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COMPOST-ASSISTED PHYTOREMEDIATION OF MINE TAILINGS AND FOOTPRINT AREAS USING *CHRYSOPOGON ZIZANIOIDES* (L) ROBERTY ENHANCED WITH MORINGA LEAF EXTRACT BIOSTIMULANT IN THE WITWATERSRAND GOLDFIELDS OF SOUTH AFRICA: A SUSTAINABILITY INITIATIVE

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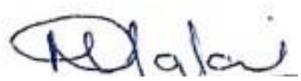
A thesis submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the PhD Studies.

DECLARATION

I, Nkanyiso Mlalazi, declare that this thesis is my own work. It has been submitted to the Faculty of Science of the University of the Witwatersrand, Johannesburg, for the fulfilment of the requirements to obtain a PhD in Geography, Archaeology and Environmental Studies. It has not been submitted to any other institution for examination prior to this submission.

Signed: At Wits University, Braamfontein, Johannesburg



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ABSTRACT

In the Witwatersrand goldfields of South Africa, mine tailings and footprint areas are significant environmental problems because they are major sources of toxic metals. These metals can leach into soils, and both surface and ground water, causing serious risks to human, animal, and plant life. In this study, the compost-assisted phytoremediation of tailing storage facilities (TSFs) and footprint soil using *Chrysopogon zizanioides* (vetiver grass) enhanced with moringa leaf extract (MLE) was investigated.

A greenhouse experiment was conducted to identify the most favorable parameters, and was followed by a field study to test the optimized parameters under real-environment settings. For the greenhouse experiment, a 3×2×2 fully crossed factorial design was used to determine the optimum variables. Vetiver growth was assessed under three compost concentrations (0%, 30% and 60%), two types of MLE (laboratory extracted MLE and commercial MLE) and two application regimens (once a week and twice a week) were used. The biomass and metal concentrations in the vetiver grass roots and leaves were measured after sixteen weeks followed by a two-way ANOVA analysis and the post-hoc tests. All the vetiver that was planted in 0% compost died within four weeks regardless of the MLE treatment. Vetiver grass planted on the 60% compost amendments and sprayed with laboratory extracted MLE had the highest biomass production, followed by plants grown in 30% compost amendments and sprayed with commercial biostimulant. However, the heavy metal removal or uptake data by the plant was inconclusive, as most of the toxic metals were not removed by vetiver grass which was attributed to the effect of compost. Based on biomass data, the 30% compost amendment and commercial bio-stimulant was the ideal treatments for the phytoremediation of gold mine tailings using vetiver grass. Although metal accumulation by plants is one of the attributes considered in phytoremediation, it is not the most significant factor in the phytostabilisation process. Plant growth and biomass production are the most significant, therefore it is concluded that vetiver, MLE and compost can be used in the phytostabilisation of gold mine tailings, however reduction in compost may be considered in future to improve the accumulation of metals in the roots for improved results.

Following the conclusion of the greenhouse study, a field study was conducted during the rainy season of 2021. Two field experiments were carried out concurrently at two sites: the footprint area (that was used as a rock dump) and the tailings storage facility (TSF 4). A split-plot design was used in this study. The experiment at each site assumed a 3×1×2 factorial design, with

three levels of compost treatment (0%, 15% and 30%), 1 level of vetiver cultivar (*Chrysopogon zizanioides*), and 2 levels of MLE treatment (commercial MLE and tap water, both sprayed once a week). Three blocks measuring 1 m × 2 m, each with 20 holes filled with equal amounts of soil amended with the different compost levels were prepared in triplicates. A single vetiver grass slip was planted in each hole. The blocks were then divided into 2 sections, each with 10 holes, and commercial MLE was sprayed on one section, while only water was sprayed on the other section once a week. After sixteen weeks, three plants were harvested from each section and the number of leaves, leaf length, number of tillers, biomass for roots and leaves and element concentrations were measured. Data analysis was done using two-way ANOVA.

The footprint area results showed that the application of 15% and 30% compost significantly improved vetiver growth parameters. There was no significant difference in biomass between vetiver grass grown on 15% compost amendment and that on 30% compost. Application of MLE led to a significant increase in the uptake of As, Cu, and Mn into the leaves and Cr, Cu, Mn, and Ni into the roots of vetiver grass grown on 0% compost-amended footprint soils. However, there was no significant difference in metal uptake by vetiver grass grown in 15% or 30% compost-amended soil due to MLE application.

The tailings study showed that adding 15% or 30% compost to tailings significantly improved vetiver grass growth, while plants grown on unamended tailings died within 4 weeks. However, there was no significant difference in biomass between vetiver grass sprayed with MLE and unsprayed vetiver grass on 15% or 30% compost-amended tailings. MLE application led to a significant decrease in the concentration of all metals investigated in vetiver leaves, except for Mn, which increased in concentration in vetiver plants grown on 15% compost-amended tailings. There was no significant difference in the concentrations of As, Cr, or Cu in vetiver roots between plants treated with MLE and untreated plants grown on 15% compost amended tailings. However, the concentrations of Mn and Ni increased due to MLE application. Application of MLE on vetiver grown on 30% compost led to increase in the concentration of As and Mn but not Cr, Cu, or Ni in the roots.

In conclusion, although compost improved the growth of vetiver grass, it is not recommended for use in the footprint area because it lowers the phytoextraction of metals by vetiver grass. This reduces the chance for the cleanup of the footprint area. Only MLE is recommended in enhancing the phytoextraction of contaminants on the footprint area. In the future, it may be worth exploring alternative growth enhancers that can be used together with MLE to improve vetiver grass growth and metal extraction on the footprint area.

On the contrary, compost, and MLE have the potential to effectively remediate gold mine tailings, with potential for phytoproducts and services. However, further research is needed to quantify the biomass production, bioenergy production, and carbon sequestration potential of vetiver grass as well as the economic viability of this remediation process. This study can help determine the full value chain of vetiver grass for gold mine tailings remediation. In addition, the extraction process for laboratory extracted MLE should be optimized to maximize its efficacy as a biostimulant at lower compost amendment levels. This would further improve the economic benefits of the process and could support local communities.

