	H_2PO^{4-} (also present in other forms)
Sodium	Na	Na^+
Sulphur	S	SO_4^{2-}
Zinc	Zn	Zn^{2+}

The metal contents in soil profiles are essential for evaluating the soil's toxicity and acidity potentials that influence plant growth and the soil's ability to defer erosion and acid leaching. The concentrations of metals in soils are largely related to weathering and the charges of the specific parental materials (RÊGO et al., 2016).

The carbon/nitrogen (C:N) levels in soils tend towards equilibrium and is an indication of the decomposition rate for organic materials (Valarini et al., 2003). Optimum C:N ratios enable protein synthesis and enhance biological transformations (such as microbial proliferation) that are necessary for seed germination and consequently plant growth. Thus, reductions in N directly prompts a loss of C, which induces a decrease in SOM. The nutrient contents of a technosols is also influenced by the amendments that are added to the parental materials. In studies where Technosols were amended with sewage sludge, the phosphorus and organic matter concentrations in the engineered soil increased, whereas increased calcium, magnesium, manganese and boron levels emanated from incorporating slag into the technosols (Weiler et al., 2018).

Other significant characterisation methods for soil chemical properties include pH, humic acid content, total organic carbon (TOC), sulphur speciation, and electrical conductivity. The humic acid content, and humic acid to fulvic acid ratio, are direct indications of the stability of the OM content in a soil since humus is the largest organic fraction in soil, coal, peat and ash (Sekhohola and Cowan, 2017). The TOC of a soil is often used to evaluate the rate of plant material decomposition in the soil. A low TOC could be as a result of low levels of available plant biomass in the soil and intermittent precipitation (Rego et al., 2016). The sulphide, sulphate and pyritic content of a soil, especially in a technosols, is important to determine ARD potential. A slow rate of pyrite oxidation in soil is desirable since the chemical and biological reactions transform the sulphur species to compounds that are accessible and beneficial for plant growth and microbial proliferation (Weiler et al., 2018).

The physical structure and the quality of a soil are interdependent factors. Seed germination, microbial proliferation, good aeration, water percolation and plant and root growth are determined from the framework provided by the physical properties of the soil (Botta, 2015).

Soil aggregation (the natural porous compounds that form between soil particles of sand, silt, clay and OM) is a soil parameter used to analyse the soil's ability to perform primary soil functions, as well as the quality of the soil. Additionally, soil bulk density, soil porosity, water holding capacity (WHC) and soil texture are commonly characterised when analysing soil physical properties. According to the review on the declining soil quality in South Africa by Mills and Fey (2004), WHC has been the single most popular indicator of soil quality owing perhaps to the simplified techniques of measuring the water infiltration rate of a soil. However, in this review its effectiveness as a tool for developing management strategies for the OM content in a soil is questioned since the WHC is not directly proportional to the soil organic material (SOM) content (Mills and Fey, 2004).

Amendments are incorporated into the formulation of a technosols to minimize chemical, physical or biological deficiencies. For example, to increase the organic matter content or to improve water permeation (Jordán et al., 2017). The WHC (%) for the technosols with a coal waste to native soil ratio (CW/NS) of 3:1 in the study by Amaral Filho et al. (2020) was more than 10% higher than for the technosols constructed from the same ratio of CW and NS by Sekhohola and Cowan (2017) . This is a direct result of the addition of the 2% (w/w) malt residue which acted as a physical ameliorant (Amaral Filho et al., 2020).

Soil microbiota mediate soil ecosystem functions and is essential for sustaining agricultural production and plant diversity (Suzuki et al., 2005). A biologically active soil ensures biophysical transformations and contaminant degradation (Botta, 2015). The microbiome population also plays a role in phytostabilization through interacting with plants to decompose mineral complexes and disturb organic layers to mobilize previously inert nutrient materials (Osmond, 1961; Zornoza et al., 2017) . Useful indicators of soil biological properties are microbial biomass, basal soil respiration, earthworm populations, decomposition of OM and activities related to various soil enzymes (Suzuki et al., 2005; Thiele-Bruhn et al., 2020) . The established microbial communities assist soil aggregation by breaking down rocks into the parental material (Osmond, 1961). Investigations into the function and dynamic structure of a technosols microbiome facilitate understanding of soil pedogenesis and is a useful approach to assess the ability of a technosols to be self-sustaining.

Phytostabilization, the process of remediating metals in soils to below hazardous levels by means of organic material amendments and vegetation, is carried out by microorganisms in the rhizosphere. Microbiome functions include carbon and nitrogen sequestration, precipitation of metals by bacterial and root surfaces and exudates, decomposition of organic matter, and the degradation of pollutants (Huang et al., 2012; Novo et al., 2013).

The soil biota is extremely diverse in structure, function and population. Core species (such as nitrifiers and methanogens) are defined as groups of organisms that influence mechanisms in specific ecosystem functions (Suzuki et al., 2005). For plant growth, microorganisms of significance are arbuscular mycorrhizal fungi (AMF) (Thiele-Bruhn et al. (2020), ectomycorrhizal fungi (EcM) and bacteria (Thavamani et al., 2017) which are both unique to and abundant in the rhizosphere. It is commonly categorised as plant growth promoting rhizobacteria (PGPR). These papers suggest that mining processes have a negative impact on the diversity of the AMF and saprotrophic communities, which are responsible for nutrient and metal accumulation.

Dangi et al. (2012) identified gram-positive and gram-negative bacteria, eubacteria, fungi, AMF, and actinomycetes in reclaimed mine soils, while Thavamani et al. (2017) reported on the prolific nature of acidophiles (archaea and bacteria such as *Acidobacterium capsulatum*) in soils with acid drainage issues. The research provided an overview of the soil microbial diversity in mined sites. It included nitrospira (iron-oxidizing bacteria such as *Ferroplasma acidiphilum*), bacteroidetes (e.g. *Flavobacterium sp.*), proteabacteria (alpha-, beta- and gamma-proteabacteria such as *Acidithiobacillus spp*. and *Acidiphilium spp.*), actinobacteria (e.g. *Ferrimicrobium acidiphilum*) and firmicutes (includes iron-reducing and sulphur-oxidizing bacteria such as *Sulfobacillus acidophilus* and *Alicyclobacillus pomorum*).

The microbial diversity of Technosols, especially that of CW amended-Technosols, is ill-defined. The microbiome structure and function will vary from native mine soils, but the microorganisms identified by Thavamani et al. (2017) provide a good framework to build upon when undertaking this investigation. Microorganism proliferation is a challenge in metalliferous soils; therefore, by employing bioaugmentation to incorporate selected efficient microbes in the form of a soil microbial inocula (SMI) it could potentially result in microbial colonization and sustainable remediation of degraded mine soils. Moreira-Grez et al. (2019) and Thavamani et al. (2017), both suggested an inoculum comprised of PGPR, nitrifiers and phosphate-solubilizing (e.g. *Pseudomonas* and *Azotobacter*), AMF, and phosphate- and potassium-solubilizing bacteria for soils fabricated from mining waste. However, the selection of a specific inoculum must be based on the pollutants that need to be targeted for degradation.

Botta (2015) suggests that an acidic pH leads to a decreased rate of microorganism proliferation; thus, reduced microbial activity. High concentrations of nitrogen and organic carbon are an indication of active microorganisms (Dangi et al., 2012; Herran Fernandez et al., 2016). Therefore, when investigating soil biota, pH measurements, electrical conductivity and elemental analyses should also be performed to provide supporting evidence for microbial activity results. The microbial composition, function and diversity data should be related to the results from environmental assessments to evaluate the impact of environmental and technical processes on soil biota (Thiele-Bruhn et al., 2020).

3 SETTING OUT THE STUDY – RESEARCH APPROACH AND METHODS USED

3.1 Scoping the Project

The experimental approach used in this study was developed from the proof-of-concept studies presented in WRC K5/2231, entitled 'An Industrial Ecology Approach to Sulphide-containing Mineral Wastes to Minimise ARD Formation' (Harrison et al. 2020) in which fabricated soils were made using mixtures of coal waste and native topsoil as the main substratum (parental material). In the current study, we expand our investigation beyond proof of concept to focus on the following:

- The required characteristics of the waste coal fractions used to ensure a successful fabricated soil, with associated characterisation of these coal waste materials
- The refinement of the soil fabrication procedure
- The characterisation of the fabricated soils to assess their physico-chemical structure, their potential to maintain regenerative conditions and their microbial consortia and activity to ensure a fertile soil with good nutrient accessibility and cycling
- The preliminary environmental and economic considerations of the potential of fabricated soil use in mine site rehabilitation

In addressing these, the following objectives were laid out for the project:

- Comprehensive characterisation of coal waste, occurrence of ARD and associated leaching of heavy metals and other potentially toxic elements
- Recovery of values and removal of risk-bearing components from coal waste using mineral processing techniques
- Evaluation of the repurposing of different coal waste streams for fabricated soils
- Identification of the aspects related to the effect of different fabricated soil mixtures on resultant soil quality and productivity
- Evaluation of the need for addition of alkaline amendments in fabricated soil produced with acid generating material
- Study of the environmental factors associated with fabricated soils in terms of the presence and mobility of contaminants in the soil-plant system in different FabSoil mixtures
- Development of a procedure for a top soil fabrication using coal waste and organic amendments according to national mined land rehabilitation guidelines
- Study of the behaviour of a single plant species over time
- Preliminary consideration of the environmental and economic benefits of soil fabrication
- Provision of a basis on which to build the demonstration scale and tech-transfer aspects of soil fabrication in collaboration with SMEs and industry, as next step to be undertaken

For this study, coal ultrafine waste samples from two collieries located in the eMalahleni area have been selected for use as main material component from which to build the soils. One sample corresponds to a flotation tailings stream and a second sample to an ultrafine downstream slurry which has not been further processed. Virgin and stockpiled soils are used as auxiliary parental material and controls. Further, in the final study (Section 5.2), a fertile potting soil is used as the positive control. Additional potential amendments and auxiliary materials, available near the mine and in sufficient quantity, to provide economically feasible components to address the soil structure, provision of humic materials, permeability and porosity, nutrient provision and availability, amongst others, are considered.

3.2 An Overview of the Experimental Approach

In this project, two amendments have been used in soil fabrication. The first, compost, is widely available in the mining region and can readily be manufactured in the vicinity using agricultural residue, waste from living areas and biomass growing on the mine site. The second, malt residue, is available from local brewing facilities and is recognised as a rich source of nutrients. The characterisation of degraded soil in the region has also been undertaken. Following selection of coal process streams as samples for this study, test work was undertaken to refine the separations for recovery of values (saleable coal) and removal of sulphur. Environmental characterisation of coal waste as well as characterisation of the products obtained from the two-stage separation technique has been carried out in Section 4. These were assessed, together with characterisation of the amendments and degraded soil, to ascertain the best approach to soil fabrication.

In the first stage of this investigation coal waste, degraded topsoils from Mpumalanga, compost, organic fractions, nutrient sources and physical ameliorant selection have been considered, taking into account the results obtained in the proof of concept for fabricated soils performed at UCT during 2017 (Amaral et al., 2020; Harrison et al., 2020b) (Amaral et al., 2020; Harrison et al., 2020b) as well as the defined final soil characteristics required as determined through literature review and associated studies. In addition to the nutrient profile, chemical constituents and presence of carbon macro-molecules and microorganisms, the design of the soil is required to consider its permeability, hydrodynamics and the capacity of water retention. This required careful selection of methods.

Physicochemical analysis was performed on the new fabricated soils to assess the impact of the biological, physical and chemical characteristics on soil fertility and sustainable plant development. Tests were conducted to allow plant growth studies. Two control experiments using 100%coal waste, 100% topsoils sourced in a local nursery are also included.

The growth experiments were carried out in the greenhouse at UCT under controlled growth conditions; however, no temperature control was used. The drainage rate and water retention of the soil was measured to confirm whether manipulation of the approach to soil fabrication impacts retention of water achieved; this was also compared to natural soils. The growth vegetation studies used a typical grass from South African mine sites for validation, *Eragrostis tef*. In particular, this was selected to be suited to the eMalahleni (Witbank) region in this study and the Waterberg region in subsequent studies should this show promise. Propensity for metal accumulation is considered.

Monitoring and characterisation of fabricated soils was undertaken in terms of micro-biochemical characteristics (microorganisms, development in the root zone, pH, macro and micronutrients), physical parameters (porosity, permeability, density, field capacity and electric conductivity), and environmental factors (available metals, evaporation rate). Plant size and biomass production (above and below ground plant tissue) as well as plant health were measured and compared during and at the end of the experiments. All the studies were conducted in replicates according to the number of growth cycles with and without cultivation, for verification of the development of the soil structure with and without cultivation.

While initially flotation was used for fractionation of fine coal streams where required, in accordance with the suggestion in the “WRC Evaluation Decision Letter" from 1 December 2017, further investigations were carried out without further processing of the coal waste samples to assess feasibility while reducing cost and complexity. Technical and economic aspects will be considered to make sure it is suitable for commercial realities. For this, fabricated soil experiments were performed using ultrafine coal waste ‘as received’ in parallel with the two-stage separation studies.

Figure 3-1 presents a schematic flowchart of the materials and operation units for the project.

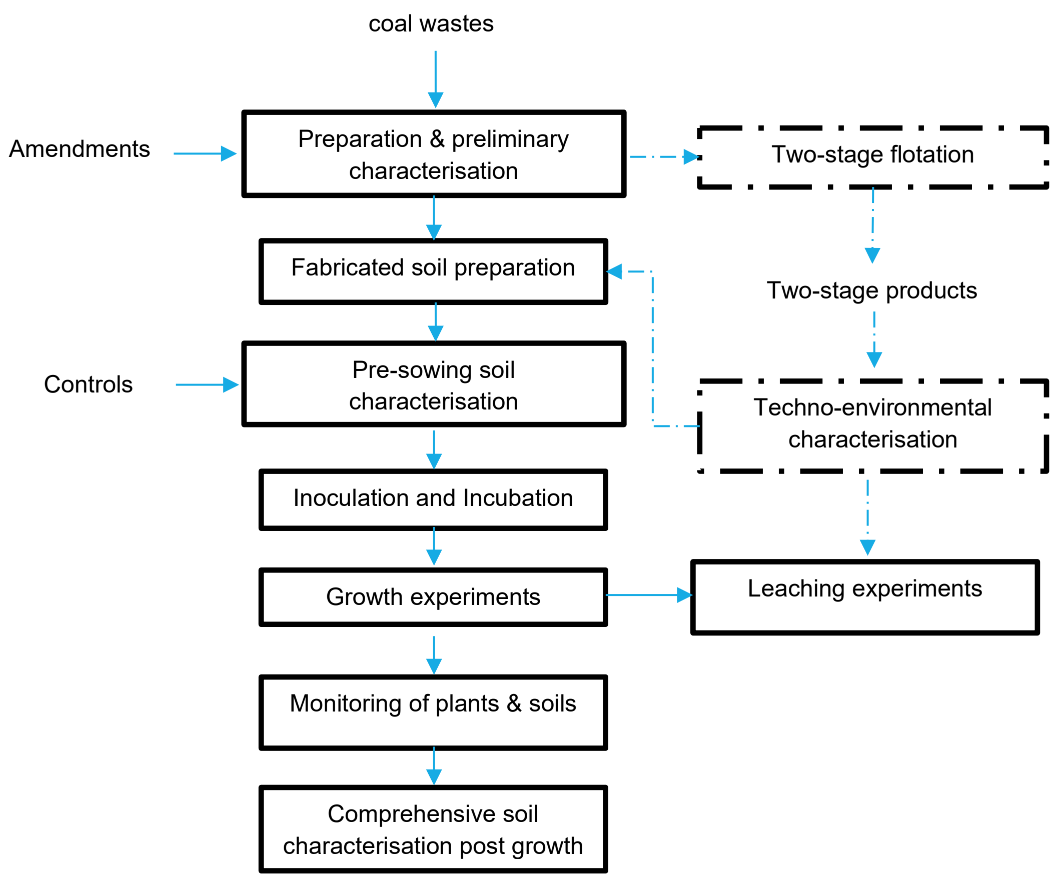

Figure 3-1 Schematic flowchart of materials and unit operations conducted during the experiment.

3.3 Two-stage flotation

Two ultrafine coal wastes from different collieries were used in the froth flotation studies:

- Flotation tailings coming from an operating a flotation circuit; and
- Slurry coming from a slurry dam.

In this phase of the study, first stage flotation experiments were conducted on both samples to evaluate the potential to recover coal. Owing to the non-acid generating capacity of the coal tailings sample, the 2nd stage sulphide flotation step was carried out only for the slurry samples.

Technological characterisation was conducted on the ultrafine samples according Table 3-1. The flotation conditions used in this work are based on the work reported in Kazadi Mbamba (2011) and Iroala (2014), shown in Table 3-2. Table 3-3 shows the three different dosages of the collectors and the dosages for reagents used for the preliminary test-work of two-stage flotation

Table 3-1 Pre-flotation coal waste characterisation conducted in the slurry and tailings ultrafine coal waste samples, from two collieries in eMalahleni.

Test	Method
Particle size distribution	laser diffraction scattering
Ash content	ASTM D3174 12
Total CHNS	ASTM D 4239
Elemental analysis	x-rays fluorescence & inductively coupled plasma-mass spectrometry
Specific Gravity	ASTM D854 14
Static ARD tests	Modified ABA and NAG tests

Table 3-2 Experimental flotation conditions and reagents to be used for the two-stage flotations experiments

Tested Flotation Conditions	
Coal collector	Oleic acid
Sulphide collector	PAX
Coal depressant (2nd stage)	None and Dextrin
Frother	MBIC
Pulp density	
1st stage*	7%
2nd stage**	7%
Pulp pH	
1st stage*	Natural
2nd stage**	Natural
Fixed Flotation Conditions (Iroala, 2014; Kazadi Mbamba, 2011)	
Air flow rate	5-6 L min^{-1}
Impeller speed	1 500 rpm
Flotation time	
1st stage*	5 minutes
2nd stage**	20 minutes

*coal flotation; **sulphur flotation

Table 3-3 Three reagent dosages used for two-stage flotation performed

coal flotation step			
Collector: Oleic acid (kg/t)	0.7	1.4	2.79
Frother: MIBC (kg/t)	0.28	0.28	0.28
sulphide flotation step			
Collector: PAX (kg/t)	1.4	1.86	2.33
Frother: MIBC (kg/t)	0.11	0.11	0.11
Depressant: Dextrin (kg/t)	0.93	0.93	0.93

3.4 Fabricated Soils Growth Trial

Figure 3-2 summarizes the eight stages in the experimental plan that have been set out to achieve the project objectives. The first stage is material selection, followed by the preparation thereof for characterisation which is the third step of the experimental plan. Subsequently, soils were fabricated based on procedures described by Weiler et al. (2020a) for bioaugmented coal based Technosols. Prior to conducting plant growth trials, the technosols were characterised for physiochemical conditions and soil samples were collected for soil microbiome analysis. To investigate the potential of the technosols to function as self-sustaining topsoils, plant growth trials using *Eragrostis tef* were performed in a greenhouse (sixth stage of the experimental plan) and pre-determined parameters were monitored throughout the trials. After this, plant biomass and soil were characterised accordingly, technosols microbiomes were analysed and profiled using microbial techniques FDA measuring microbial activity, SIR, DNA extraction and qPCR for microbial species identification.

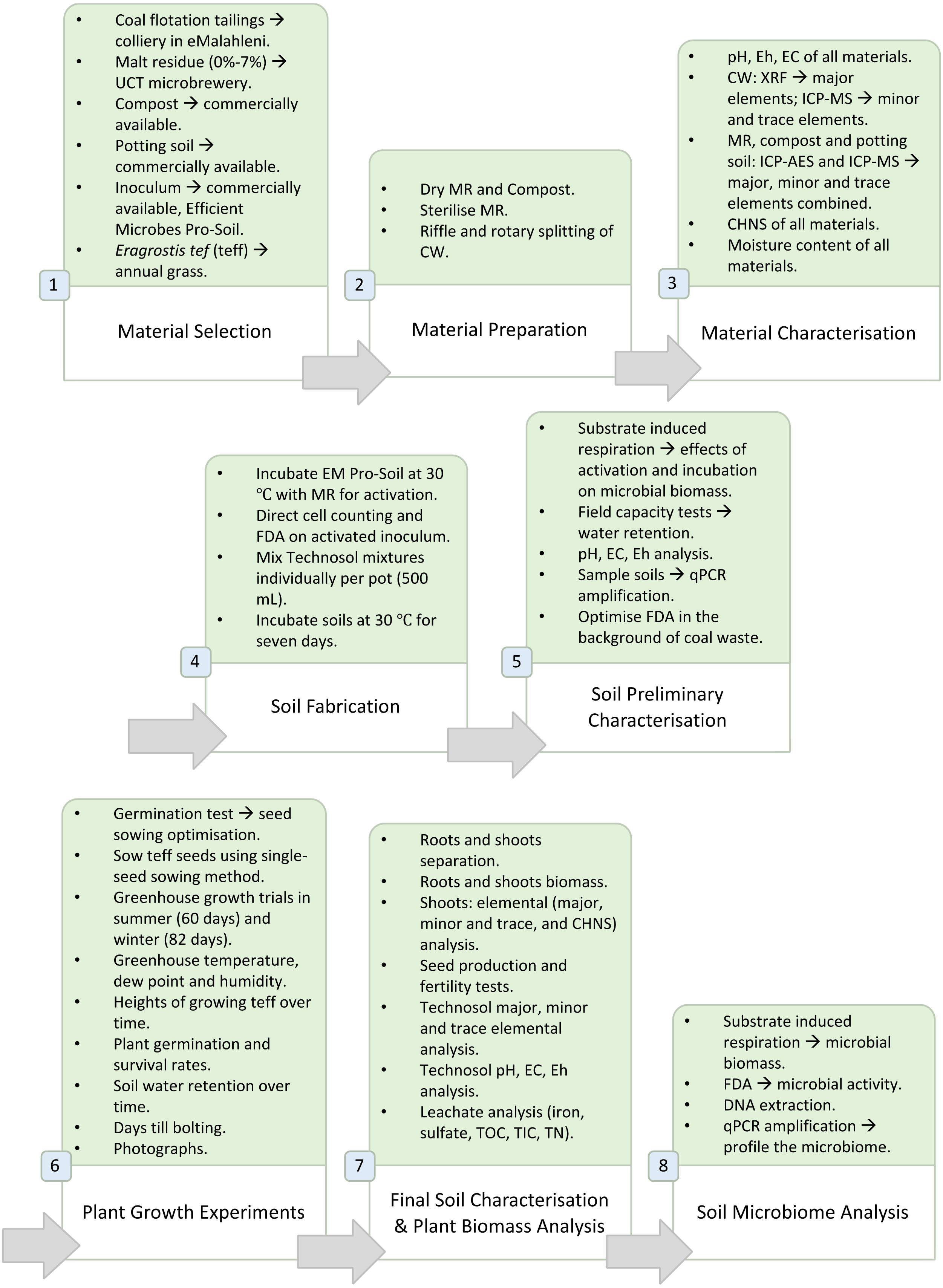

Figure 3-2 Experimental plan for FabSoils studies

According to Food and Agriculture Organization of the United Nations (2015), soil textures are defined by the relation of particulate size fractions, namely sand (0.063-2 mm), silt (also termed coarse silt) (0.002-0.063 mm) and fine particles such as clay or fine silt (<0.002 mm). In terms of texture classification (Figure 3-3), the coal ultrafine wastes have up to 4 times higher content of materials classified in line with silt and clay compared to the native soil samples used by Amaral et al. (2020) hence are classified as sandy loam materials while the native soil is classified as fine to medium sand. Despite its very fine texture, usually sandy loam soils do not restrict root growth; however, they are highly susceptible to mechanical compaction.

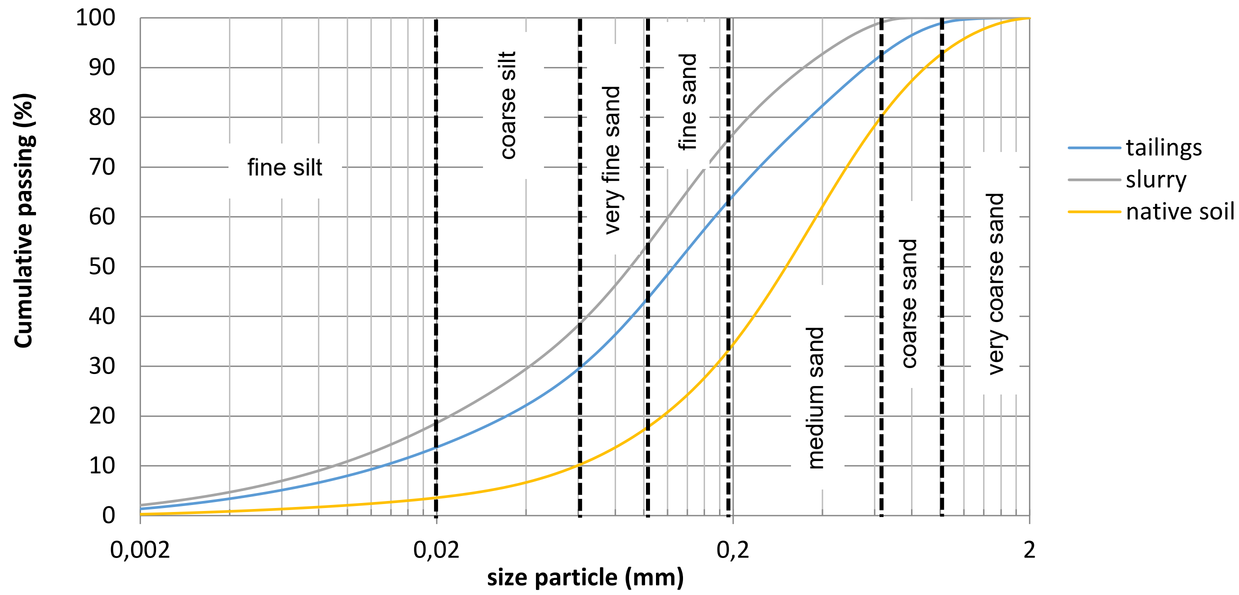

Figure 3-3 Particle size distribution against soil texture, according to (Food and Agriculture Organization of the United Nations, 2015(Food and Agriculture Organization of the United Nations, 2015) for coal waste and native soil samples. Slurry = D_{50}~0.053 mm; Tailings = D_{50}~0.106 mm; Native soil = D_{50}~0.285 mm

The amendment selected for soil fabrication in this project was malt residue. to provide components to address the soil structure, provision of humic materials, permeability and porosity, nutrient provision and availability, amongst others. Malt residue or also called spent grain, from a brewing process, is the most produced by-products from brewing processes. This material consists of the barley (or other cereal) grain husks obtained as solid residue after the production of liquid medium for brewing, wort, being rich in fibre and protein and, to date, the main option for final disposal of this by-product has been as an animal feed (Lynch et al., 2016).

3.4.1 Material Characterisation

Coal waste tailings were characterized using a combination of XRF for major elements (Al, Ca, Fe, K, Mg, Mn, Na, P, Si, Ti) and ICP-MS for minor and trace elements (Sc, V, Cr, Co, Ni, Cu, Zn, Rb, Sr, Y, Zr, Nb, Mo, Cs, Ba, La, Ce, Pr, Nd, Sn, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Hf, Ta, Pb, Th, U) to determine if the quantified values would result in engineered soils that are within national legislative limits for arable land (Botta, 2015). The major, minor and trace elements combined (Na, Mg, Ca, K, P, Si, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Hg, Pb) were analysed in MR, compost and potting soil by total digestion followed by ICP-AES and ICP-MS, the latter carried out in the Central Analytical Facilities at Stellenbosch University. Total carbon, hydrogen, nitrogen and sulphur content was determined using a Perkin Elmer 2400 CHNS Analyzer. The elemental contents of materials are characterised to determine their applicability in soils fabricated for vegetational growth and for environmental considerations (Herran Fernandez, et al., 2016). Material bulk densities defined as dry weight per unit volume of soil, considered both the solids and pore space for compaction analysis. Dry weight and moisture content analyses were performed as defined in the SSSA protocols (1996).

The physiochemical parameters of all materials; pH, EC and Eh, were analysed (deionised water to material ratio of 5:1) according to soil standard procedures described by Tedesco et al. (1995). A calibrated Jenway 3510 pH meter (Jenway; Staffordshire, UK), an AZ 86555 probe (AZ instrument corporation; Taichung City, Taiwan), and a Metrohm 827 pH Lab probe (Herisau, Switzerland) were used for pH, EC and redox measurements, respectively. Measurements were conducted in triplicate.

Field capacity was determined by placing 100 mL of Topsoil or Fabsoil samples into a measure cylinder. After 100 ml of water was carefully poured over the soil, avoiding preferential ways. Drainage was collected into a measuring cylinder. The final volume of water in the measuring cylinder after 24 hours was recorded. The field capacity could then be calculated as the difference between the initial 100 ml of water and the final volume collected after 24 hours. The results are presented in percentage of water retained in the soil. The drainage profile of the samples were determined using the same method as the field capacity experiment

3.4.2 Soil Fabrication – inoculum development, soil fabrication and incubation

To ensure physiological adaptation and growth of the microbial community in bioaugmented Technosols, *the inoculum preparation process* was completed over seven days at 30°C (Schiraldi & De Rosa, 2014). Based on the methodology described by (Weiler et al. (2020), EM Pro-Soil was added in volumes of 250 mL to 2.5 g of dried MR per sterilised 250 mL Erlenmeyer flask, covered with cotton wool and aluminium foil. All flasks were placed on a platform shaker (Labcon; Mogale City, Gauteng, South Africa) at 120 rpm and 30°C for seven days. Samples of 5 mL were taken daily to evaluate the effect of incubation on microbial diversity (through direct cell counting, described as follow) and activity (through FDA analysis, refer to Section 0).

Direct cell counting was performed with an Olympus CX40 Biological Upright Phase Contrast oil immersion microscope and haemocytometer (or counting chamber) (Olympus Corporation; Shinjuku City, Tokyo, Japan). A standard microscopy protocol was followed (ASTM, 1985). The cell concentration per volume of suspension was determined from the following equation.

$$x_{cells} = \frac{x_{quadrant} \times 1000\ mL^3}{(4 \times Q_t) \cdot V_{quadrant}} \times DF \qquad [2]$$

where, x_{cells} is total cell count of the suspension, $x_{quadrant}$ refers to number of cells counted, Q_t is the number of squares per quadrant, $V_{quadrant} = 0.004\ mm^3$ is the volume of one quadrant, and DF is dilution factor. technosols Fabrication and Incubation

Technosols *were prepared* per pot by mixing the raw materials (weight based on 500 mL pot volume, bulk densities, and correction factor) with a hand spade in a plastic tray until a homogeneous mixture had been achieved. The mixtures trialled as fabricated soils are detailed in Table 3-4. Fabricated soils and agricultural soils without the addition of EM Pro-Soil were used as controls. Inoculum was added to the experimental soils at 0.09 mL per gram technosols. To corroborate previous research by Weiler et al. (2020) on EM Pro-Soil in MR-amended coal-based soils, the inoculum was added at 2.5 x 10^7 cells per gram technosol.

Soil incubation followed soil fabrication to further ensure physiological adaptation of soil microbes (Ntougias, et al., 2006). Soil incubation followed soil fabrication to evaluate the effects of inoculation on various Technosols and to kickstart microbe-mediated processes, especially nitrogen fixation (Chenu, et al., 2015). All Technosols and control soils were packaged in individual A4 plastic bags (Ziploc; San Diego, CA, USA) and placed in a 30°C temperature controlled room for seven days similarly to Weiler et al. (2020). Each bag was mixed and aerated daily to reduce fungal growth and minimise clumping.

After incubation, triplicate samples of all treatments were collected in Eppendorf tubes (2 mL) and stored at -20°C. These samples were used during soil microbiome analysis.

3.4.3 Soil Preliminary Characterisation

Before plant growth experiments were conducted, Technosols and the controls were characterised for physiochemical and biological conditions. These properties provided information on the ability of the fabricated soil to withstand abiotic stresses and support plant growth (Richter, 2007; Valarini, Diaz Alvarez, Gasco, Guerrero, & Tokeshi, 2003). In addition to, providing information on the influence of amendment dosage on soil quality (Herran Fernandez, et al., 2016). Substrate induced respiration experiments (described in Section 3.6) were performed to determine the initial microbial biomass present within Technosols, DNA extraction and qPCR amplification for initial microbiome profiling (Section 3.6). Field capacity tests were done to determine water holding capacity (Mills & Fey, 2004; SSSA, 1996), and pH, EC and Eh measurements were taken (refer to 0).

Field capacity were determined using 100 g of each fabricated soil, placed onto separate water-wetted filtration paper in a funnel on 250 mL Erlenmeyer flasks. The weight of the dry and wet filtration papers were recorded for each sample. Subsequently, 250 mL of tap water measured in a volumetric cylinder was poured over each soil filled funnel. After a 24h drainage period, the volume of water collected in each flask was measured with a volumetric cylinder. The difference between the volumes of water initially added and drained was determined (water retention). From this, the FC per pot of technosols was determined using initial soil weights.

3.4.4 Plant Growth Experiments

Evaluation of fabricated soils typically makes use of plant growth experiments to evaluate soil health and quality (Firpo, et al., 2021; Prado, et al., 2020; Deeb, et al., 2017). Following soil fabrication, pots were seeded and initially monitored in the laboratory (24-h-day) at constant abiotic conditions to ensure seedling emergence (Sayuti & Hitchmough, 2013). Whereafter, pots were moved to a greenhouse located on the University of Cape Town’s Upper Campus.

Plant growth experiments were conducted in a greenhouse at controlled conditions (16-h-day/8-h-night cycle) to simulate endemic site conditions. Relative humidity, temperature and dew point were automatically recorded every hour for the duration of each plant growth trial. Fabricated soils and controls were potted into 500 mL Polyvinyl Chloride (PVC) pots with drainage holes. All soils were transferred directly from the incubation bags (minimal moisture loss) into individual pots after mixing. It was assumed that negligible amounts of micro-plastics were transferred to soils and that the effects on plant growth were negligible compared to other abiotic variables. The pots were randomly placed onto a single-tier stainless steel plant rack. This ensured that specific Technosols were not negatively affected by spatial variation within the greenhouse, which aligns with the assumption made throughout the study. Pots were perpendicular to the greenhouse racks and were not rotated during the trials to mimic stationary plant growth in agriculture.

To evaluate the *E. tef* growth performance in the fabricated soils, *E. tef* growth trial was conducted during autumn and winter. The structure of the final plant growth trial is summarized in Table 3-4. An optimised irrigation strategy proceeded from the results in the initial trial, accompanied by a better suited control soil; potting soil, a precedent for technosols performance.

Each treatment was performed in quadruplicate and these were evaluated with and without *E. tef*. No *E. tef* controls were used to evaluate the effect of plants in nutrients availability and uptake. In addition, all treatments except pure potting soil were evaluated with and without the inoculum to investigate soil related benefits gained from inoculation.

Table 3-4 *E. tef* growth trial in bioaugmented and biostimulated coal waste tailings (CW-T)-based Technosols. Here, I represents inoculated and NI the non-inoculated treatments.

	Components (wt.%)			Bioaugmentation	Greenhouse	
technosols ID	CW-T	MR	Potting Soil	EM Pro-Soil	With *E. tef*	No *E. tef*
CW-T100%; I	100	0	0	Yes		
CW-T100%; NI				No		
CW-T+MR2.5%; I	97.5	2.5	0	Yes		
CW-T+MR2.5%; NI				No		
CW-T+MR5%; I	95	5	0	Yes		
CW-T+MR5%; NI				No		
CW-T+MR7%; I	93	7	0	Yes		
CW-T+MR7%; NI				No		
Control	NA	NA	100	No		

3.4.5 Seed Sowing

Eragrostis tef was chosen as a suitable plant species for vegetation in coal-based Technosols as it is a pioneer species which is commonly found in the eMalahleni area and used in seed mixtures for rehabilitation of mine sites (Amaral Filho, et al., 2020). It is known for its durability and tolerance to saline soil conditions (Dame, 2020). An initial small scale test on *E. tef* germination in the various Technosols showed that the soil structure changed with different dosages of MR and altered the germination rate of *E. tef* in fabricated soils. Hence, a single-seed sowing technique was developed and employed for the two growth trials to reduce structural impedances on seed germination. Sowing seeds on the soil surface and optimising the germination thereof would be beneficial when considering implementation processes of this rehabilitation scheme. The sowing process is illustrated in Figure 3-4.

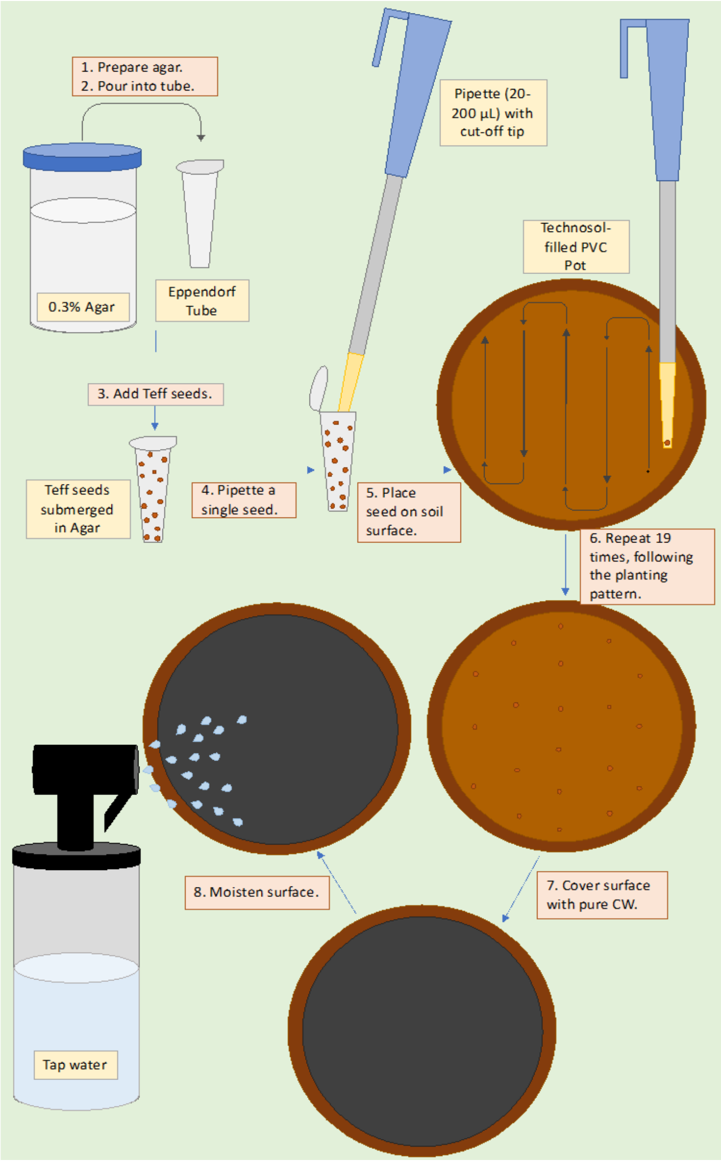

Figure 3-4 Single-seed sowing process.

E. tef seeds were suspended in 0.3% (w/v) agar. From which, individual seeds were placed with a micropipette (20-200 µL) on the surface of each fabricated soil. Sown seeds were covered with a thin (4 mm) layer of pure CW and moistened with tap water. Twenty seeds were sown per pot to ensure sufficient seedling emergence.

3.4.6 Seedling Emergence and Plant Survival Rates

Daily monitoring included seedling quantification for seedling emergence and plant survival rates. Seedling emergence assays were conducted in the first 31 days after planting analogously to Weiler et al. (2020a). Whereafter, the number of plants that were able to grow until maturity in all pots placed within the greenhouse were quantified every day. After 19 days of experiments, the number of plants in each pot were normalized to 6. A plant growth cycle was monitored from seed planting until all shoot heights stabilized, inflorescence had been initiated and maturity had been reached in all plants, similarly to the approach of Weiler et al. (2020a).

3.4.7 Irrigation and Evapotranspiration Analysis

Crop irrigation in greenhouse plant growth studies requires daily assessment (Nikolaou et al., 2019). Consequently, in this investigation all pots were watered daily to sustain plant growth. During the seedling emergence period, sprouts are very fragile and susceptible to pests, fungi and dehydration. Therefore, the pots were kept in the laboratory for seven days until the seedling emergence rates had stabilised (Sayuti and Hitchmough, 2013). Here, pots were placed in tap water-filled trays (15 mm) and covered with plastic film. Small holes were made in this film to allow necessary oxygen transfer whilst minimizing evaporation. The layer of water created a humid environment ideal for seedling germination. Seedlings were quantified each day and moisture levels were adjusted with a water spray-bottle containing lab tap water (pH between 7.6 and 8.6).

Greenhouse irrigation was done on a field capacity basis. Each pot was weighed daily, and rain water (collected in a water tank outside of the greenhouse) was added to keep all soils in pots at the respective 50% field capacity level as suggested by Doran et al. (2013).This ensured optimised irrigation (no dehydration or over-watering) of *E. tef*. Daily irrigation data was used to determine evapotranspiration rates for Technosols and estimations on water requirements for implementation.

3.4.8 Plant Heights and Growth Rates

Throughout each plant growth trial, plant heights were recorded to determine growth rates in Technosols. Once *E. tef* shoots reached a measurable height, the number of plants per pot were carefully reduced to 6, or maintained if already less than 6, to minimize competition between plants. Seedlings were removed with garden tweezers to prevent disruption of root growth in adjacent plants. This occurred on day 21 and day 30 since planting for the initial and final trials, respectively.

Plant heights were measured from the soil surface to the longest apical meristem similarly to Weiler et al. (2020). Shoot heights of all *E. tef* per pot were recorded at the start and end of every working week. Heights were measured with a stainless steel 60 cm ruler. Representative plants for all Technosols were photographed once weekly for a visual diary of *E. tef* development within all Technosols.

3.5 Soil and Plant Biomass Characterisation

3.5.1 Above and Below Ground Plant Biomass Yields

Upon termination of a growth trial, all pots were transferred back to the laboratory for analysis. The pots were weighed and the final number of plants per pot were quantified. As per the standard operating procedure, visible plant biomass (shoots) were cut and dried in a constant flux oven at 60°C for 48 hours to determine the above ground plant dry biomass per pot and *E. tef* yield per technosol.

Soil-like substrates were dried for seven days (room temperature; 22°C) until all moisture had been lost (Amaral Filho, et al., 2020) before *E. tef* roots were removed by hand and rinsed with water similarly to (Weiler et al., 2020a). From the standard operating procedure, roots were dried in a 60°C constant flux oven for 48 hours, after which it was weighed to quantify the yield of below ground biomass per technosols.

3.5.2 Plant Biomass Characterisation

To investigate the feasibility of vegetation in coal-based technosols as topsoils and in support of technosol characterisation, collected *E. tef* biomass were characterised according to major, minor and trace elements. Results inferred on phytoremediation potential of *E. tef*, soil biogeochemical processes and the potential of using *E. tef* as cattle feed. Dried plant biomass (shoots and roots) were analysed for Na, Mg, Ca, K, P, Si, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Sn, Sb, Ba, Hg, and Pb using ICP-MS. CHNS analyses on the dried plant shoots were also performed with a Perkin Elmer 2400 CHNS Analyzer. Elemental analysis was outsourced to the centre for analytical facilities (CAF) at the University of Stellenbosch (SUN).

3.5.3 Seed Production, Yield and Fertility

E. tef seeds were collected to evaluate grain yield and fertility thereof. As *E. tef* grains are high in nutrition and used in the food and beverage industry, as well as for cattle feed (KETEMA, 1991; ZHU, 2018); a high seed production is desired. The seed collection process consisted of placing the shoots in plastic bags and rubbing it to release the seeds within all spikelets. Shoots and seeds were transferred to a sieve (mesh size 20; 840 microns) and rubbed through repeatedly, allowing all grains and some biomass to pass. Subsequently, seeds and remaining shoot biomass were placed onto a sheet of paper. Using the electrostatic forces between paper and plastic, remaining fine plant biomass were carefully separated from the heavier seeds. The seeds per plant per pot of technosol were weighed and placed into clean, labelled Eppendorf tubes (1.5 mL) for additional fertility tests.

To evaluate the extended effects of mine waste-based soils and commercial inoculums on grain fertility and quality of collected *E. tef* seeds from both trials, small scale germination tests were conducted for 14 days (OECD, 2006). Seeds from both trials were sowed (using the single-seed sowing method) according to their original labelled pot number, onto pure CW in open petri dishes. CW as medium eliminated variability that could be introduced from natural soil due to varying physiochemical characteristics and microbial communities. Petri dishes were placed in trays containing a 5 mm layer of tap water, and plastic film were placed on top to create an ideal environment for germination. The surface of the petri dishes were moistened and monitored daily. Seedling emergence was recorded.

3.5.4 Leachate Analysis

The microbial-meditated cycling of Fe and S elements are interlinked; thus, ferrous and total iron and sulfate leaching analysis were performed. Leachates were collected from final soil field capacity tests.

Ferrous and total iron concentrations in the collected leachates were determined by spectrophotometry with 1-10 phenanthroline following standard laboratory procedures (CeBER, 2018). Roughly; for ferrous iron concentrations; leachate samples were filtered to remove suspended solids. Whereafter, 2 mL ammonium acetate buffer solution (stock solution), 2 mL 1-10 stock phenanthroline solution and 1 mL leachate sample were added to each empty, clean test tube and vortexed for 1 minute. Three blanks (2 mL acetate buffer, 2 mL 1-10 phenanthroline solution and 1 mL deionised water) were prepared for auto-zeroing of a Genesys 10S UV-Vis spectrophotometer (Thermo Fisher Scientific; Waltham, Massachusetts, United States). Five minutes were allowed for the chelation reaction between ferrous iron and phenanthroline to form orange-red complexes. After which, test tubes were vortexed and 1 mL per sample was transferred to a clean cuvette to record absorbance measurements (510 nm). A standard curve ranging from 0-50 mg/L Fe^{2+} was prepared to ensure proportionality.

For total iron concentrations in soil leachates, the reaction volumes (including blanks) were returned from the cuvettes to test tubes with one micro-scoop hydroxylamine, and vortexed (30 s) to ensure all hydroxylamine had dissolved. To allow for Fe^{3+} reduction by hydroxylamine, samples were left for 5 minutes. Finally, samples were vortexed (30 s) and poured into individual, clean cuvettes for absorbance measurements (510 nm). If absorbance measurements of blanks were above 0.04, samples were contaminated and fresh blanks had to be prepared.

Sulfate turbidimetric analysis was performed based on the standard protocol (APHA – American Public Health Association, 2005). Roughly; leachate samples were filtered to remove suspended solids of which 5 mL of appropriately diluted sample (1 to 4 dilution with deionised water) was added to a test tube with 0.25 mL of stock conditioning solution and one micro-scoop of finely ground (20 to 30 mesh) barium chloride crystals.

Three blanks (5 mL deionised water, 0.25 mL conditioning solution and one micro-scoop of $BaCl_2$ crystals) were prepared. All samples and blanks were vortexed for 1 minute, before transferring 1 mL sample to a clean cuvette for absorbance measurements (at 420 nm) in a Genesys 10S UV-Vis spectrophotometer (Thermo Fisher Scientific; Waltham, Massachusetts, United States). Absorbance measurements were correlated to sulfate concentrations from the prepared sulfate standard curve (0-50 mg/L SO_4^{2-}).

3.5.5 Soil Physiochemical Analysis

Triplicate samples of soil in each pot were collected in Eppendorf tubes (2 mL) and stored at -20°C. These samples were used in the soil microbiome analysis. Technosols and control soils were dried at room temperature (22°C) in the respective pots for seven days. Additional leachate TOC tests were performed using a Multi N/C 3100 TOC analyser (Analytik Jena; Reinhach, Switzerland). The soil CEC, potential acidity (H + Al), T value, OM, and elemental content (C, H, TN, S, P Bray II, K, Ca, Mg, Na, Cu, Zn, Mn, B, Fe) were performed by Bemlab Soil Analysis, Somerset West. Soil field capacity, pH, EC, and Eh were measured as previously detailed in section 0. The results addressed the agricultural potential of using Technosols as topsoils. Table 3-5 describes the sols analysis conducted in this study.

Table 3-5: Soil fertility characterisation analysis to be conducted in the Fabricated Soils (AgriLASA, 2004)

Parameter	Description
pH	Indication whether the soil is acidic or alkaline
Resistance	Indication of the soil salinity. Usually soil with resistance <300 Ω is regarded as saline
Na	Non-essential basic nutrient which destabilises soil structure
K	Essential nutrient Increases crop yield and improves quality. Required for numerous plant growth processes, including maintenance of turgor, reducing water loss and wilting
Ca	Essential nutrient that stabilises soil structure. Essential for good growth and structure. Insufficient Ca levels lead to deterioration of the cell membrane
Mg	Essential element for many critical physiological and biochemical processes. Especially photosynthesis in plants is adversely affected by Mg deficiency.
P Bray II	Essential nutrient, immobile in soil and vital to plant growth and found in every living plant cell. Involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars, nutrient movement in plant and transfer of genetic characteristics across generations.
Bulk Density	Defined as dry weight per unit volume of soil, taking into account both solids and pore space. Particle density considers only the mineral solids.
Cation exchange capacity (CEC)	A measure of fertility and nutrient retention capacity. Indicates a soil's capacity to protect groundwater from nutrient contamination and to ensure nutrient bio-availability.
Stone fraction	The volume of stone in the soil reduces the reactive volume of the soil. It must be taken into account when lime, gypsum and P-fertilisation requirements are calculated.[ASTM D6913]
Fe	Essential micro-nutrient for plants. Abundant in soil, but not necessarily bio-available for plant uptake. Fe acts as catalyst to chlorophyll production. Essential for protein production.
Mn	Essential micro-nutrient for plants. Plays a direct role in chlorophyll synthesis & photosynthesis

Parameter	Description
Cu	Essential metal for plants. At excessive levels in soil, inhibits microbial activity. In plants Cu plays a key role in photosynthetic and respiratory electron transport chains, cell wall metabolism and stress protection.
Zn	Essential micro-nutrient for plants. Zinc deficiencies stunt growth and cause chlorosis and smaller leaves, increasing crop maturity period, sterility and inferior quality of harvested products
B	Essential micro-nutrient for plants, but difference between deficient and toxic B concentrations is very small. Boron impacts cell wall strength and development, cell division, fruit and seed development, sugar transport and hormone development in plants
C organic (walkley-black)	Organic matter in soil improves microbiological activity, soil water holding capacity and fertility (cation exchange capacity (CEC)) as well as soil structure
Electric conductivity	Indicative of ability of an aqueous solution to carry an electric current. Plants are detrimentally affected by excess salts in some soils and by high levels of exchangeable sodium in others
Base saturation	Percentage of the CEC occupied by the basic cation

3.6 Soil Microbiome Analysis

The methods chosen to investigate the technosol microbiology is supported by literature on soil microbiology. Biological soil properties were evaluated to support physical and chemical characteristics that discern the effects of amendment dosages and bioaugmentation on plant-microbe interactions to perform soil processes such as nitrogen cycling and carbon sequestration.

3.6.1 Microbial Biomass through Substrate Induced Respiration

Microbial biomass through substrate induced respiration experiments based on the protocol by Jaggi (1976) were performed prior to and after plant growth as an indication of the rate and persistence of microbial proliferation in the various technosols. Microbial function, specifically OM decomposition, is discerned from induced microbial respiration in soils (Graca & Abelho, 2020).

All samples were performed in triplicate with four blank samples. Roughly; 60 mg glucose was added to 20 g technosols in a 250 mL Erlenmeyer flask and incubated (22°C) for two hours. Subsequently, an alkali trap was made by placing a 50 mL plastic beaker containing 10 mL of NaOH (0.125 M) in each of the soil and glucose filled flasks, sealing with parafilm for incubation (4 hours at 22°C). Finally, the NaOH was titrated with HCl (0.125 M). Using the titrate volume per sample, the carbon (mg) per gram of soil was determined with the following equation (Jaggi, 1976):

$$C_{mic} = 30(BL - SA) \times \frac{C_{HCl} \times k \times 1000}{\rho_{CO_2} \times SW \times 4}$$

Where, C_{mic} represents the carbon content in microbial biomass (mg C per kg soil), BL is the mean of the titrated HCl volume of the blanks (mL), SA is the titrated HCl volume of a sample (mL), C_{HCl} represents the HCl concentration, k corresponds to 22 mg carbon dioxide per 1 mL of 1 M HCl, 1000 converts g soil to kg, ρ_{CO_2} is carbon dioxide density (mg/mL) at 22°C, SW is sample weight (g) and 4 is the conversion factor of 4h to 1h incubation.

3.6.2 Microbial Hydrolytic Activity through Fluorescein Diacetate Assay

Microbial enzymes are effective bioindicators of soil health and disturbances thereon (Karaca, Cetin, Turgay, & Kizilkaya, 2010). Therefore, analysis on FDA (easily hydrolysed to fluorescein by various enzymes) was a kinetic and rapid method for measuring total microbial activity (Schumacher, et al., 2014).

FDA analysis through spectrometry was optimised for Technosols in the background of CW. All samples were performed in triplicate to ensure statistically relevant results. Soil samples (10 mg soil with 9.90 mL 1xPBS in a 50 mL Centrifuge/falcon tube) were prepared. In an organics fume-hood, a 2 mg/mL FDA/Acetone solution was prepared by adding 10 mL pure acetone to 0.02 mg stock fluorescein diacetate in a 50 mL falcon tube. 1 mL of this solution was added to 49 mL of 1xPBS in a sterilised 50 mL Falcon tube to prepare a FDA/PBS (50 µg/mL) solution. Subsequently, 0.2 mL of the FDA/PBS solution was added to each of the soil samples that were placed on a rotary shaker for incubation (10 min at 37°C). Sub-samples (500 µL) were taken into 2 mL Eppendorf tubes. After 10 mins, hydrolysis was terminated by pipetting 500 µL chloroform into each tube, closing the lids and vigorously shaking all tubes containing the sub-samples (working in an organics fume-hood). All samples were centrifuged at 13 krpm for 10 min in a Universal 320 centrifuge (Hettich; Tuttlingen, Germany). From which, 300 µL of each supernatant was syringe filtered into a qPCR tube. Fluorescence (RFU) was measured with a Quantus™ Fluorometer (Promega; Madison, Wisconsin, USA), and correlated to the amount of soil per sample form dry weight measurements.

3.6.3 DNA Extraction and Quantification

Quantification of soil extractable gDNA was performed to evaluate changes in the technosols microbiome structure due to amendment dosage, bioaugmentation and *E. tef* growth. DNA extraction on soil samples (collected upon soil fabrication and post-plant growth) were performed by using the Machery-Nagel NucleoSpin® Soil kit (Düren, Germany) as per manufacturer's description. Initial nucleic acid quantification and quality was assessed with the NanoDrop® Spectrophotometer (Thermo Fisher Specific; Waltham, Massachusetts, USA). Preceding dilution of the DNA for qPCR analysis, the DNA was also quantified using the Quantus™ Fluorometer (Promega; Madison, Wisconsin, USA) system as described by the manufacturer for double stranded DNA. DNA aliquots containing 1 ng/µl gDNA were prepared and used for subsequent qPCR analyses.

3.6.4 Profiling of Microbial Community through qPCR amplification

The total bacteria, archaea and fungi represented within the gDNA was determined by 16S rRNA RT-qPCR analysis to profile the soil microbial community changes with plant growth in coal-based soils as recommended by Sansupa et al. (2021). Each sample was analysed in triplicate through three separate DNA extraction samples. KAPA SYBR® FAST qPCR Master Mix (2X) Universal (KAPA Biosystems; Cape Town, South Africa) was used to carry out the analysis in Rotor-Gene Q equipment (Qiagen; Hilden, Germany). Total bacteria analysis was performed under the following optimised conditions: 10 min at 95°C, followed by 45 cycles of denaturation at 95°C for 3 s, annealing for 15 s at 63°C, and fluorescence measurement at 80°C. Universal archaea and total fungal analysis followed the same process with annealing at 60°C. Every extraction was conducted in a 15 µL volume containing 1 µL of gDNA template (1 ng/ µL), 200 nM of each primer, and 7.5 µL of the master mix. The specific primer pairs used for total bacteria, universal archaea and total fungal are referenced and summarized in Table 3-6 and Table 3-7.

Table 3-6: Universal primers for bacteria, archaea and fungi in RT qPCR analysis.

		Bacteria	Archaea	Fungi
Forward Primer	Name	27F	Arch787F	5.8F
	Sequence	AGR GTT YGA TYM TGG CTC AG	ATT AGA TAC CCS BGT AGT CC	GAT GAA GAA CGC AGC GAA ATG
Reverse Primer	Name	1492R	Arch1043R	28R
	Sequence	TAC GGY TAC CTT GTT ACG ACT T	GCC ATG CAC CWC CTC T	ATT GAT ATG CTT AAG TTC AGC GGG
Positions (nt)		20	272	163
Amplicon size (bp)		8-27	787-806	74-94
Reference		(Frank, et al., 2008; Bomberg, et al., 2019)	(Fischer, et al., 2016; Yu, et al., 2005)	(White, et al., 1990; Bergman, et al., 2007)

Standard curves for total bacteria, archaea and fungal were produced from 16S rRNA gene standards (10 ng/ µL) for total bacteria, archaea and fungi. Ten-fold serial dilutions of known copy numbers of the plasmid DNA (the range 10^2 to 10^7 copy numbers) were analysed in triplicate to produce the standard curves. Amplification efficiencies were greater than 97% and the coefficient of determination (R^2) for standard curves were above 0.98. All results were processed with the QIAGEN Q-Rex software.

When investigating pedogenesis in fabricated soils, analysis of microbial functions such as nitrogen cycling is necessary to understand shifts in microbial communities elicited by amendment dosages, bioaugmentation, and plant growth (Hafeez, et al., 2012). Primers targeting genes encoding the catalytic enzymes responsible for nitrogen-fixation (nifH), and denitrification (nirK, nirS, nosZ) similar to those applied by (Gupta, et al., 2012) were considered. The gene name and primer pairs are referenced and summarized in the table below. The oligonucleotides (primers) were purchased from Inqaba Biotec (Menlo Park, South Africa).

Table 3-7: Nitrogen cycling primers for RT qPCR analysis.

Gene		nirS	nirK	nosZ	nifH
Protein/Function		Cytochrome cd1 nitrite reductase – denitrification	Nitrite reductase (Cu containing)-denitrification (nitrite to nitric oxide)	Nitrous oxide reduction – denitrification	Nitrogen fixation
Forward Primer	Name	nirS_916F	nirk876	nosZ2F	nifH112F
	Sequence	GTS AAC GTS AAG GAR ACS GG	ATY GGC GGV CAY GGC GA	CGC RAC GGC AAS AAG GTS MSS GT	GGI TGY GAY CCN AAV GCN GA
Reverse Primer	Name	nirS_1332R	nirk1040	nosZ2R	nifH482R
	Sequence	GAS TTC GGR TGS GTC TTG A	GCC TCG ATC AGR TTR TGG TT	CAK RTG CAK SGC RTG GCA GAA	GCR TAI ABN GCC ATC ATY TC
Positions (nt)			876-1040		112-482
Amplicon size (bp)		409	184	267	390
Reference		(Pereg, et al., 2018; Throback, et al., 2004; Wang, et al., 2019)	(Pereg, et al., 2018; Henry, et al., 2004; Bru, et al., 2011)	(Henry, et al., 2006; Pereg, et al., 2018)	(Widmer, et al., 1999; Levy-Booth & Winder, 2010)

The operating protocol for the nirS primers set was optimised at: 10 min at 95°C, followed by 45 cycles of denaturation at 95°C for 3 s, annealing for 20 s at 62°C, extension at 72°C for 20 s, and fluorescence measurement at 80°C. Analysis on nirK, nosZ and nifH genes followed the same protocol; however, annealing (for 20 s) occurred at 64°C, 68°C, and 58°C, respectively. All the results were processed with the QIAGEN Q-Rex software. Each extraction was conducted in a 15 µL volume containing 1 µL of gDNA template (1 ng/ µL), 800 nM of each primer, and 7.5 µL of the master mix. Plasmids containing nirS, nirK, nosZ, or nifH gene fragments were used to generate the standard curves for each. Standard curves were produced similarly to that of the general primers. Amplification efficiencies were greater than 94% and the coefficient of determination for the standard curves were above 0.98.

4 FLOTATION STUDIES

4.1 Preliminary characterisation for flotation test-work

According to Table 4-1, the coal waste slurry sample selected has a particle size distribution (PSD) in which 50% of the material has a particle size lower than 0.09 mm, i.e. D_{50} (D_{10} 0.01 mm; D_{90} 0.35 mm), whilst the flotation tailings sample presented a D_{50} of 0.13 mm (D_{10} 0.01 mm and D_{90} 0.56 mm). The coarser PSD of the fine coal waste tailings is consistent with samples from different collieries which use different separation equipment as well as different process management approaches. The specific superficial area results presented in Table 4-1 are consistent with the size particle distribution.

Table 4-1 Particle size distribution and specific superficial area results for the two ultrafine coal waste samples conducted by means light scattering method (instrumental)

Sample	Particle size distribution (mm)						Specific superficial area ($g.m^{-2}$)
	D_{10}	D_{20}	D_{50}	D_{80}	D_{90}	D_{98}	
Slurry	0.01	0.02	0.09	0.23	0.35	0.57	0.33
Tailings	0.01	0.03	0.13	0.36	0.56	0.91	0.23

Table 4-2 presents the results in terms of mass within each size class as well as ash and total sulphur content by size for the ultrafine samples used in this investigation. Size distribution is consistent with the results obtained from the light scattering method. The ash and sulphur distribution by size increased with decreasing particle size in the slurry sample. In contrast, the tailings coming from the flotation circuit presented a higher sulphur and ash content in the fractions in size classes higher than 0.425 mm despite the small contribution in terms of total mass. For smaller fractions (<0.425 mm), the trend followed that reported for the slurry.

Table 4-2: Ash and total sulphur content by size in the ultrafines (slurry and tailings). Particle size distribution by manual sieving

Max. aperture (mm)	Slurry			Tailings		
	mass (%)	ash (%)	S_{total} (%)	mass (%)	ash (%)	S_{total} (%)
1.00				1.6	38.1	3.4
0.425	2.9	27.1	0.5	12.5	24.0	1.5
0.212	10.2	32.1	0.5	17.5	21.0	0.6
0.15	8.8	35.6	0.6	10.4	20.2	0.5
0.106	8.5	37.4	0.8	13.1	22.8	0.5
0.075	9.9	43.2	0.9	13.6	25.7	0.7
<0.075	59.7	50.1	1.2	31.3	32.1	1.0

The characterisation in terms of total ash and sulphur, specific gravity and natural pulp pH is presented in Table 4-3. In Table 4-4, elemental characterisation in terms of major and minor elements for both samples is presented. As observed, the samples have similar total sulphur content, in contrast, the presence of higher amounts of iron and lower amounts of calcium in the coal waste slurry samples relative to the tailings (Table 4-4), indicates the higher presence of pyrite and lower presence of carbonate. This is confirmed by the pulp pH results shown in Table 4-3.

Table 4-3 Characterisation in terms of total ash and sulphur, specific gravity and natural pulp pH

	Slurry	Tailings
Specific gravity ($g.cm^{-3}$)	2.1	1.5
Ash content (%)	43.7	25.5
Total Sulphur (%)	1.1	0.9
Sample $pH_{water\ (1:1)}$	5.0	7.8

The results in Table 4-4 show relatively high concentrations of the major elements Si and Al for coal slurry samples; this is indicative of the higher content of ash minerals, such as kaolinite and quartz. On the other hand, the results show the coal tailings samples to be higher in Mg and Ca, suggesting a higher presence of acid neutralizing minerals when compared to the slurry samples. The higher carbon content observed in the tailings samples is consistent with the ash content results and indicates even after flotation a considerable amount of coal is still not recovered.

Table 4-4 Major and Minor elements in the ultrafine samples used in for the two-stage flotation test-work.

element (%)	UF tailings	UF slurry
Major elements in wt % ash basis		
Al_2O_3	4.71	13.16
CaO	2.45	0.83
Fe_2O_3	0.92	2.34
MgO	0.31	0.20
K_2O	0.17	0.34
SiO_2	7.82	22.51
TiO_2	0.28	0.77
C	53.9	41.25
H	3,09	2.62
N	1,45	1.13
Minor elements in ppm		
Ba	376.85	942.5
Mn	200.00	102.9
Na	200.00	241.7
P	1700.00	1084.3
Sr	384.05	480.8
Zr	83.8	248.0

The results of the static ARD tests conducted on the bulk samples are summarised in Table 4-5and Figure 4-1. As indicated, a sample is classified as PAF when it has a NAG pH<4.5 and NAPP>0 and as NAF with NAG pH>4.5 and NAPP<0. Samples are classified uncertain when there is an apparent conflict between the NAG pH and NAPP results.

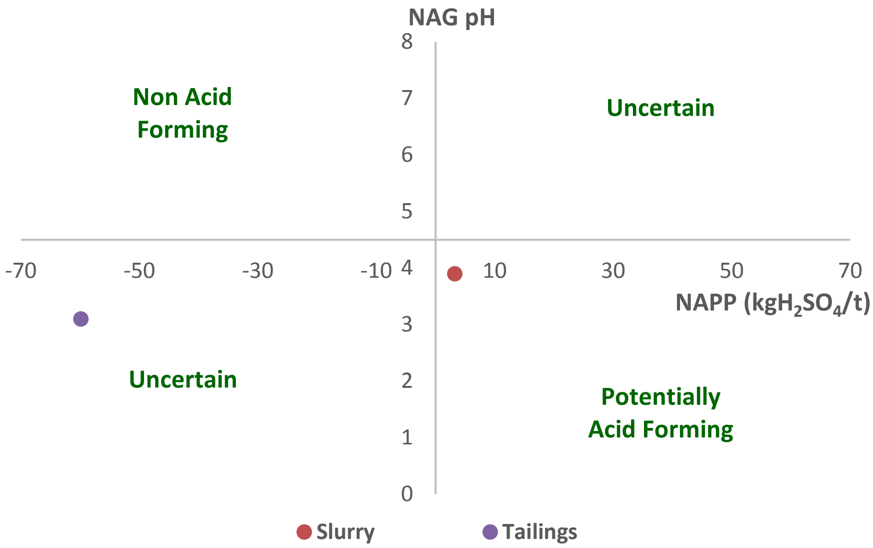

Figure 4-1 Classification of ARD potential for ultrafines coal waste samples by ABA and NAG tests

Slurry samples presented ANC results of 29.1 kg-H_2SO_4 /t. In contrast, the flotation tailings presented an ANC of 87.4 kg-H_2SO_4 /t. Considering the NAPP -59.9 kg-H_2SO_4 /t and the single stage NAG pH results, the flotation tailings sample was classified as uncertain. However, the slurry sample, with higher sulphur content indicated by an MPA of 32.4 kg-H_2SO_4 /t, was classified as PAF.

The coal fines slurry has a total sulphur content of 1.1%, a total high ash content of 44.6%, and neutralizing capacity of 29.2 kg H_2SO_4 /t and was classified as 'Potentially Acid Forming'. The tailings, however, contains only 20% ash and 0.9% sulphur, with a high neutralizing capacity of 87 kg H_2SO_4 /t, and was classified as Uncertain.

Table 4-5: Static Acid Rock Drainage characterisation test results

Sample	ABA				NAG			
	Total S %	MPA	ANC kg-H_2SO_4/t	NAPP	pre-boil NAG pH	after-boil NAG pH	ext boil NAG pH	ARD Classification
Slurry	1.1	32.4	29.2	3.2	2.5	3.9	5.2	PAF
Tailings	0.9	27.5	87.4	-59.9	3.0	3.1	5.5	Uncertain

4.2 Flotation test-work

Two-stage flotation tests, using oleic acid as a coal collector and PAX as a sulphide collector, were carried out on the ultrafine samples as described in the Section 3.3. The results of coal flotation (first stage), and sulphur flotation (second stage) are presented in Table 4-6 in terms of concentrate quality and recovery and tailings composition.

A comparison of results for coal recovery in the ultrafine samples indicates a much lower mass yield of coal in the case of the slurry samples (<6% of 1st stage feed) which means that this stream is not suitable for the coal recovery stage. In contrast, an 83.5% yield of coal concentrate was found for the flotation tailings sample with a coal concentrate containing 20% ash and 0.6% total sulphur, thus representing a usable product. Hence the first stage flotation results indicated that, using the selected flotation conditions, the flotation tailings were suitable for preparation of a coal concentrate to feed into South African coal power stations as these accept coal with an ash content less than 35% and a sulphur content less than 1%. However, the high dosages of collector used in this study are a likely constraint to application at an industrial scale and require improvement. The high yield in the flotation tailings as well as the substantial coal content (~25% of ash) in the feed sample indicates that a significant amount of coal is still present in the flotation tailings and a “scavenger” step after the current flotation process used in the respective colliery should be considered.

The low yield of slurry samples could be explained to its low pulp pH. Although coal is naturally hydrophobic, floating well over a wide range of pH, the optimum pH for low grade coal, with high content of mineral matter, flotation is nearly that of a neutral solution. Near pH of 7, the coal surface will have a small negative charge, and as the pulp is made slightly acidic; hydrogen ions will be adsorbed so that the charge on the coal particles will become zero and the hydrophobicity of the surface will be a maximum. In a more acidic pulp, the coal surface will acquire a positive charge. Working with Australian coals, studies found the optimum pH for coal recovery to lie between 6.0 and 8.0. Alkaline solution is known to depress pyrite.

The results in Table 4-6 affirm that the coal flotation yields and recoveries are heterogeneous and differ considerably across the samples studied. Hence optimisation must be performed on the particular sample of interest as the results vary on application of the same conditions across different coal waste streams with differing characteristics.

The 2nd flotation stage for desulphurization was carried out on the slurry samples. This resulted in a final tailings comprising 62.7% of the original waste by mass, with a sulphur content of 0.6%. Further 51.3% of the total sulfur present in the feed was recovered to the 2nd stage concentrate with a total sulfur content of 1.1%. High sulphur concentrates are beneficial in terms of downstream processing and utilisation. Selective recovery has been demonstrated as technically feasible and further investigations are being performed to improve the understanding to the second stage process to maximize the benefits already found.

Table 4-6 Composition of the first and second stage products for flotation tailings and slurry samples.

	Concentrate					Tailings				
pH natural	Ash (%)	S_{total} (%)	Yield (%feed)	Sulfur recovery (%)	Comb. recovery (%)	Ash	S_{total}	Yield (%feed)	Sulfur recovery (%)	Comb. recovery (%)
1st stage										
Flotation Tailings	20.0	0.6	83.5	62.6	88.6	48.1	1.1	16.5	22.7	10.5
Slurry	45.6	0.4	5.6	3.2	5.6	44.8	0.7	94.4	82.6	75.4
2nd stage										
Slurry	43.0	1.1	37.3	51.3	27.1	39.5	0.6	62.7	47.0	41.8

The ARD static tests were conducted on the streams generated through the two-stage flotation to verify the acid generating capacity of the streams generated after coal recovery from the flotation tailings and sulphide recover from the slurry stream. These results are presented in Table 4-7. In both cases, despite being classified as uncertain in terms of acid formation, the coal and sulphur flotation resulted in final tailings with a significantly higher acid neutralizing capacity (ANC) results, indicating that most of the acid generating material had reported to the flotation concentrates while the neutralising capacity remained in the bulk sample.

Table 4-7 Static Acid Rock Drainage prediction tests results for the coal waste pre flotation and waste streams generated after froth flotation.

Final product streams from flotation study	ABA				NAG			
	Total S	MPA	ANC	NAPP	pre-boil NAG pH	after-boil NAG pH	ext boil NAG pH	ARD Classification
	%	kg-H_2SO_4/t						
Flotation Tailings (coal flotation)	1.1	33.7	246.7	-213.1	5.2	3.1	N/D	uncertain
Flotation Tailings (pre flotation)	0.9	27.5	87.4	-59.9	3.0	3.1	5.5	uncertain
Slurry (sulphide flotation)	0.6	18.4	36.2	-17.8	2.6	3.3	N/D	uncertain
Slurry (pre flotation)	1.1	32.4	29.2	3.2	2.5	3.9	5.2	potentially acid forming

The PSD of the feed and product streams following flotation are shown in Figure 4-2. As observed, the final tailings streams for both samples have increased particle size distribution after being processed by flotation. The D_{50} of the slurry and flotation tailings samples have increased from 0.126 to 0.353 mm, and from 0.907 to 1.323 mm, respectively. These results indicate that further processing of the flotation tailings not only led to the recovery of coal for power generation, but also improved the texture of the material to be used in soil fabrication and hence the texture of the resulting technosol.

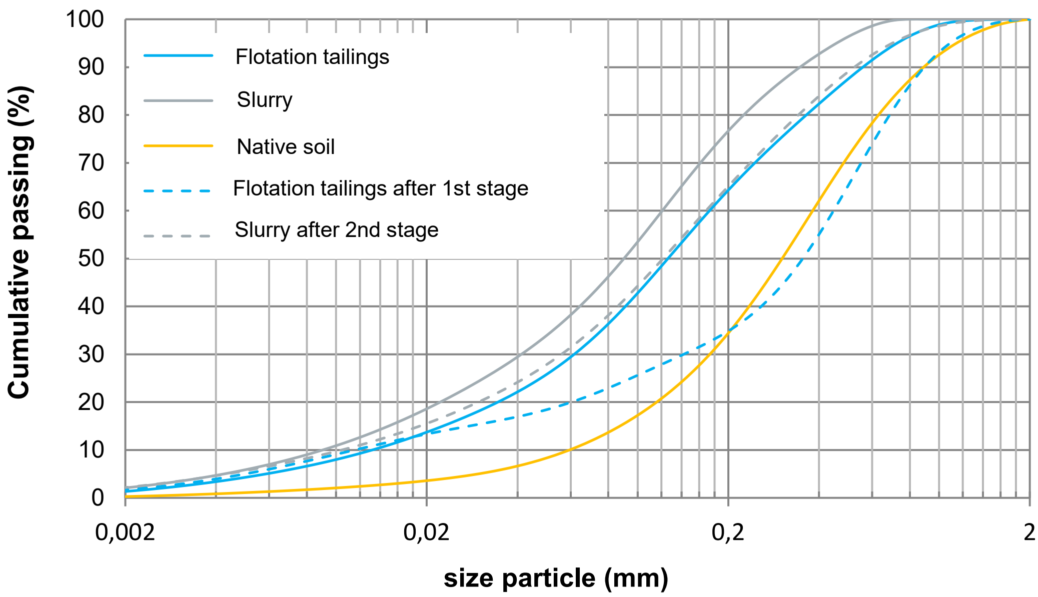

Figure 4-2 Particle size distribution of the ultrafine coal processing waste samples before and after two-stage flotation.

5 FABRICATED SOILS AND PLANT GROWTH STUDIES

5.1 Characterisation of Components for Soil Fabrication and their Early-stage Characteristics

In this section, the characteristics of the components for soil manufacture are determined to allow appropriate mixtures to be designed for soil fabrication. Further, the properties of the raw manufactured soil, prior to its application, are considered.

Coal waste streams

Samples have been obtained from the mine sites of interest and have undergone analysis, of which a sub-set of samples selected for use are presented here. Characterisation of further samples used in related studies are presented in the final report of the Water Research Commission project WRC K5/2761 (Kotsiopoulos et al., 2023The combined characterisation has been used to inform final sample selection.

In Table 5-1 and Table 5-2, we present the characterisation test results from the three samples studied in the WRC K5/2761 in terms of ash, sulfur, calorific value and particle size distribution for the ultrafine samples. ARD static tests results are presented in the Table 5-2 Static Acid Rock Drainage prediction tests results. Static tests used: Modified Acid Base Accounting method (ABA H2O incremental) and Net Acid Generation method (extended boil NAG). NAPP = net acid producing material, MPA = maximum potential acidity, ANC = acid neutralising potential, PAF = potentially acid forming, NAF = non-acid forming. Samples 2A (slurry) and 3A tailings) are the same streams as those used in the flotation studies in Section 4. Based on the very fine aspect related to the Sample 1A and its low ash content and high calorific value this sample was not considered suitable for soil manufacture. Sample 3A (Tailings) was considered for soil manufacture. The samples has no aggregate market value and has already passed through further processing in a flotation unit.

Samples 2A (slurry) and 3A (tailings) are the same streams as those used in the flotation studies in Section 4. Based on the very fine aspect related to the Sample 1A and its low ash content and high calorific value this sample was not considered suitable for soil manufacture. Sample 3A (tailings) was selected for soil manufacture. The samples has no aggregate market value and has already passed through further processing in a flotation unit.