Key words

Phytoremediation, phytoextraction, phytostabilisation, moringa leaf extract, biostimulant, compost, sustainability, mine tailings,

DEDICATION

This study is dedicated to my two handsome sons, Bevan Siphesihle, and Brendon Tafara………

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ABRREVATIONS

BCF	Bioconcentration factor
DEFF	Department of Environment, Fisheries and Forestry
DET	Department of Environment and Tourism
DMR	Department of Mineral Resources
DMR	Department of Minerals Resources
EEB	European Environmental Bureau
GDARD	Department of Agriculture and Rural Development
GDP	Growth Domestic Product
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
MLE	Moringa Leaf Extract
MPRDA	Mineral and Petroleum Resources Development Act
NEMA	National Environmental Management Act
NEMBA	National Environmental Management: Biodiversity Act
NEMWA	National Environmental Management: Waste Act 59 of 2008
NRC	National Research Council
NWA	National Water Act 36 of 1998
SSV	soil screening values
TF	Translocation factor
USEPA	United States Environmental Protection Agency

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Environmental degradation and pollution have significant negative impacts on the environment and have raised global concerns. This is predominantly due to unsustainable anthropogenic activities such as mining, which cause irreversible destruction of ecosystems and the release of a significant amount of waste to the environment (United States Environmental Protection Agency (USEPA), 1987). Mining waste-related problems are in fact the second largest problems after global warming and ozone depletion (European Environmental Bureau (EEB), 2000). According to Hooke et al. (2012), mining activities have disturbed approximately 4×10^5 km^2 of global land, with annual mine tailings production worldwide exceeding 10 billion tonnes (Adiansyah et al. 2015). Some scholars believe that mining has also perturbed social inequality (Loayza & Rigolini, 2016; Schueler et al., 2011), climate change, biodiversity loss (Sonter et al., 2017) and health problems (von der Goltz & Barnwal, 2019).

The Department of Mineral Resources (DMR) has reported a total of 6152 mines in South Africa (DMR, 2010a). These mines are accompanied by large environmental footprints. For example, in 1997, 200,000 hectares of land were converted for mining operations, and 47,000 hectares were used to deposit 471 million metric tons of slime and waste rock dumps (Department of Environment and Tourism, 2008). The Gauteng Province alone hosts 374 mine residue areas, most of which are linked to gold mining (Department of Agriculture and Rural Development (GDARD), 2012).

The negative effects of pollution caused by mining led to public demands for immediate cleanups, which in turn led to the creation of the remediation industry in the 1970s to fix these problems using conventional (physical and chemical) cleanup solutions (National Research Council (NRC), 2005). Even though the remediation industry had good intentions, the shortcomings of the physical and chemical remediation measures become apparent when they fail to achieve the desired level of cleanup over a large area and over a long period of time (NRC, 2005). In addition, the conventional methods are very expensive (Danh et al., 2009; P., 2012), often rely on non-renewable energy sources (Wang et al., 2019), have a high environmental footprint (Hou et al., 2018) and need to be monitored constantly due to environmental safety concerns. Furthermore, some conventional remediation processes generally lead to the formation of other secondary waste products that require further treatment and proper disposal (Simate & Ndlovu, 2014). An example of conventional remediation is a "dig and haul" approach in the remediation of a single brownfield in New Jersey (USA) which produced 2.7 million tons of carbon dioxide, estimated to be equal to 2% of the annual carbon dioxide emissions for the entire state (Garon, 2008).

Due to the reasons stated above, an alternative plant-based technique known as 'phytoremediation' has been adopted by contaminated land management experts for sustainable and less expensive detoxification and contaminant removal from soil (Berti and Cunningham, 2000). The consensus is that, compared with conventional methods, phytoremediation is an eco-friendly alternative (Meagher, 2000). Its ornate features include biophilia and carbon neutrality (Khan et al., 2023), high social acceptance, little environmental footprint, and low operational costs (O'Connor et al., 2019), which makes phytoremediation a preferable option (Shmaefsky, 2020; Prabakaran., 2019)

In South Africa, the mining industry is the pioneering proponent of phytoremediation, primarily for restoring surface stability (Carbutt & Kirkman, 2022). This approach is largely guided by the 2007 guidelines for the remediation of impacted environments, which is considered a "go-to" manual in the design and implementation of remediation strategies. The current technology combines chemical, physical, and/or other biological processes with phytoremediation. The 2007 guidelines emphasize revegetation to restore surface stability and forage production and attempt to "return the land to premining state", with zero net loss of biodiversity (Vicklund, 1999). The process is initiated by substrate preparation, which involves the application of lime and superphosphate fertilizer to the substrate. A layer of cladding material is sometimes applied, depending on the physicochemical properties of the substrate under question (Cowan et al., 2016; Mentis, 2006). This process is followed by seeding with a cocktail of grass seeds.

The common grasses used for rehabilitation in South Africa include indigenous grasses such as *Cynodon dactylon* (couch grass, also called Bermuda), *Eragrostis teff* (Teff grass), and *Chloris gayana* (Rhodes grass) (Loch et al.,2004); *Eragrostis curvula* (Wheeping Love Grass); *Chrysopogon zizanioides* (vetiver grass); *Digitaria eriantha* (Smuts Finger Grass); *Medicago sativa*; *Paspalum notatum;* and *Cenchrus clandestinus* (Kikuyu). While vetiver grass is mentioned as one of the grasses being used in the revegetation of mined land, its use is limited due to its nonnative status.

In recent years, there has been a paradigm shift to consider historical species assemblages, biodiversity conservation and carbon sequestration (Carbutt & Kirkman, 2022). Climate change and carbon sequestration has become one of the top priorities worldwide. Most of the indigenous plant species used in phytoremediation do not possess high carbon sequestration potential and this necessitate the consideration of non-indigenous plant species in the phytoremediation of mine impacted environments. For example, Truong (2003) and Danh et al. (2009) argues that vetiver grass is one of the best species for phytoremediation due to its ability to survive in a wide pH range and climatic conditions amongst other things. In addition, it has high carbon sequestration potential, can be used for bioenergy production, its phytoproducts have valuable applications of socio-economic benefits and most importantly it is not an invasive species. Therefore, the benefits of using vetiver grass in South African landscape outweigh the potential downsides that may arise due to its use as it has no significant impact on biodiversity.