Table 5-1 Ash and total sulphur content by size in the ultrafines (slurry and tailings) from 3 collieries: Colliery 1 (sample 1A), Colliery 2 (sample 2A), Colliery 3 (sample 3A) – adapted from WRC k5/2761

	Ash (%)	Sulphur (%)	Specific gravity	Pulp pH	CV ($MJ.kg^{-1}$)
Sample 1A	17.5	0.5	1.3	8.3	22.4
Sample 2A	43.6	1.0	2.1	5.0	16.3
Sample 3A	25.0	0.9	1.5	7.8	20.9

Table 5-2 Static Acid Rock Drainage prediction tests results. Static tests used: Modified Acid Base Accounting method (ABA H2O2 incremental) and Net Acid Generation method (extended boil NAG). NAPP = net acid producing potential; MPA = maximum potential acidity; ANC = acid neutralising potential; PAF = potentially acid forming. NAF = non-acid forming

Sample	ABA				NAG			
	Total S	MPA	ANC	NAPP	pre-boil NAG pH	after-boil NAG pH	ext boil NAG pH	Combined ARD Classification
	%	kg-H_2SO_4/t						
Sample1A	0.5	15.3	72.4	-57.1	2.6	2.8	5.6	NAF
Sample 2A	1.1	32.4	29.2	3.2	2.5	3.9	5.2	uncertain
Sample 3A	0.9	27.5	87.4	-59.9	3.0	3.1	5.5	NAF

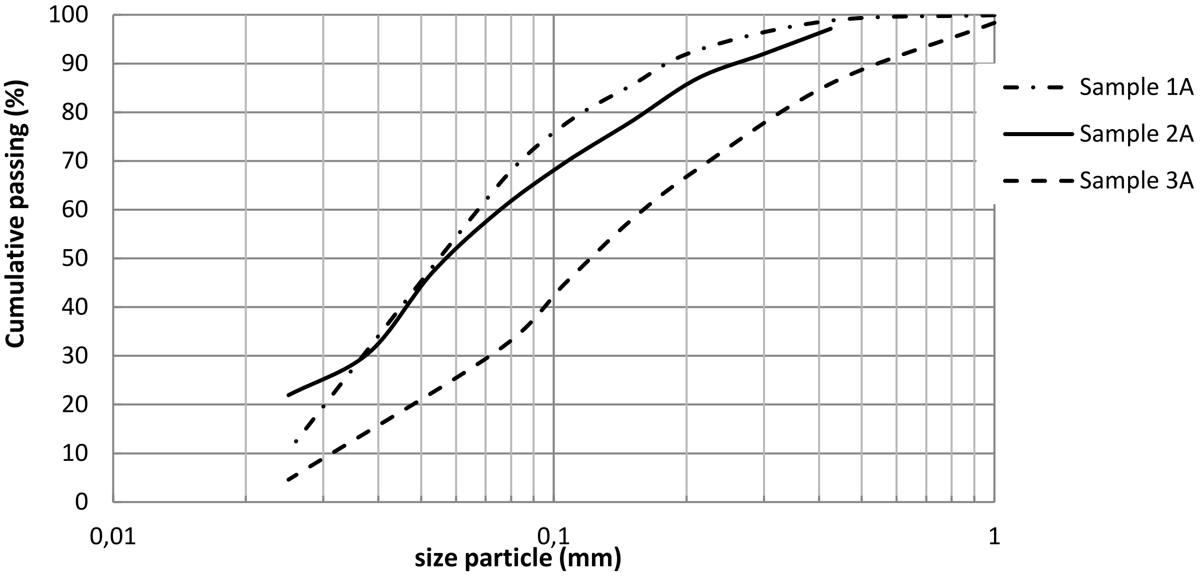

Figure 5-1 Size particle distribution in coal waste samples. Sample 1A (slurry) D50 ~0.053 mm; sample 2A (slurry) D50 ~0.053 mm; sample 3A (tailings) D50 ~0.106 mm

Soil fabrication components

Characterisation results in this section are provided for the materials used in this study, according to kind and functionality of each component. This includes the mine waste stream, the degraded soil component and the soil amendments used.

Drainage profiles are presented for the parental materials in

Figure 5-2 ; these include the ultrafine coal streams (slurry (2A) and flotation tailings (3A), the virgin soil and the stockpiled soil. The flotation tails as well as the local topsoils reached their respective drainage plateau after 2 minutes, retaining approximately 40-45% of the water applied. The slurry showed a higher rate of drainage reaching the final plateau before 1 min and retaining only 50% of the water applied. This is due of the coarser PSD than the flotation tails and differences in hydrophobic nature. In terms of water holding capacity (field capacity) it is observed the topsoils and tailings presented similar results varying from 40 to 45% while that of the fine coal slurry was higher at 50%.

The physiochemical conditions of the raw materials used for technosol fabrication and controls are tabulated in Table 5-3. These properties strongly influence the characteristics of the fabricated soils. They inform the required amendment dosage and type to be incorporated into the engineered soils.

The sulphur speciation, presented in Table 5-4, revealed pyrite as the major form of sulphur in these ultrafine coal processing wastes, forming roughly 50% of the total sulphur. The characterisation also showed the presence of high contents of low-risk sulphur and non-acid sulphate in the tailings samples; these are consistent with the uncertain potential for this sample. Gypsum, epsomite and jarosite are common sulphates with contents varying with the degree of oxidation of the samples (Kazadi Mbamba et al., 2012; Kotelo, 2013).

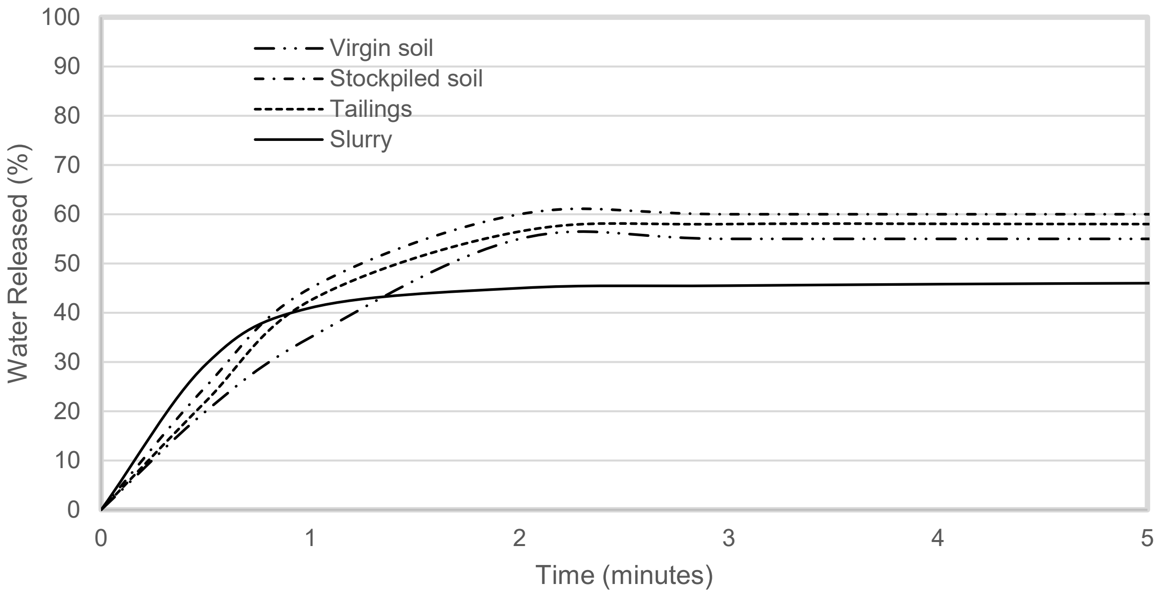

Figure 5-2 Drainage profiles of the ultrafine coal processing wastes and control over 5 minutes using mL of water. Control =100% native soil

Table 5-3 Physiochemical analysis of raw materials: CW-T (coal waste tailings from flotation plant), MR, Compost and Potting Soil for technosol fabrication.

Parameter	**Unit**	**CW-T**	**MR**	**Compost**	**Potting Soil**
pH		7.20	5.71	8.09	6.05
EC	µS/cm	2030	690	797	893
Eh	mV	185	190	236	337
Moisture Content$_d$	%	2	7	4	6
Field Capacity	%	48	44	39	49
Bulk Density	g/mL	0.94	0.18	0.71	0.98
C	%	53.9	45.3	21.0	10.3
H	%	3.09	6.92	3.12	2.06
N	%	1.45	3.11	0.58	0.31
S	%	1.19	BDL	BDL	BDL
Al	ppm	33500	7.56	7700	14800
Fe	ppm	18200	97.0	5760	19000
Ca	ppm	29100	1960	13500	15500
K	ppm	2300	298	4580	10700
Mg	ppm	3000	1500	1810	32900
Na	ppm	400	121	1320	192
P	ppm	900	4860	1440	2800

Parameter	Unit	CW-T	MR	Compost	Potting Soil
Si	ppm	Nq	767	712	1650
B	ppm	Nq	3.15	14.3	6.24
V	ppm	40.7	0.0378	11.6	27.7
Cr	ppm	36.4	0.751	11.1	63.8
Mn	ppm	200	34.6	61.1	179
Co	ppm	3.57	0.357	1.61	19.6
Ni	ppm	12.6	0.167	3.55	43.7
Cu	ppm	10.9	10.1	16.8	40.3
Zn	ppm	9.96	82.0	99.0	43.7
As	ppm	BDL	0.185	6.60	3.66
Se	ppm	BDL	0.0675	0.349	0.415
Sr	ppm	384	7.61	83.2	78.6
Mo	ppm	1.07	0.931	0.324	1.02
Cd	ppm	BDL	0.0319	0.122	0.147
Sn	ppm	BDL	0.0267	1.166	0.830
Sb	ppm	BDL	0.0107	0.331	0.147
Ba	ppm	377	7.84	34.2	158
Hg	ppm	BDL	0.0116	0.0315	0.0462
Pb	ppm	8.32	0.0677	10.6	10.6

Table 5-4 Sulphur speciation results for the tailings and slurry samples

Sulphate	Slurry (2A)	Tailings (3A)
Pyritic (%)	51.4	48.0
Acid Sulphate (%)	0.9	-
Non-Acid Sulphate (%)	17.8	10.0
Total Sulphate (%)	18.7	10.0
Low risk (%)	29.9	42.0
Total	100.0	100.0

Semi-quantitative results from XRD analysis, shown in Table 5-5, confirmed kaolinite and quartz to be the major constituents for all parental material and topsoils. The coal slurry stream contained 57% kaolinite and 20% quartz while the coal tailings sample had 52% and 15% respectively. In contrast the topsoils presented quartz as major crystalline phase, at 70% in virgin soil and 73% in stockpiled soil. Despite the high amount of iron determined in the elemental results (18 000-19 000 ppm in the coal waste and soil samples), no iron related mineral was reported in the XRD results. The results also showed the coal tailings to have higher amounts of carbonates, in the form of dolomite and calcite which contribute to the higher pH of the tailings stream compared to the slurry.

Table 5-5 XRD semi-quantitative results from major mineral phases of the ultrafine wastes used on this study

Mineral	Tailings 3A	Slurry 2A	Stockpile soil	Virgin soil
Quartz - SiO_2	15	20	73	70
Pyrite - FeS_2	2	1	-	-
Kaolinite - $Al_2Si_2(OH)_4$	52	57	24	26
Muscovite - $KAl_2(F, OH)_2$, or $(KF)_2(Al_2O_3)_3(SiO_2)_6$	12	12	3	4
k-Feldspar - $KAlSi_3O_8$	4	2	-	-
Spinel - $MgAl_2O_4$	2	2	-	-

Mineral	Tailings 3A	Slurry 2A	Stockpile soil	Virgin soil
Gypsum - $CaSO_4.2H_2O$	4	4	-	-
Anhydrite - $CaSO_4$	-	<1	-	-
Calcite - $CaCO_3$	5	<1	-	-
Dolomite - $CaMg(CO_3)_2$	3	-	-	-

In Table 5-6, soil analysis for the virgin and stockpiled soils is presented and compared to the fabricated soil (FabSoil) reported by Amaral Filho et al. (2020). The pH of the local topsoils were acidic in contrast with the FabSoils pH of 7.4. Higher pH values lead to lower bioavailability of micronutrients and phosphorus; conversely lower pH values decrease the bioavailability of macronutrients and increase aluminium toxicity if pH drops below pH 5.5 (Doran et al., 2013). Bulk density and coarse stone material content were similar across the three soils and in accordance to soil texture and plant needs (USDA, 2019). The stockpiled soil had a higher bulk density, correlating with its low organic matter content, high ash content, and higher voidage. It is noted that the bulk density of fabricated soils changes over time as aggregation and soil organic matter incorporation change (Arriaga et al., 2017; Bi et al., 2014). Comparisons of soils show P, N, Ca, K nutrient concentrations in superior concentrations for the FabSoils. The results indicate that the topsoils used for rehabilitation require the addition of chemicals fertilizers to provide condition to promote a sustainable plant growth, while FabSoils using coal waste and organic residues provide enough conditions to sustain plant growth.

Table 5-6 soil analysis for the topsoils used in this study compared with a FabSoil from Amaral Filho et al. (2020).

Parameter	FabSoil*	Virgin Soil	Stockpiled	Reference Values for Arable Soils #
Soil	Sand	Loam	Loam	
pH_{KCl}	7,4	3,6	3,4	6.5-7.5
bulk density ($kg{\cdot}L^{-1}$)	1,3	1,5	1,7	<1.6 g/cm^3
Stone (vol %)	1	1	1	
Resistance (Ohm)	320	3570	1830	>400
EC ($mS{\cdot}m^{-1}$)	192	100	200	100-250
CEC_{pH7} - $cmol_c{\cdot}kg^{-1}$	7,4	3.1	1.7	
$Na_{exch.}$- $cmol_c{\cdot}kg^{-1}$	0,4	0,03	0,04	>1.2
$K_{exch.}$ - $cmol_c{\cdot}kg^{-1}$	0,2	0,08	0,02	
$Ca_{exch.}$ - $cmol_c{\cdot}kg^{-1}$	13,8	0,33	0,35	2.00-6.00
$Mg_{exch.}$ - $cmol_c{\cdot}kg^{-1}$	1,7	0,21	0,12	0.50-2.00
$C_{organic}$ - %	5,1	0.8	0.2	0.8-1.5
N - %	0,4	0.04	0.02	0.06-0.15
P - $mg{\cdot}kg^{-1}$	57	7.09	3.88	30
Cu - $mg{\cdot}kg^{-1}$	6,7	0.68	0.45	5-25
Zn - $mg{\cdot}kg^{-1}$	8,8	0.7	0.42	
Mn - $mg{\cdot}kg^{-1}$	46,2	5.9	2.3	5-60
B - $mg{\cdot}kg^{-1}$	0,5	0.16	0.09	1-3
$S_{Am.Acet.}$ - $mg{\cdot}kg^{-1}$	307	82.4	209.8	20-200

*Amaral Filho et al. (2020) - technosols made by mixing 3 parts fine coal waste with 1 part native soil with specified Amendments (3% compost and 2% malt residue). After 90 days of growth.

https://www.larssa.co.za/sites/default/files/LaRSSA_Rehab%20Guideline_FINAL_August2019_0.pdf
http://www.bemlab.co.za/uploads/GENERIC%20SOIL%20ANALYSIS%20NORMS_a.pdf

Characterisation of newly fabricated soils

Physico-chemical characterisation of the fabricated soil mixes for Round 1 are presented in Table 5-7 indicates pH and EC of fabricated soils were higher than those of the stockpiled and virgin soils. The EC of the fabricated soils points to their salinity. Both pH and EC decreased over time, following fabrication, incubation and planting. In contrast, the low redox potential of the coal waste-based fabricated soil with compost or malt residue amendments (relative to virgin and stockpiled soils) increased following incubation and planting. All pots showed a higher redox potential, following planting and early plant growth (ORP 330 to 485 mV), suggesting a higher microbial activity in the soils at this point.

Table 5-7 Physico-chemical characterisation pre growth experiments for topsoils and control. CWT = tailings; CWS = slurry; SS= stockpiled soil; MR = malt residue; C = compost; (5%) = 5% of organic material w/w; (2.5%) = 2.5% of organic material w/w.

	pre incubation			week 6			week 12			10 day after planting		
	pH	EC (mS/cm)	Eh (mV)	pH	EC (mS/cm)	Eh (mV)	pH	EC (mS/cm)	Eh (mV)	pH	EC (mS/cm)	ORP (mV)
Virgin soil	4,4	0,1	392	4,4	0,1	429	4,3	0,1	354	4,4	0,1	452
Stockpiled soil	4,0	0,2	428	4,2	0,2	419	4,2	0,1	383	4,2	0,1	456
Tailings	7,9	1,8	303	8,2	1,4	297	7,1	1,6	194	7,4	0,1	207
CWT+SS+MR(5%)	7,0	1,4	303	3,1	1,0	208	7,9	1,2	233	6,8	0,9	329
CWT+C(5%)	7,9	1,6	133	8,1	1,3	295	7,6	1,3	270	7,2	1,0	485
CWT+MR(5%)	7,0	1,6	149	8,1	1,1	242	7,6	1,3	235	7,5	0,9	384
CWT+C(2,5%)	7,8	1,7	134	8,1	1,4	310	7,4	1,3	264	7,5	1,0	380
CWS+C(5%)	5,4	2,2	170	4,7	1,9	379	5,0	2,1	404	3,8	1,7	448

Figure 5-3 Pots placed in the UCT/CeBER greenhouse.

5.2 Growth Trial on technosols, including focus on the Soil Microbiome

The agricultural potting soil (control soil) used in this growth trial provided information on *E. tef* growth and its performance in naturally occurring soils with optimised fertility and quality as a benchmark for the anthropogenic soils. Plant growth was also evaluated in 100% CW-T (both inoculated (I) and not inoculated (NI)), including the previously cultivated CW-T Technosols. This informed on the value of biostimulation with MR as an OM amendment and structural ameliorant to CW-T. In addition, the difference between physical, chemical and biological properties of vegetated and unvegetated fabricated soils were determined in this trial.

Plant Development & Performance

The average rate of seedling emergence (germination period of 31 days) per treatment type is presented in Table 5-8 Table 5-8 and Figure 5-4. Plant growth and development measurements included *E. tef* shoot heights, water retention, number of plants per pot, and days till bolting which were recorded until the *E. tef* plants had matured. Seasonal changes were measured through daily greenhouse temperature, dew point and relative humidity data. This growth cycle lasted 82 days during autumn and winter.

Table 5-8 Average rate of *E. tef* seedling emergence (expressed as percentage) per coal-based technosols and potting soil as the control relative to the number of seeds initially planted per pot (20) during the final growth trial in winter. Dotted lines indicate major changes; the relocation from the laboratory to the greenhouse (day 8) and seedling removal (day 20). I represents inoculated and NI is non-inoculated treatments.

	100% CW-T		**CW-T+MR2.5%**		**CW-T+MR5%**		**CW-T+MR7%**		**Control**
Day	**I**	**NI**	**I**	**NI**	**I**	**NI**	**I**	**NI**	**NI**
0	0%	0%	0%	0%	0%	0%	0%	0%	0%
1	0%	0%	0%	0%	0%	0%	0%	0%	0%
2	0%	0%	0%	0%	0%	0%	0%	0%	0%
3	0%	0%	0%	0%	0%	0%	0%	0%	0%
4	0%	0%	0%	0%	0%	0%	0%	0%	0%
5	0%	0%	0%	0%	0%	0%	0%	0%	0%
6	94%	89%	83%	93%	88%	96%	74%	85%	95%
7	94%	90%	83%	91%	89%	95%	68%	85%	95%
8	91%	76%	81%	86%	85%	95%	51%	84%	95%
9	90%	69%	81%	85%	83%	95%	44%	83%	92%
10	90%	69%	81%	85%	83%	95%	44%	83%	92%
11	90%	69%	81%	85%	83%	95%	44%	83%	92%
12	90%	69%	81%	85%	83%	95%	44%	83%	92%
13	90%	69%	80%	85%	81%	95%	40%	83%	93%
14	88%	69%	74%	85%	79%	95%	36%	80%	93%
15	83%	69%	74%	84%	78%	95%	35%	81%	93%
16	83%	69%	70%	81%	75%	94%	35%	81%	93%

Day	100% CW-T		CW-T+MR2.5%		CW-T+MR5%		CW-T+MR7%		Control
	I	NI	I	NI	I	NI	I	NI	NI
17	83%	66%	70%	81%	75%	94%	35%	80%	93%
18	83%	66%	70%	81%	75%	94%	35%	80%	93%
19	83%	66%	70%	81%	75%	94%	35%	80%	93%
20	83%	66%	100%	100%	100%	100%	88%	100%	94%
21	80%	66%	100%	100%	100%	100%	88%	100%	94%
22	80%	66%	100%	100%	100%	100%	88%	100%	94%
23	80%	66%	100%	100%	100%	100%	88%	100%	94%
24	80%	66%	96%	100%	100%	100%	88%	100%	94%
25	80%	66%	96%	100%	100%	100%	88%	100%	94%
26	80%	66%	96%	100%	100%	100%	88%	100%	94%
27	80%	66%	96%	100%	100%	100%	88%	100%	94%
28	80%	66%	96%	100%	100%	100%	88%	100%	94%
29	80%	66%	96%	100%	100%	100%	88%	100%	94%
30	80%	66%	96%	100%	100%	100%	88%	100%	94%
31	100%	66%	96%	100%	100%	100%	88%	100%	94%

As seen in Table 5-8, the inoculated 100% CW-T showed the highest rates for seedling emergence in the experiments containing coal waste, presenting an average of 91.5% of the total planted seeds germinating by the day 6. This was anticipated as the loam-like soil texture of the coal tailings presented no structural impedance to germination. As hoped for, germination was effective in the potting soil, with an average germination rate of 95% after 5 days.

E. tef germination rates within bioaugmented and biostimulated fabricated soils were marginally slower compared to that in only biostimulated soils. Firpo et al. (2015); Herran Fernandez et al. (2016); and Novo et al. (2013) underlined that pedogenesis in anthropogenic soils differ from natural soils, as there is at times an inherent exotic distinction between parental materials and amendments to the natural environment. Material degradation often must occur first, consequently delaying other soil functions (e.g. supporting seed germination) and the process of reaching equilibrium between soil health, quality and fertility (Rivas-Pérez et al., 2016).

Inoculated CW-T+MR7% demonstrated poor performance in supporting seed germination as seen in Figure 5-4 and Table 5-8 . After five days, the average emergence rates for inoculated and non-inoculated CW-T+MR7% were 74% and 85%, respectively. This is 1.2- and 1.1-fold lower than that of inoculated and non-inoculated CW-T+MR5%. It continued to decrease with time due to persistent fungal growth issues. Fungal growth occurred predominantly on the surface of the incubated, fabricated soils with a high nitrogen content from the applied 7 wt.% MR. The humid conditions (covered, moistened soils), carbon and nitrogen-rich MR and nutrient-dense *E. tef* endosperms provided feedstock and favourable conditions for fungi development, whilst bacterial communities lagged (Rousk and Bååth, 2007).

Relocating the pots to the greenhouse minimised fungal growth and eventually completely ceased. On day 8, the average number of seedlings that have emerged in CW-T+MR5% Technosols were 89% in inoculated and 95% in non-inoculated, compared to 81% and 86% in I and NI treatments of

CW-T+MR2.5%, suggesting that a 95:5 ratio of CW-T:MR seems optimum for *E. tef* growth within a bioaugmented-technosols. From Figure 5-4, inoculated 100% CW-T treatments showed potential in supporting *E. tef* growth, with 83% by day 19 compared to 66% in its non-inoculated control.

Once in the greenhouse, the number of plants per pot declined in all treatments. The change in photosynthesis period (from a 24h-day to 16h-day/8h-night) and abiotic conditions (lower temperatures and undulating humidity) resulted in plant deaths. *E. tef*, although resilient, is best grown at temperatures above 18°C (van Delden et al., 2012). A significant decline in temperature was recorded from day 7 (20.3°C average) till day 20 (14.9°C average) which delayed seedling growth. The greenhouse conditions during this growth trial are presented in Figure 5-5. Nematodes and flies preceded in this growth cycle. Fly larvae growth ensued from the fluctuations in humidity caused by sporadic rain storms during the trial. Fly strips were put up in attempt to mitigate flies/larvae from feeding off *E. tef* endosperms. Nonetheless, *E. tef* seedlings in Technosols with high dosages of MR were adversely affected. Inferring seedling deaths as seen in Figure 5-6.

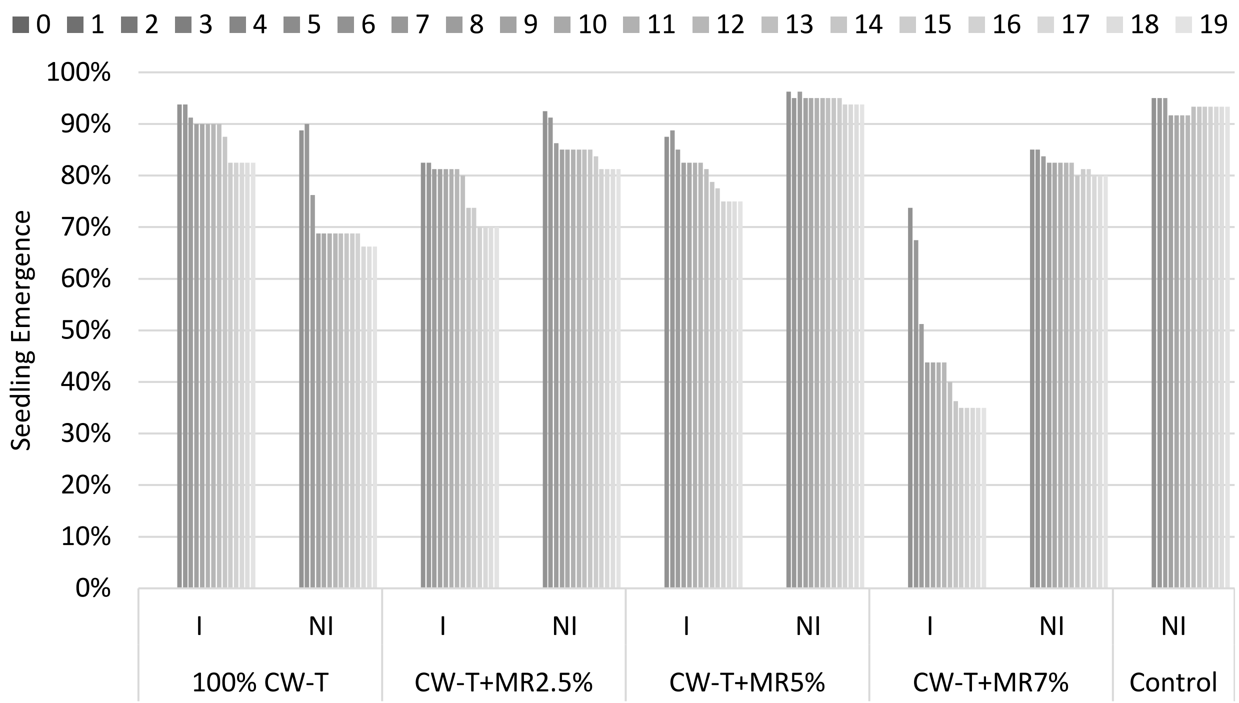

Figure 5-4: Average rate of *E. tef* seedling emergence per coal-based technosols and potting soil as control, for 19 days before the number of seedlings per pot were reduced to six, during the final plant growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

The rate at which seedlings emerged had stabilised by day 19 as listed in Table 5-8, and, the number of flies and nematodes were declining daily. To minimise competition for resources, the number of *E. tef* per pot were reduced to 6 on day 20. Hence, the increase in the seedling emergence rate seen after this day. Interestingly, at day 24 the percentage of plants in bioaugmented CW-T+MR2.5% slightly decreased from 100% to 96%, perhaps just as an anomaly. The number of plants that have established or died by the end of the growth cycle relative to the number of seeds planted, expresses the fabricated soil's ability to function as a topsoil for revegetation. At this stage, the potential of CW with biostimulation of 2.5% and 5% (w/w) MR as a topsoil is clear. Inoculated CW-T+MR5% and non-inoculated CW-T+MR2.5% were able to consistently support all *E. tef* plants for a growth cycle in unfavourable conditions. Evaluating the soil microbiome health in these fabricated soils should provide insight regarding the self-sustaining aspect as topsoils.

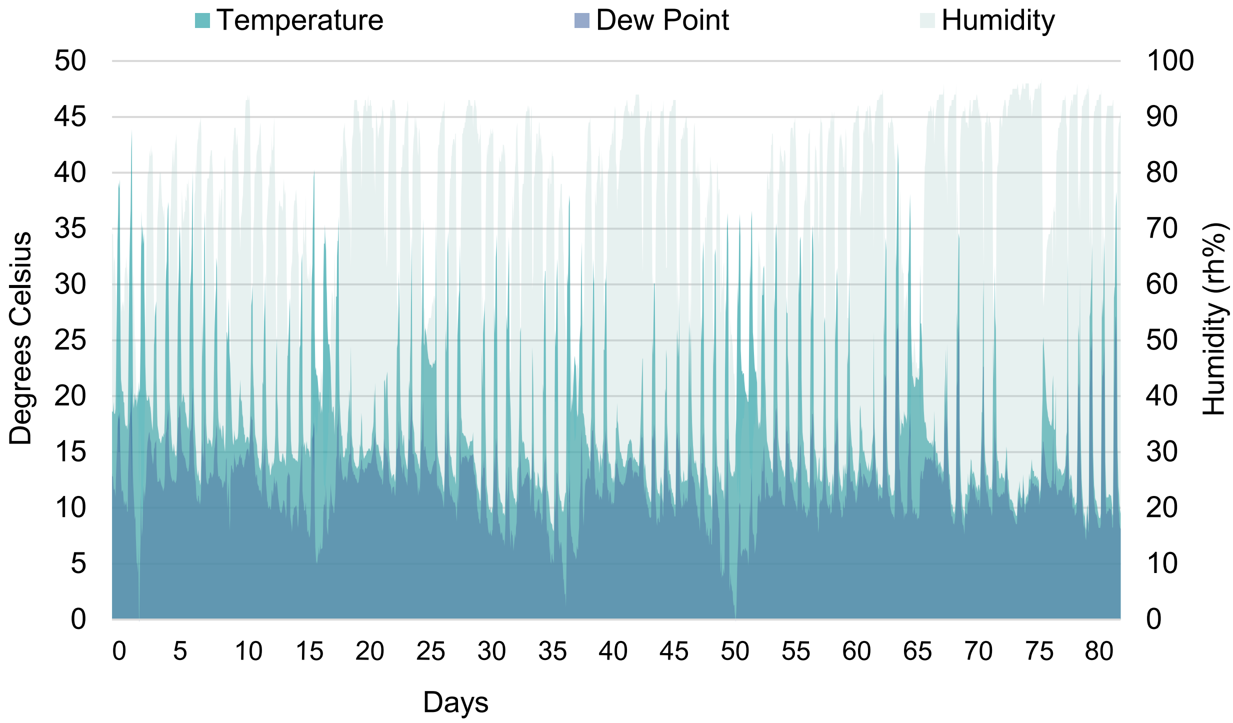

Figure 5-5 Daily greenhouse temperature, dew point and humidity conditions during the final plant growth trial in winter.

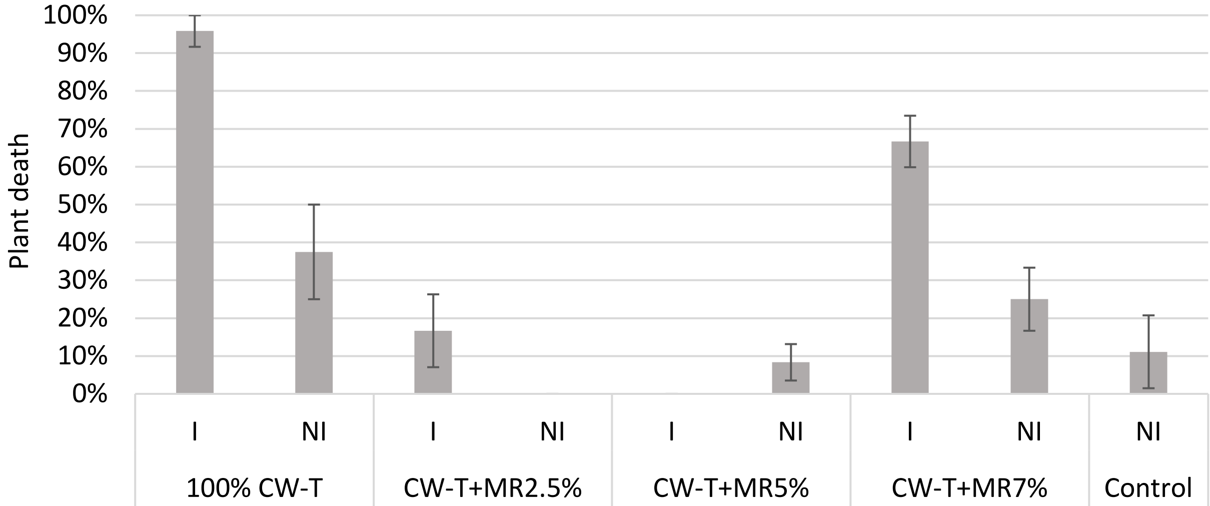

Figure 5-6: Average percentage of plant deaths in coal-based technosols and potting soil as the control during the final plant growth trial in winter. I represents inoculated and NI is non-inoculated treatments.

The results support the findings of Bakhoum et al. (2012) and the conclusions from the initial trial that the effects and outcomes of bioaugmentation are dependent on the soil type, and thus on the dosage of MR. Plant growth and development in 100% CW were also limited, with only 4% *E. tef* at the end of the trial in inoculated pure CW-T. The inadequate nutrient availability of CW-T cannot entirely sustain plant growth. As observed, the poor germination results for the soils containing 7% MR in coal waste suggest that an increased ratio of organic material as amendment does not result directly in better plant growth in a bioaugmented-technosols.

Shoot lengths of all plants were routinely recorded commencing after the 31-day germination period until the plants reached maturity. Mature plants showed visible signs of inflorescence and negligible increase in shoot height. The instantaneous growth (cm/day) of the plants per pot were determined as per the initial growth trial, and illustrated as cumulative growth in Figure 5-7. As observed, it is clear that *E. tef* cultivated in the potting soil control performed the best, and *E. tef* growth in 100% CW-T and CW-T+MR7% was very poor. Of the Technosols, the greatest plant growth was achieved in coal-based Technosols amended with 2.5% MR, reaching an average final plant height of 57.3 cm in the non-inoculated treatment. *E. tef* grown in CW-T+MR5% were taller than that in CW-T+MR2.5% during the initial growth cycle. Recurringly, a higher percentage of organic material as amendment did not improve plant growth in a bioaugmented-technosols. Of the inoculated fabricated soils, the tallest *E. tef* shoots were in CW-T+MR2.5% with an average height of 55.1 cm, compared to 47.5 cm in inoculated CW-T+MR5%. In the control soil, *E. tef* grew to an average height of 56.3 cm. Therefore, plant development obtained within the best performing Technosols (CW-T+MR2.5%; NI and CW-T+MR5%; I) were resemblant to that achieved within the control soil.

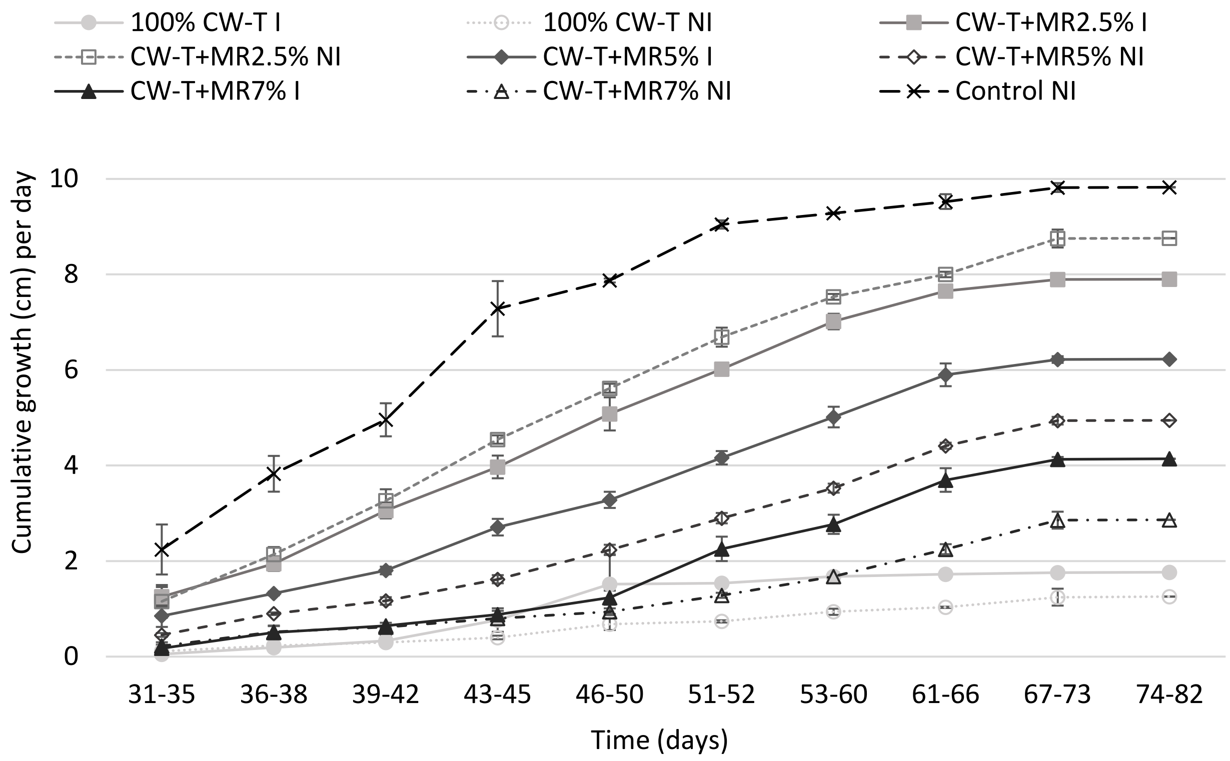

Figure 5-7: Cumulative above ground *E. tef* growth (cm) per day in coal-based Technosols and potting soil as the control, during the final plant growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

Significant growth occurred between days 36-42 and 51-60, where seedlings had established and extended their root networks. During these periods, greenhouse conditions were stable and there was no competition for resources. Average daily greenhouse temperatures ranged between 20.6-18.1°C between days 36-40, and 18.2-18.4°C between days 51-58 (refer to Figure 5-5). The optimum conditions during these periods resulted in good shoot growth. Whereas, after these periods, temperatures continuously dropped with 14.6°C on day 45, and 12.2°C on day 70. Representative pots of each technosols type after the growth cycle, are shown in Figure 5-8 (bioaugmented) and Figure 5-9 (without bioaugmentation).

Control	CW-T+MR2.5%; I	CW-T+MR5%; I	CW-T+MR7%; I	100% CW-T; I

Figure 5-8: Representative pots illustrating the final above ground *E. tef* growth achieved in each of the bioaugmented coal-based Technosols relative to the control soil (potting soil) in the final plant growth trial in winter.

The representative pots for bioaugmented fabricated soils accurately portray the plant development and performance achieved. It is evident that CW-T amended with 7% (w/w) MR and those receiving no added OM, were not able to effectively support *E. tef* growth. Whereas, *E. tef* within the mine waste soils amended with 2.5% and 5% MR had long, hair-like stems and multiple panicles; similarly to those in the potting soil. Visually, the plants in non-inoculated soils differ from the *E. tef* cultivated in the inoculated soils. In CW-T+MR7% and 100% CW-T, less panicles and leaves branch off the stems; pointing to lower biomass production. *E. tef* grown in inoculated CW-T+MR5% seemed more vibrant, healthy and lush in appearance. This correlates to the good biomass production achieved in this technosols.

Control	CW-T+MR2.5%; NI	CW-T+MR5%; NI	CW-T+MR7%; NI	100% CW-T; NI

Figure 5-9: Representative pots illustrating the final above ground *E. tef* growth achieved in each of the non-inoculated coal-based Technosols relative to the control soil (potting soil) in the final plant growth trial in winter.

High biomass generation and healthy, developed root systems are an indication of the soil’s ability to accommodate phytoextraction(Hrynkiewicz et al., 2018). A healthy soil microbiome stimulates both root proliferation and shoot development through nutrient cycling, carbon and nitrogen sequestration, and the degradation of organic material (Novo et al., 2013). Therefore, soil health and agricultural potential was determined from yields for above and below ground plant dry biomass after the final growth trial.

In Figure 5-10, *E. tef* in potting soil (control) had the highest above ground dry biomass production (0.72 g per 100 g soil). Root structures in the control were long and fibrous; creating a large surface area for mass transfer between plant organs, microbial cells and soil matrix. Hence, correlating to the good *E. tef* growth and performance seen in the control. Variation in biomass yields between pots were significant in *E. tef* shoots cultivated in CW-T+MR2.5%-I, CW-T+MR5%-I and in the control, due to the interdependence between biotic and abiotic factors during soil processes (Sauer, 2015) .

Non-inoculated CW-T+MR2.5% generated the highest above ground dry biomass (0.54 g per 100 g soil) in Technosols; corroborating the previously presented seedling survival and *E. tef* height results for this engineered soil. As previously found, a higher ratio of OM added as amendment did not enhance plant growth in a bioaugmented coal-based technosols as the average dry biomass production was the highest in treatments with only 2.5 wt.% MR. The results are comparable with those presented by Amaral et al. (2020), Firpo et al. (2015), and Weiler et al. (2020), where the addition of 2-5 wt.% OM enhanced the shoot and root productivity in the fabricated soils. Here, the added OM also acted as a physical ameliorant.

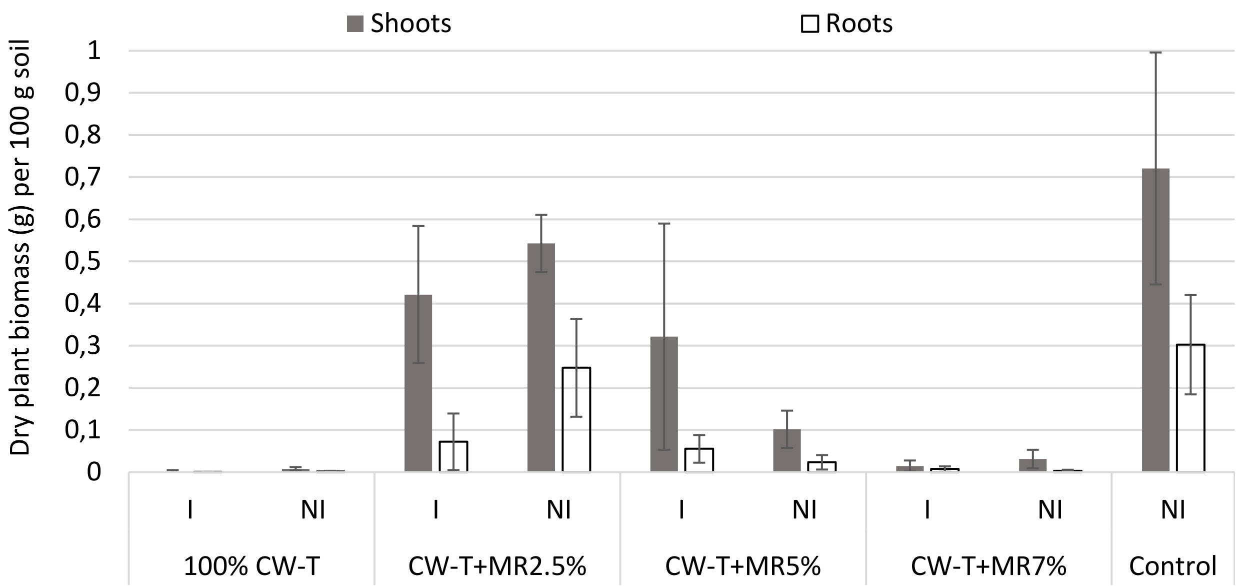

Figure 5-10: Average above (shoots) and below (roots) ground *E. tef* dry biomass produced per 100 gram soil (Technosols and potting soil as the control) in the final plant growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

As anticipated, there is a positive correlation (R^2 = 0.91) between above and below ground *E. tef* dry biomass production since leaf growth and seed production ensues root evolution(Tiwari et al., 2021). Non-inoculated CW-T+MR2.5% performed the best (root dry biomass of 0.25 g per 100 g soil), compared to potting soil with 0.30 g per 100 g soil. As determined from the initial growth cycle, 100% CW-T and CW-T+MR7% were not able to sustain *E. tef* growth and produced negligible yields. This can easily be seen from Figure 5-11 for the root structures in all bioaugmented Technosols after the final growth trial. Species diversity is maintained by root exudate (Thavamani et al., 2017). Thus, results suggest that bioaugmentation and biostimulation with 2.5% and 5% MR (wt.%) supported soil microbes to establish, to develop root structures that enhanced soil water retention. The soil microbiomes were profiled to distinguish between the persistence of the different soil microorganisms.

100% CW-T	CW-T+MR2.5%; I	CW-T+MR5%; I	CW-T+MR7%; I

Figure 5-11: Visual representation of *E. tef* root development in bioaugmented coal-based Technosols after the final plant growth trial in winter.

Overall, higher biomass (above and below) yields were achieved in the initial trial (summer leading into autumn) (data not shown) indicating that warmer conditions were more favourable for *E. tef* growth compared to those in the winter trial (data shown). During summer (data not shown), inoculated CW-T+MR5% outperformed the other fabricated soil types in terms of *E. tef* germination, soil quality, shoot heights and biomass production. However, in winter, non-inoculated CW-T+MR2.5% showed the most potential in supporting germination, seedling growth, root development, biomass production and shoot heights. It can be assumed that if performance was adequate in suboptimal conditions, it would also suffice (and improve) in favourable conditions.

In the final stage of plant growth, small florets form in dense spikelets to produce grains (seeds). The daylight hours progressively shortened during the final, autumn to winter, growth trial. The attenuated exposure to light encouraged flowering since *E. tef* is a short-day plant and the developmental response thereof is affected by photoperiodism. However, the continuous drop in temperatures and rise in humidity during the winter plant growth phase (seen in Figure 5-5) were unfavourable and significantly delayed inflorescence compared to the summer trial (data not shown). Here, bolting occurred seven days later in bioaugmented CW-T+MR5% compared to the initial growth trial.

The days till bolting were correlated to the average weight of seeds produced per pot of all Technosols, and graphically summarized in Figure 5-12. Seed production was the highest in NI and I CW-T+MR2.5%. Of the bioaugmented Technosols, CW-T+MR2.5% were able to best support inflorescence and seed production, and bolting was initiated on day 45 (5 days later than in the initial, summer growth trial) along with *E. tef* in potting soil. Three days later, flowering occurred in *E. tef* in CW-T+MR5%, followed by CW-T+MR7% and then 100% CW-T. This trend of inflorescence and seed production in Technosols were congruent to the summer growth trial results. A higher dosage of organic material as amendment does not result in improved plant growth.

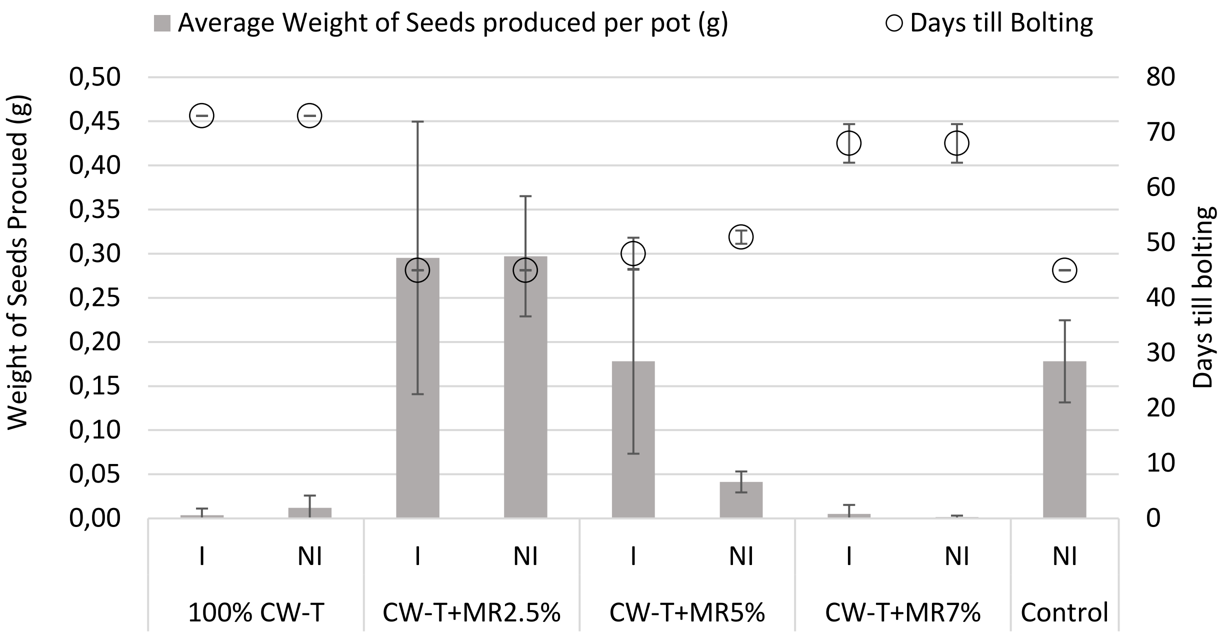

Figure 5-12: *E. tef* grain yield (g) and days till bolting in all coal-based Technosols and potting soil as the control in the final plant growth trial in winter. Where, I represents inoculated and NI is non-inoculated treatments.

The correlation between plant dry biomass and seed production for *E. tef* cultivated in Technosols during the final growth trial is presented in Figure 5-13. From the coefficient of determination (R^2 = 0.9), it is evident that higher above ground plant biomass resulted in higher seed production. The biomass and seed production in coal-based Technosols amended with 2.5% (w/w) MR were comparable to that obtained in potting soil (grain yield outweighs that of the control). Additional fertility tests were conducted to investigate the effect of soil health on grain fertility.

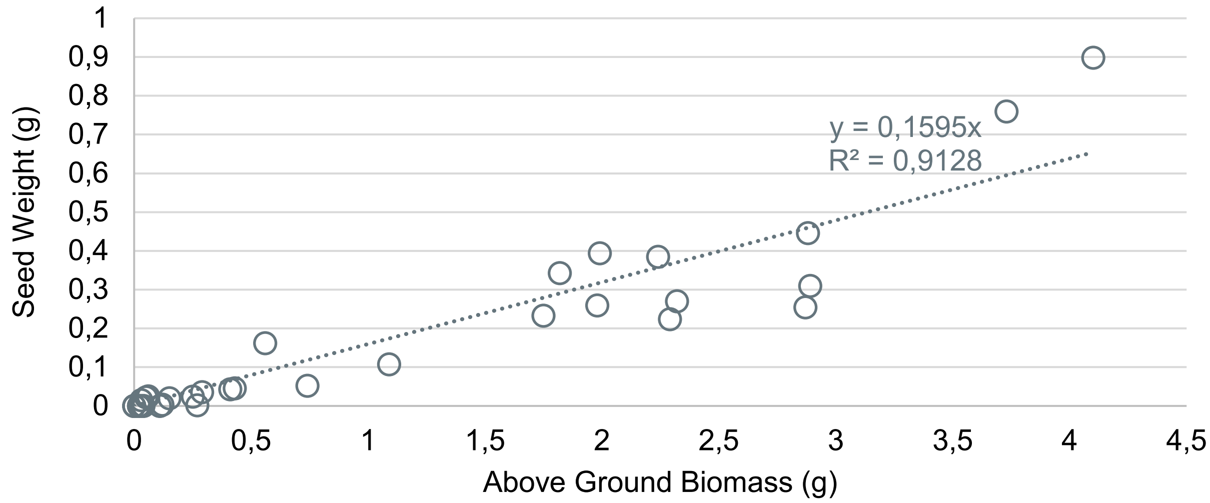

Figure 5-13 *E. tef* seed production (g) as a function of above ground *E. tef* dry biomass in all coal-based Technosols with increasing dosages of MR as amendment (2.5%, 5%, 7%) in the final plant growth trial in winter.

With the *E. tef* seeds collected from shoots cultivated in each of the coal-based Technosols, germination potential was evaluated in comparison with the market obtained seeds. The collected seeds were sown under identical conditions, and germination rates monitored for 14 days. The results are presented in Figure 5-14. Seeds collected from CW-T+MR2.5% were most fertile, with final germination values of 91% and 90% in inoculated and non-inoculated treatments, respectively. This is followed by CW-T+MR5%, with 81% and 71% in the inoculated and non-inoculated treatments, respectively. The control yielded 92% germination of seeds collected in the potting soil. Here, the effect of bioaugmentation on the agricultural potential of Technosols was evident. Interestingly, seeds collected from *E. tef* cultivated in non-inoculated Technosols germinated faster than those from bioaugmented fabricated soils. By day two since planting, only 59% and 36% of the *E. tef* seeds from inoculated CW-T+MR2.5% and CW-T+MR5%, respectively, had germinated. However, by the same day, 73% and 51% of the seeds from the non-inoculated versions of these fabricated soils (CW-T+MR2.5% and CW-T+MR5%, respectively) had germinated. Yet, by day 14, germination rates of seeds from shoots in the inoculated treatments for CW-T+MR2.5% and CW-T+MR5% exceeded those of the *E. tef* originally sown in these engineered soils. This strengthened confirmation of the benefit of including 2.5-5 wt.% MR as biostimulation in bioaugmented Technosols on *E. tef* cycle-to-cycle growth stability.

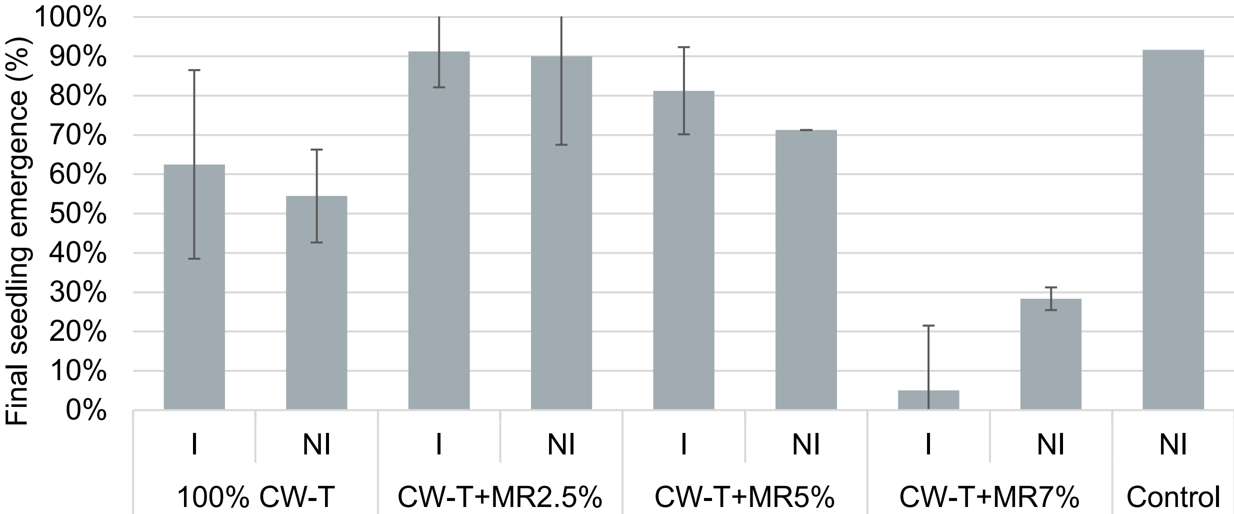

Figure 5-14 *E. tef* seedling emergence (expressed as percentage) relative to the number of seeds sown that was achieved after 14 days in pure coal waste. Seeds were collected from above ground *E. tef* biomass cultivated in all coal-based Technosols in the final plant growth trial. Where, I is inoculated and NI represents non-inoculated treatment types.

Water requirements vary based on soil type. The daily water requirements from soil field capacity data infer water usage demands of plants and soil microorganisms for evapotranspiration rates. Figure 5-15 cumulatively summarizes the water requirements per kilogram of fabricated soil over a 30-day period.

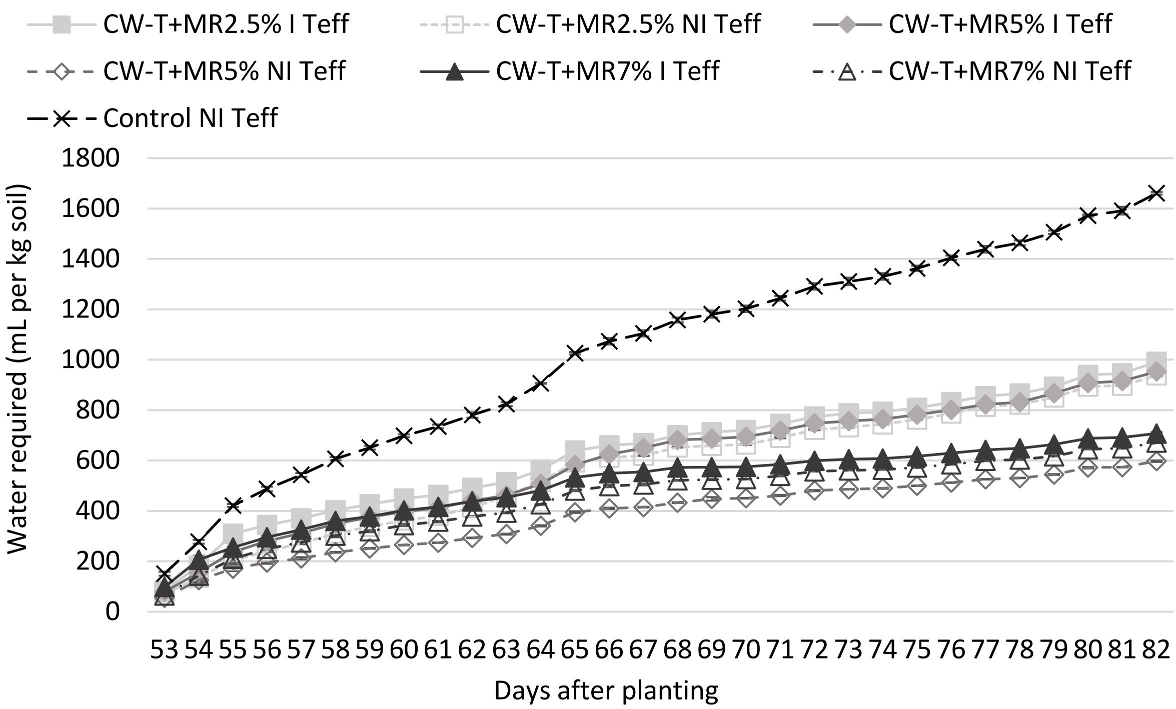

Figure 5-15 Cumulative water used to maintain the soils under 50% field capacity per kilogram of vegetated amended coal-based fabricated soils compared to the control (potting soil) over a 30-day period. Where, I refers to inoculated and NI refers to non-inoculated treatments.

MR as a structure ameliorant is expected to minimize water percolation (Lamb et al., 2014) and improve soil water content by which microbial activities are controlled (Paul et al., 2003). Hence, it was anticipated that Technosols with the highest ratio of MR (7 wt%) would have the lowest average daily water consumption, provided that plant growth is maintained. This trend remains, especially within fabricated soils with bioaugmentation. It can be concluded that water requirements were reduced in CW-T-based Technosols relative to the control. To sustain 6 *E. tef* plants in bioaugmented CW-T+MR2.5% and CW-T+MR5%, 19.8 mL and 31.8 mL water per 500 mL of fabricated soil were respectively required every day, compared to 33.2 mL in the control. From the plant development and performance results, *E. tef* cultivation within bio-stimulated and bioaugmented coal-based Technosols showed significant potential in terms of seed germination, survival rate, and above ground height and biomass.

Characterisation of Technosols

Plant performance demonstrated that an increased ratio of MR to coal based bioaugmented-fabricated soils, beyond a critical value, did not lead to successful seedling emergence, prolonged plant growth, nor to superior plant biomass yields. It was determined that a CW-T:MR ratio of 95:5 (w/w) provided the best performing soil matrix for *E. tef* growth. To further investigate the soil quality in these bioaugmented fabricated soils, physiochemical conditions were analysed as detailed in Chapter 3.5.

Crop cultivation and yield are directly influenced by soil pH. The results for average pH values of the Technosols before and after the winter plant growth trial are graphically presented in Figure 5-16 for non-vegetated and in Figure 5-17 for vegetated soils. Leaching tests were performed after *E. tef* growth. The pH, Eh and EC of all soil leachates are summarized according to the soil conditions.

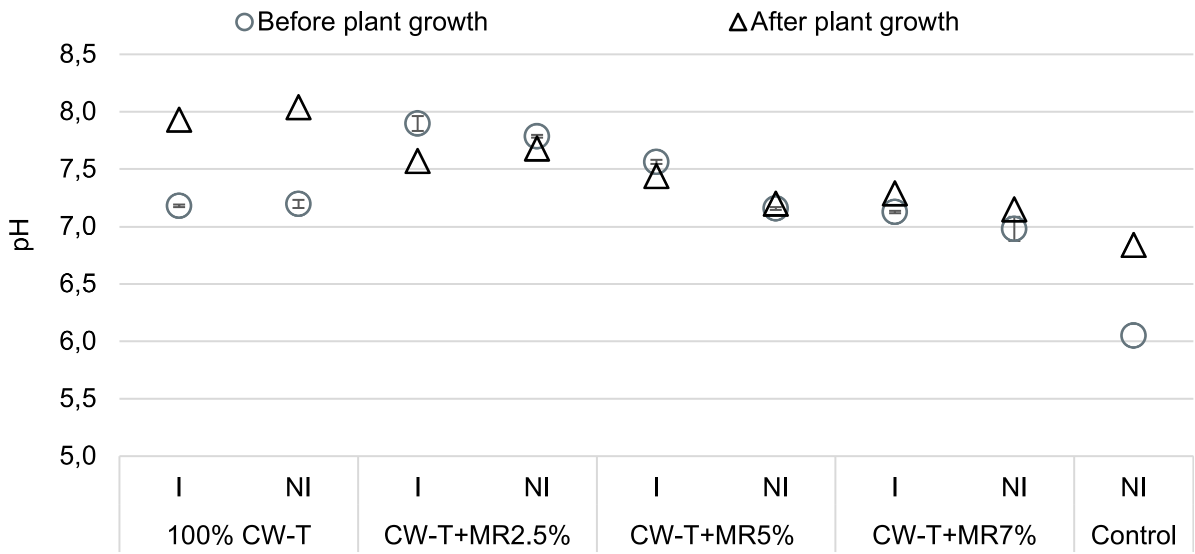

Figure 5-16: Average pH values for non-vegetated coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

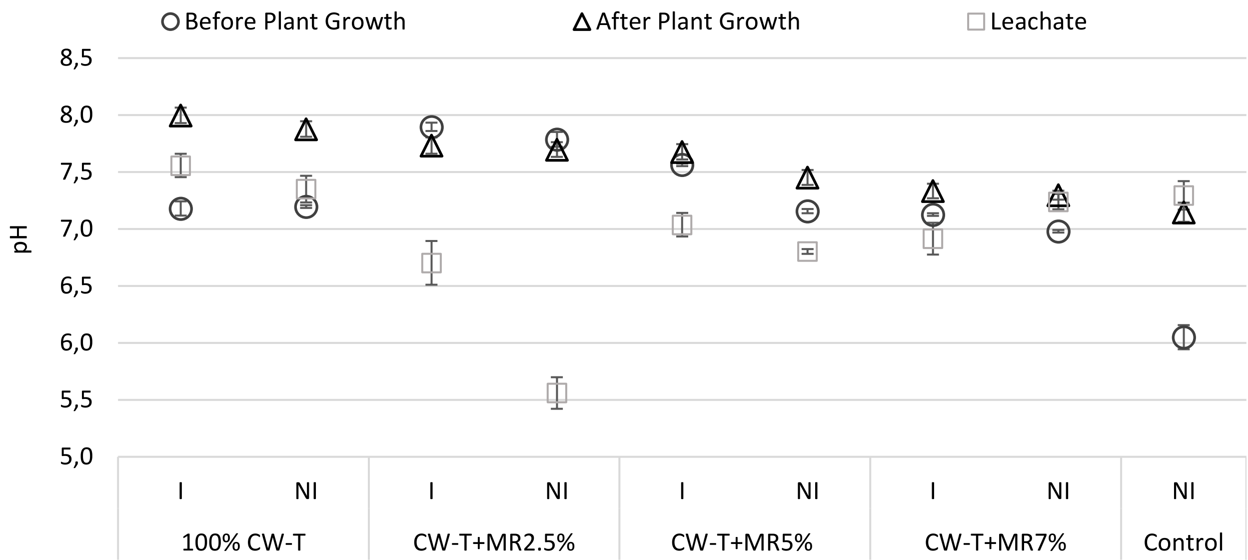

Figure 5-17: Average pH values of vegetated coal-based Technosols and potting soil as the control, and of associated leachates, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatments.

As previously shown, *E. tef* growth was exhibited in the control which had the most acidic (6.05) initial soil pH of the studied treatments. This is in line with the preferred soil pH conditions for *E. tef*. A declining pH with increasing OM levels were expected as described by Husson (2013). The initial technosols pH values are all moderately alkaline (between 7 and 7.90). The addition of 2.5% MR as amendment (pH of 5.71) increased the initial acidity. As dosage of MR increased, initial pH decreased. This is corroborated by the reduction in pH observed whenever MR was used in coal-based Technosols for plant growth experiments by Amaral Filho (2020). This trend, along with the increase in pH in the presence of the inoculant compared to the control treatments, correlates to the biogenic regulation of soil pH through MR application as biostimulant for microbial respiration (Neina, 2019; Sánchez-

Clemente et al., 2018) . Interestingly, upon initial amendment application of 2.5% MR, soil pH increased from 7.2 in bioaugmented 100% CW-T to 7.9 in bioaugmented CW-T+MR2.5%. However, the corresponding decrease in Eh for this alkalinization illustrates the interdependence between pH-Eh in pedogenesis, and is perhaps indicative of microbial respiration from bioaugmentation or initial soil compaction upon MR application (Husson, 2013).

Soil pH modification is dependent on initial soil pH, amendment application and application rate, C:N ratio, and rate of material decomposition within the soil. Yet, pH also alters with biotic factors. Biological functions in the rhizosphere induce soil pH changes to enhance proton motive forces across microbial cell membranes, by ensuring membranal diffusion of formic acid, acetic acid, and other short-chain fatty acids, and to promote microbial growth (Neina, 2019). Thus, it is expected that the microbial populations introduced through inoculation would be able to establish and proliferate in these initially alkaline Technosols (enhanced proton motive force), especially those with increased MR dosages capable of supporting *E. tef* growth (CW-T+MR2.5% and CW-T+MR5%). Nutrient uptake, microbial respiration and root exudation occur within the rhizosphere; thereby, causing corresponding pH changes to maintain electro-neutrality (Atlas & Bartha, 1998). During nitrogen sequestration when nitrate uptake is dominant, hydroxyl or bicarbonate ions are released by plants to regulate ion potential (Atlas and Bartha, 1993). This increases the soil pH. This phenomenon corresponds to the slight increase in soil pH after *E. tef* growth in Technosols with 5% and 7% (w/w) MR and in the control. The soil pH increased with *E. tef* growth as during anaerobic respiration, consumption of protons during reduction reactions resulted in a pH increase (Prado et al., 2020). Additionally, the storage of rainwater (used for irrigation) with more rainstorms, contributed to pH alkalinity adjustments. Profiling the soil microbiomes for nutrient cycling genes was performed in support. According to Prado et al. (2020), the alkalising interaction of *E. tef* growth in MR amended CW-T-based Technosols are specific to *E. tef* as a pioneer species. Suggesting a phytoremediating capability of *E. tef*, i.e. rehabilitating soil conditions (such as, pH alkalisation) based on the specific soil requirements through ion uptake into plant organs.

The trends in change in soil pH with time in vegetated and non-vegetated pots are similar, revealing that technosols pH was not independently regulated by *E. tef* growth. Thereby, eluding the significance of abiotic variables (temperature, irrigation, exposure to light) and of microbiota on pH regulation as suggested by Atlas and Bartha (1993), the randomised pot placement in the greenhouse ensured equal exposure to sunlight, and all pots were maintained at 50% FC. Thus, eliminating variation in technosols pH from abiotic variables. The neutralising effect on pH seen in inoculated Technosols with 2.5% MR along with good *E. tef* growth (suggesting a stable and well-functioning soil microbiome) were expected as most bacterial cells are neutrophiles and acidophiles (Atlas and Bartha, 1993). Conversely, minimal microbial abundance is expected in the evidently poor performing soils (100% CW-T and CW-T+MR7%). Husson (2013) and Mills & Fey (2004) suggest that pH is the primary parameter influencing soil microbial diversity and richness. Thus, it is expected that the soil microbiome structures will vary between the fabricated soils with varying pH values.

The soil pH, especially that of metal-rich mine-based soils, is also regulated by the release of cations from Ca, Mg, K and Na and Al during weathering with plant growth (Prado, et al., 2020). This is evident in the difference of these cation concentrations (cmol/kg) between vegetated and non-vegetated Technosols (refer to Table 5-9 and Table 5-10, respectively). From Figure 5-17, maximum biomass production in coal-based engineered soils occurred when the pH was closer to 7.5 (all CW-T+MR2.5% and inoculated CW-T+MR5%). This was expected as higher pH values lead to a higher bioavailability of macronutrients and reduced aluminium toxicity (Amaral Filho, et al., 2020).

Leachate pH values are noticeably lower than the final technosols pH after *E. tef* growth. Except, pH values of leachates and Technosols were similar in 100% CW-T and CW-T+MR7%. Implying that the better performing Technosols (based on *E. tef* performance) contained more water-soluble nutrient ions from microbial-meditated material decomposition; hence, the reduction in leachate pH. The optimised irrigation in this growth trial could have contributed to this compared to the initial trial where irrigation to maintain 50% FC had not yet been regulated. Leaching results showed that only 41% and 26% for inoculated and non-inoculated CW-T+MR2.5%, respectively, of the water entering, leached out. This coincides with the extensive root structures seen in Figure 5-11. (Li et al., 2010) described that the release of heavy metals (e.g. Cu and Zn) from soils are influenced by climatic changes (temperature fluctuations), soil pH and irrigation. Thus, it would be valuable to investigate metal leaching with time in the coal-based Technosols containing different ratios of amendments.

From literature, it is expected that a higher CW-T content would increase the concentrations of metal and mineral leached (Komonweeraket et al., 2015). None of the substrates were strongly acidic or basic, implying a lower probability of metal(loid) leaching that follow amphoteric leaching patterns (Mahedi et al., 2019). The pH of technosols leachates were slightly above final soil pH values, except for inoculated CW+MR2.5% and the control.

The overall change in redox conditions within vegetated and non-vegetated coal-based Technosols and for associated leachates are graphically presented in Figure 5-18 and Figure 5-19, respectively. Redox values were enhanced with an increased dosage of MR and with *E. tef* transpiration (Prado, et al., 2020). The increased OM content provided more accessible and labile substrates for energy in metabolic processes that govern electron transport within the soil environment (Husson, 2013). The initial redox potentials align with the material characterisation where MR was characterised by a redox potential (190 mV) higher than that of CW-T (185 mV). As previously discussed, the initial decrease in soil redox value upon 2.5% MR addition compared to 100% CW-T was expected as literature suggests reduced Eh levels when OM levels increase (Husson, 2013). Conversely to pH, inoculation resulted in a decline in redox potential (also seen in the first growth trial) since bacterial growth (CO_2 emissions during respiration) is associated with a decrease in redox potential (Reichart et al., 2007). This trend remains throughout the growth trial. However, initial redox potential of inoculated CW-T+MR2.5% were 153 mV compared to 149 mV of the non-inoculated treatment. Yet, here the standard error is more significant than the non-inoculated soil-like substrate.

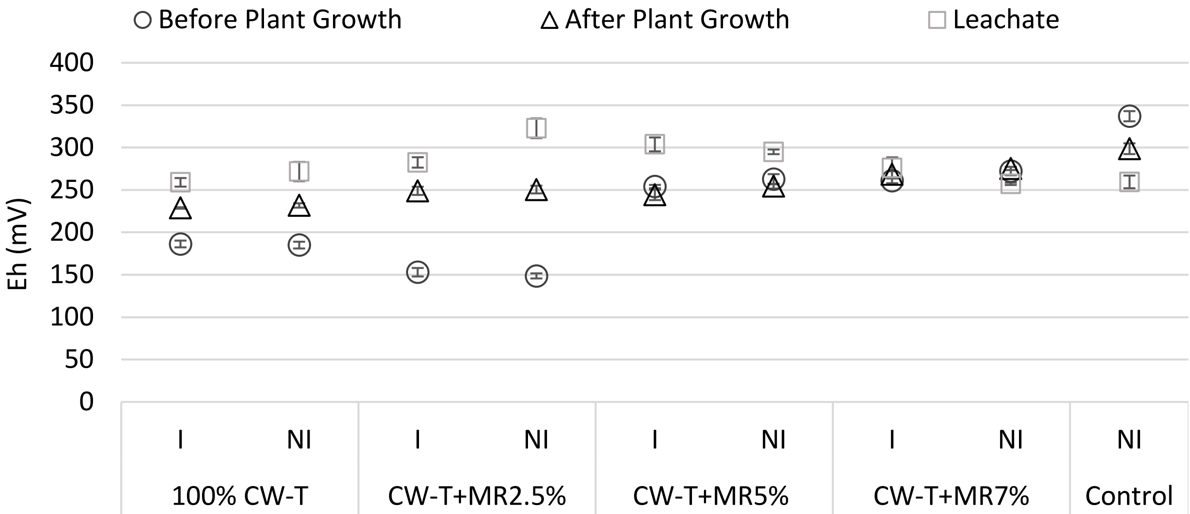

Figure 5-18: Average redox potential (Eh) values for vegetated coal-based Technosols and potting soil as the control, and of associated leachates, before and after the final plant growth trial in winter. I is inoculated and NI represents non-inoculated treatments.

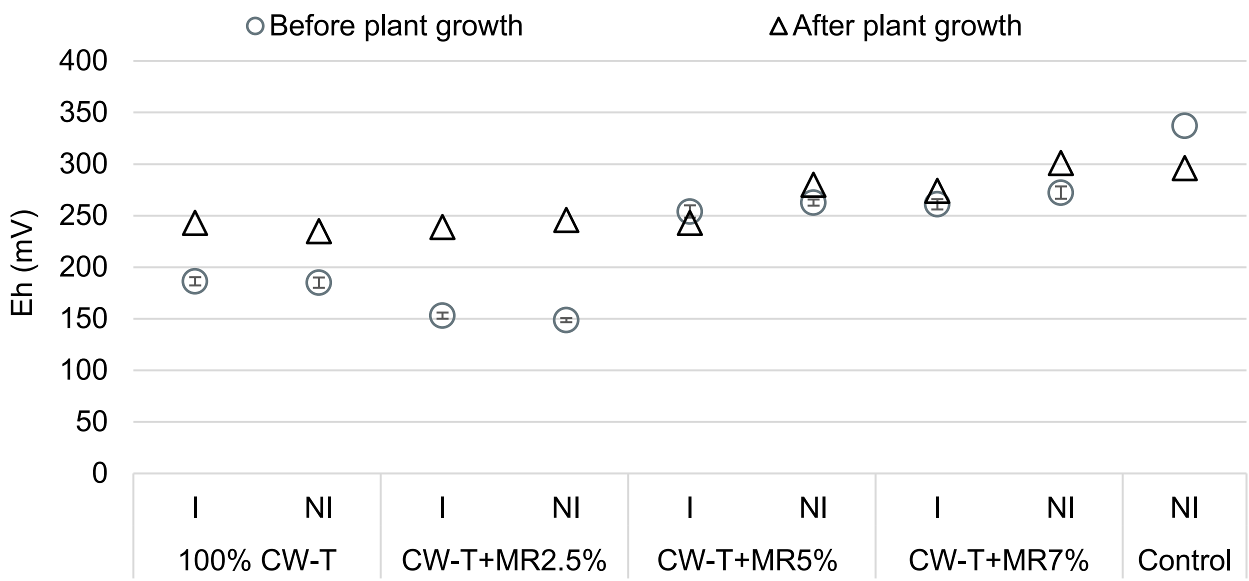

Figure 5-19: Average redox potential (Eh) values for non-vegetated coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter. I is inoculated and NI represents non-inoculated treatment types.

If the Technosols were aerobic (identified by redox values above 800 mV (Husson, 2013), pyrite would oxidize and generate sulfuric acid. Thereby, reducing the soil pH to 4.0 or less in the absence of neutralizers such as limestone. Under these acidic conditions, aluminium cations are liberated that are toxic to plant growth. Thus, the slightly anaerobic conditions (much lower Eh) were an indication of slow biological pyrite oxidation, thereby minimizing ARD potential. The results were supported by (Dong et al., 2020) who found undetectable pyrite oxidation at 650 mV even with associated acidophilic bacteria. Consequently, adding to the feasibility of coal-based Technosols as a topsoil for degraded coal mine land.

The increase in redox potential in CW-T+MR2.5%, and the decrease in CW-T+MR5% after *E. tef* cultivation seen in Figure 5-18, directly resemble the results after the initial growth trial. However, as with pH values of Technosol leachates, the leachate redox conditions of this growth round differed from the initial trial. Here, leachates of the best performing Technosols had higher Eh values than the corresponding soil conditions. The trend in Eh increase is supported by the leachate pH values that decreased, as previously discussed.

As seen with pH, soil reduction processes (photosynthesis, nitrogen fixation, etc.) modify the soil electro-neutrality (Husson, 2013). Yet, microbial diversity, especially bacterial, is controlled by changes in pH and Eh (Neina, 2019; Husson, 2013). Therefore, as previously mentioned, differences in soil microbial abundances were expected between the different types of fabricated soils with varying pH and Eh conditions.

Technosols were also characterised according to electrical conductivity as it affects the soil-water balance and microbial performance (Yan et al., 2015). The results for non-vegetated soils are illustrated in Figure 5-20 and for vegetated soils in Figure 5-21.

Initially, the salinity between the treatment types varied significantly. Before plant growth commenced, technosols EC was positively influenced by the amendment dosage and negatively influenced by bioaugmentation (when comparing fabricated soils of the same type). It addresses the amalgamated contribution of parental materials and associated amendments to the overall soil characteristics as described by Jordan et al. (2017), Novo et al. (2013), and Herran Fernandez et al. (2016). In terms of soil structure, clay-like soils commonly have higher salinity due to enhanced surface area between soil particles (Cervantes et al., 2011). This was observed in both greenhouse trials in Technosols with 7% (w/w) MR where soil particles clumped together making it impervious to water. The sand-like 100% CW-T had the lowest initial EC since cation concentrations and moisture content were low. technosols EC was also influenced by soil-water content (initially higher in Technosols with higher percentages of MR as seen in Figure 5-15) and OM content (higher EC when more OM) (Othaman, et al., 2020).