The mining industry can improve its sustainability by reassess mine waste as a valuable resource and embracing the principles of a secondary resource economy by harnessing value from mine waste (Golev et al. 2016). Phytoremediation of the mine waste with vetiver grass will not only be a mechanism to control pollution but a strategy for carbon sequestration, bioenergy production and other benefits. Despite the acceleration of phytoremediation research with the aim of fostering phytoproducts and services this past decade (Hou & Al-Tabbaa, 2014; Montanarella & Vargas, 2012), there is need for the legislation to maintain the pace with innovation and adopt the circular economy approach. Haywood et al. (2019), argues that South Africa's legislation is limiting. Novel technologies that can drive sustainability are limited by legal parameters, and this could be detrimental to sustainable development agendas. A disconnection between the current environment, social and economic sustainability and the legislation is noted.

1.2 PROBLEM STATEMENT

Although environmental pollution is the primary problem of mining, other challenges, such as resource depletion, social inequality, food insecurity, global warming, and climate change also emanates. A more holistic approach, considering the far reaching economic, social, and environmental consequences of mining is essential for effectively mitigating the negative impacts of mining activities. An approach that considers a full range of challenges and opportunities, combines technological innovation and systems thinking is key to effectively addressing mining impacts. This study therefore assumed an interdisciplinary approach by considering multiple fields of knowledge in decision pertaining to remediation.

As a point of departure, this study explored the use of vetiver grass in the phytoremediation of mine tailings. Vetiver grass is an ideal candidate for phytoremediation due to its unique properties. It can potentially grow without inorganic fertilizers. Instead, environmentally friendly compost and biostimulant such as moringa leaf extract (MLE) can improve its survival and growth. Several authors have highlighted that *Moringa oleifera* (Lam.) extracts improve plant growth in several crops because of their high protein, sugar, mineral, cytokinin, amino acid, antioxidant, proline, auxin, and gibberellin contents (Elzaawely et al., 2017; Zulfiqar et al., 2019). Hypothetically speaking, MLE can be extracted (using simpler techniques) , and compost can be made by the local communities. This would provide a source of income for local communities and could lead to the development of an entire industry. This approach could also reduce the need for packaging and transportation of compost and MLE, thereby reducing waste and carbon emissions in conformity with sustainable development goals. However, this approach should be based on the viability of the process in achieving the phytoremediation goals and objectives. The ultimate goal of this phytoremediation approach is to harness the potential of vetiver grass, boosted by environmentally friendly amendments, to address the interconnected issues of environmental pollution, energy production, climate change and sustainability topromote a more resilient and sustainable future.

1.2.1 Research Questions

In view of the above, this research was guided by the following questions.

(i) To what extent can locally produced MLE (laboratory extracted MLE) yield similar results as commercially available MLE and at what application rates?

(ii) Does MLE and compost application improve the phytoremediation potential of Vetiver grass?

(iii) Is there any difference in the performance of vetiver grass at the tailings and footprint areas?

(iv) What are the overall implications of the current study?

1.3 RESEARCH AIM AND OBJECTIVES

This study aimed to investigate MLE and compost could enhance the growth of vetiver grass with a view to determining its phytoremediation potential on mine tailings and footprint substrates.

i. To compare the efficacy of laboratory extracted and commercial MLE for enhancing vetiver grass growth under different compost amendments.
ii. To determine the phytoremediation potential of vetiver grass in different environments under the influence of MLE and compost
iii. The overall implications of this study in relation to sustainability initiatives.

1.4 METHODOLOGY AND EXPERIMENTAL DESIGN

According to Leedy and Ormrod (2015), collecting, analyzing, and interpreting data in a systematic process helps in understanding a subject of interest. This study therefore had to address several considerations prior to and during the research process to generate a substantial amount of data and ensure that the research design could be replicated. Several limitations were encountered during the design of this study; therefore, the study methodology was modified accordingly.

For effective data collection and analysis, a three-step research design was used to address the research questions, aim and objectives of the study. The first step was a desktop literature review of phytoremediation techniques in South Africa, including the latest studies on novel technologies that are honing towards sustainability initiatives. Secondly, it was determined that engaging mining houses to obtain a study site was a lengthy process, and at the same time, abandoned mining sites posed many safety risks when conducting field studies. As a result, the tailings material for greenhouse studies was obtained from an abandoned historic mine site and the goal was to determine the input variables that would yield optimum results. The third and final stage was the small-scale field study that was conducted at Sibanye Stillwater's tailings storage facility 4 (TSF4), an active TSF, and the footprint area study was performed at Sibanye's Driefontein rock dump 6 (DRD 6). A literature review and greenhouse and field observations were vital components of this study, and the study involved a large amount of quantitative data from the study variables.

1.5 STUDY SCOPE AND LIMITATIONS

This study assessed the use of compost and MLE for enhancing the phytoremediation of gold mine tailings and footprint areas using *Chrysopogo*n *zizanioides* (vetiver grass) in the Witwatersrand goldfields of South Africa. Inexhaustible studies on how vetiver interact with harsh environments such as tailings have already been performed globally as well as in South Africa. This study, however, assessed metal accumulation by vetiver grass and vetiver growth characteristics under the influence of environmentally friendly ameliorants, particularly MLE.

The current study is limited to vetiver's phytoremediation potential in the presence of compost and MLE. This study briefly covered the literature on the legal framework governing mine remediation in South Africa to establish the position of vetiver use from a legal point of view. Finally, the overall implications of the study were discussed.

1.6 PROPOSED THESIS OUTLINE

This thesis is composed of five chapters. The information presented in each chapter builds up the study's final conclusions. The first chapter highlights the frames of reference that were considered for this study. It gives the background of the study; the study questions, aim, and objectives; and a brief overview of the research design. Chapter two follows with the main theoretical considerations to support the study. This chapter covers the literature on the mining industry globally and in the South African context, including different approaches to environmental management, as well as the specific environmental remediation methods. Further, emerging trends in line with sustainability and circular economy related to the use of plant species that can provide value added benefits over and above phytoremediation, with attention to *Chrysopogon zizanioides* is discussed. Lastly, the substitution of inorganic fertilizers by biostimulants and compost to enhance plant growth is presented with attention to Moringa leaf extract.