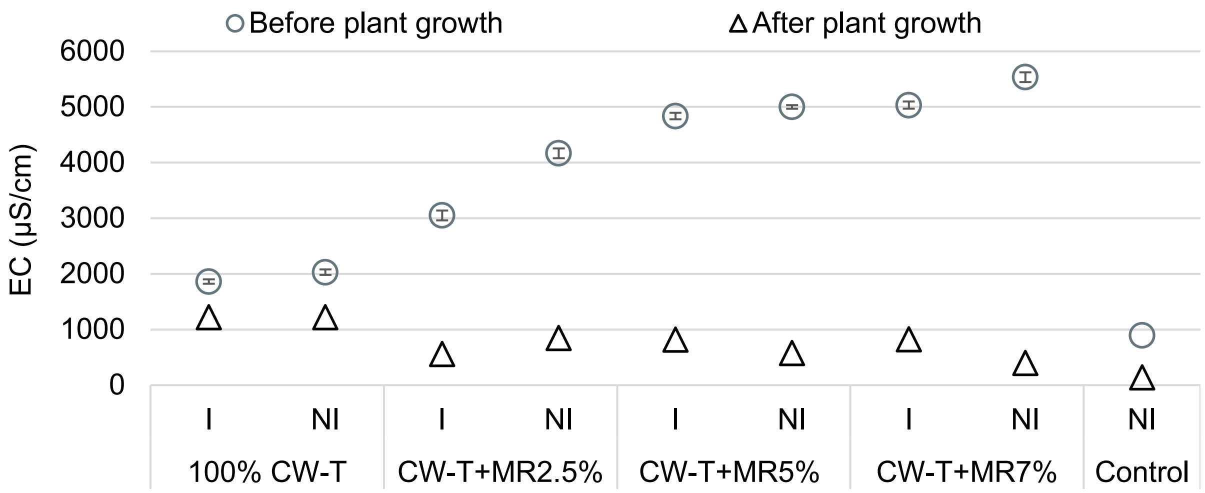

Figure 5-20 Average electrical conductivity (EC) values for non-vegetated coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter.

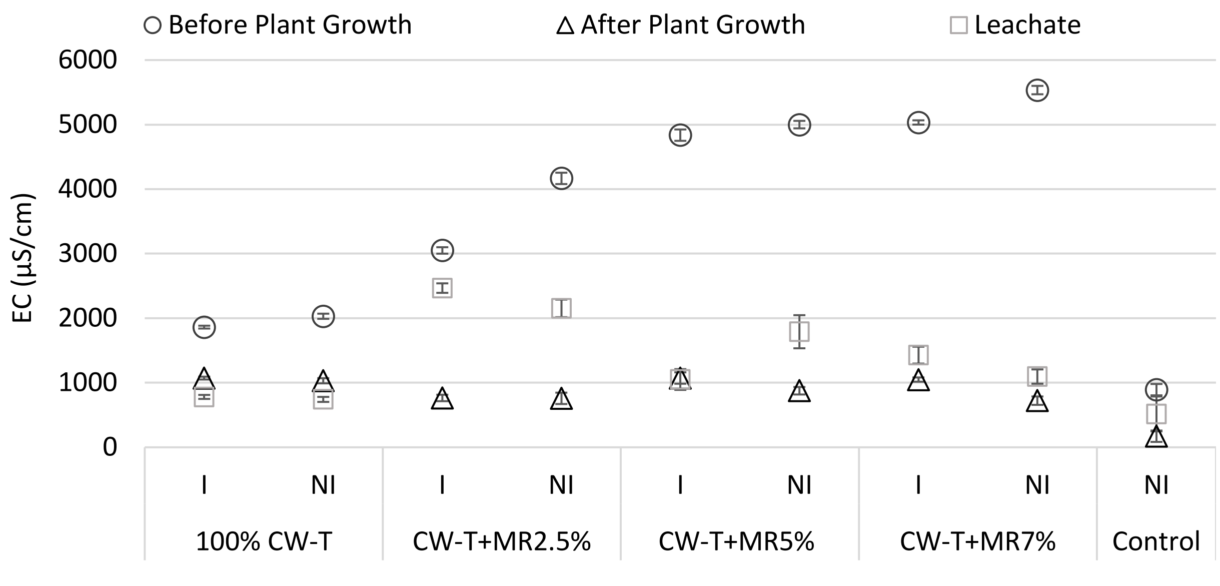

Figure 5-21: Average electrical conductivity (EC) values for vegetated coal-based Technosols and potting soil as the control, and of associated leachates, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatments.

Non-inoculated CW-T+MR5% and all CW-T+MR7% treatments were initially characterised as very saline. High salinity (EC values above 5000 µS/cm) is deleterious to microbial growth and activity(Boyrahmadi and Raiesi, 2018). This corroborates the poor *E. tef* growth and performance achieved in the aforementioned Technosols. Soil EC values between 1000-2500 µS/cm are acceptable by standard (USDA, 1954) when values increase, soil sodicity (high accumulation of sodium salt relative to other cation salts) increases (Amaral Filho, et al., 2020). Based on the agricultural standards, the initial EC of fabricated soils before plant growth were unsuitable (except for 100% CW-T). Even so, conditions in all Technosols were improved with *E. tef* growth. EC had stabilised in all treatments to levels below 1100 µS/cm; an indication of nutrient and metal(loid) uptake into plant organs, photosynthesis, root exudation and pollutant degradation processes that have occurred to control soil salinity (Cervantes et al., 2011). Thereby, contributing to the feasibility of coal-based Technosols and *E. tef* cultivation in these soils. As expected, by the end of the trial the control had the best *E. tef* development and performance with the lowest EC of 171 µS/cm, compared to the worst performing treatment, inoculated 100% CW, with the highest EC of 1072 µS/cm.

From Figure 5-21, there are no trends in the salinity of technosols leachates. Yet, those of CW-T+MR7% remain relatively high (above 1000 µS/cm) subject to the high initial soil salinity. The relatively high EC in CW-T+MR2.5% leachates, support the acidity thereof (presented in Figure 5-17) and the proposed reasoning that leachates were concentrated in nutrient ions and salts from active soil microbes and maximised irrigation.

The water uptake and cycling within soils are pivotal to biogeochemical transformations, plant and microbial growth, and soil-plant mass transfer (Lowery, et al., 1996). WHC of every treatment type was evaluated before and after the final growth trial. The results for vegetated and non-vegetated pots are graphically presented in Figure 5-22. As seen in the graph, initial WHC were all averaged near 50%. However, WHC in 100% CW-T was poor (inoculated: 21%; non-inoculated: 17%), yet foreseeable as ultrafine coal tailings are homogeneous in structure, sand-like in texture and have a very small particle size (<2 mm). Soil structural stability is also influenced by nutrient content, soil microbiota and vegetation, which was assumably lacking in all pots with pure CW-T. Noticeable water channels were observed in 100% CW-T Technosols during the greenhouse trial which formed as a result of reduced soil porosity when aggregates are continuously exposed (Mills and Fey, 2004b). Implementing 100% CW-T as a topsoil would result in extensive water run-off, crusting or gully erosion. Thereby, emphasizing the significance of amendments to Technosols and the correct dosage thereof to ameliorate soil structure, as suggested by Macia et al. (2014) and Jordan et al. (2017).

From Figure 5-22, WHC increased with an increasing ratio of MR. The results are consistent to Amaral Filho et al. (2020), Firpo et al. (2015), and Weiler et al. (2018), where the addition of OM benefitted biomass production and soil structure performance. Yet, inoculated CW-T+MR5% were best able to retain water at 55% initial WHC.

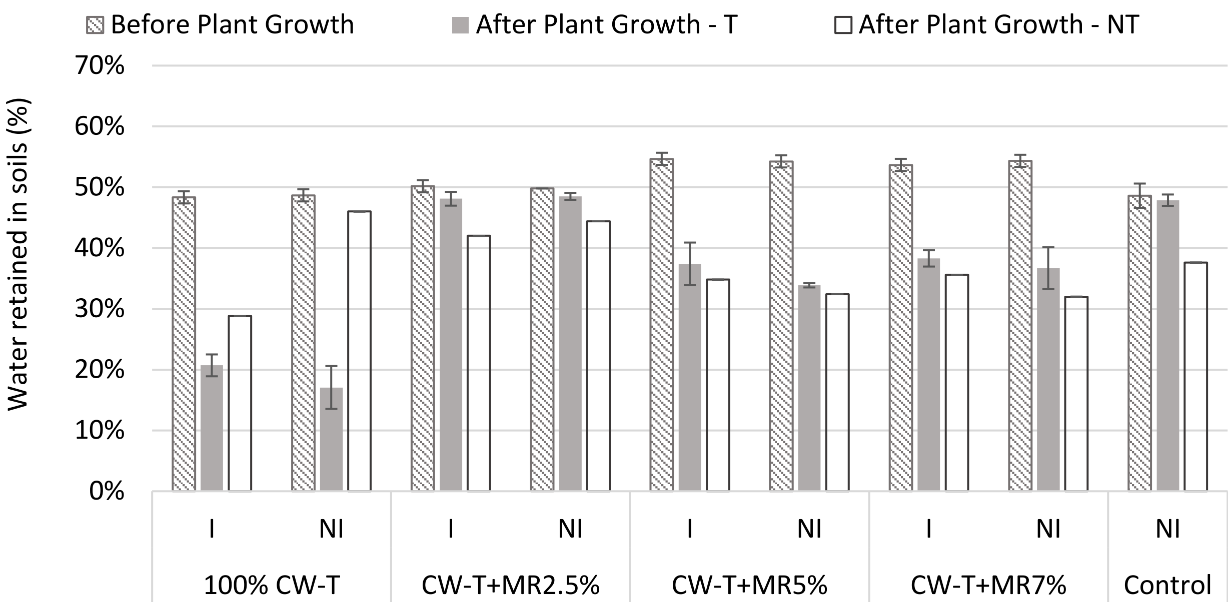

Figure 5-22: Average percentage water retained in vegetated and non-vegetated coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types, T is for *E. tef* growth and NT is no *E. tef* growth.

In this greenhouse study, 50% FC in all pots were maintained. Pedogenesis, soil aggregation, and SOM transformation with plant growth alter the bulk density and soil water holding capability (Amaral Filho, et al., 2020). Hence, the decrease in WHC seen in Figure 5-22 at the end of the growth trial. The average percentage of water retained in CW-T+MR2.5% were the highest amongst all Technosols, with 48% for inoculated and 49% for non-inoculated treatments (matching the potting soil's final WHC with 49%). Here, adequate water retention suggests good mass transfer within the technosols for oxygen diffusion, cation translocation, carbon sequestration and nitrogen fixation(Lowery et al., 1996). It also correlates to good shoot and root development in Figure 5-11. These results are consistent with those by Amaral Filho et al. (2020) who reported that a mixture of CW-T and native soil (in w/w ratio 3:1) amended with a lower ratio of 2% (w/w) OM resulted in better water percolation than the technosols constructed from the same ratio of CW-T and NS without OM by Sekhohola and Cowan (2017).

The soils amended with 7% MR had higher WHC (38% for I and 37% for NI) than those with 5% (37% for I and 34% for NI) as a direct consequence of the compact, clay-like structure that formed by high MR dosages to CW-T. As previously mentioned, Technosols with 7 wt.% MR were more prone to compaction with decreased water drainage and high salinity that inhibited root development. The exposed OM in CW-T+MR7% was oxidised that attenuated microbial activities and nitrogen levels. Leaching results on 100% CW-T and CW-T+MR7% support the previously presented results. 79% and 75% of the water entering inoculated and non-inoculated 100% CW-T, respectively, leached out. Whereas, 62% and 63% of the water entering inoculated and non-inoculated CW-T+MR7%, respectively, were leached. This was expected from the initial growth trial results and from literature on the effect of OM (when absent and in very high concentrations) on water drainage and infiltration (Lowery, et al., 1996; Atlas & Bartha, 1998).

WHC of simultaneously biostimulated and bioaugmented Technosols were superior to only biostimulated Technosols. An indication of structural stability introduced by microbes performing OM decomposition and soil aggregation as suggested by (Atlas and Bartha, 1993). Interestingly, WHC of non-vegetated pure coal-based Technosols were higher than those with vegetation, as a result of water channels that formed around *E. tef* plants in the ultrafine coal tailings.

Macronutrients (C, N, S, P Bray II, K) are pertinent to soil development (Botta, 2015). Soil metal(loid) profiles influence plant growth and soil toxicity (Da Silva Rego, et al., 2016). Characterising a soil accordingly, provided information on the soil potential. Soil fertility measured after plant growth for vegetated and non-vegetated Technosols are shown in Table 5-9 and Table 5-10, respectively.

Table 5-9: Physiochemical characteristics of vegetated coal-based technosols and potting soil as the control, after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types. Technosols were analysed for macronutrients and metal(loid)s; Ca, Mg, Na, Cu, Mn, Zn, Fe, B.

Parameter	**Unit**	**100% CW-T**		**CW-T+MR2.5%**		**CW-T+MR5%**		**CW-T+MR7%**		**Control**
		I	NI	I	NI	I	NI	I	NI	NI
C	%	50	46	50	50	52	50	51	50	15
TN	%	1	1	1	1	1	1	1	1	0
P (Bray II)	mg/kg	14	8	32	30	58	54	106	104	35
K	mg/kg	209	32	140	25	141	45	196	47	319
S	mg/kg	760	1076	709	732	648	709	1093	1037	136
Ca^{2+}	cmol/kg	33.1	35.4	31.7	32.8	29.8	28.9	24.8	25.3	23.8
Mg^{2+}	cmol/kg	1.9	1.7	1.7	1.7	1.9	1.8	2.3	1.9	10.4
K^{+}	cmol/kg	0.5	0.1	0.4	0.1	0.4	0.1	0.5	0.1	0.8

Na^+	cmol/kg	0.6	0.2	0.5	0.2	0.5	0.2	0.6	0.2	0.6
Ca	%	91.6	94.8	92.7	94.2	91.5	93.1	88.2	91.8	65.9
Mg	%	5.3	4.4	4.9	4.9	5.9	5.8	8.0	6.9	28.8
K	%	1.5	0.2	1.1	0.2	1.1	0.4	1.8	0.5	2.3
Na	%	1.6	0.5	1.4	0.7	1.5	0.7	2.0	0.8	1.6
Cu	mg/kg	2.0	2.4	3.6	3.3	3.8	3.5	3.1	3.4	4.0
Zn	mg/kg	1.3	1.2	2.5	2.3	3.9	3.1	4.5	4.5	14.0
Mn	mg/kg	23.9	22.5	23.2	23.6	23.5	23.1	23.1	24.1	67.0
B	mg/kg	0.4	0.3	0.5	0.5	0.4	0.6	0.7	0.6	0.6
Fe	mg/kg	34.2	33.8	41.2	33.9	62.1	54.2	56.8	60.5	273.7
S Am.acet	mg/kg	3185	3335	3145	3183	2883	2683	2653	2475	121
Resist.	(ohm)	278	413	350	403	280	315	240	313	623
T Value	cmol/kg	36	37	34	35	33	31	28	28	36
CEC	mg/kg	13	11	13	13	12	11	13	15	19
Stone	Vol %	1	1	1	1	1	1	1	1	2

Table 5-10 Physiochemical characteristics of non-vegetated coal-based technosols and potting soil as the control, after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

		100% CW-T		**CW-T+MR2.5%**		**CW-T+MR5%**		**CW-T+MR7%**		**Control**
Parameter	**Unit**	I	NI	I	NI	I	NI	I	NI	NI
pH	KCl	7.5	7.3	7.2	7.2	6.8	7.3	6.5	7.1	5.8
Resist.	(ohm)	320	400	280	380	310	290	320	310	680
T Value	cmol/kg	36	36	36	37	32	30	27	30	38
CEC	mg/kg	13	10	13	10	17	12	14	14	19
Stone	Vol %	1	1	1	1	1	1	1	1	2
C	%	50	45	47	46	51	54	51	54	35
TN	%	1	1	1	1	1	1	1	1	0
P (Bray II)	mg/kg	13	7	40	39	84	39	116	64	51
K	mg/kg	184	28	210	34	200	47	152	51	387
S	mg/kg	884	1360	768	722	1160	630	1080	677	75
Ca^{2+}	cmol/kg	33.2	34.4	32.4	34.4	29.0	27.6	24.0	28.0	24.6
Mg^{2+}	cmol/kg	1.6	1.7	2.0	1.8	2.3	2.0	1.9	1.8	10.8
K^+	cmol/kg	0.5	0.1	0.5	0.1	0.5	0.1	0.4	0.1	1.0
Na^+	cmol/kg	0.4	0.2	0.6	0.2	0.6	0.2	0.3	0.2	0.4
Ca	%	93.0	94.6	91.2	94.2	89.6	92.2	90.1	92.9	65.6

Parameter	Unit	100% CW-T		CW-T+MR2.5%		CW-T+MR5%		CW-T+MR7%		Control
		I	NI	I	NI	I	NI	I	NI	NI
Mg	%	4.5	4.7	5.6	4.9	7.1	6.7	7.1	6.0	28.8
K	%	1.3	0.2	1.5	0.3	1.6	0.4	1.5	0.4	2.6
Na	%	1.2	0.5	1.7	0.7	1.7	0.7	1.3	0.7	1.2
Cu	mg/kg	2.0	2.6	3.8	3.1	2.9	3.7	4.1	4.2	4.1
Zn	mg/kg	1.7	1.4	2.6	2.3	3.2	3.1	5.7	5.1	13.7
Mn	mg/kg	23.5	21.9	23.2	23.9	23.5	23.4	23.7	24.6	69.6
B	mg/kg	0.3	0.3	0.7	0.7	0.7	0.8	0.7	0.6	0.3
Fe	mg/kg	28.7	24.3	47.4	32.8	48.7	63.0	63.4	78.7	258.0
S Am.acet	mg/kg	3280	3280	3360	3280	2890	2510	2460	2440	67

By comparing the technosols in Table 5-9, it is apparent that C, P, K, and Ca nutrient concentrations where in superior concentrations whenever MR was applied. MR is a source of key nutrients, including N, Ca, K, Zn and P whilst also imparting structure to the technosols. Magnesium content was similar in all bioaugmented Technosols but 1.6-fold higher in CW-T+MR7%.

Calcium, magnesium, potassium and sodium are necessary for plant tissue development and enzyme activation (Ca and K), and chlorophyl production (Mg and Na)(Mwende Muindi, 2019; Rawat et al., 2016). Thus, it was expected that higher concentrations of these ions would be cycled in soils (Technosols and control) that showed good *E. tef* growth and corresponding healthy soil microbiomes. The results support this provided that inoculation improved soil microbial and associated plant performance, as after *E. tef* growth Ca levels were slightly less, and Mg, K, and N contents slightly more in bioaugmented-treatments than those with only biostimulation. The soil cation concentrations correspond to the higher electrical conductivity seen in Figure 5-21 in bioaugmented technosols compared to non-inoculated controls and potting soil. The control had the highest concentrations of K, Mg, Cu, Zn and B, which supports the high cation exchange capacity and T-value, implying that there are more nutrients in the soil matrix and less probability of nutrient loss.

The P (Bray II) contents are graphically presented in Figure 5-23. The graph illustrates the nutrient contribution by MR additives; as P concentrations increase proportionally to MR dosage. The P content of potting soil is 34.9 mg/kg which has been engineered in terms of optimum nutrient content for agricultural purposes. It is important to note that the P content in bioaugmented CW-T+MR2.5% (32.0 mg/kg) is the most similar to potting soil. Here, EM Pro-Soil and MR additives to CW-T generated soil-like conditions with high phosphorus availability and soil charge capable of capturing and releasing the necessary nutrients during plant growth, whilst minimizing nutrient loss and metal leaching (Mwende Muindi, 2019). The rhizosphere microbes from inoculation enhanced P solubility (Campbell, 1985). Inoculated 100% CW-T contained only 0.4-fold that of the control, whereas inoculated CW-T+MR5% and CW-T+MR7% had 1.7-fold and 3-fold that of the control, respectively. Comparing the biomass produced in each treatment to their respective fertility results, it is clear that bioaugmented CW-T+MR2.5% outperformed the higher dosages of MR without inoculation.

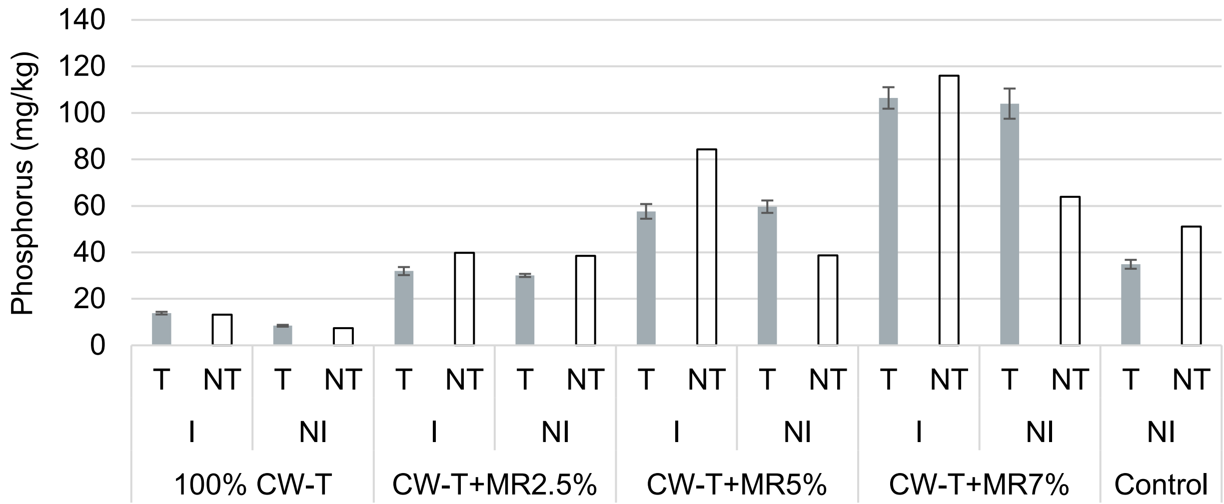

Figure 5-23 Concentration of phosphorus (P Bray II) in non-vegetated (NT) and vegetated (T) coal-based technosols and potting soil as the control, after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

Insufficient K delays plant maturity and increases susceptibility to pestilence (Rawat et al., 2016). This was discernible in the *E. tef* grown in non-inoculated 100% CW-T (K: 31.8 mg/kg) and CW-T+MR7% (K: 47.0 mg/kg) in the final greenhouse study. Treatments with bioaugmentation outperformed those without EM Pro-Soil in terms of K cycling, where inoculated CW-T+MR2.5% had accumulated 5.6-fold that of the non-inoculated treatment (Figure 5-24). Initial amendment application of 2.5% (w/w) MR decreased the relatively high K content (0.23%) of the ultrafine coal tailings. Furthermore, the effect of biostimulation on soil fertility is evident as K levels increased with MR dosage.

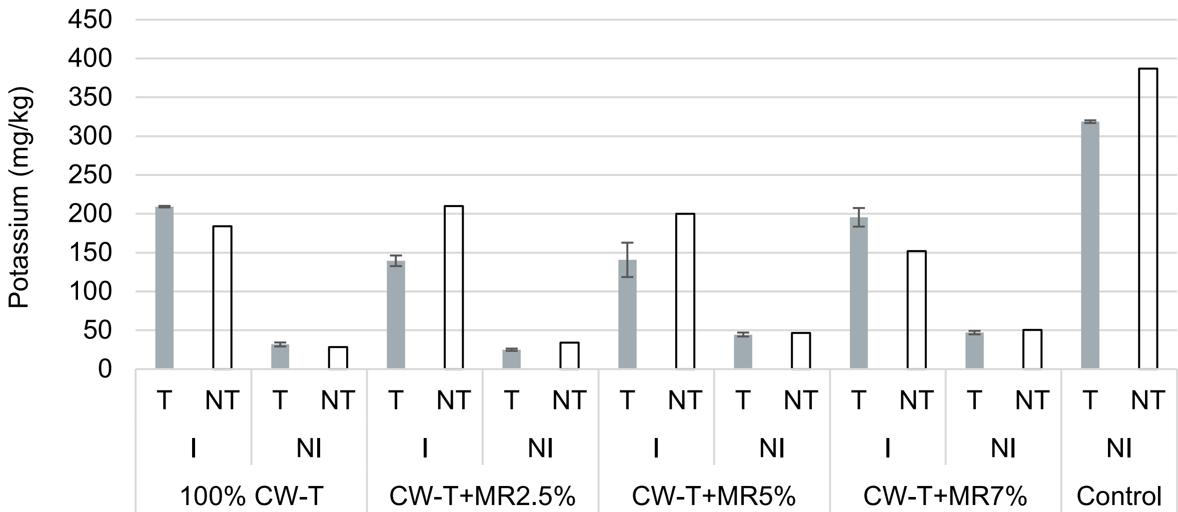

Figure 5-24 Concentration of potassium (K) in vegetated (T) and non-vegetated (NT) coal-based Technosols and potting soil as the control, after the final plant growth trial in winter where I is inoculated and NI represents non-inoculated treatment types.

Total sulfur after plant growth is graphically presented in Figure 5-25. Pure CW-T was characterised by 1.19% S. Therefore, high S levels in Technosols even after plant growth were expected in comparison to the control. The results indicate that bioaugmented and biostimulated (with 2.5% and 5% MR) Technosols were best able to remediate soil S to suitable levels (709 mg/kg and 648 mg/kg respectively) with *E. tef* growth. The S concentrations were below the maximum national screening values for soils according to the South African National Environmental Management Waste Act from 2014. Whereas, S in 100% CW and CW+MR7% were eminent (1.7-fold the reference value). These results are in favour of simultaneous bioaugmentation and biostimulation of coal-based Technosols but further research is required to evaluate soil S levels and pyrite oxidation with successive plant growth cycles. The sulfur speciation on coal tailings indicated primarily pyritic content of 48.0%. The low soluble iron and sulfur results are supported by the low redox potentials and low iron and sulfate levels in leachates, to suggest that pyritic reduction occurred in all Technosols. A slow rate of pyrite oxidation in soil is desirable since sulfur species are transformed to compounds that are accessible and beneficial for plant and microbial growth and microbial proliferation (Weiler, et al., 2020; Dong, et al., 2020). However, analysis on the technosols elemental content prior to plant growth is necessary for validation.

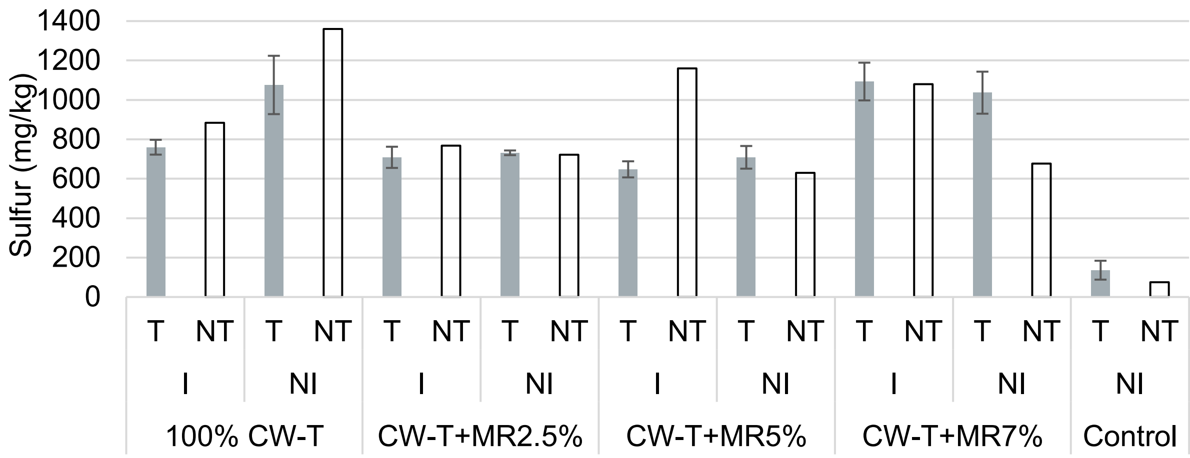

Figure 5-25: Concentration of sulfur (S) in vegetated (T) and non-vegetated (NT) coal-based Technosols and potting soil as the control, after the final plant growth trial in winter, where I is inoculated and NI represents non-inoculated treatment types.

To evaluate environmental effects of coal-based Technosols as a topsoil, leaching of sulfate and ferrous iron were analysed in all treatment types. The results for ferric iron are presented in Figure 5-26, ferrous iron in Figure 5-27, and sulfate in Figure 5-28. Ferrous and ferric iron concentrations in all leachates were negligible (all ferrous below 0.6 mg/mL, and all ferric below 0.35 mg/mL). According to Mahedi et al. (2019), iron leaching patterns are cationic and decrease with an increase in soil pH conditions. As the investigated Technosols all had pH levels between 6.98 and 7.90, iron leaching is not a concern. Fe concentrations in Technosols were high compared to other metals, the results are thus promising for minimized ARD during implementation when cultivating *E. tef*. Sulfate is generated in Technosols during sulfur sequestration with added S from MR (0.67% S), irrigation with rainwater, leaf litter decomposition, and atmospheric deposition (Tabatabai, 1987). It is expected that bioaugmentation and amendment application will reduce the pyritic sulfur content, as was the case in CW-T amended with 5% OM where pyrite sulfur content decreased by 50% with *Medicago sativa* (alfalfa) growth in research by Weiler et al. (2020). The results for sulfate leaching (refer to Figure 5-28) suggest a corresponding increase in the sulfate form which is auspicious to results by Weiler et al. (2018, 2020). Sulfate production followed exposure of pyrite to aerobic conditions with microbes, and plant absorption of sulfur. Leaching of SO_4^{2-} was induced by the alkaline technosols conditions with Ca, K and Mg cation dominance. The acidic profile of potting soil resulted in improved SO_4^{2-} absorption.

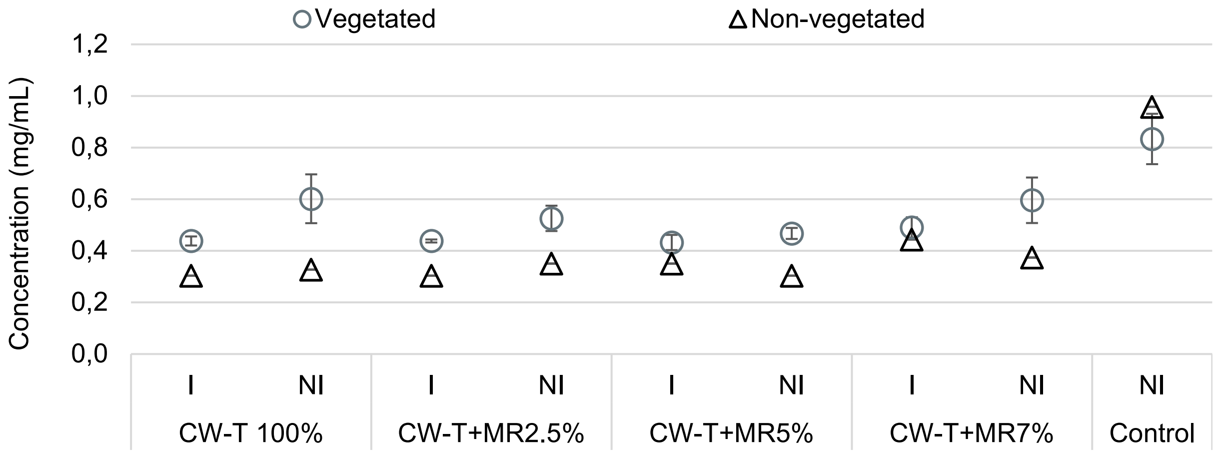

Figure 5-26: Average concentration of ferric iron (mg/mL) within leachates collected from vegetated and non-vegetated coal-based Technosols and potting soil as the control, in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

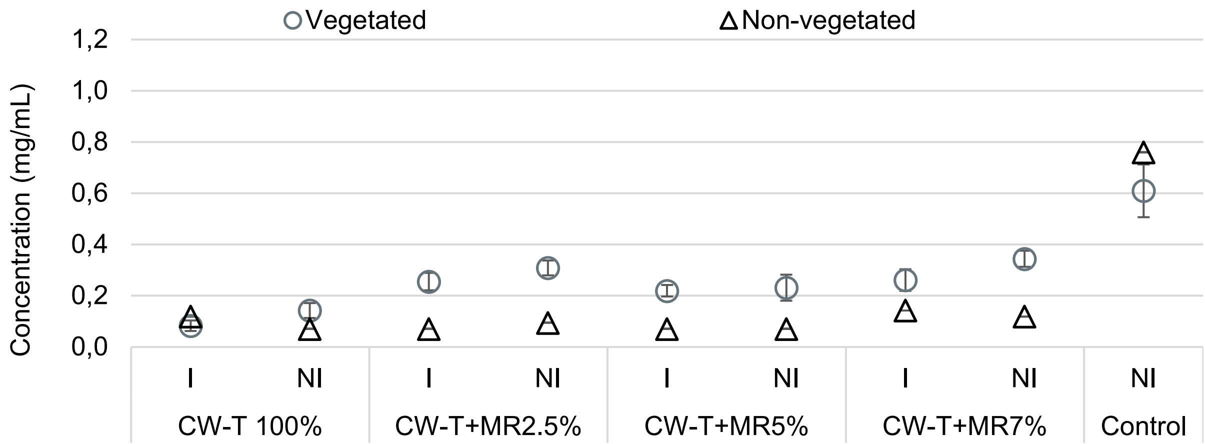

Figure 5-27: Average concentration of ferrous iron (mg/mL) within leachates collected from vegetated and non-vegetated coal-based technosols and potting soil as the control, in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

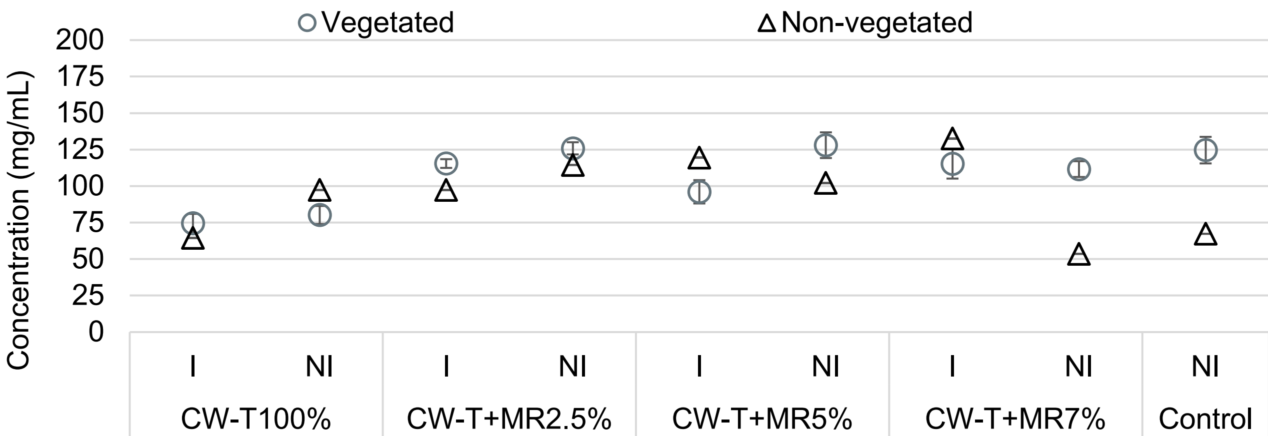

Figure 5-28: Average concentration sulfate (mg/mL) within leachates collected from vegetated and non-vegetated coal-based technosols, in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

Technosols metal(loid) concentrations after *E. tef* growth are illustrated in Figure 5-29. From this graph, Fe and Mn levels were superior compared to other metals. Microorganisms in the rhizosphere enhance the availability and solubility of Fe and Mn to plants by producing chelating agents (as described by Atlas & Bartha (1993). Nonetheless, all metal(loid)s were below the national screening values according to the South African National Environmental Act from 2014. While Cu concentrations were similar across all engineered soils in the presence of MR, it was 1.5-fold lower in the absence thereof. Mn concentrations were similar across all treatment types but 3-fold more in the potting soil. All analysed metal(loid)s were more abundant in the control, with 4.5-fold more Fe than the highest bioaugmented technosols, CW-T+MR7%.

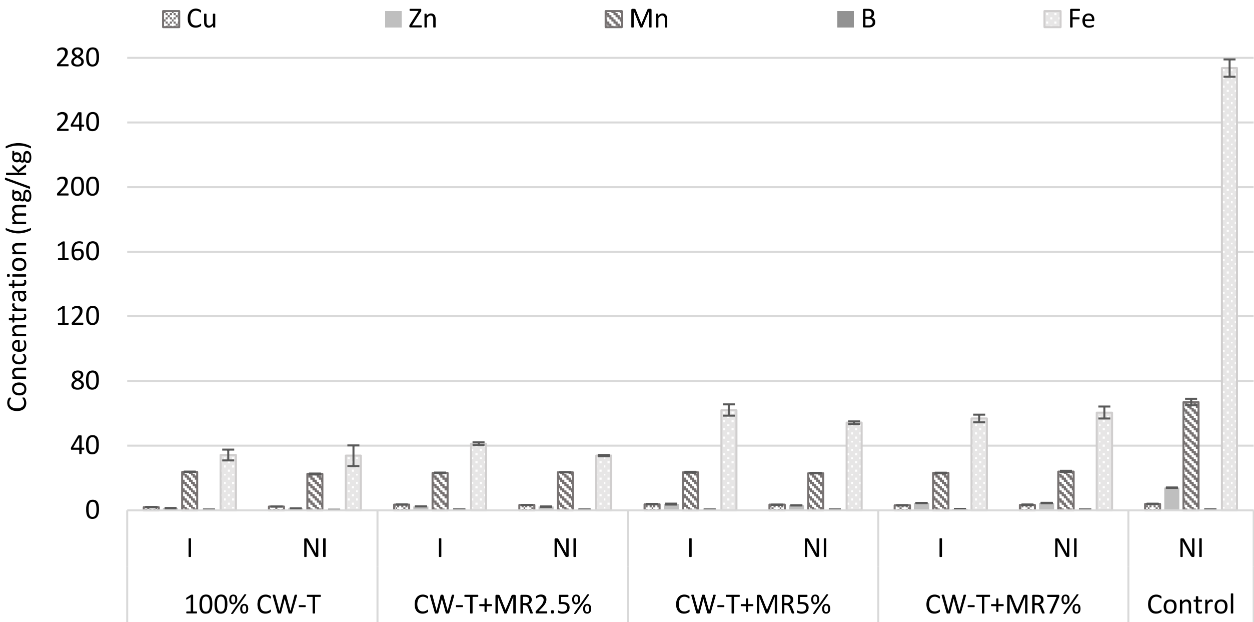

Figure 5-29: Metal(loid) (Cu, Zn, Mn, B, Fe) concentrations in vegetated coal-based technosols and potting soil as the control after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

Figure 5-29 eludes to the beneficial phytoremedial potential of *E. tef* in mine waste-based soils. Metal(loid) levels within the plant dry biomass corroborated this. *E. tef* roots and shoots grown in the best performing CW-T+MR2.5% and CW-T+MR5% Technosols were analysed for major, minor and trace elements, and compared to those cultivated in the control, potting soil. The results are summarized in Table 5-11.

As shown in the initial growth round, the macro elements were Na, Mg, Ca, K and P. Of which all were more concentrated in the shoots compared to the roots, except Ca. Higher concentrations of calcium in plant roots are indicative of microbial respiration in the rhizosphere as high concentrations of carbon dioxide increase Ca solubility and availability (Atlas & Bartha, 1998). Interestingly, shoots and roots of *E. tef* in inoculated CW-T+MR2.5% and CW-T+MR5% had lower concentrations of Ca than the non-inoculated controls. But CW-T+MR5%-I had a higher concentration of calcium cations (29.8 cmol/kg) in the soil matrix than its control treatments (28.9 cmol/kg). Nonetheless, in the initial growth trial, all *E. tef* below ground biomass from inoculated treatments contained more Ca than the non-inoculated controls. Suggesting, the influence of seasonal variation on microbial functions for metal uptake.

Na, Mg, Ca, K and P are vital to plant growth (Atlas and Bartha, 1993; Campbell, 1985). The abundance thereof in inoculated CW-T+MR2.5% and CW-T+MR5% compared to the control validate the potential value of cultivating *E. tef* in a revegetation scheme with Technosols for otherwise nutrient-deficient coal mining waste (Rawat et al., 2016; Truter, 2007). Once again, Al and Fe concentrations were relatively high in the root structures as a result of the initial high content of these metals within the parental material, and increased Fe solubility from microbial mechanisms (Hrynkiewicz et al., 2018).

Table 5-11: Elemental characterisation of above (shoots) and below (roots) ground *E. tef* dry biomass cultivated in coal-based technosols amended with 2.5% and 5% (w/w) MR against potting soil as the control in the final growth trial in winter where, I is inoculated and NI represents non-inoculated treatment types.

		CW-T+MR2.5%				**CW-T+MR5%**				**Control**	
		Shoots		**Roots**		**Shoots**		**Roots**		**Shoots**	**Roots**
Symbol	**Unit**	I	NI	I	NI	I	NI	I	NI	NI	NI
Na	g/kg	0.546	0.492	1.47	0.321	0.608	0.675	2.23	1.64	0.274	0.340
Mg	g/kg	3.65	4.04	3.29	2.22	3.43	4.28	4.69	7.93	3.40	19.8
Ca	g/kg	5.32	6.21	14.1	19.8	6.50	9.71	14.8	17.4	3.59	14.6
K	g/kg	14.9	7.69	1.85	0.988	15.5	7.79	2.55	1.20	18.1	6.55
P	g/kg	3.27	1.39	0.848	0.736	2.64	1.71	1.12	1.07	2.74	0.969
Si	g/kg	1.62	1.76	0.273	0.187	2.41	2.87	0.229	0.566	1.21	0.372
B	mg/kg	9.46	5.00	21.7	24.4	7.87	9.30	23.2	33.5	7.31	12.9
Al	g/kg	0.144	0.151	18.7	27.4	0.159	0.318	16.8	13.6	0.0868	11.9
V	mg/kg	0.169	0.162	18.0	25.6	0.175	0.325	16.3	14.4	0.177	26.5
Cr	mg/kg	1.92	6.48	33.4	39.1	1.55	1.07	27.9	20.6	0.630	184
Mn	mg/kg	202	104	134	143	117	98.5	126	109	22.2	210
Fe	g/kg	0.0951	0.0845	7.71	11.8	0.117	0.172	7.00	5.20	0.0683	11.1
Co	mg/kg	1.098	0.477	7.95	8.67	0.564	0.416	6.70	5.87	0.0395	33.0
Ni	mg/kg	1.393	1.68	22.3	26.6	1.05	1.62	19.1	17.0	0.803	96.6
Cu	mg/kg	14.4	11.9	33.9	33.4	14.2	13.4	31.8	31.5	8.10	45.5
Zn	mg/kg	86.2	39.3	41.1	26.3	76.0	46.8	45.1	31.2	81.4	53.9
As	mg/kg	0.127	0.130	2.53	3.66	0.156	0.322	2.39	2.70	0.260	1.51
Se	mg/kg	0.135	0.0798	0.808	0.966	0.0790	0.0810	0.735	0.678	BDL	0.551
Sr	mg/kg	70.2	76.9	208	270	63.8	85.5	205	250	25.9	62.4
Mo	mg/kg	0.736	0.538	1.32	0.693	0.804	0.695	1.76	2.70	1.49	2.12
Cd	mg/kg	0.364	0.0955	0.381	0.135	0.172	0.0900	0.300	0.290	0.785	0.663
Sn	mg/kg	0.0299	0.0282	0.954	1.335	0.0330	0.0440	0.861	0.741	0.0341	0.912
Sb	mg/kg	0.0584	0.0346	0.028	0.0166	0.0190	0.0250	0.045	0.071	0.0164	0.0632
Ba	mg/kg	13.0	5.25	211	292	12.1	9.77	191	157	34.3	101
Hg	mg/kg	0.0137	0.0165	0.136	0.178	0.0210	0.0280	0.113	0.097	0.0166	0.0492
Pb	mg/kg	0.143	0.149	5.75	8.42	0.152	0.248	5.12	4.56	0.170	12.4

The elemental analyses showed favourable results for *E. tef* grown in bioaugmented CW-T+MR5%, with K concentrations in *E. tef* shoots similar to those in the control and 1.4-fold more than shoots in CW-T+MR2.5%-I. Phosphorus levels were 1.3-fold higher in the roots of CW-T+MR5%-I compared to CW-T+MR2.5%-I, suggesting enhanced P solubility in the soil-like substrate. Campbell (1985) described greater rates of phosphate uptake associated with plants in soils with rhizosphere microbes compared to sterile soils. The microbes produce acids that dissolve the mineral group, apatite, which releases soluble forms of phosphorus (Atlas and Bartha, 1993). Thus, linking to the augmented P (Bray II) levels in the soil matrix of inoculated CW-T+MR5%, compared to the non-inoculated control in Table 5-11. These results support that of the initial greenhouse growth trial.

E. tef shoots in CW-T+MR5%-I had the highest Al (16 800 ppm) and Fe (97 000 ppm) content compared to bioaugmented CW-T+MR2.5%. However, this treatment type had 1.5-fold more Fe in the soil matrix compared to inoculated CW-T+MR2.5%. Nonetheless, iron leaching was prevented as previously discussed. Minor and trace metal(loids) such as Ni, As, Sr, and Hg, were present in lower concentrations compared to the inoculated technosols with 2.5% MR. Hence, indicating that 5% amendment in conjunction with bioaugmentation, were able to sufficiently reduce metal(loid) solubility. The above ground *E. tef* dry biomass were analysed for CHNS to further investigate the effect of dual biostimulation and inoculation. The results are illustrated in Figure 5-30. The carbon content of the shoots in the final growth round are all similar and above 40.7%, and the sulfur contents are negligible. This compared well with the results for the first cycle of *E. tef* growth. From Figure 5-30, it is clear that bioaugmentation in soils amended with 2.5% and 5% (wt.%) MR increased nitrogen and phosphorus uptake into plant above ground organs. Perhaps as an indication of associations between *E. tef* and myrcorrhizal fungi. For further research when investigating plant-microbe interactions to determine nutrient absorption from soil and plant tolerance to abiotic variables, it is recommended to microscopically look at a cross section of the *E. tef* rootlet to determine which mycorrhizal associations (ectotrophic or endotrophic) have established after a growth cycle in the coal-based Technosols (Atlas & Bartha, 1998).

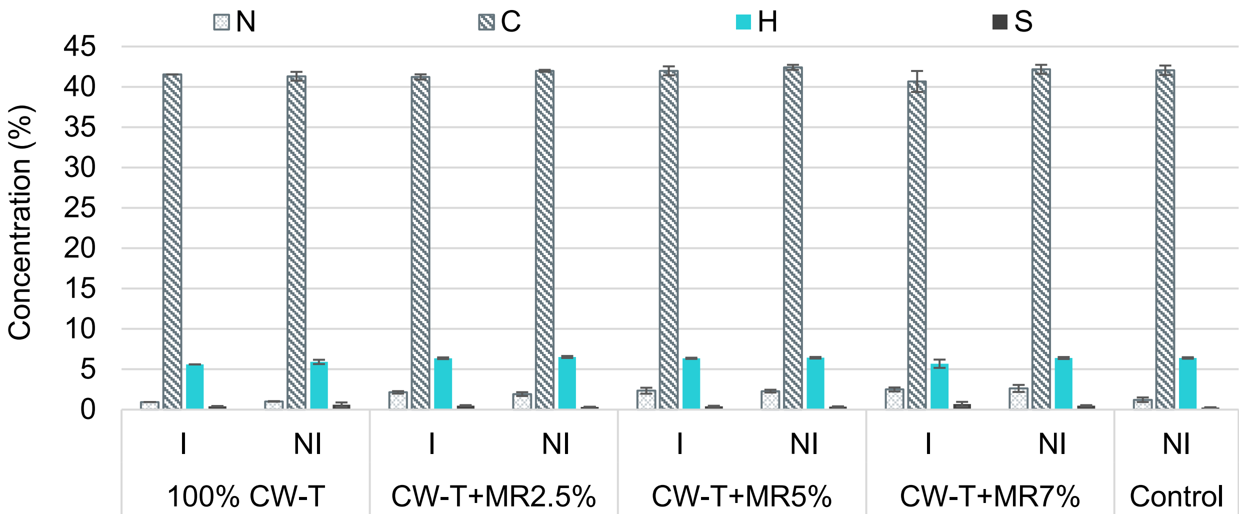

Figure 5-30 CHNS characterisation (expressed as percentage) in above ground *E. tef* dry biomass cultivated in coal-based Technosols and potting soil as the control in the final growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

Collected leachates from all Technosols were analysed for TOC, TIC and TN (refer to Figure 5-31 and Figure 5-32). TN in technosols leachates followed the same trend as in the soil matrix where nitrogen contents increased with MR dosage. Adding MR to CW-T accelerated the breakdown of organic material, resulting in more TOC and TN in leachates of MR-amended Technosols. TN in bioaugmented CW-T+MR7% was 12-fold that of 100% CW-T. The effect of biostimulation is evident since TOC in 100% CW-T with no amendments were almost 5-fold less than inoculated CW-T+MR2.5%. TOC in potting soil leachates were superior; 3.3-fold that of inoculated CW-T+MR2.5%. Potentially as a result of enhanced C sequestration (implying enhanced microbial activity) in the control. TN in potting soil leachates were similar to that of bioaugmented CW-T+MR2.5%; corroborating the plant growth results highlighting this fabricated soil above other types. Low TOC and TN leachates results in bioaugmented CW-T+MR2.5% and CW-T+MR5% compared to their non-inoculated controls and compared to

CW-T+MR7% treatments, indicate that the good *E. tef* growth (high biomass productivity) through bioaugmentation enhanced C and N mineralisation and the uptake of nutrients into plant organs. However, the low TOC in 100% CW-T indicated low microbial activity and inadequate OM mineralisation (Cervantes, et al., 2011).

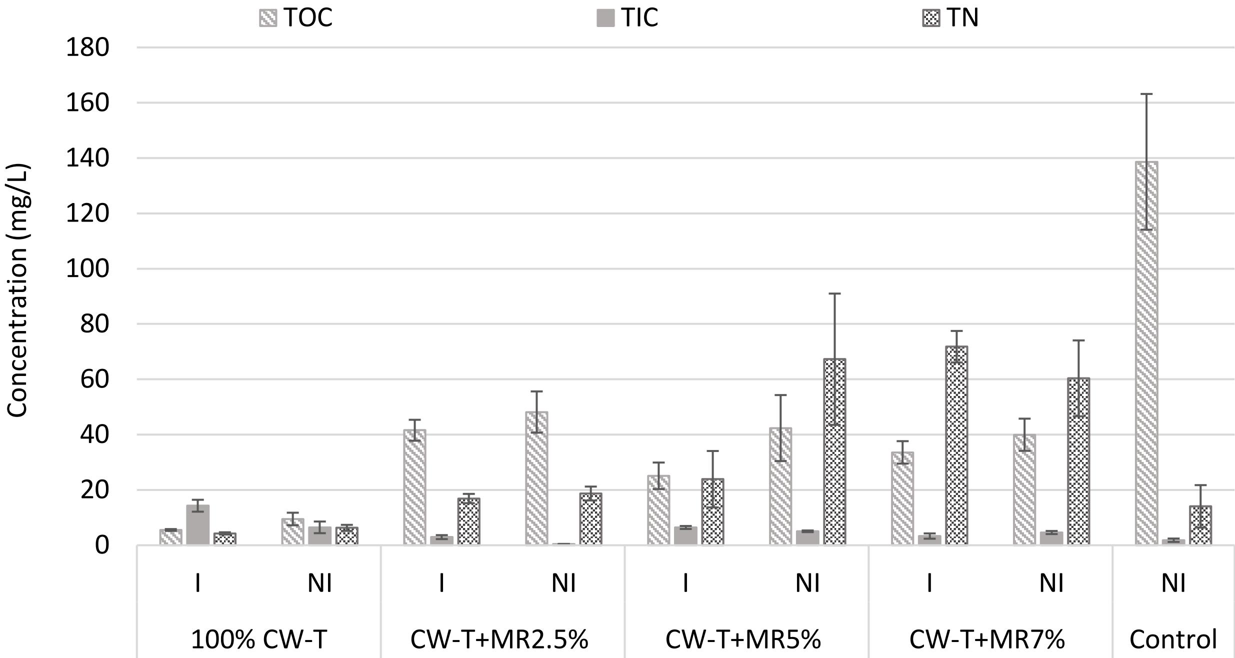

Figure 5-31: Total organic & inorganic carbon and total nitrogen concentrations (mg/L) in leachates from vegetated coal-based Technosols and potting soil as the control, collected from the plant growth trial. Where, I is inoculated and NI represents non-inoculated treatment types.

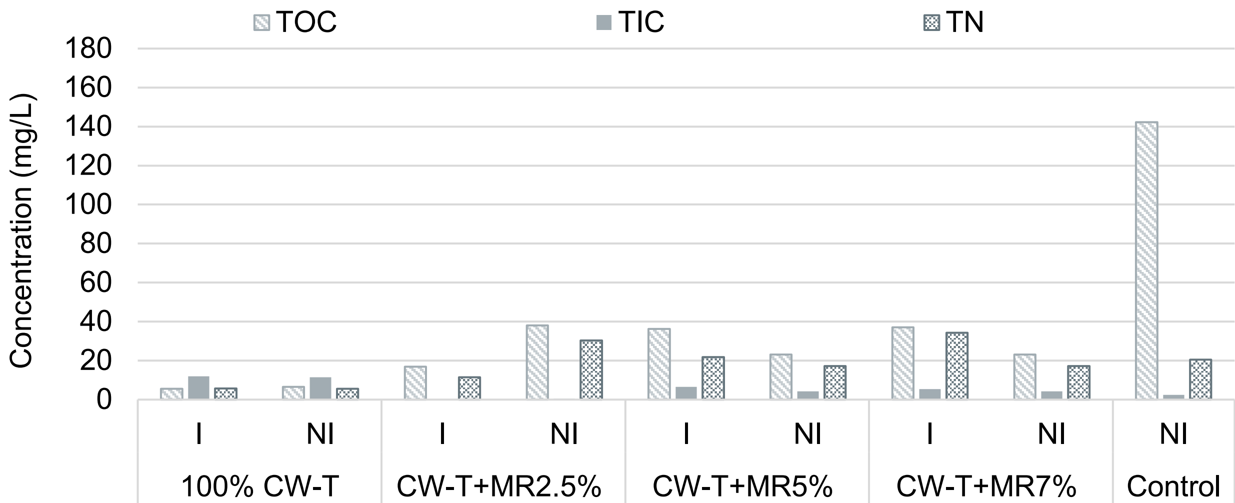

Figure 5-32 Total organic & inorganic carbon (TOC, TIC) and total nitrogen (TN) in non-vegetated coal-based Technosol leachates in the plant growth trial. Where, I is inoculated and NI represents non-inoculated treatment types.

Thus far, the best performing soils were determined from the fertility and plant development results. However, a single cycle of well-maintained plant growth does not imply long-term soil fertility, quality and health (Dangi et al., 2012a). This study employed biomolecular techniques to analyse technosols microbiomes in terms of structure and function to identify: 1) the effects of bioaugmentation and biostimulation on soil fertility and plant growth, and 2) which soil composition can ensure sustained fertility in a mine waste derived soil.

Soil Microbiome Analysis

Owing to the importance of the soil microbiome in achieving active, regenerative soils, its analysis was undertaken with particular focus on its nutrient cycling capacity.

Cell counts were performed in parallel with FDA analysis (a measure of metabolic activity) to evaluate microbial diversity and proliferation. Cell counts after the seven-day incubation period showed a 1.45-fold increase in the microbial population during the activation process. As a result, a lower volume of activated inoculum was required (1.5-fold less than non-activated EM Pro-Soil) for bioaugmentation per gram of technosols, thereby lowering associated costs.

The activation process was further evaluated with FDA and SIR. The metabolic activity measured through FDA is summarized in Figure 5-33 to Figure 5-37. A direct correlation between concentration of microbial cells (cells/mL) and activation period was found through fluorescence (RFU) measurements. Microbial activity increased by 1.1-fold with the incubation period. These results were consistent with the substrate-induced respiration (SIR) results as shown in Figure 5-38.

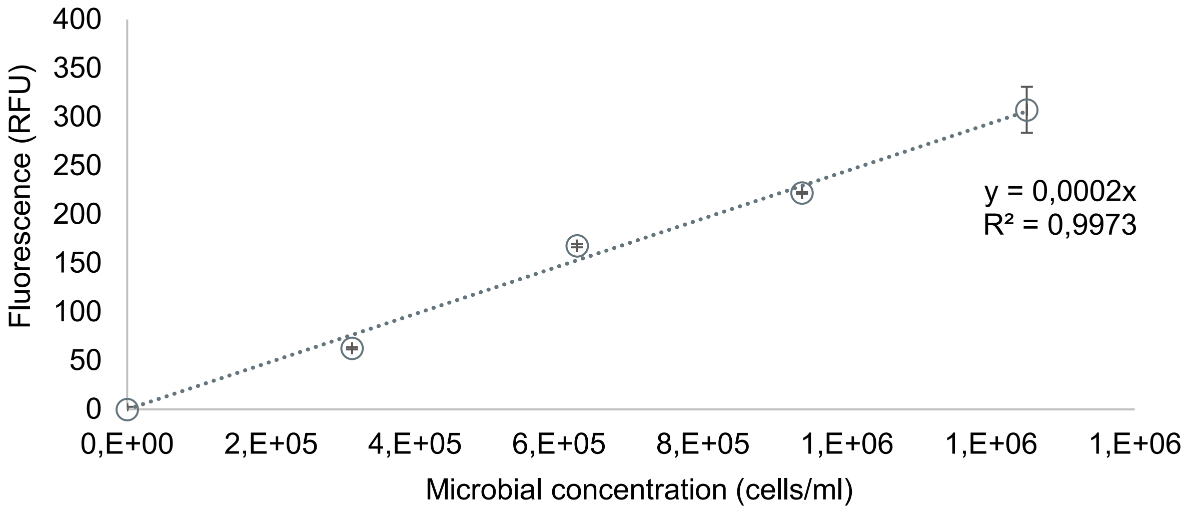

Figure 5-33 Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 0 of inoculum activation as measured through FDA analysis.

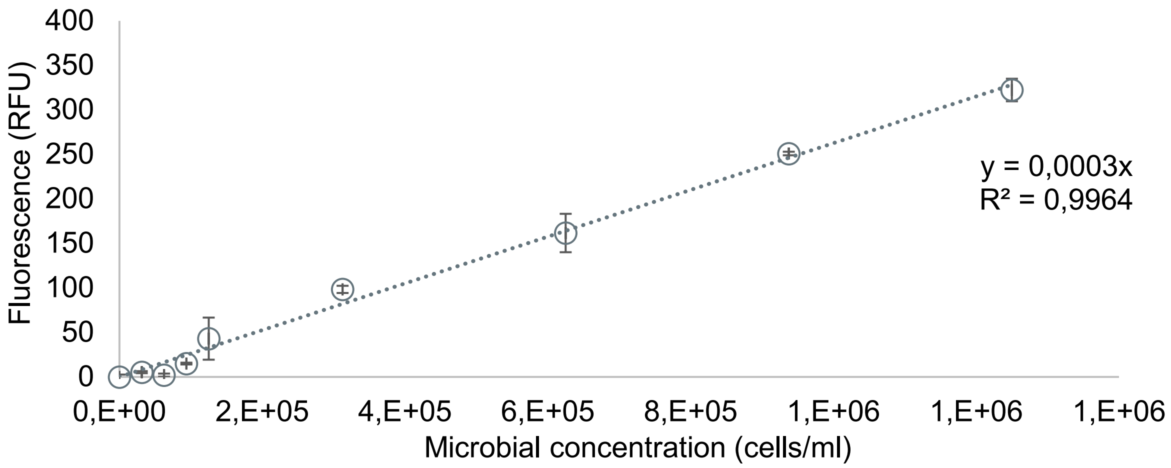

Figure 5-34: Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 1 of inoculum activation as measured through FDA analysis.

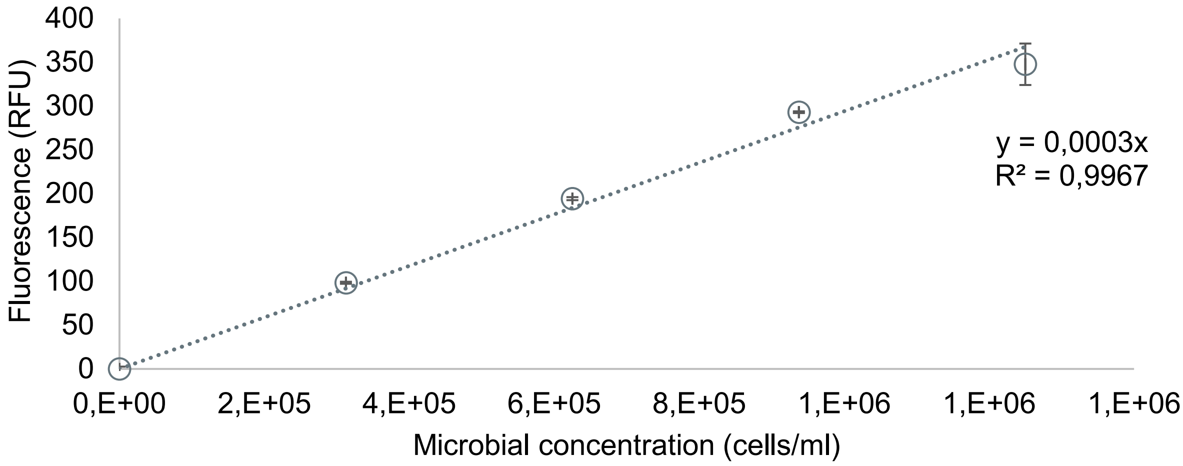

Figure 5-35: Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 3 of inoculum activation as measured through FDA analysis.

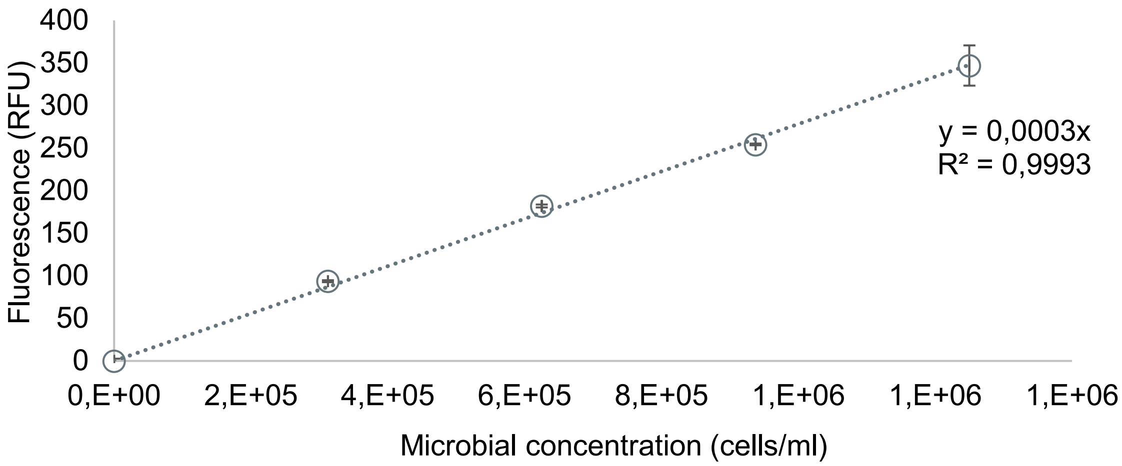

Figure 5-36: Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 5 of inoculum activation as measured through FDA analysis.

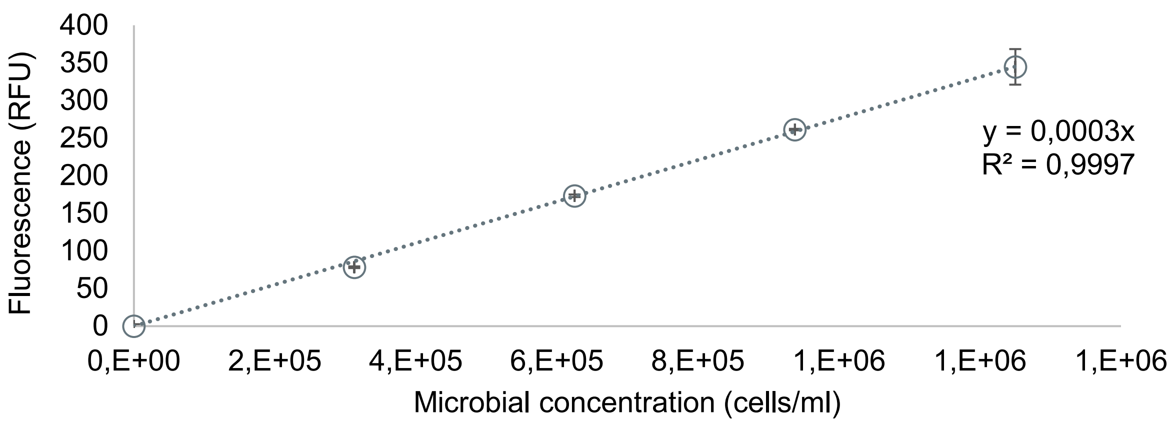

Figure 5-37: Average fluorescence (RFU) emitted by microorganisms present in EM Pro-Soil on day 7 of inoculum activation as measured through FDA analysis.

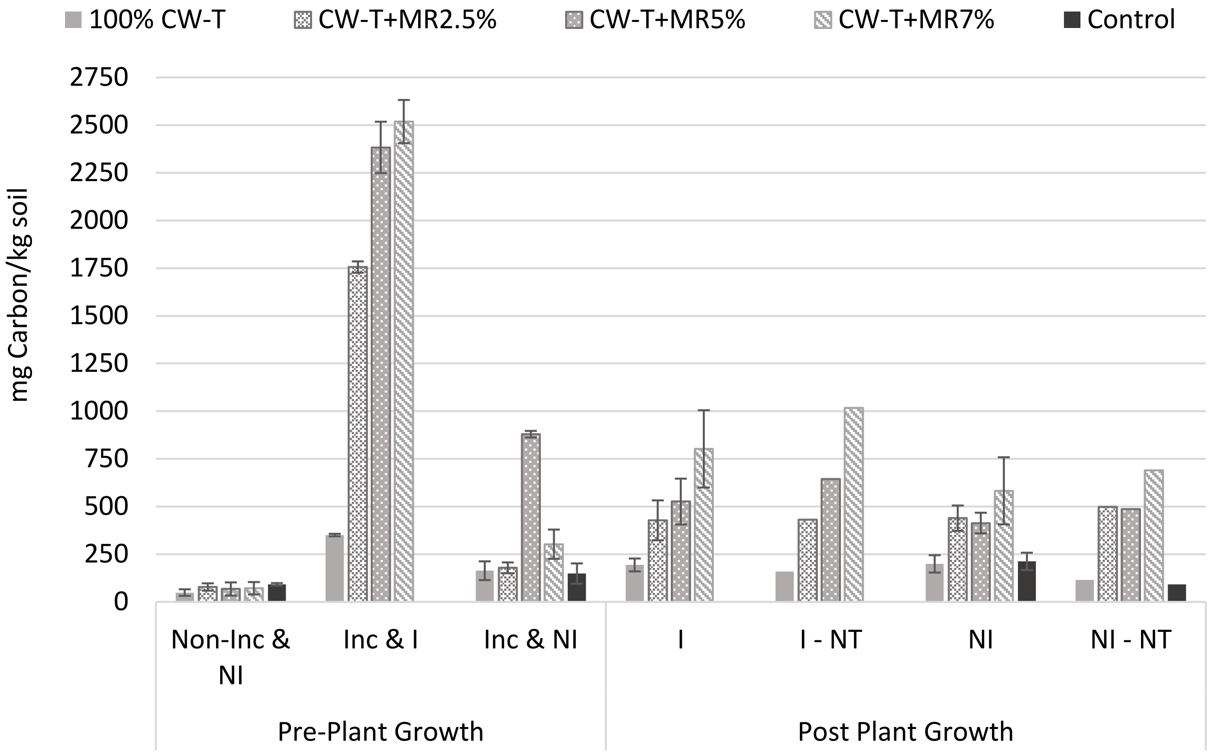

Figure 5-38: Microbial biomass in vegetated and non-vegetated (NT) coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter. Where, Non-Inc is non-incubated, NI is non-inoculated, Inc represents incubated, and I refers to inoculated treatment types.

Inoculation and incubation resulted in a 2.7-fold increase in microbial biomass in CW-T+MR5%, and a tenfold increase in CW-T+MR2.5%. This is consistent with research by Weiler et al. (2020). As expected, inoculation resulted in significant increased microbial biomass relative to non-inoculated soils before plant growth. In CW-T+MR5%, bioaugmentation increased the biomass by 2.7-fold, and 9.8-fold in CW-T+MR2.5%. Figure 5-38 also visually shows greater microbial biomass in inoculated treatments after *E. tef* growth. The carbon content per weight of technosols in bioaugmented CW-T+MR5% was 21.5% more than non-inoculated CW-T+MR5%, 16.6% more than non-inoculated CW-T+MR2.5%, and 59.5% more than the control by the end of the greenhouse study.

The immediate contribution of MR to soil organic carbon content was discerned from the SIR analysis and supported by MR characterisation. Even though the results seem promising for CW-T+MR7%, microbial biomass here included fungal biomass. As previously discussed, mould growths in CW-T+MR7% treatments were detrimental to *E. tef* germination and development. Bacterial growth is delayed or terminated by osmotic stress from large relative conductivities between medium (soil) and cell (community). As CW-T+MR7% was a saline soil-like substrate (Figure 5-21), it was anticipated that initial bacterial community development would be less abundant in these Technosols with high EC. To distinguish between microbial abundances, qPCR analysis was performed. Nevertheless, the SIR results clearly indicate that the inclusion of amendments to coal mine waste benefit soil fertility (in terms of microbial biomass) in conjunction with bioaugmentation.

As the first growth round was conducted in favourable *E. tef* growth conditions, it was expected that the microbial biomass results would resemble that of the high plant biomass yields that were achieved. Perhaps an indication of the influence of irrigation levels (maintaining 50% of the maximum water retention) on soil pH and consequently on soil microbial abundances as suggested by literature (Li et al., 2021), showed that soil bacterial communities' structure and function were more strongly affected by irrigation than nitrogen fertilization in *Triticum aestivum* (winter wheat) grown in a semi-arid environment (also prevalent to eMalahleni from this study), as soil pH is directly determined by soil water content, and is the primary driving force in soil microbial community structure (Neina, 2019).

FDA analysis after plant growth were performed to further investigate if OM as amendment enhanced microbial activity in a bioaugmented-technosols. FDA was optimised for coal-based Technosols. The effectiveness of using chloroform to terminate hydrolysis was corroborated by Schumacher et al. (2014), and it was assumed that all living organisms were removed from MR through sterilisation. It was determined that microbially-emitted fluorescence in the background of CW-T was directly proportional to microbial cells per mL soil for a 10-minute incubation period. Thus, FDA results for soils after the final growth cycle were presented accordingly in Figure 5-39.

Rhizodeposition and OM degradation in the rhizosphere influence the microbial community composition in vegetated soils (Nannipieri, et al., 2003). Consequently, there is a direct relationship between soil concentrations of organic carbon and nitrogen with FDA hydrolysis. The microbial activity and function were expected to be superior in Technosols with improved *E. tef* growth compared to the poor performing fabricated soils (100% CW-T and CW-T+MR7%) as soil enzyme levels fluctuate with soil OM and microbial composition.

It is apparent in Figure 5-39 that microbial concentrations per volume of soil increased with bioaugmentation and biostimulation. Adding amendments that are easily metabolizable to CW-T enhanced microbial growth in treatments without vegetation compared to the Technosols with vegetation and no amendments (100% CW-T). MR as a source of carbon was utilised for microbial growth (Chessa et al., 2016) and plant development. Thus, in the no-vegetation controls, microbial activities were higher with no limiting OM substrates. Research by (Weerasekara et al., 2017), similarly found decreased FDA in loam and sand-like soils with plant growth. MR-application to CW-T increased technosols WHC (Figure 5-15), improved plant growth , and resulted in augmented P, Ca and Mg soil contents (refer to Table 5-9), that supported microbial activities. This is in agreement with (Bandick and Dick, 1999) who described higher enzyme activities in soils that received added organic input compared to controls without added OM after plant growth.

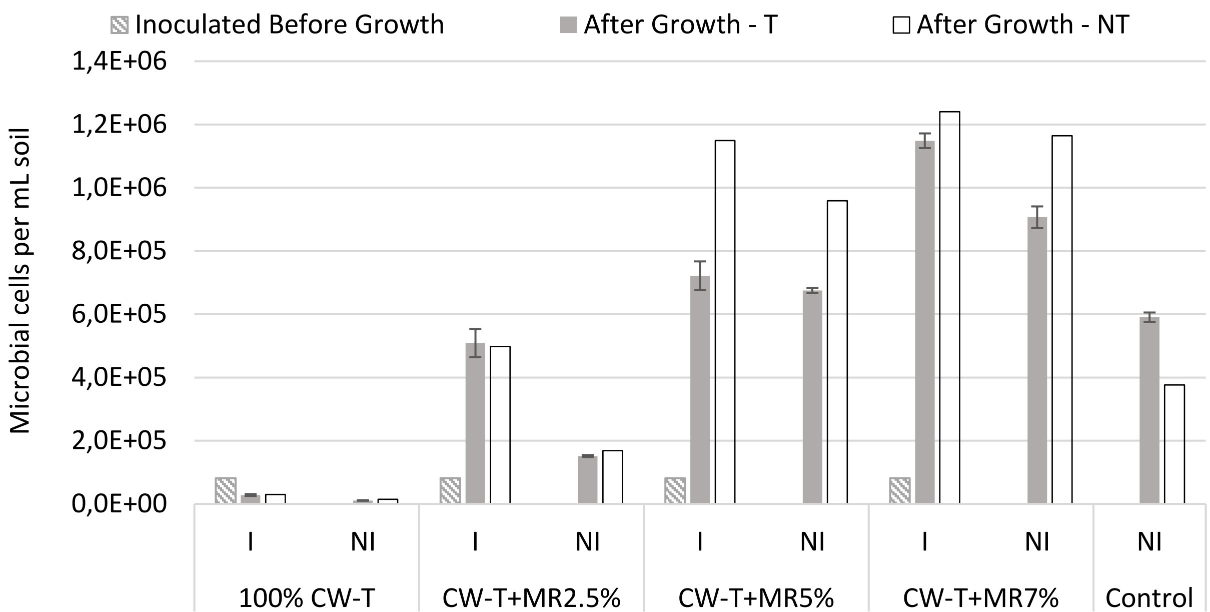

Figure 5-39: Concentration microbial cells per coal-based technosols with *E. tef* (T) and without *E. tef* (NT) after the final plant growth trial in winter. Where, I is inoculated and NI is non-inoculated treatments; T represents soils with *E. tef* growth and NT is the no *E. tef* control of each treatment type.

Microbial activities in inoculated CW-T+MR2.5% were comparable to that in the control, potting soil. Furthermore, the WHC of these soils after plant growth were comparable (both at 48% from Figure 5-15) as well as the number of plants that survived by the end of the growth study. Thereby, suggesting adequate performance of the bioaugmented CW-T+MR2.5% and its feasibility as a topsoil. Nonetheless, as previously discussed, K and P concentrations were significantly lower and sulfur concentrations considerably higher in this fabricated soil compared to the potting soil. Through FDA analysis, Bakhoum et al. (2012) concluded that the effect of bioaugmentation and shifts within soil microbial communities are dependent on soil origin and type. Hence, highlighting the effect of biostimulation with MR on bioaugmented Technosols.

Hydrolysis through FDA analysis encompasses activity of several microbial enzymes, e.g. esterases, proteases, and lipases (Schumacher et al., 2015). Soil metal(loid) concentrations are deleterious to microbial enzymes. Therefore, augmented microbial activity from FDA analysis in bioaugmented-soils amended with 5% MR (wt.%) suggests reduced metal(loid) solubility which is in support of the ameliorated soil characteristics as previously discussed (increased nutrient content, improved WHC, increased pH and decreased salinity). Nonetheless, CW-T+MR2.5% also showed improvements of soil physical and chemical properties but did not concur with microbial activity assessments thus far (SIR and FDA). In this investigation, amplification of 16S rRNA gene sequences by qPCR was used to further investigate these effects of simultaneous bio-augmentation and -stimulation to ultimately determine which and if a technosols can perform as a self-sustaining topsoil.

Results in Figure 5-39 concur with SIR analysis in Figure 5-38, where the highest microbial activity was in Technosols amended with 7% (w/w) MR. However, from plant growth results, higher dosage of OM added as MR resulted in fungal growth (hence, results for high fungal biomass and activity) and did not improve soil health and quality. Thus, high activity seen in CW-T+MR7% with SIR and FDA represents total microbial activity (Adam and Duncan, 2001), and does not necessarily suggest a more fertile rhizosphere. Low microbial activity in 100% CW treatments from FDA results were due to poor water retention in these soils and corroborate the results by(Schumacher et al. (2015). Furthermore, it supports the reasoning behind the low TOC results obtained in these Technosols (Figure 5-32).

Molecular methods were used as a way of looking at species abundance and the persistence of the microbial communities in Technosols of the final growth study. From DNA extraction and 16s rRNA gene amplification, to infer microbial community persistence and function in the soils solely based on the microbial community profile and what literature suggests. The results for quantified double stranded DNA (dsDNA) extracted from each technosols treatment before and after the final growth trial are in Figure 5-40, as a summary of microbial community change with amendment dosage and bioaugmentation in soil subjected to *E. tef* growth.