Chapter three documents the methodological approach of the study. The methodology chosen is argued, and its opportunities and limitations are presented. This chapter further substantiates the chosen research design. The adopted research design for data collection and analysis is presented, including the study sites. The sampling methods used are described, and the details of how the experiments were set up are given. Finally, the analysis method is described.

The empirical evidence of the present study is presented in chapter four in the form of manuscripts. Chapter four highlights the findings from the methodology that this study followed. All the quantitative findings relevant to the study are presented in the form of figures, tables, and images. A discussion of the findings is also presented in chapter four, where the results are scrutinized to help in meeting the objectives of the study. These findings are further related to the findings of previous studies on similar areas of interest. Finally, the overall implications of the study, recommendations, shortfalls, limitations, and room for improvement are presented in chapter 5.

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CHAPTER TWO
LITERATURE REVIEW

2.1 INTRODUCTION

Mining plays an equivocal role in the world and has become an absolute necessity for the economy in most countries (Walser, 2002). While mining creates optimism, it dominates the list of activities that pose vastly grievous environmental degradation worldwide. Understanding the severity of the negative impacts of mining has evolved slowly despite awareness as early as the 16th century (Jarrige and Le Roux, 2020). As a result, mining has left a global legacy of both large and small tailings disposal sites that require remediation (Munshower, 2018; Li, 2006). According to Sutton & Weiersbye (2007a) the abandonment of mines in South Africa was a result of the lack of pollution control measures, as the emphasis of mining was its economic gains.

Given the escalating concerns about the negative impacts of mining, environmental remediation has been added to most countries' mining legislation. Following the promulgation of the constitution of South Africa and other legislations that hold mine rights holders liable for environmental harm (Swart, 2003), mining companies are now forced to remediate their impacts on the environment. As a result, there has been increasing interest in developing technologies to remediate contaminated sites (Bolan et al., 2014). Nissim et al. (2023) however, argues that there is a need to integrate ecosystem services, resilience to global climate change, biodiversity, carbon sequestration, bioenergy, added-value chemicals, and cultural benefits into decisions pertaining to remediation. Therefore, pursuing innovative methods that achieve multiple benefits in an environmentally friendly manner is more important than ever.

2.2 MINING IN AFRICA

Africa is increasingly recognized as a world natural resource hub. It is rich in gold, diamond, crude oil, ore, cement, and phosphate, among other mineral resources (Odoh et al., 2019). The mining, exploration, and trade of mineral resources are significant sources of revenue for many African governments. In the last five decades, most foreign direct investment in Africa has gone to the natural resource mining sector (Twerefou, 2009). In South Africa, mining contributed 7.56% and 7.53% to the growth in domestic product (GDP) in 2021 and 2022, respectively (https://www.mineralscouncil.org.za/industry-news/publications/facts-and-figures). It is therefore an absolute necessity for the economy. However, there are concerns that the environmental impacts of natural resource extraction and mining by multinational companies are often underestimated due to weak regulations and a lack of transparency from production companies (UNEP, 2011; Okonkwo & Etemire, 2017). Weiersbye (2007) noted that the legacy of contamination in Africa is severe due to the long period of mining, the chemical nature of some mineral deposits, and a history of poor controls. The main concerns are the impacts of mining on soils, groundwater, and water channels (Naicker et al., 2003; Sutton et al., 2006) and the bioaccumulation of hazardous contaminants in plants, animals, and humans (Weiersbye et al., 1999; Tutu et al., 2005). Agricultural land and many local communities are exposed to mine-impacted environments around South Africa's Witwatersrand goldmines (Durand, 2012). There are concerns on health especially in people living around the mining areas and the effect of occupational exposure (McSwane et al., 2015).

2.3 MINE TAILINGS, REHABILITATION AND REMEDIATION

Mine tailings are waste products that remain after ore beneficiation and are an important type of mine waste (Gil-Loaiza et al., 2016). Mine tailing disposal sites are prevalent, especially in northern Mexico, the western United States, Chile, Peru, Australia, South Africa, southwestern Spain, and western India, where extensive mining occurs (Tordoff et al., 2000). According to Adiansyah et al. (2015), the amount of mine tailings produced every year is more than 10 billion tonnes. They can spread via water and wind across tens of hectares (Morris et al., 2003; U.S. Environmental Protection Agency (USEPA), 2004; Warhurst 2000) and are the main metal sources in surrounding areas (Lu & Wang 2012). This is supported by the findings of Sheoran & Sheoran (2006), who reported that approximately 1.15 billion tons of metals, including copper (Cu), zinc (Zn), lead (Pb), cobalt (Co), cadmium (Cd), and chromium (Cr), have been mined.

An account of how the mine tailings were disposed of in the past was provided by González & González-Chávez (2006) and Schwegler (2006). The tailings were either returned to the mining site; dumped into an ocean, stream, or lake; or placed in a receiving pond. Alternatively, in arid and semiarid regions, tailings can be dried, spread, and compacted through a process known as dry stacking. Dry-stacked tailings are unstable and prone to wind and water erosion, with the potential to spread over tens of hectares and cause significant global impacts (González & González-Chávez, 2006; Csavina et al., 2011; Root et al., 2015).

Within the mining sector, terms such as rehabilitation and remediation has been used interchangeably to argue or support the idea of phytoremediation. As documented in literature, rehabilitation refers to restoring the land disturbed and polluted by mining activities back to a more sustainable and usable state (Ali et al., 2013). Remediation is defined as *"a physical, chemical or biological action to remove contaminants with the goals to reduce and manage the risks to human beings posed by contaminated sites"* (Beames et al., 2014). In this study, remediation and rehabilitation were used interchangeably. Mine rehabilitation can be a long-term process, and it is now recommended that mining companies begin rehabilitation during the planning phase of mine development (Sutton & Weiersbye, 2007b). Rehabilitation of mine-impacted environments is performed mostly through conventional physical and chemical methods, phytoremediation, or a combination of the two strategies.