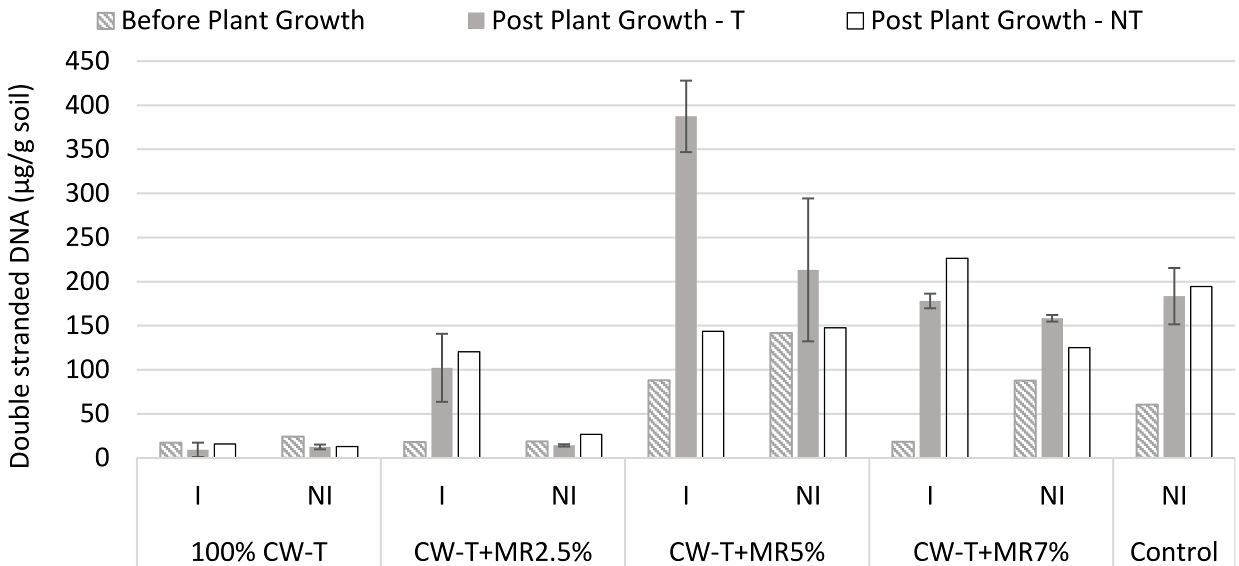

Figure 5-40: Concentration double stranded DNA per gram of soil in coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter. Where, I is inoculated, NI is non-inoculated, T is with *E. tef* growth and NT represents no *E. tef* growth.

CW-T+MR5%, primarily the inoculated treatment, contained the most quantified dsDNA per gram of soil. Containing 25-fold more than inoculated 100% CW-T which evidently could not support *E. tef* growth. Thereby, supporting the previously detailed results for CW-T+MR5%-I on improvement in physiochemical soil conditions and plant development in both plant growth trials. Non-inoculated CW-T+MR5% had similar quantities of dsDNA to the control, which further motivates the use of 5% MR as amendment to CW-T. The contribution of bioaugmentation to soil extractable DNA is evident in Figure 5-40 , where all inoculated treatments had more amounts of dsDNA after *E. tef* growth than the non-inoculated controls. This contribution is most pertinent in CW-T+MR2.5%, where the bioaugmented treatments showed a 7.3-fold increase in dsDNA per gram soil.

Interestingly, before plant growth, inoculated CW-T+MR5% had on average 4.9-fold more dsDNA per gram of technosols compared to the other treatments with EM Pro-Soil. This is unexpected as all treatments were initially inoculated with the same number of cells per gram of technosols. However, a relatively high initial concentration of dsDNA was also in both non-inoculated CW-T+MR5% and CW-T+MR7%, and the control (potting soil). The results for the control were expected as pedogenesis had already been initiated in the agricultural soil; a higher concentration of initial microbial biomass was evident in Figure 5-38. Bacterial contamination during DNA extraction could have led to these results; however, the SIR results for initial microbial biomass in non-inoculated CW-T+MR5% and CW-T+MR7% coincide with the DNA extraction results. Thus, eliminating the probability of bacterial contamination during the extraction process. Inoculum activation for all soil types were controlled and identical; therefore, the assumption was made that no additional, unexpected benefits (i.e. higher microbial biomass) could have resulted from inoculation of fabricated soils. Hence, it could only be accounted for in the soil fabrication process. Suggesting that the higher concentration of labile OM in soils amended with 5% and 7% MR resulted in rapid microbial growth during soil incubation (at optimum mesophilic growth conditions). Thereby, corroborating with (Lebrun et al. (2021) and Bakhoum et al. (2012) in that bacterial populations are strongly dependent on the amendments that alter soil properties. Profiling the soil microbiomes would provide valuable information regarding this observation.

The soil microbiome facilitates 90% of all soil processes (Burns et al., 2009). Therefore, the investigated coal-based Technosols' abilities to perform as a self-sustaining topsoil were also investigated from its microbial diversity. technosols microbiomes were profiled in terms of bacteria, fungi and archaea, and nutrient cycling genes through qPCR amplification of 16S rRNA genes. The relative abundance of bacteria, fungi and archaea before and after plant growth in each of the Technosols are presented in Figure 5-41.

From this figure, it is clear that CW-T+MR5% (both I and NI) had the highest initial and final bacterial populations of the investigated Technosols. Inoculated CW-T+MR5% had 1.46E+07 bacterial gene copies per gram of soil, whereas the control had 1.52E+08 bacterial 16S rRNA gene copies per gram soil before *E. tef* growth. This suggests that the bacterial population from inoculation were able to best establish and proliferate in the engineered soils amended with 5 wt.% MR which is in agreement with Weiler et al. (2020). The high initial soil bacterial abundance in CW-T+MR5%-NI and CW-T+MR7%-NI corroborate the SIR and FDA results, thereby, coinciding with the previously suggested enhanced bacterial growth rates in soils with higher SOM. It also concurs with research by Shen et al. (2019) who found that bacterial populations increased from OM addition to coal waste in stacks compared to the control soils. In CW-T+MR2.5% and CW-T+MR5%, final bacterial abundances were more than archaea abundances, implying that nitrification was governed by bacteria rather than archaea. This correlates to research by Hafeez et al. (2012).

It was expected that the highest microbial diversity would be found in Technosols with a higher pH and WHC, and lower metal(loid) availability compared to Technosols with more acidic pH and higher metal(loid) concentrations (Lebrun, et al., 2021). However, there are no apparent trends in the microbial diversity of the investigated Technosols with *E. tef* growth. In addition, the effects of increasing amendment dosage or inclusion of bioaugmentation are not clear from the microbiome profiles. However, fungal populations, with respect to bacteria and archaea, were lower in treatments with inoculation before plant growth. This trend does not remain after plant growth. Instead, the relative abundance of fungi increased with plant growth in all soils, including the control.

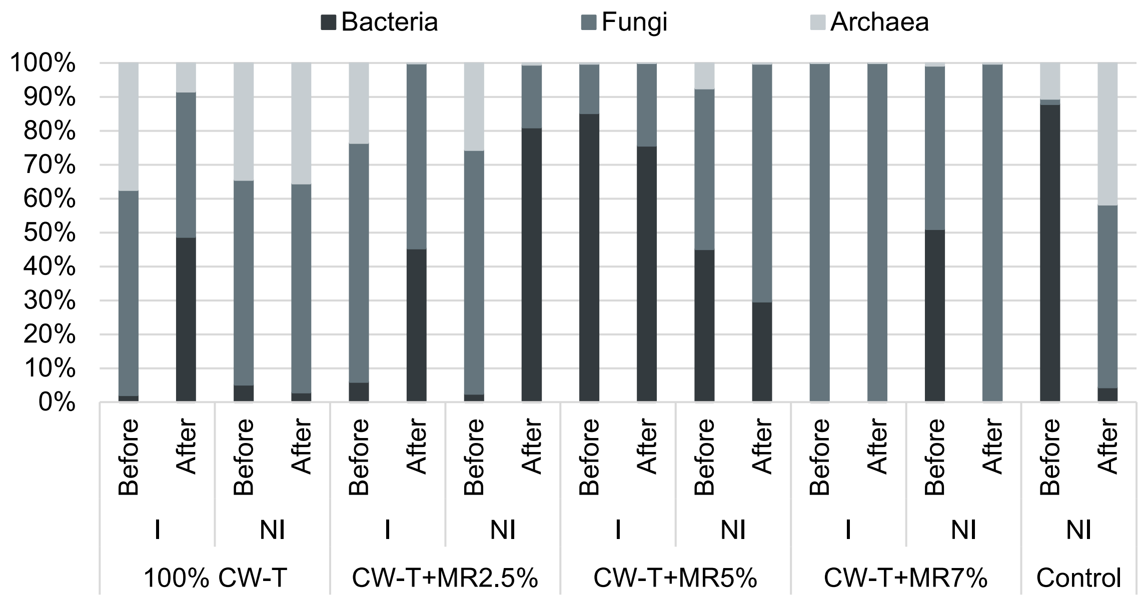

Figure 5-41: Relative abundance (expressed as percentage) of bacterial, fungal and archaeal gene copy numbers within vegetated coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter. Where, I represents inoculated treatments and NI is non-inoculated soils.

Additionally, bacterial communities from the inoculum (as outlined in Chapter 3), were only enhanced in CW-T+MR2.5% and CW-T+MR5% during vegetation. Of the fungal populations in the aforementioned engineered soils, *Sacchoromyces cerevisiae* were introduced by EM Pro-Soil. This yeast plays an important role in soil aggregation, nutrient breakdown and nutrient cycling (Botha, 2011). Subsequently, supporting the previously presented results on *E. tef* growth and physiochemical soil parameters that concluded promise and best performance (relative to the other fabricated soils) in bioaugmented CW-T+MR2.5% and CW-T+MR5%. Moreover, the enhanced WHC and *E. tef* growth (increased growth rates and grain yields) in these Technosols, were most likely from fungal populations of AMF and EMF that created mycorrhizal network to support root development, water uptake and carbon sequestration (Atlas and Bartha, 1993; TIWARI et al., 2021).

As expected, inoculated CW-T+MR7% initially had large fungal populations (5.37E+07 fungi gene copies per gram soil. Although bacterial growth is deterred by high soil salinity (as seen in all CW-T+MR7% for both plant growth trials), fungi are less affected. Thus, fungi persisted in CW-T+MR7% as seen in Figure 5-41. Suzuki et al. (2005) suggested a positive correlation between soil fungal community abundance and soil fertilization; supporting the high fungal gene copy numbers per gram soil found in technosols with higher concentrations of OM as MR, especially in CW-T+MR7%. In corroboration, many studies have shown that fungal biomass and associated activity are higher in substrates with larger soil macro-aggregates. In this investigation, CW-T+MR7% had the largest soil particles due to the addition of 7 wt.% MR. Furthermore, corroborating the very high microbial biomass and activity results for this technosols. Microbial respiration and nitrification decline with an increase in EC (as discussed with SIR results); hence, the reduction seen in bacterial abundance in CW-T+MR7%, and the abundance of nifH genes (genes regulating nitrogen fixation) are expected to be very low.

Non-inoculated CW-T+MR2.5% had very small amounts of extractable soil dsDNA in Figure 5-40; amounts similar to that of 100% CW could not support *E. tef* growth. This was unanticipated as the above and below ground biomass production was exceedingly high in this technosols and all of its planted *E. tef* survived in the final growth trial during unfavourable growth conditions. Profiling the Technosols' microbiome indicated that bacterial, fungal and archaeal abundances were in the range of that in 100% CW-T; where, both bacteria and fungi gene copies per gram of CW-T+MR2.5%-NI were 4 orders of magnitude less, and archaea was 3 orders of magnitude less than in CW-T+MR2.5%-I. This was expected from inoculating the fabricated soils with EM Pro-Soil. In non-inoculated substrates amended with 5% and 7% (w/w) MR, bacterial and archaeal 16S rRNA gene copies per gram soil were also less than in the inoculated substrates. However, in both CW-T+MR5% and CW-T+MR7%, fungi copies per gram soil were one order of magnitude higher in non-inoculated than the inoculated, with the highest of all treatments in CW-T+MR7% after *E. tef* growth. Suggesting not only that soil fungal abundances increased with amendment dosage, but also increased in the absence of inoculation with lower abundances of bacterial and archaeal copy numbers when available OM concentrations are high.

Archaeal diversity decreased with *E. tef* growth in all but non-inoculated 100% CW-T and the control. Potting soil had the highest archaeal gene copy numbers (2.43E+06) per gram soil after *E. tef* growth but with final TN lower than the Technosols (Table 5-9). (Sun et al., 2020) found that 90% of archaea genera in coal soils were unidentifiable, thereby implying valuable yet unknown archaeal resources in the mining soils. And, SCG, a chemoautotrophic ammonia oxidising archaea that participates in nitrogen cycling, was the dominant phyla in the coal mining soils (Sun, et al., 2020). This coincides with (Sterngren et al., 2015) who reported that nitrogen fixation through archaea is more significant in poor quality soils with grass compared to fertile soils. This could have played a role in N cycling in CW-T+MR5%-NI that showed the highest initial archaeal gene copy numbers (3.82E+06) per gram technosols.

Non-planted controls showed low microbial diversity with high fungal gene dominances throughout technosols types after 82 days in the greenhouse. In addition, low copy numbers of bacteria and archaea genes, especially in CW-T+MR5% and potting soil. Therefore, PGPR and other endophytic microbes were assumed to be less abundant or even absent in some fabricated soil types. PGPR and endophytes synthesize phytohormones and siderophores for metal mobilization (Tiwari, et al., 2021). Hence, in their absence, higher metal(loid) concentrations would be expected in all non-vegetated and non-inoculated Technosols. This was predominantly seen in CW-T+MR5%, where Cu, Fe, Mg, Mn, Ca and Zn soil content was higher in those without plants compared to vegetated treatments (Figure 5-42). And, to a lesser extent in CW-T+MR7%. No differences in soil metal(loid) concentrations were observed in NI and I soils amended with 2.5 wt.% MR after the final growth trial. Thus, as previously discussed, bioaugmentation in a coal based-fabricated soil does result in enhanced metal(loid) uptake (achieving the first objective); however, only with vegetation and higher dosages (more than 2.5 wt.%) of MR as amendment. Here, the benefit of plant-microbe interactions is pertinent. Therefore, the soil bacterial communities structure, diversity and activity especially in the rhizosphere of the Technosols depend on their ability to adapt, proliferate and take advantage of the environment (soil and vegetation) (Bakhoum, et al., 2012).

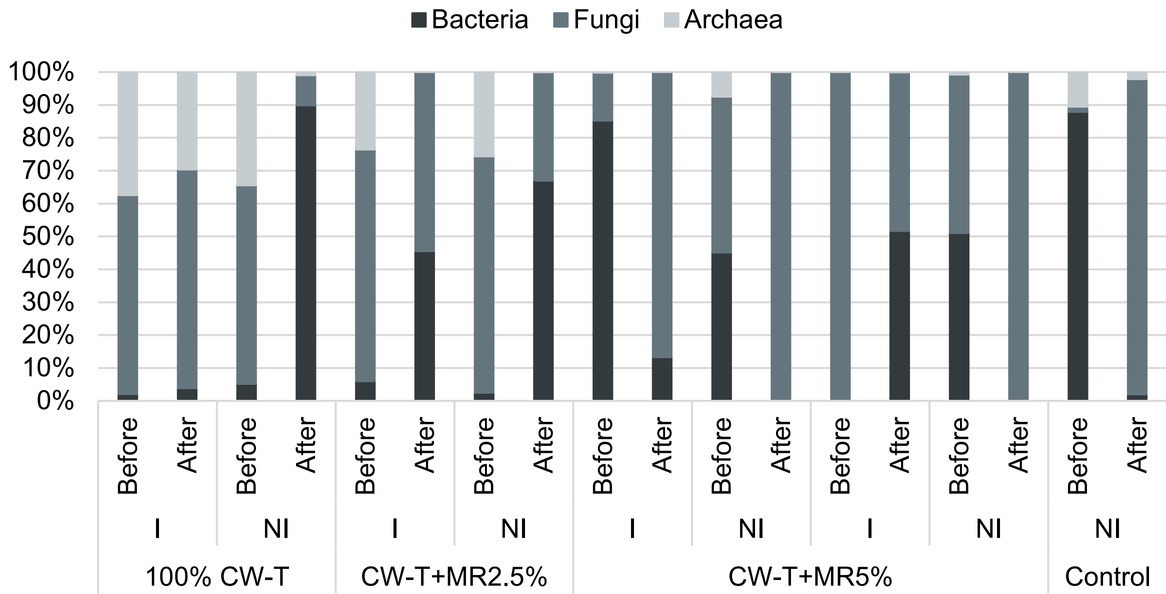

Figure 5-42 Relative abundances (expressed as percentage) of bacteria, archaea and fungi gene copy numbers in non-vegetated coal-based Technosols before and after the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

When considering fabricated soils and the influence of amendments, it is important to understand how the structural modifications (i.e. soil water retention) influence the soil microbial-facilitated functions (Hafeez et al., 2012), such as nutrient nitrogen cycling. Nitrification (nifH gene) and denitrification (nirS, nosZ, and nirK genes) are important steps in the nitrogen cycle detailed in Chapter 2. The genes were amplified through real-time qPCR of which the results are graphically presented in terms of relative abundance in Figure 5-43. As expected, nifH genes are initially more abundant in Technosols before plant growth commenced, of which non-inoculated CW+MR5% contained the most copies (3.48E+07) per gram soil. Nonetheless, the relative abundance of this nitrification gene was the highest in bioaugmented CW-T+MR5% (52.41% rel. abundance) as the inoculum contained *Rhodopseudomonas palustris* and *R. sphaeroides,* known for their soil nitrification capabilities (Knowles, 1982).

The N cycling genes diversity in all Technosols differ significantly between the inoculated and non-inoculated treatments. Fabricated soils amended with 5% and 7% MR (wt.%) had the highest copy numbers. This is auspicious to literature on the positive correlation between soil available organic carbon and denitrifier community abundance (Knowles, 1982). Here, abundances of the nirK gene encoding the nitrite reductase denitrification enzymes were dominant after *E. tef* growth with values ranging from 1.63E+09 to 4.09E+09 (5.25E+09 in the control). While, nosZ followed by nirS genes copy numbers were slightly lower in CW-T+MR5% and CW-T+MR7%). This coincides with Hafeez et al. (2012) who found highest copy numbers of nirK genes in wasteland Technosols with alfalfa; however, nirS gene abundances were similar to nirK. Nonetheless, the majority of published research have not been able to interconnect soil denitrifiers' functions and structures (Hafeez, et al., 2012).

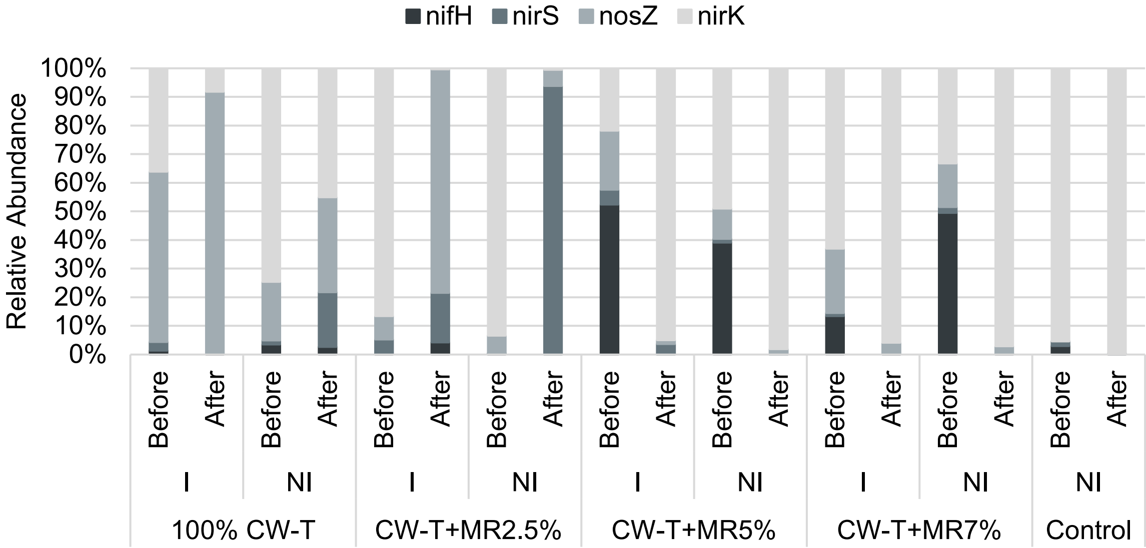

Figure 5-43: Relative abundances (expressed as percentage) of nitrogen cycling genes (nifH, nirS, nosZ and nirK) in vegetated coal-based Technosols and potting soil as the control, before and after the final plant growth trial in winter where I represents inoculated treatments and NI represents non-inoculated treatments.

Enwall et al. (2010) found that the abundance and distribution of denitrifiers were correlated to certain soil parameters; soil pH for nirS and soil copper content for nirK genes, indicating a dependence of denitrifying microbes on soil structure. However, the presented results for this study eluded no similar correlations ($R^2 < 0.40$; not presented). Additionally, Enwall et al. reported similar abundances between nirS and nirK (varied within 1 order of magnitude). In the current investigation, this was only seen in the bioaugmented CW-T+MR5% treatments (before plant growth, nirS: 3.16E+06 copies/g soil and nirK: 1.33E+07 copies/g soil; after plant growth, nirS: 1.42E+08 copies/g soil and nirK: 3.67E+09 copies/g soil) with *E. tef*, and in all non-planted controls of 100% CW-T. Yet, as expected the 100% CW-T controls demonstrated very low gene copy numbers of denitrifiers, as denitrifying bacteria transform nitrates back into nitrogen by forming asym- and symbiotic relationships with plants which were absent in the controls. For CW-T+MR5%-I, it is an indication of effective biostimulation with 5% MR for the specific inoculum to structurally support plant growth (as detailed in section 0) and the microbial community ecology for N cycling. Denitrifying microbes that facilitate the reduction of nitrate into diatomic nitrogen in abundance have been reported at environments with low nitrous oxide (third most significant greenhouse gas) emissions (Philippot et al., 2009). Thus, the abundance of such microbes are desirable in mining waste-based Technosols and have been incorporated into the inoculum, of which *Rhodopseudomonas sphaeroides* have the capacity to regulate both nitrogen fixation and denitrification (Knowles, 1982). This is in agreement with the previously discussed result for highest nifH gene copy numbers in this fabricated soil.

Enwall et al. (2010) delineated a negative correlation between the abundance of nirS and nirK genes and extractable soil P and K. Whereas, that link could not be made in this investigation. CW-T+MR5% and CW-T+MR7% had the highest P concentrations (Figure 5-23) and greatest abundance of denitrifiers, perhaps due to plant-mycorrhizal associations which enhance P and N uptake (Atlas & Bartha, 1998). Furthermore, the study also found a positive correlation between clay-like soil structure and nirS gene abundance (Enwall et al., 2010). This supports the high nirS gene copy numbers in CW-T+MR7% (I: 1.26E+06 copies/g soil; NI: 4.42E+06 copies/g soil) as the fabricated soils exhibited poor water infiltration in both greenhouse trials due to high MR content that resulted in clay-like soil substrate.

It is evident from the low abundances of nifH gene copy numbers in the non-planted controls (Figure 5-44), that plant-microbe interactions contributed to nitrogen fixation within the vegetated bioaugmented-technosols. In all soils except CW-T+MR5%, nifH gene copy numbers per gram soil increased; however, the relative abundances compared to nirS, nosZ and nirK decreased tremendously. Yet, a rise in nifH abundances was seen in bioaugmented CW-T+MR2.5% after the growth cycle in non-planted treatments. At the same time, nirS and nosZ gene copy numbers were augmented in the technosols over time. As seen in Technosols with *E. tef*, nirK gene abundances were dominant after 82 days in the greenhouse, especially in engineered soils amended with 5% and 7% MR, and the control soil.

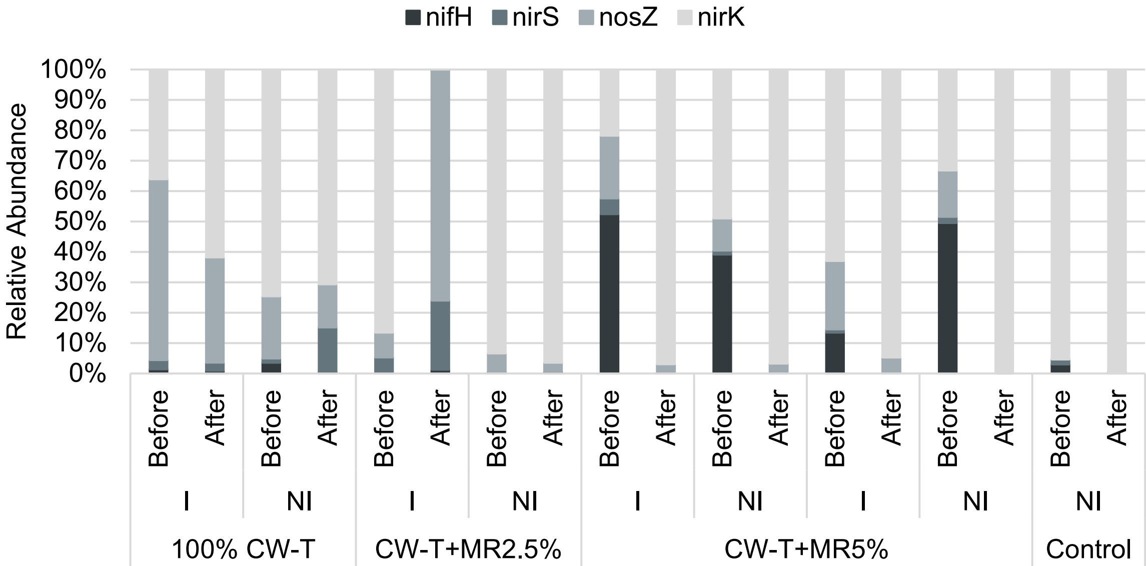

Figure 5-44 Relative abundances (expressed as percentage) of nitrogen cycling genes (nifH, nirS, nosZ, nirK) in non-vegetated coal-based technosols before and after the final plant growth trial in winter. I is inoculated and NI represents non-inoculated treatment types.

6 EVALUATING SOURCES OF AMENDMENT

Depending on location, a range of different organic material sources can be considered for use as amendment. Here key considerations are quality and characteristics of the amendment, availability and volumes available, and proximity to the location of soil fabrication and associated transport costs. We consider a number of potential amendments in this section.

The Mpumalanga province is known for its diverse agricultural production, thus, associated wastes are widely available, both from cropping and from animal husbandry. Additional resources in a close proximity to the eMalahleni colliery that have been used as amendments successfully in similar applications elsewhere are: biosolids (e.g. sewage sludge (Weiler, et al., 2018; Jordan, et al., 2017)), leaf litter (Lebrun, et al., 2021), urban waste (Prado, et al., 2020) and landfill waste (Weiler, et al., 2020; Herran Fernandez, et al., 2016).

According to the 2013 survey reported by (Nkosi et al., 2013), some 42 million m^3 of general waste were generated per year in South Africa at that time, with the Mpumalanga province being responsible for 10% or 4.2 million m^3. While the distribution of South Africa's waste at that point was as follows: 71% mining waste, 6.7% fly ash, 6.1% agriculture waste, 4.5% urban waste and 3.6% sewage sludge, this distribution will vary regionally, requiring data to be sourced more specifically on Mpumalanga and, particularly, the region within 150 km of eMalahleni. Notably, the National Waste Management Strategy of 2020 highlighted that 60% of waste sent to landfill in South Africa is organic, including the 30% of food produced in South Africa that is wasted. It is estimated that 10 million tons of organic waste was disposed in 2018, equivalent to 51% of the organic waste produced. These significant volumes, together with the mandate to divert 100% waste from landfills with a target of 75% diversion by 2035, indicates that the re-purposing of organic waste to amendments for soil manufacture is highly relevant currently. It is necessary to identify the most promising organic waste streams, availability of substantial volumes and proximity to the coalfields to realistically assess their contribution to manufacture of technosols from mine waste.

In the sections below, we consider the potential of varied organic sources to soil manufacture in the eMalahleni region.

Manure from animal husbandry

Volumes of agricultural waste can be estimated from similar environments globally. Considering animal husbandry, in India, 5 tons of organic manure/ha pastureland are generated per year, which is estimated to contain 100 kg NPK/ha/year; however, currently these wastes are not used efficiently (Mandal, et al., 2018). Thus, opportunities exist to incorporate animal manure collected from surrounding farms as amendments into technosols fabrication as suggested by Chessa et al. (2016).

The Statistics South Africa report of 2018 indicates that Mpumalanga is home to 2.47 million hectares of agricultural land, with some 0.62 million hectares under cropping. This implies some 1.85 million ha of pastureland, comprising 661 farms, with the cattle herds growing from 584 350 in 2007 to 637 459 in 2017. Based on the output of 5 tons organic manure per hectare of pastureland and an estimate of 1.85 million hectares of pastureland, Mpumalanga can be estimated to produce 9.25 million tons of organic manure annually. Estimation that each dairy cow produces 7.3 kg manure per day (Font-Palma, 2019) 2.66 tons annually, we can estimate a manure production in Mpumalanga annually of 1.70 million tons per annum. The density of cow manure is reported to range from 420 to 655 kg/m^3 (Khater, 2012). Assuming the midpoint value of 537.5 kg/m^3, a manure addition of 10% (v/v) in fabricated soils correlated to 269 ton manure per hectare. Using the more conservative estimate of manure production of 1.70 million tons per year, this suggests that manure could support the production of technosols to cover some 6350 hectares in mine rehabilitation, or 2 100 hectares if we assume one third of the manure in Mpumalanga is collectable. Using the higher estimate and again assuming one third collectable, this expands to 11 500 hectares annually with the co-processing of 52 million m^3 coal waste.

Organic waste from cropping and leaf litter

As indicated above, Mpumalanga hosts some 0.62 million hectares of cropping land and 1.85 million hectares of pastureland ((Statistics South Africa, 2020) of the cropping land, 422 843 hectares were planted with maize and 207 149 hectares with soya beans in 2017. Leaf litter and plant waste from the cropping land and harvesting of grasses from the pastureland can be composted for inclusion as the organic amendment in soil manufacture.

In addition, substantial organic waste is available as garden litter and garden waste from the nearby cities, especially in Gauteng. While these form copious volumes, transport costs for the 140 km haulage will need to be met.

Sewage sludge

Webb (2004) found that sewage sludge as amendment, fertilised the soil and increased the total biomass production of red oat grass (*Themeda triandra*) during a rehabilitation study on open cast coal mines specifically in Mpumalanga. However, sewage sludge is often associated with a high heavy metal content (Herran Fernandez, et al., 2016). Moreover, Truter et al. (2007) investigated soil amelioration with application of class F fly ash and sewage sludge to disturbed soils surrounding coal mines in Mpumalanga. Results suggested augmented macro- and micronutrients in soils and enhanced growth of indigenous grasses such as *E. tef*.

While amendment with sewage sludge will require attention to destruction of pathogens and management of heavy metals, potentially prior to application, availability of sewage sludge is advantageous. It is estimated that each person in South Africa produces 100 g as dry weight of faeces per day ((Burton et al., 2009)). The population of Emalahleni was estimated at 455 228 in the 2016 census. At 3% growth, it is expected to reach over 700 000. Across the province of Mpumalanga, the population is estimated as 4.4 million in 2022 while neighbouring Gauteng houses 16.1 million people. Hence sewage sludge production in Emalahleni can be estimated at 16 615 ton per year, while within a 160-200 km radius, it can be estimated at 748 250 ton per annum, with potential for repurposing as an amendment for soil fabrication. Owing to its energy content of 15 MJ/kg dry mass (Burton et al., 2009), ideally this would be preceded by anaerobic digestion for biogas production, leaving the fibrous and complex organics available to contribute to soil structure. The anaerobic digestion is also known to neutralise the resultant sludge. Demonstration of anaerobic digestion sludge as an ameliorant for soil fabrication is already reported by Amaral Filho et al. (2020).

Domestic organic waste

When considering alternative amendments, domestic waste from the coal mining community must be considered (Prado, et al., 2020; Weiler, et al., 2020). An average of 0.65 kg domestic waste per capita per day with an OM content of 64% (wt.%) is generated throughout Africa (TWB, 2012). Thus, when assuming compost as a substitute for MR with similar effects and a 40% OM to compost transition ratio (Diaz, et al., 2002), the domestic waste of ultimately 3800 people per annum can be used to fabricate one hectare of technosols amended with 5% of this urban OM. These volumes can be augmented by composting of agricultural residue or purpose-grown grasses. Consequently, the feasibility of using coal-based bioaugmented Technosols as topsoils are further enhanced by optimising amendment material selection to incorporate local urban or agricultural waste sources.

Brewery malt residue (spent grain)

We have demonstrated the use of malt residue (MR) from the brewing process as an efficient amendment for soil fabrication, being sufficient on addition of 2.5 to 5% by volume to fine coal waste. This both impacts soil structure and availability of nutrients. MR can be obtained from commercial breweries in reasonable scale and from microbreweries at lower scale. The two large commercial breweries closest to the Emalahleni region, both owned by Ab InBev through the SA Breweries holdings, are the Alrode and Rosslyn breweries. Alrode Brewery, the largest brewery in South Africa, produces 30% of SA Breweries' domestic volume at 8.8 million hL of beer annually (176 000 hL per week). In comparison, Rosslyn Brewery, near Pretoria, produces 128 000 hL beer per week, equating to 6.7 million hL annually. To service this, Rosslyn Brewery has 27 malt silos of 330 tons a piece (Cluett, 1987). From the literature, the litres of beer produced per kg of malt residue generated: in average is 9.1 (Chetrariu and Dabija, 2020; Lynch et al., 2016; Raniero et al., 2022). Using these, it can be seen that Alrode and Rosslyn breweries generated some 1320-3520 ton and 960-2 560 ton malt residue per week respectively or, on average 101 063 and 73 500 ton malt residue per annum respectively. Should this be fully used in soil fabrication, it supports the fabrication of soils to rehabilitate 913 ha per annum at 5% loading and 1828 ha at 2.5% loading.

Residue from paper mills

Paper mills produce significant fibrous organic wastes which could be used as amendments in soil manufacture. The types of biosolids produced in pulp and papers mills are detailed in Table 6-1. These are generally separated from the wastewater streams and de-watered where repurposing is feasible. The 'rejects', 'wastewater treatment sludge', 'primary sludge from wastewater treatment' and 'secondary sludge from wastewater treatment' are the most relevant for use in soil fabrication. While (Harrison et al., 2023) has presented the volumetric flows of the wastewaters associated with the process, further work is required for full definition of the solids waste streams. This analysis should be focused on those milla within suitable proximity to the coal fields.

Three paper mills are situated in Mpumalanga: the R&F tissue mill in Middelburg, the Ngodwana mill near Mbombela and the MPact mill near Piet Retief. The R&F tissue mills is situated in Middelburg, Mpumalanga produces 6 000 tons each of recycled jumbo reels and virgin paper annually. It is within 30 km on the coal mines. The Ngodwana mill, situated along the N4, 50 km from Mbombela, was built as a paper mill with partial conversion to dissolving pulp in 2013. It produces 320 000 tons paper pulp, 255 000 tons dissolving pulp and 380 tons paper, both newsprint and kraft linerboard, annually. It is some 160 km from the coal mining region . The MPact mill is more than 250 km from the coal fields and so not considered to minimise transport costs.(https://www.sappi.com/ngodwana-mill).

Table 6-1. The types of solid waste produced in pulp and paper mills (reproduced from Harrison et al. 2023, and developed from Monte et al., 2009)

From pulp mills	
Rejects	Rejects from virgin pulp consist of sand, bark and wood residues. They typically have low moisture content and high heating values and can be easily dewatered. They are thus generally burnt in the mill's bark boiler for energy recovery
Green liquor sludge, dregs and lime mud	Inorganic sludge was obtained from the chemical recovery section of the plant. They are usually dewatered and dried before being sent to a landfill
Wastewater treatment sludge	Usually comes from two sources: primary and secondary sludge. They are combined and dewatered to a 25–40% dry solid content. The solids can be combusted for energy recovery or sent to the landfill
Chemical flocculation sludge	Comes from water treatment and is usually disposed of in a landfill due to high inorganic content and water
From paper mills	
Rejects	Consists of impurities, fibres, metals and paper constituents such as fillers and sizing agents. Has a relatively low moisture content, high heating value and can be easily dewatered. Generally incinerated or disposed of in a landfill.
De-inking sludge	Contains mainly short fibres, coatings, fillers, ink particles, extractive substances and de-inking additives. It has a low heating value and is typically reused in other industries (e.g. cement, ceramics)
Primary sludge from wastewater treatment	Generated in primary clarification steps. It consists mainly of fines and fillers and is relatively easy to dewater. It is usually combined with de-inking or secondary sludge
Secondary sludge from wastewater treatment	Generated in the biological treatment units. It is usually thickened, dewatered and either incinerated or disposed of in a landfill

7 FEASIBILTY CONSIDERATIONS FOR IMPLEMENTATION

The feasibility of technosol manufacture and use must take into account technical, economic, and environmental criteria (Zongo, et al., 2012). While a full assessment is required when considering implementation, early stage feasibility studies are essential to direct the research and development to focus on the most critical aspects impacting techno-economic-environmental feasibility. Here we present an initial feasibility study to assess the potential of technosols built from fine coal waste, based on the results for seasonal plant growth experiments that were performed. This study also investigates the benefits of including biostimulation and bioaugmentation in coal-based technosols. Technical considerations included plant growth development, grain yield, biomass production, soil physiochemical quality and profiling the technosol microbiome to evaluate soil function.

An economic analysis considers the costs related to the production, implementation and monitoring processes of the technosols. For this investigation, initial economic predictions based on the amount of resources required, the volume of water required for vegetation, alternative options for amendment materials, and the grain and biomass production per annum were made to consider the implications of scaling up. The environmental criterion refers to the sustainability of the system when implemented. In this study, we briefly addressed the effects of seasonal variation on technosol performance, the benefits of using otherwise-wasted resources and the influence of technosols on the surrounding environment and community.

7.1 Material Requirements and Associated Costs

Material requirements and cost estimations regarding bioaugmented soil fabrication were performed as part of the techno-economic and environmental feasibility. Sample calculations for cost estimations are shown in Appendix A: Economic Analysis Assumptions & Calculations.

Traditional coal mine rehabilitation strategies are focused on revegetation with 'new' topsoils at a depth of 50 cm to ensure vegetational growth (Coaltech et al., 2019). To cover one hectare with conventional potting soil at a depth of 50 cm, 4450 t are required at an estimated cost of ZAR 2.475 million (Appendix A). Considering one specific mine site, it is estimated that 500 ha require rehabilitation, needing 2.5 million m^3 of soil. Furthermore, high overhead costs for excavation and transportation are associated with the burrowed topsoils (Cowan, et al., 2016). On using burrowed topsoils, costs associated with excavation, transport, fertiliser and lime addition are described in Appendix A and estimated at ZAR 0.95 million per hectare where stockpiled soils available within 3 km are used and ZAR 1.4 million per hectare where burrowed soils are transported 10 km (no allowance for rehabilitation of burrow pits; 1.67 ha require rehabilitation per 1 ha mine site rehabilitation). Further to rehabilitation costs, waste disposal and ARD treatment facilities also cost where coal waste is not re-purposed (Kazadi Mbamba et al., 2012). The expense of purchasing or borrowing natural soil is reduced when implementing Technosols. Alternatively, we have demonstrated the manufacture of technosols from fine coal waste. Under the experimental conditions reported, to produce 1 m^3 of bioaugmented CW+MR5%, it was necessary to mix 681 kg CW with 82 kg MR and 62 mL EM Pro-Soil. The soil-like substrate has an apparent specific gravity of 0.76 kg/L (0.94 kg/L for CW and 0.18 kg/L for MR). Therefore, one can estimate that about 3 624 tonnes CW, 191 tonnes MR, and 323 kilo-litres EM Pro-Soil would be necessary to produce the 5 000 m^3, equivalent to 3 815 ton of technosols needed to cover one hectare of soil with a depth of 50 cm (Appendix A).

To improve the rate of generation of regenerative soils with active microbiomes, inoculation is preferred during soil fabrication; however, commercial inocula are expensive. The estimated cost of applying the activated EM Pro-Soil inoculum per hectare technosols (50 cm depth) is ZAR 15.9 million, based on the desired number of microbial cells per mL soil recommended by Weiler et al. (2020). In addition, considering the annual chemical fertilization for traditional South African soils at a rate of 72 830 kg/ha

(FAO, 2018), fertiliser costs are estimated at approximately ZAR 680 130 per hectare per annum (Agritech, 2013). Chemical fertilizers can be deleterious to the environment where not appropriately balanced or over-applied. As an alternative, establishment of appropriate communities of soil micro-organisms can contribute to sustaining soil fertility, quality and health for arable land after mine closure. Further investigation into alternative, commercially available inocula that are more financially viable with similar plant growth and soil quality enhancing capabilities to EM Pro-Soil must be considered, as must on-site generation of inocula using a simple aerated bioreactor system. Through these interventions, reductions in cost can be expected.

The use of amendments in technosols play a significant role in soil quality and structure (Herran Fernandez, et al., 2016) as shown in the greenhouse studies of this investigation. Malt residue (MR) was sourced from SA Breweries Ltd. in the Western Cape, South Africa, a part of AB InBev. SA Breweries Rosslyn Brewery and Alrode Brewery in Gauteng are the nearest to the eMalahleni area, at a proximity of 138 and 144 km respectively. Therefore, for the use of MR, the costs associated with transportation to the mining site must be taken into account. Further, its availability is limited, limiting the ratio in which it can be added. Its alternative use against which it must be costed is in animal feed.

Using these data, the costing of fabricated technosols is estimated in Table 7-1. The high cost of EM ProSoil for soil inoculation is noted. It is noted that in the absence of this inoculum, the cost of the technosols is substantially lower than stockpiled (<70%) or burrowed soils (<50%) owing to the extended haulage and fertiliser needed. It is noted that the benefits of the inoculum can be achieved far more cost-effectively through onsite production of custom-built inocula and further investigation and costing of this is recommended.

Table 7-1 Calculation of the cost of technosols, manufactured from coal waste tailings (95%) and malt residue (5%), with and without inoculation with EM Pro-Soil

Component	technosols CW95:MR5 without inoculation	technosols CW95:MR5 inoculated with EM ProSoil
Coal waste tailings -assume zero cost and available onsite	3 624 tonnes CW Assume no cost	3 624 tonnes CW Assume no cost
Malt residue	191 tonnes MR	191 tonnes MR
Malt residue haulage	Bulk density 0.18 ton/m^3 Vol = 191 / 0.18 = 1061 m^3 ZAR 616.46 per m^3 for 75 km Haulage = ZAR 0.654 million	Bulk density 0.18 ton/m^3 Vol = 191 / 0.18 = 1061 m^3 ZAR 616.46 per m^3 for 75 km Haulage = ZAR 0.654 million
EM ProSoil		323 kilo-litres EM Pro-Soil ZAR 15.9 million
Fertiliser	NPK sufficient without fertiliser	NPK sufficient without fertiliser
Total cost per hectare at 50 cm depth	**ZAR 0.654 million**	**ZAR 16. 55 million**

7.2 Water Requirements and Biomass Production

High soil moisture availability enhances photosynthesis and leads to increased *E. tef* aboveground biomass (Hilemicael & Alamirew, 2017). Therefore, biomass predictions of *E. tef* in technosols with the associated water requirements are useful for financial feasibility studies. Predictions regarding water requirements for *E. tef* growth in the best performing technosols (CW-T+MR2.5% and CW-T+MR5%)

were made from data collected in the final greenhouse study. Initial soil weights, material bulk densities, cumulative water requirements (summarised in Table 7-2), and above ground biomass and grain yields per technosol were utilised. The conditions of the final growth trial were most similar to the year-round climate in eMalahleni: average annual temperature of 16.3°C and average annual precipitation falls of 760 mm (Climate-Data, n.d.). The results are presented in Table 7-2. Sample calculations are summarised in : Economic Analysis Assumptions & Calculations. it must be noted that yields are expected to be enhanced following multiple growth cycles in the technosol and its associated maturation and pedogenenis development.

Table 7-2. Estimated total volume of water required per *E. tef* growth cycle and approximate dry biomass and grain yields per hectare of technosols with a 50 cm depth.

Technosol Type	Inoculated (I) or non-inoculated (NI)	Water Required (KL)	Above ground dry biomass (t)	Grain Yield (t)
CW-T+MR2.5%	I	9 494	16.0	2.51
CW-T+MR5%	I	9 574	12.2	1.31
	NI	5 943	3.81	0.32
Potting soil	NI	19 368	32.0	6.73

The land-use activities (coal mining, agricultural practices, and energy production facilities) in the area have rendered eMalahleni a water stressed municipality. Coal mining and associated ARD in the area have prohibited exploitation of underground water resources without treatment. Thus, to extend the circular economy approach and further reduce the financial impact of the rehabilitation scheme, run-off water from the mine, treated mine wastewater, or on-site collected rainwater can be used for plant irrigation. Additionally, from Table 7-2, implementing bioaugmented CW-T+MR5% would utilise 49% less water than the agricultural soil.

E. tef is known for its agricultural value in the food and beverage industry, and for livestock feed as described in Chapter 6. It was estimated that a total of 16 tonnes of *E. tef* dry biomass can be cultivated in inoculated CW-T+MR2.5% and 12 tonnes in bioaugmented CW-T+MR5%, compared to 32 tonnes in the control soil, per hectare. These predictions seem unrealistically high compared to literature on *E. tef* farming that described 4-8 tons of dry *E. tef* shoots per hectare per season (AGT Foods Africa Pty Ltd. , n.d.). Optimised yields were obtained in the controlled, small-scale experiment of this study. Yet, the economic benefit of *E. tef* production in bioaugmented technosols is clear from the estimated price per hectare of technosol as shown in Table 7-3. The financial benefits of *E. tef* cultivation in technosols extend to the coal mining communities for job opportunities (seed sowing, maintenance and biomass processing, potentially inoculum development), empowerment through skills development, increased public welfare, and relationship strengthening between the involved parties.

Table 7-3 Estimated economic benefit from *E. tef* cultivation per hectare technosols at a depth of 50 cm.

Technosol Type	Inoculated (I) or non-inoculated (NI)	Estimated price (ZAR) of *E. tef* hay*	Estimated price (ZAR) of *E. tef* grain**	Estimated price (ZAR) of *E. tef* flour**
CW-T+MR2.5%	I	718 972	50 860	60 279
	NI	924 393	50 208	59 506
CW-T+MR5%	I	549 241	26 495	31 402
	NI	171 638	6 573	7 790
Natural soil	NI	1 441 800	136 185	161 405

*Based on the average market price for *E. tef* hay in South Africa (Farmer, 2022).

**Based on FAO Analysis of price incentives for *E. tef* (FAO, 2015).

The health benefits of *E. tef* grains and the multiple applications thereof are widespread. Ethiopia farms more than 90% of the *E. tef* in the world, with more than 24%, or 3 017 914 ha of the grain area dedicated to *E. tef* production (Lee, 2018); a testament to the ever-growing *E. tef* market in Africa. As previously discussed, a positive correlation ($R^2 = 0.91$) was found between grain yields and above ground *E. tef* biomass. Therefore, similar trends in biomass production and grain yield, and in associated prices were seen in Table 7-2 and Table 7-3. Expectedly, *E. tef* cultivated in agricultural soil would result in the greatest financial benefits due to augmented biomass and grain yields within this soil type. However, predictions were made from potting soil results for *E. tef* biomass. It can be assumed that commercial topsoils that have been exposed for extended periods to chemical fertilisation, would have lower productivities. Furthermore, when considering the cost of 4450 tonnes of agricultural soil per hectare, the 38% increased price margin of *E. tef* hay per hectare over that in inoculated CW+MR5% becomes less significant.

7.3 *E. tef* as Cattle Feed

To ensure that the cultivated *E. tef* shoots contain the necessary nutritive values for animal feed and acceptable metal levels, *E. tef* macro- and micronutrients contents from the best performing Technosols and control soil were compared to literature for *E. tef* hay as cattle feed. The results are presented in Table 7-4.

It is clear that the non-inoculated technosols resulted in *E. tef* shoots with higher Na, Mg and Ca contents compared to the *E. tef* grown in inoculated treatments. However, the above ground dry biomass from bioaugmented technosols CW+MR2.5% and CW+MR5% were characterised by higher K, P, Mn, Cu, Zn and Mo compared to biomass from non-inoculated fabricated soils. Metal translocation and nutrient cycling were enhanced by exogenous microorganisms (Margerison, et al., 2020) that were more abundant in these soil-like substrates (Table 7-5.) From Table 7-4, *E. tef* cultivated in coal-based technosols had augmented nutritive values relative to the standard values for cattle feed. Of the bioaugmented fabricated soils, both CW+MR2.5% and CW+MR5% showed good prospect for *E. tef* hay production, with higher Na, Mg, Ca, Zn, and Fe levels compared to the reference values.

Table 7-4: Nutritional values of *E. tef* cultivated in Technosols and agricultural soil compared to *E. tef* hay for cattle feed by Vinyard et al. (2018).

Variable	Unit	CW-T+2.5% MR		CW-T+5% MR		Natural soil	Cattle Feed
		I	NI	I	NI	NI	*E. tef* Hay
Na	%	0.05	0.05	0.06	0.07	0.03	0.04
Mg	%	0.36	0.40	0.34	0.43	0.34	0.2
Ca	%	0.53	0.62	0.65	0.97	0.36	0.51
K	%	1.49	0.77	1.55	0.78	1.87	2.38
P	%	0.33	0.14	0.26	0.17	0.27	0.27
Mn	mg/kg	202	104	117	98.5	22.2	33
Fe	mg/kg	95.1	84.5	117	172	68.3	230
Cu	mg/kg	14.45	11.9	14.2	13.4	8.10	10
Zn	mg/kg	86.2	39.3	76.0	46.8	81.4	33
Mo	mg/kg	0.74	0.54	0.80	0.69	1.49	2

Table 7-5 Universal bacteria, archaea, fungal, and nitrogen cycling (nirS, nirK, nosZ, nifH) 16S rRNA gene copy numbers in vegetated coal-based Technosols before and after *E. tef* growth in the final plant growth trial in winter. Where, I is inoculated and NI represents non-inoculated treatment types.

			Bacteria	Archaea	Fungi	nirS	nirK	nosZ	nifH
Before plant growth	CW+MR2.5%	I	1.23E+03	4.83E+03	1.45E+04	1.57E+03	2.66E+04	2.51E+03	5.30E+01
		NI	4.50E+02	4.64E+03	1.30E+04	2.84E+01	3.59E+04	2.38E+03	1.33E+02
	CW+MR5%	I	1.46E+07	3.33E+04	2.50E+06	3.16E+06	1.33E+07	1.25E+07	3.19E+07
		NI	2.31E+07	3.82E+06	2.42E+07	1.17E+06	4.36E+07	9.40E+06	3.48E+07
	CW+MR7%	I	1.27E+04	9.02E+03	5.37E+07	4.24E+02	2.47E+04	8.82E+03	5.27E+03
		NI	3.75E+06	5.68E+04	3.54E+06	5.79E+03	9.47E+04	4.34E+04	1.41E+05
	Control	NI	1.52E+08	1.82E+07	2.70E+06	1.67E+06	9.79E+07	3.72E+03	3.06E+06
After plant growth	CW+MR2.5%	I	1.52E+08	2.16E+05	1.83E+08	5.93E+06	1.14E+05	2.68E+07	1.46E+06
		NI	5.46E+04	3.22E+02	1.25E+04	5.79E+05	3.19E+03	3.51E+04	1.95E+02
	CW+MR5%	I	4.47E+08	3.89E+04	1.44E+08	1.42E+08	3.67E+09	5.08E+07	5.05E+05
		NI	4.77E+07	3.09E+05	1.12E+08	1.92E+06	4.09E+09	7.63E+07	2.05E+06
	CW+MR7%	I	4.83E+06	5.50E+05	1.11E+09	1.26E+06	1.63E+09	6.57E+07	2.14E+06
		NI	7.38E+04	1.18E+06	5.08E+08	4.42E+06	1.91E+09	4.76E+07	4.95E+06
	Control	NI	2.54E+05	2.43E+06	3.15E+06	1.75E+06	5.25E+09	1.81E+07	4.17E+06

7.4 Environmental Considerations

Research on Technosols as topsoils, such as this investigation, contribute to the development of mine waste management strategies that include the circular economy principle. However, when considering the sustainability of implementing Technosols, the performance thereof with seasonal changes, and the impacts and advantages on the surrounding environment must be considered.

On considering the impacts on the surrounding environment, fabricating soils from CW and otherwise-waste resources as amendments reduces resource requirements and prevents waste accumulation. Thus, the environmental impact of mining on the mining communities, landscape opportunities and future land use are minimized. Implemented technosols accumulate SOM over time which ensures carbon, nitrogen and sulfur (building blocks of SOM) sequestration. It conserves the structure of the pedoderm (Mills & Fey, 2004), ensures organic nutrient availability and is vital to soil microbial activity (Botta, 2015). Revegetation in bioaugmented technosols amended with 5% MR reduces the probability of ARD by inhibiting iron leaching and ensuring soil organic sulfur content below the South African maximum screening values for soils. Frameworks on coal rehabilitation provide scope for land and waste management to identify areas or zones specifically vulnerable to degradation. Here, technosol revegetation strategies can be optimised based on the identified issues or areas of improvement. Various plant species can be cultivated after mine closure for optimal land use, augmented economic benefit and improved local biodiversity.

Revegetation techniques have been reported to improve health concerns associated with untreated coal mining waste dumps, alleviate the aesthetic quality of the community through vegetational growth, increase the local biodiversity of fauna surrounding the mine, and create job opportunities (Lamb et al., 2014). The engineered soils align with the SDGs by utilising waste resources, mitigating land degradation and by promoting the development and implementation of environmentally sound technologies (Department of Statistics South Africa, 2019). These contribute to the social aspect of sustainable mine rehabilitation.

7.5 Broadening the spectrum of plants for study of growth in technosols from fine coal waste

While the research presented in this study has focused on *E. tef* as the model grass for first stage revegetation on mine site rehabilitation, it is well recognised that multi-species vegetation adds to the resilience of revegetation, providing protection to perturbations and enhancing biomass production. Increasingly, mine rehabilitation teams are introducing a mixture of plants for rehabilitation, including both early-colonising annuals and perennial species to avoid rehabilitation (Mentis, 2006; Mentis', 1999). In south Africa most of the Examples of the extended range of species used include:

*Chloris gayana, (*Rhodes grass)
*Cenchrus ciliaris (*Buffalo grass)
*Cynodon dactylon (*Kweek)
*Digiteria eriantha, (*Smuts finger grass)
Eragrostis curvula (Love grass)
*Eragrostis teff (*Teft)
Medicago sativa .(Lucerne)
Eragrostis chloromelas (Krulblaar)
Eragrostis plana (Taaipol)
Hyparrhenia hirta (Thatch grass)
Pennisetum clandestinum (Kikuyu)

The ratio of the seed mix used for re-vegetation is usually specified in the mine's Environmental Management Programme (EMP).

Most of the EMPs recommended the following seed cocktail mix for rehabilitation and re-vegetation in South Africa (Coaltech et al., 2019): *Eragrostis tef*, *Digitaria eriantha*, *Cynodon dactylon*, *Cenchrus ciliaris* and *Chloris gayana*. While the initial revegetation is selected to stabilise the environment, long-term rehabilitation includes the establishment of tree, bushes and grasses. The properties of a selection of common rehabilitation species are shown in Table 7-6.

It may also be preferable to select plant species based on those that regularly colonise the surfaces of tailings storage facilities. Mudenda (2022), explored this for hard rock tailings from copper mining on the Zambia CopperBelt. He proposed a range of plants, including *Andropogon eucomus*, *Cynodon ductylon*, *Conzya floribuda*, *Cypernus alternifolius*, *Hyparrhenia filipendula* and *Bidens steppia* and provided evidence of their capacity for selective metal uptake. Several of these illustrate good ability for metal uptake, adding to rehabilitation potential.

Table 7-6 Properties of a selection of common rehabilitation species

Name	Planting time	Germination time	Picture	comments	source
Rye Grass (Annual)	mid of August to the end of September – Seeding up to mid-October is possible	7-10 days		Ryegrass prefers fertile, well-drained loam or sandy loam soils, but establishes well on many soil types, including poor or rocky soils. It tolerates clay or poorly-drained soils in a range of climates and will outperform small grains on wet soils	http://ryegrasscovercrop.com/researc/plantingarg/ http://www.sare.org/Learning-Center/Books/Managing-Cover-Crops-Profitably-3rd-Edition/Text-Version/Nonlegume-Cover-Crops/Annual-Ryegrass
Eragrostis curvula (Perennial)	spring or early summe	≈12 days		Prefers sandy loams and well drained fertile soils, but will grow in a wide range of soils. It prefers a pH of 7.0-8.5; Grows on low-fertility soils.	http://www.tropicalforages.info/key/Forages/Media/Html/Eragrostis_curvula.htm
Digitaria eriantha	from October to late February	3 days		Pangola grass can grow on a wide range of soil types from sands to heavy clays In warm moist environments vegetatively planted swards establish rapidly and in general weeds are suppressed.	https://www.daf.qld.gov.au/plants/field-crops-and-pastures/pastures/digit-grass

Eragrostis teff (Annual)	Mid-October to mid-January.	3 to 5 days		Under optimal growing temperatures and moisture, Teff germinates quickly and is ready for early boot stage harvest in 45 to 55 days after seeding	http://www.kingsagriseeds.com/blog/wp-content/uploads/2014/12/Teff-Grass-Management-Guide.pdf

8 CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

Coal mining processing contributes to global economics; however, the associated activities and processing are deleterious to the surrounding environment. The impacts of coal mining processes and the liabilities of past mining on the environment can be minimized through the successful implementation of sustainable mine rehabilitation schemes. To achieve this, strategies need to result in sustained soil quality, health and fertility. The interdependence between the physical structure of the (fabricated) soil, plants and the microbiome needs to be better understood. Research on the rehabilitation of degraded mine land to achieve appropriate soil quality has been underway for some years, but remains observational (Thavamani, et al., 2017). Fabrication of technosols to achieve appropriate physical structure, organic content, nutrient availability and a well-functioning and regenerative soil microbiome is still in its infancy, with much potential. In addition to its role in rehabilitation, it can play a major role in waste minimisation where coal waste and other waste materials are re-purposed, in line with the circular economy principles.

To investigate the fabrication of coal waste-based technosols through both soil quality studies and *E. tef* cultivation, the physical, chemical and biological properties were assessed and related to productivity.

The primary objective for the investigation was to explore variables in construction of an amended coal-based technosols that improved soil performance (plant development, soil fertility, soil physiochemical characteristics). To meet this objective, the core characteristics of fertile soils were assessed. Technosols were constructed to meet these characteristics as closely as possible, characterised plant growth studies with *E. tef* were performed in a greenhouse. *E. tef* was selected as the model plant for these studies as it is an “early coloniser” of rehabilitated lands and provided ready comparative analysis of studies conducted in pots. It is well-recognised that at the next scale up stage it will be preferable to sow seed mixes of 5 to 10 plant types to enable the establishment of a poly-culture with enhanced resilience and to allow the tracking of plant proliferation with time.

In this study, technosols fabricated from CW-T (both thickener slurry and flotation tailings streams) were amended with different ratios of MR (2.5%, 5%, 7%). In some studies, the technosols were inoculated with a mixed microbial inoculum to enable more rapid establishment of a functional microbiome. The favourable plant growth results with supporting soil characteristics and soil fertility results showed that CW-T+MR-based technosols are capable of supporting plant growth, and that bioaugmentation with effective microorganisms in a 5% MR amended fabricated soil improved overall soil health, quality and fertility.

It was concluded that initial salinity of coal-based technosols was directly proportional to the amendment dosage, and inversely proportional to bioaugmentation. Nonetheless, plant-microbe relationships developed with time to release root exudates, degrade pollutants and enhance cation uptake that stabilised the salinity of all fabricated soils (Cervantes, et al., 2011).

MR acted as a structural ameliorant, as WHC increased with higher MR dosages. In addition, bioaugmentation improved soil structure by soil agglomeration. Moreover, WHC of simultaneously biostimulated and bioaugmented technosols were superior to only biostimulated technosols due to structural stability introduced by microbes performing OM decomposition and soil aggregation (Atlas &

Bartha, 1998), thereby emphasizing the significance of inoculation in mine waste-based soils to prevent erosion from metal leaching or ARD generation.

Soil organic C, P, K, Ca and metal contents, with leachate TN, TOC and TIC were directly proportional to the weight percentage of MR. Bioaugmentation with biostimulation increased soil macronutrient contents through nutrient cycling (Tiwari, et al., 2021), corroborating research by Weiler et al. (2020). Carbon and sulfur sequestration occurred in vegetated fabricated soils, as overall percentages of organic carbon and sulfur were higher than in non-vegetated Technosols.

Pure compost could not support plant growth and was not a good control. Levels of phosphorus and potassium were inadequate for *E. tef* germination when compared to the potting soil, as excessive K inhibited the uptake of other cations into plant organs (Rawat, et al., 2016). The alkalinity of compost decreased the availability of many soil nutrients that are important for plant development (Prado, et al., 2020). Furthermore, compost was characterised by poor water retention. Conversely, potting soil was a good control, with an average germination rate of 95% after 5 days. The best shoot growth rate per day was achieved in potting soil, corresponding to the tallest *E. tef* (56.3 cm) of *E. tef* cultivated in the colder climate study.

Pure coal waste sustained plant growth poorly due to inadequate phosphorus and potassium concentrations. The sand-like texture and small particle size of the tailings presented the lowest initial EC from very low nutrient cation concentrations and poor WHC. It was concluded that implementing 100% CW as a topsoil would result in extensive water run-off, crusting or gully erosion. This emphasises the significance of amendments to Technosols and the correct dosage thereof to ameliorate soil structure.

The investigation of soil physiochemical and biological properties of CW-T+MR2.5% and CW-T+MR5% presented favourable results. It was concluded that *E. tef* growth was supported in both summer and winter in bioaugmented CW-T+MR5% and non-inoculated CW-T+MR2.5% substrates. The similar above ground *E. tef* dry biomass results for Technosols amended with 2.5% and 5% MR (w/w) were comparable with those presented by Amaral Filho et al. (2020), Firpo et al. (2015), and Weiler et al. (2018), where the addition of 2-5 wt.% OM enhanced the shoot and root productivity in the fabricated soils. The earliest signs of inflorescence were in CW-T+MR2.5% and CW-T+MR5%; 40 days since planting in summer and delayed by 5 days in winter.

Bioaugmented and biostimulated (with 2.5% and 5% MR) Technosols were best able to retain water and remediate soil S to suitable levels with *E. tef* growth, whereas, S in 100% CW-T and CW-T+MR7% were 1.7-fold the maximum screening value for South African soils. The abundance of macronutrients Na, Mg, Ca, K and P in inoculated CW-T+MR2.5% and CW+MR5% technosols validated the feasibility of *E. tef* cultivation in Technosols as topsoils for nutrient-deficient coal mining waste (Truter, 2007).

This investigation, supported by previous research on coal-based Technosols, concludes the potential for implementation of technosols. This is further motivated by the reduction in capital costs associated with commonly implemented commercial topsoils, minimised ARD generation and SOM production, sustained plant growth with temporal effects, economic benefits of *E. tef* cultivation and options for reusing up to 9574 kilo-litres of mine site water for irrigation. Furthermore, it was concluded that the *E. tef* cultivated in inoculated CW-T+MR5% are suitable for cattle feed with improved Na, Mg, Ca, Zn, and Fe contents.

Comparison of costs associated with fertile topsoil, or with the use of burrowed or stockpiled soils to those of soil fabrication, considering the raw material and transport costs only, suggests the techno-economic feasibility of technosols for mine site rehabilitation. However, the cost of the commercial inoculum, EM Pro-Soil, is very high, negatively affecting economic feasibility. Alternative inoculums, on-site inoculum generation or reduced dosages with similar effects are viable alternatives and must be considered. Previously low cost on-site production of microbial consortia has been demonstrated for enhancement of soil and plant growth (Largier, personal communication); this should be applied to soil fabrication. Further, the availability of sufficient amendment may require the selection of multiple amendments to be used in combination or as a mixture. The added benefit of manufacturing of technosols is the repurposing of 4500 m^3 coal waste per hectare rehabilitated, thereby reducing coal disposal requirements.

The biostimulated and bioaugmented fabricated soils target SDGs 8, 9, 11, 12, 15 and 17.

8.2 Research Recommendations

The following recommendations apply to future investigations. When considering implementation of technosols as a mine rehabilitation strategy, amendment sources in close proximity to the mine site should be investigated. Animal manure, agricultural waste, sewage sludge and the organic fraction of municipal waste are recommended as these wastes would contribute directly to waste management and the social sustainability of technosols in the eMalahleni area, and are available in reasonably large amounts. The relative transport distances are an important consideration in these studies as illustrated in Chapter 7. This research would improve feasibility assessments on South African mine waste-based soils. It is recommended to investigate plant growth and soil fertility when various amendments are used in conjunction with one another, in addition to investigating amendment application rates.

The ability of the coal-based technosols to support other valuable and viable plant species and mixtures of plant species should be investigated. It is recommended to evaluate plant species from four perspectives: 1) legume growth with associated N fixation, 2) industrial crops, including energy crops, 3) multi-species grass crops for resilient pastureland, and 4) metal-accumulating plant species for metal mobilisation, uptake and recovery. Furthermore, the technosol quality when mixed plant species are cultivated should be investigated. Particularly, it is of interest to investigate long term vegetation (several growth cycles) in coal-based technosols with seasonal variation, as well as the effects thereof on sulfur bioaccumulation. *In situ* growth studies are recommended to refine economic predictions of water requirements and biomass production at the specific implemented mine site. This research would extend the applicability of mine waste-based technosols and therefore their feasibility.

The commercially available inoculum has been used across multiple applications with a reputation in improved plant growth, however, it is a major cost component in bioaugmented technosols. It is recommended to evaluate the minimum required dosage of EM Pro-Soil for inoculation that would result in an economically viable strategy with sustained soil fertility. Furthermore, less expensive commercially available alternatives to EM Pro-Soil should be investigated, as should the on-site production of the inoculum as is done in a number of organic farming environments.

The effects of bioaugmentation and amendment dosages on the soil biodiversity (i.e. the presence and abundance of invertebrates such as, earthworms) should be investigated. Subsequently, the addition of earthworms to coal-based fabricated soils and their corresponding effects on soil structure, microbial biomass and activity, and plant performance should be evaluated. Deeb at al. (2017) described improved soil aggregate stability in technosols from earthworms, and that the benefits of compost as amendment were only observed in the presence of plants or earthworms.

With regards to soil properties, drainage over time and soil bulk densities in coal-based technosols with different amendment dosages should be investigated as faster drainage results in less water retention

(Prado, et al., 2020), and higher bulk density reduces total soil porosity (Hattingh, et al., 2018). These parameters are valuable for evaluating soil compaction over time with associated adverse effects on plant growth and land use.

Expanded techno-economic and environmental analysis is recommended. Here attention should be given to possible trends in cost and availability of waste resources as its potential for re-purposing is understood, the potential for reduced usage of water, for averting extended environmental impact. Further, it will be valuable to further assess the detailed costs of technosols against the detailed cost of excavation and transport of soils for revegetation, the cost of responsible disposal of wastes and the opportunity cost of the “as is” situation.

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9 APPENDIX A: ECONOMIC ANALYSIS ASSUMPTIONS & CALCULATIONS

9.1 Estimation of water requirements for *E. tef* cultivation

Using water retention and water uptake determination from lab-scale and greenhouse studies, the water requirements for *E. tef* cultivation are estimated.

Table A-9-1Total volume of water required per kg soil for 90 days.

Technosol Type	Inoculated (I) or non-inoculated (NI)	*E. tef* (T) or no *E. tef* (NT)	Trendline Equation	R^2
CW-T+MR2.5%	I	T	$y = 26.152x + 216.75$	0.968
		NT	$y = 27.635x + 143.5$	0.967
	NI	T	$y = 14.369x + 160.85$	0.960
		NT	$y = 8.6545x - 24.054$	0.844
CW-T+MR5%	I	T	$y = 27.124x + 172.62$	0.962
		NT	$y = 20.88x + 139.41$	0.971
	NI	T	$y = 16.413x + 130.58$	0.957
		NT	$y = 4.0635x - 30.518$	0.968
Control - Potting Soil	NI	T	$y = 46.896x + 306.6$	0.972
		NT	$y = 30.979x + 157.86$	0.971

Price estimations for *E. tef* grain and flour based on recent FOA analysis of *E. tef* prices; 1.35 USD/kg for *E. tef* grains and 1.60 USD/kg for *E. tef* flour (FAO, 2015) in in April 2022. Current conversion rates in in April 2022 between USD and ZAR were used; 1 USD = 15.01 ZAR. Cost estimations on EM Pro-Soil were based on current market prices in in April 2022; 1228.00 ZAR per 25 L of Efficient Microbes Pro-Soil (Efficient Microbes, 2006).

To predict the total volume of water required per kilogram of Technosol when cultivating *E. tef*, the following assumptions were made:

- *E. tef* plants are grown for 90 days per growth cycle.
- Four growth cycles per annum (one yield per season).
- The germination period lasts 14 days.
- During germination, the water requirement per day is half the volume required per pot per day for the rest of the growth trial.
- Soils are maintained at 50% field capacity.
- Seasonal effects are negligible.
- The number of *E. tef* plants are kept maintained at 14 plants per kilogram soil, or 857 plants per 1 m^2 soil.

9.2 Sample calculations for technosols components and associated cost at implementation scale of 1 hectare at a depth of 50 cm

The sample calculations are based on the fabricated soil: CW+MR5%.

Apparent technosol density:

$$\rho = \frac{m}{V} = \frac{619.4\ g}{472.5\ mL} = 0.763\,\frac{g}{mL} = 0.763\ \frac{kg}{L} * \frac{L}{dm3} * \frac{1000\ dm3}{m3} = 763\ \frac{kg}{m3}$$

Thus, for 1 ha of technosols at 50 cm deep and constructed from 95% CW-T and 5% MR (CW+MR5%), the total mass of soil required is estimated as:

$$m_{CW+MR5\%} = V * \rho = (5\ 000\ m^3) * \left(763\,\frac{kg}{m^3}\right) = 3\ 815\ 000\ kg$$

Therefore, mass of CW required is estimated as:

$$m_{CW} = m_{CW+MR5\%} * weight\ fraction = (3\ 815\ 000\ kg) * 0.95\ = 3\ 624\ 250\ kg$$

and the mass of MR added:

$$m_{MR} = m_{Total} - m_{CW} = 3\ 815\ 000\ kg - 3\ 624\ 250\ kg = 2.29E + 08\ g \approx 190\ 750\ kg$$

9.3 Sample calculations for provision of fertile topsoil or burrowed soil for rehabilitation at an implementation scale of 1 hectare at a depth of 50 cm

Outdoor fertile topsoil

For potting soil:

$$m_{Control} = 890\ \frac{kg}{m3} * 5\ 000\ m^3 = 4\ 450\ 000\ kg$$

The costing of topsoil for one hectare was based on the average market price for outdoor topsoil of ZAR 990.00 per m^3 of soil. Assuming a discount of 50% based on the large volumes needed, the cost for 4450 tons of topsoil for 1 hectare is estimated at 0.5 * ZAR 4.95 million = ZAR 2.475 million.

Rehabilitation using burrowed soils or stockpiled soils

For rehabilitation using burrowed soils, the costs of excavation, loading and haulage, shaping and levelling, fertilising and lime addition must be considered. Here these are estimated while assuming negligible cost of the burrowed or stockpiled soil itself and not taking into account the cost of rehabilitating the burrow pit.

Excavation and transport:

Excavation of burrowed soils:	ZAR 29.00 per m^3
Loading and haulage (2-3 km):	ZAR 47.66 per m^3
Additional haulage per km:	ZAR 8.39 per m^3
Shaping and levelling:	ZAR 3.91 per m^3

Chemical fertilisation cost estimations:

Market prices for NPK fertilisers in April 2022: ZAR 466.93 per 50 kg

Rate of application to South African soils: 72 830 kg/ha (FAO, 2018)

Cost of chemical fertiliser: ZAR 0.680 million per hectare

Lime addition

Market price per ton (2021): ZAR 325 per ton

Rate of application: 35 ton per ha

Based on these costs, the cost of soil for one hectare at a depth of 50 cm is calculated below:

Table A-9-2Total volume of water required per kg soil for 90 days.

Component	**Using stockpiled soils available within 3 km and not requiring excavation**	**Using burrowed soils transported a distance of 10 km**
Volume of soil required (m^3)	5 000	5 000
Mass of soil required based on a bulk density of 0.98 kg/L (Table 5-3)	4 900	4 900
Excavation cost (ZAR)		= 29.00 * 5 000 = 145 000
Loading and haulage (2-3 km) (ZAR)	= 47.66 * 5 000 = 238 300	
Loading and haulage (10 km) (ZAR)		= 238 300 + (8.39 * 7 * 5000) = 531 590
Shaping and levelling (ZAR)	= 3.91 * 5000 = 19 500	= 3.91 * 5000 = 19 500
Fertiliser application (ZAR)	= 72 380 * (466.93/50) = 675 928	= 72 380 * (466.93/50) = 675 928
Lime application (ZAR)	= 35 * 325 = 11 375	= 35 * 325 = 11 375
Total cost per ha at 50 cm depth (ZAR)	**ZAR 945 103**	**ZAR 1 383 393**

9.4 Calculation of ‘above-ground’ biomass and seed formed per hectare

E. tef above ground dry biomass predictions were based on above ground dry biomass per 100 g soil produced according to the findings of this research. The estimated grain yields following *E. tef* growth on technosols was based on the correlations between above ground dry biomass and mass of seed produced as shown in the Figure A-9-9-1.

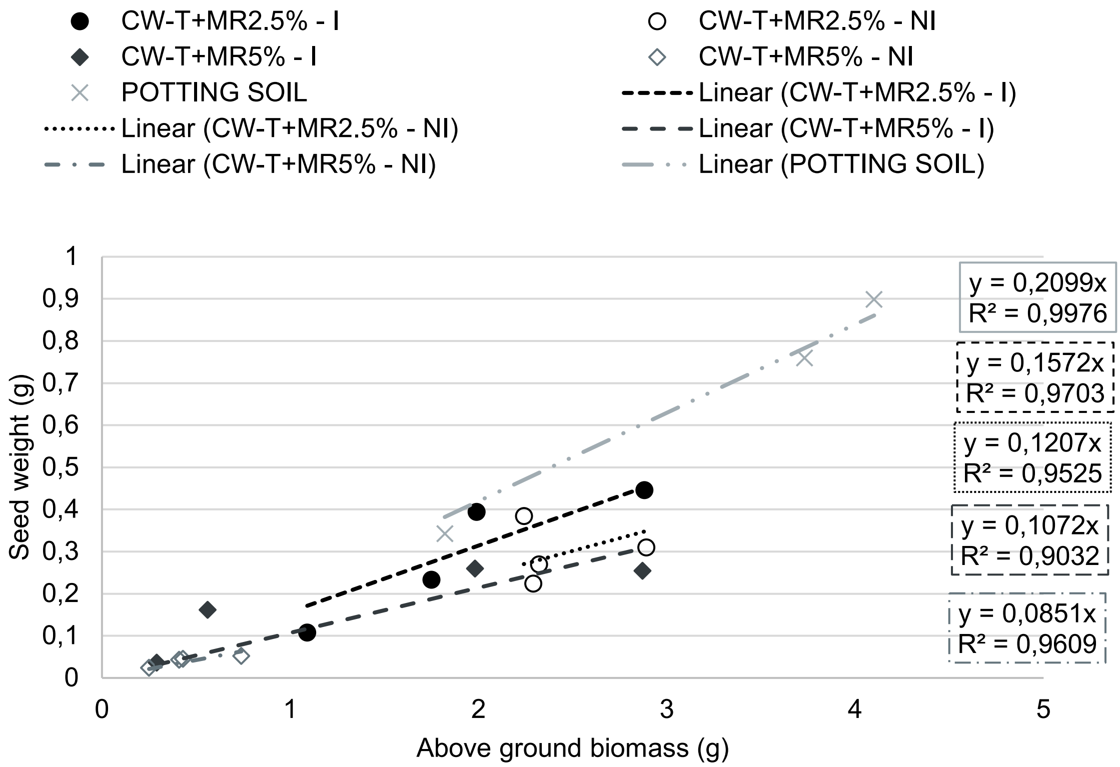

Figure A-9-9-1 Correlation between seed weight and above ground dry *E. tef* biomass achieved in the growth trial for coal-based technosols amended with 2.5% and 5% malt residue and potting soil as the control.

10 APPENDIX B: PROJECT OUTPUTS AND ACHIEVEMENTS

Capacity Development

Student Name	Discipline	Degree	Race	Sub-task title
Areesen Reddy	4th year student (graduated)	Chemical engineering	Indian male	Fabricating soils from fine coal waste – designing the soil permeability
Martin Strauss	4th year student (graduated)	Chemical Engineering	White male	
Fabio Torino	4th year student (graduated)	Chemical engineering	White male	Evaluation of soil-water relations between different technosols mixtures using South African coal waste
Michael Tatham	4th year student (graduated)	Chemical Engineering	White male	
Lisa T Kumadiro	4th year student (graduated)	Chemical engineering	Black female	Optimizing two-stage froth flotation for coal recovery and desulfurization
Mellisa T Mundida	4th year student (graduated)	Chemical Engineering	Black female	
Omphemetse Mothibi	4th year student (graduated)	Chemical Engineering	Black female	South African soil quality and opportunities for post-mining land uses in the context of gold and coal processing activities
Nontlantla Mkwenkweni	4th year student (graduated)	Chemical Engineering	Black female	
Juarez Amaral Filho	Postdoctoral research fellow	Environmental engineering	Brazilian male	On the feasibility of South African coal waste for production of 'FabSoil', a Technosol
Jessica Weiler	Visiting PhD student (graduated), (UFRGS/Brazil)	Environmental engineering	Brazilian female	Evaluation of microbial amendments in a technosol produced from South African coal processing wastes
Andani Mphinyane	PhD student (discontinued)	Geosciences	Black male	South African coal waste re-purposing as alternative for topsoil production in rehabilitation of mined areas
Cari van Coller	MSc Student (graduated)	Environmental Sciences	White Female	Eragrostis tef growth performance and persistence of microbial function in a bioaugmented coal-based Technosol

International links

This project is being conducted in collaboration with the research team of Professor Ivo Schneider from the Federal University of Rio Grande do Sul (UFRGS/Brazil).

Resources Leveraged

Preliminary studies related to the effect of different microbial communities in the FabSoil productivity and pedogenesis were supported through a project approved by Coaltech Research Association (2018/2019).

Academic Journal Articles

Amaral Filho J.R., Firpo B., Broadhurst J.L. and Harrison S.T.L. (2020). On the feasibility of South African coal waste for production of "FabSoil', a technosol. Minerals Engineering 146, 106059.

Deliveries at Conferences

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Amaral Filho J.R., Firpo B., Broadhurst J.L. and Harrison S.T.L. (2018). Fabricated soils from South African desulfurized coal processing waste. Sustainable Minerals'18. Namibia. June, 2018.