2.3.1 Conventional physical and chemical remediation methods

Conventional remediation comprises chemical and physical methods. Chemical methods include in situ vitrification, soil washing, soil flushing, solidification, and stabilization of electrokinetic systems, while physical methods include soil incineration, excavation, and disposal into landfills (Sheoran et al., 2010; Wuana & Okieimen, 2011). Solidification and stabilization technologies work by binding contaminants and preventing their release into the environment. During solidification, a binding agent is mixed with the contaminated material, causing them to bind together (Xia et al., 2017). Inorganic binding agents, such as clay, zeolite, charcoal, Fe/Mn oxides, calcium carbonate, fly ash, and cement (Fawzy, 2008; Gunatilake, 2015), or organic binding agents, such as manure, compost, and bitumen (Lim et al., 2016), can be used to fix the contaminated material and binding agent together. Both organic and inorganic binding agents can be used in combination. In situ immobilization processes are beneficial due to the low labour and energy inputs required (Singh Sidhu, 2016; Sabir et al., 2015).

Vitrification is the process of heating contaminated soil to high temperatures to immobilize heavy metals. However, this process may cause the volatilization of organic compounds (Kołaciński et al., 2019). Soil washing involves the use of solvents and mechanical methods to decontaminate soil contaminated with heavy metals (Song et al., 2017). The effectiveness of soil washing depends on the ability of the solvent to dissolve heavy metals in the soil (Dermont et al., 2008). The solvents used in this process include organic acids, chelating compounds, surfactants, and cosolvents (Maturi and Reddy, 2008). Physical stabilization involves the application of inert materials, such as mine waste, gravel, topsoil, or clay capping, to the surface of contaminated soil to reduce wind and water erosion (United Nations Educational, Scientific and Cultural Organization (UNESCO, 2011)). Another option is to excavate the contaminated soil and dispose of it in a landfill.

Physical and chemical approaches have been criticized due to their high cost, intensive labour requirements, irreversible changes in soil properties, disruption of native soil microflora, high energy demands, potential secondary pollution problems, and difficulty implementing large-scale methods (Danh et al., 2009a; Kuppusamy et al., 2017). Tordoff et al. (2000) describes both physical and chemical stabilization as temporary solutions due to the impermanence of capping, while Dada et al. (2015) argue that these conventional techniques are effective only for small, heavily impacted areas.

2.3.2 Phytoremediation

Phytoremediation is a phytotechnology that is widely studied and documented (Njoku et al., 2016). It is a novel, cost-effective, environmentally friendly, and solar-driven approach to soil and water remediation (Kalve et al., 2011; Singh & Prasad, 2011; Vithanage et al., 2012). While phytoremediation is often described as a recent technology, the basic concepts date back to the eighteenth century, when Carolus Linnaeus (1707–1778) first proposed this idea (Gawronski et al. 2011). Phytoremediation has been widely researched in the last three decades and has attracted a fair amount of interest due to its advantages over conventional chemical and physical methods.

Plants and soil microbes are utilized to limit contaminant concentrations through the uptake, transport, storage, and degradation of contaminants (Pelfrene et al., 2015; Viehweger, 2014). Additionally, phytoremediation can help improve food safety, reduce global warming, and promote renewable energy sources while contributing to sustainable land use management (Mench et al., 2009; Vangronsveld et al., 2009; Claveria et al., 2010; Bardos et al., 2016). As a result, phytoremediation has been described as a 'green' or environmentally friendly approach to land decontamination (Efe and Elenwo, 2014) and is gaining global popularity (Ali et al., 2013; Bulak et al., 2014; Lee, 2013).

Several phytotechnological techniques can be used to extract, immobilize, or degrade various types of contaminants, including salts, metals, cyanides, and sediments (Bañuelos, 2006; Shah & Nongkynrih, 2007; Fulekar 2012). Figure 2-1 demonstrates different techniques which are used by plants in the phytoremediation of contaminated environments and how each mechanism work is summarized in Table 2-1.

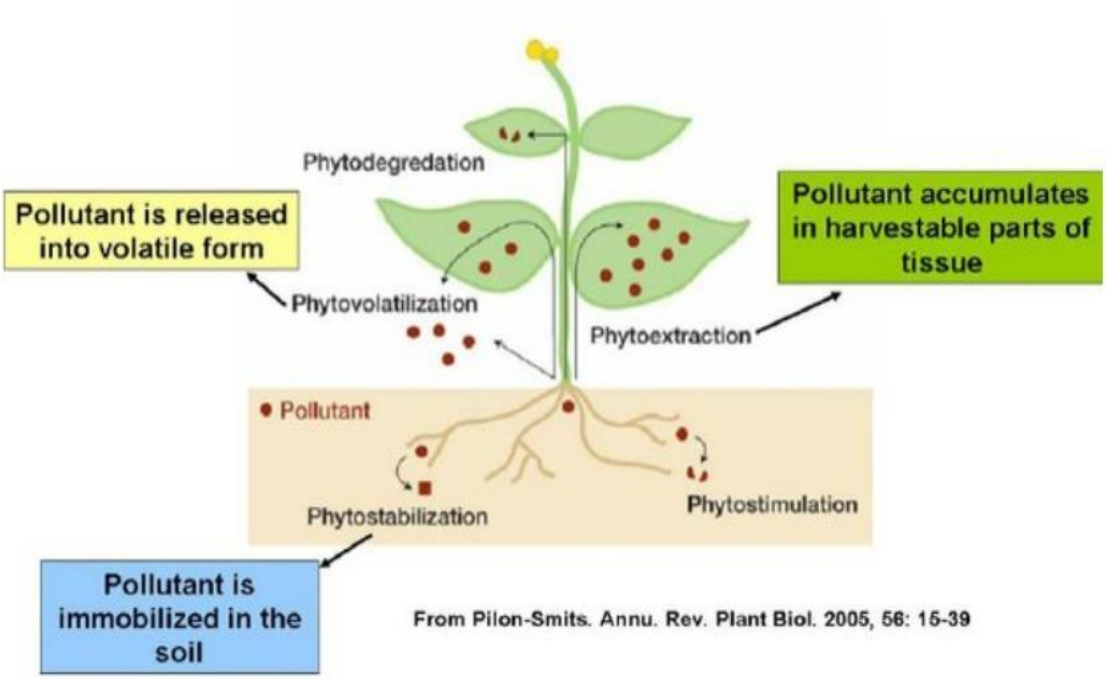

Figure 2-1: Demonstration of how plants carry out different phytoremediation technologies (From Pilon-Smits. Annu.Rev. Plant Biology. 2005, 58: 15-39)

Table 2-1: Summary of different phytoremediation techniques

Techniques	Description	Reference
Phytoextraction	Pollutants accumulated in harvestable biomass	Antonkiewicz et al. (2022)
Phytofiltration	Pollutants sequestration from water by plants	Bokhari et al. (2022)
Phytostabilization	Pollutants immobilized, adsorbed onto root, or precipitated at the rhizosphere	Visconti et al. (2020)
Phytodegradation	Plant enzymes breakdown and degrade organic xenobiotics within plant tissues	Pulford and Watson (2003)
Rhizodegradation	Rhizospheric organisms in the root zone of plants degrade organic xenobiotics.	Sivaram et al. (2020)

Techniques	Description	Reference
Phytovolatilization	Release of pollutants to the atmosphere after being converted to volatile forms	Guarino et al. (2020)
Phytodesalination	Excess salts are removed from the soil using halophytes	Lastiri-Hernández et al (2019)

Substantial achievements in phytoremediation have been reported. For example, in the USA, more than 200 sites were contaminated with radiological pollution, and approximately 25,000 sites polluted by the battery, paint, mining, chemical, coated glass, electroplating, and metal finishing industries were decontaminated via phytoremediation (Nwaichi & Dhankher, 2016; Sharma & Pandey, 2014; Sinha et al., 2011) and in Zululand, South Africa, more than 400 ha of indigenous dune forest was restored in 1977 (Cooke & Johnson, 2002). Phytoextraction and phytostabilization are the most common phytoremediation methods.

2.3.2.1 Phytoextraction

Phytoextraction is a process in which plants absorb contaminants from soil or water through their roots and transport them to their aboveground biomass (shoots), where the contaminants can be removed (Rafati et al. 2011; Tangahu et al., 2011). The extraction and transport of contaminants through the plant system can occur through cell walls (apoplastic), the cytoplasm (symplastic), membrane carriers (transmembrane). Some metals that can be readily transported to shoots include Cd, manganese (Mn), nickel (Ni) and zinc (Zn). Phytoextraction is considered the most important phytoremediation technique for removing contaminants from polluted environments (Milić et al., 2012). For instance, in the remediation of footprint areas, which are earmarked for postmining land use, such as pastureland, farming or residential areas, phytoextraction is the ideal mechanism for lowering the concentration of metals to levels that are not detrimental to animal, plant and human health. The efficiency of phytoextraction depends on factors such as soil properties, metal bioavailability, the plant species used, and metal speciation.

Metal phytoextraction can either be induced or performed naturally. When done naturally, soil amendments are not applied, whereas in induced mode, chelating agents are added to improve metal availability for uptake by plants. The chelating agents include citric acid, ethylenediaminetetraacetic acid (EDTA), ammonium sulfate and elemental sulfur (Lone et al., 2008). Lowering soil pH may also increase metal bioavailability because of the solubility of metal salts at acidic pH. However, proper care is needed when applying induced methods since these methods may cause secondary pollution problems (Zhao et al., 2011; Song et al., 2012). The characteristics of plants suitable for phytoextraction include a high rate of growth, high shoot biomass, an extensive root system, high metal tolerance, adaptation to weather conditions, pests and pathogens, easy propagation and harvest, high translocation factors, and unpalatable properties (Adesodun et al., 2010; Shabani & Sayadi, 2012; Sakakibara et al., 2011).

The determinant factors for the plant's phytoextraction potential are its shoot biomass and the metal concentration in its shoots. The bioconcentration factor (BCF) and translocation factor (TF) were used to quantify the plant phytoextraction efficiency. The BCF is the ratio of the metal concentration in different plant parts to the metal concentration in the soil (Branzini et al., 2012). The TF is the ratio of the metal concentration in the shoot to the metal concentration in the roots (Favas et al., 2014). Plant selection for phytoextraction depends on the BCF and TF (Wu et al., 2011). In addition, the selection of plant species that possess some economic added value (renewable biomass for bioeconomy and/or bio-ores) is beneficial (Escande et al., 2014; Van der Ent et al., 2013; van der Ent et al., 2013).

However, plants that have high phytoextraction potential (hyperaccumulators) suffer from low biomass production, leading to a high number of cropping cycles required to reduce total element concentrations in soils. In addition, the absence of commercially available seeds/seedlings, their selectivity (for example, most of these plants hyperaccumulate one or a few metals), a lack of knowledge related to their cultivation, climate needs or competition with other metal-tolerant plants are some of the drawbacks. High-biomass perennial or annual plant species and woody plants with moderate to high bioconcentration factors are considered viable alternatives to hyperaccumulators for phytoextraction. Food crops such as maize, cabbage, and barley have been evaluated for metal phytoextraction; however, there is a high potential for food chain contamination. Phytoextraction, like conventional remediation methods, has several limitations (Ramamurthy & Memarian, 2012). An extended period is needed for this approach to be effective because of the typically low biomass and slow rate of growth of most hyperaccumulator plants. The limited bioavailability of contaminants in the soil further restricts the practical applicability of the method (Audet & Charest, 2007).

2.3.2.2 Phytostabilization

Phytostabilization or phytoimmobilization occurs when certain plants are established in contaminated soils to stabilize contaminants (Singh, 2012). Vegetation cover reduces contaminant mobility by promoting metal accumulation in roots or by forming complexes or reducing the metal valence around the rooting zone of the plant (rhizosphere) (Wuana & Okieimen, 2011). Additionally, plants are important for preventing erosion and adding organic matter, which is important for forming soil aggregates and metal binding. They also aid in aerobic environment creation in the substrate (Meeinkuirt et al., 2012).

Phytostabilization reduces heavy metal accumulation in plants and groundwater, and it creates a vegetative cap that stabilizes tailings for a long time. Unlike phytoextraction, phytostabilization traps metals in the rhizosphere instead of extracting them from the soil. (Ernst, 2005). As a result of phytostabilization, heavy metals become less bioavailable and less likely to pose a risk to animals or humans (Cunningham et al., 1995; Munshower 2018; Wong, 2003).

Tailings often have no organic matter or macronutrients, and most have acidic pH (Krzaklewski & Pietrzykowski, 2002; Lin et al. 2003), a combination of these factors leads to a heterotrophic microbial community that is stressed (Méndez-Ortiz et al., 2007; Southam & Beveridge, 1992) and has very low species diversity (Moynahan et al., 2002). Furthermore, autotrophic iron- and sulphur-oxidizing bacteria, which are dominant in acidic tailings, are said to be the cause of plant death in tailings (Schippers and Jorgensen, 2002). Additional challenges in arid and semiarid areas, including extreme temperatures, low precipitation, and high winds, further impede plant establishment (Munshower, 2018). These conditions lead to low water infiltration and high evaporation and hence are partly responsible for very high concentrations of salts on tailings. This results in irregular vegetation growth even on the surfaces of historic tailings (Macdonald et al. 2017).

The phytostabilization process is often improved by the addition of amendments such as biosolids, lime, compost, and/or fertilizers (Clemente et al., 2012; Li & Huang, 2015; Lee et al., 2014). A single or combination of amendments is often added to the soil first to lower the concentration of plant-available contaminants and reduce plant death by causing sorption and/or precipitation of the contaminants before planting (Mench et al., 2006). Bolan et al. (2011) demonstrated that phytostabilization may be enhanced via soil amendments. The resultant vegetative cap, plant–root exudates, organic matter, and established microbial community should promote the phyto-catalyzed stabilization of inorganic contaminants in the root zone (Mendez & Maier, 2008); Santibañez et al., 2012). Furthermore, metal accumulation in aboveground biomass should be limited to prevent contaminants from spreading to nearby areas and food (Henry et al., 2013; Mendez & Maier, 2008; Pérez-de-Mora et al., 2011). A plant survey by González & González-Chávez, (2006) showed that the plant families associated with phytostabilization include *Anacardiaceae (Schinus molle* L), *Asteraceae (Asocoma veneta* (Kunth) Greene), and *Chenopodiaceae (Teloxys graveolens* (Willd.) W.A. Weber) *Euphorbiaceae (Euphorbia spp*.), and *Fabaceae (Dalea bicolor* Humb and Bonpl ex Willd). A summary of how plants work in the phytostabilization process is provided in Figure 2-2 below.

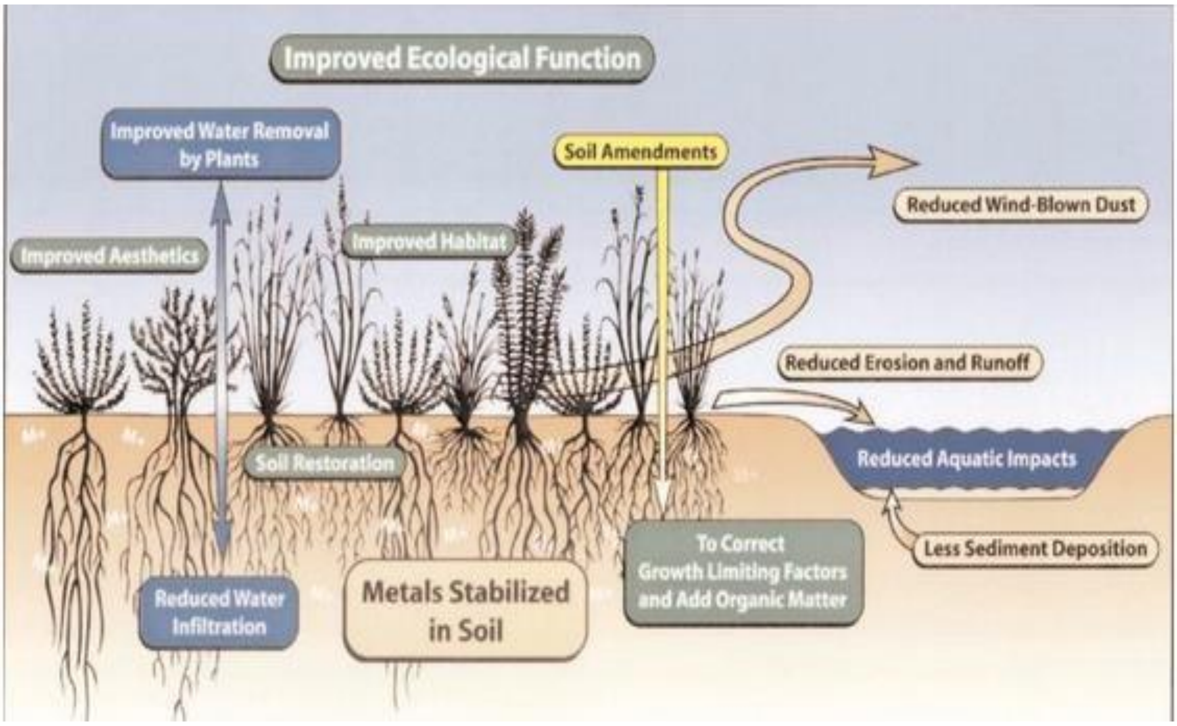

Figure 2-2: Schematic diagram showing the phytostabilization mechanisms of plants. Source: (https://knowhowtogmo.wordpress.com/tag/disadvantages-of-phytostabilization/)

The limitation of these techniques is that heavily contaminated substrates cannot sustain plant growth; therefore, the use of plants in the remediation of contaminated land is limited to sites with low to moderate contamination levels (Audet & Charest, 2007).

2.4 FACTORS INFLUENCING PLANT SPECIES SELECTION FOR PHYTOREMEDIATION

Plant species selection is an essential part of phytoremediation program. Every plant species has unique phytoremediation abilities (Barwise & Kumar, 2020; Cresswell et al., 2019; Gastauer et al., 2020). It is crucial to choose species that are appropriate for specific contaminants and site conditions. Fast-growing, high biomass production, deep-rooted, easily propagated plants with high metal tolerance and accumulation capacity are important (Rodríguez-Vila et al., 2015). The phytoremediation potential of grasses has been extensively studied. Some of the most studied grasses include vetiver, switchgrass, and Indian mustard (Elekes, 2014) All these grasses have unique characteristics that make them useful for phytoremediation. The high rate of biomass production, fast rate of growth, resistance, and applicability of these grasses in the remediation of different types of soil make them important subjects for research (Elekes, 2014). Grasses provide quick cover, which limits particle dispersion by wind (Williams & Currey, 2002). In South China, revegetation of Pb/Zn using *Vetiveria zizanioides, Cynodon dactylon, Typha latifolia* and *Festuca rubra* has been successful owing to the deep root systems of these grasses, which abet erosion control (Wong, 2003).

Another important attribute of phytoremediation agents is the ability to adapt to the physical, chemical, and climatic conditions of contaminated sites, and these agents must be stress tolerant (Odoh et al., 2019; Efe and Elenwo, 2014). Laghlimi et al. (2015) suggested that the architecture of the plant roots, contaminant characteristics, physical and chemical properties of the substrate and weather conditions of the region are important determinants of phytoremediation. The failure of plants to adapt may result in alterations in physiological and metabolic processes, which may lead to interference with the photosynthesis and respiratory activities of several key enzymes (Hossain et al., 2009; Villiers et al., 2011; Volland et al., 2014). As a result, metal toxicity associated with oxidative damage caused by reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radicals (.OH), may lead to biotoxicity and ultimately plant death (Mittler, 2002). Therefore, to combat the toxicity of high metal concentrations, phytoremediation agents should employ strategies such as chelation, metal uptake and transport restrictions and tolerance mechanisms (Anjum et al., 2016).

2.4.1 Metallophytes

Metallophytes can adapt and thrive in soils containing high levels of heavy metals (Bothe, 2011; Sheoran et al., 2010). The use of metallophytes either alone or in combination with microorganisms is highly attractive and is widely accepted (Bothe, 2011). There are three categories of metallophytes: metal hyperaccumulators, metal excluders, and metal indicators.

Metal excluders limit the movement of metals from the roots where they accumulate to aboveground biomass (Sheoran et al., 2010; Malik and Biswas, 2012). Metal excluders are efficient for phytostabilization. Metal indicators indicate the levels of metals in soils by accumulating metals in aerial parts (Sheoran et al., 2010). The concentrations of metals in the aerial parts of hyperaccumulator plants far exceed the concentrations of metals in the substrate (Memon & Schröder, 2009) and as high as 100 times greater than the concentrations of metals in non-accumulator plants (Chaney et al., 2007). Consequently, the TF in these plants is above 1 (Badr et al., 2012). Hyperaccumulators are ideal for phytoextraction. In South Africa, species selection is in line with the legal framework governing land remediation, rehabilitation, and restoration.

2.5 SOUTH AFRICAN LEGISLATION GOVERNING MINING

Mining legislation is not new to South Africa, as laws placing mining impact responsibilities on mine owners date back to 1903 (Munnik et al., 2010). Despite these laws, mining companies had no regard for the environment, only economic gain mattered in any decision regarding mining prior to the Minerals Act (Act 50 of 1991) The negative impacts of mining were left for citizens to live with and for the state to deal with (Swart, 2003). The 1975 Farie Botha accord attempted to address the problem of derelict and ownerless mines whereby 100% responsibility for the mines closed before 1976 was assumed by the state. A 50:50 responsibility between the state and mine owners was adopted for mines closed between 1976 and 1986. After 1986, all mines and closures were the responsibility of mine owners (Munnik et al., 2010). Unfortunately, the 1975 Farie Botha accords did not achieve the desired results.

In 1996, the constitution of South Africa was enacted. This was followed by a set of laws and regulations governing mining in South Africa. Section 24 (Act 108 of 1996) of the constitution gives all South Africans "*the right to an environment that is not harmful to their health or well-*

being and to have the environment protected, for the benefit of present and future generations, through reasonable legislative and other measures that prevent pollution and ecological degradation, promote conservation, and secure ecologically sustainable development and use of natural resources while promoting justifiable economic and social development".

Currently, the reference for the obligations to rehabilitate mine sites is to a greater extent contained in the Mineral and Petroleum Resources Development Act 28 of 2002 (MPRDA) administered by the Department of Minerals Resources (DMR). The National Environmental Management Act 107 of 1998 (NEMA), National Environmental Management: Biodiversity Act 10 of 2004 (NEMBA), National Environmental Management: Waste Act 59 of 2008 (NEMWA) and the National Water Act 36 of 1998 (NWA) are invariably triggered by the mining related activities.

Section 43(1) and Section 38 (1) (d) of MPRDA, 2002; Section 2 (4) (p) of NEMA, 1998; and Section 28(1) of NEMA, echo the same sentiment that the person responsible for the damage to the environment remains liable and is responsible for minimizing and rectifying all the damage done to the environment. The MPRDA requires mines to develop an Environmental Management Programme Report (EMPR) containing adequate provisions for financial guarantees for rehabilitation and arrangements for monitoring and auditing. Effective pollution control and rehabilitation measures for tailing storage facilities (TSFs) and impacted sites can be implemented before a mine closure certificate can be issued. This is in line with "the polluter pays" principle.

Plant species used for phytoremediation is governed by the NEMBA. NEMBA provides for amongst others, the management and conservation of South Africa's biodiversity within the NEMA framework. For instance, the use of some alien species traditionally used in TSF vegetation has been prohibited. NEMBA states that to ensure lasting reductions in TSF emissions and achieve site closure, ecologically meaningful rehabilitation with native vegetation that minimizes site emissions to air, water, soil, and biota is required.

However, the mining industry is facing increasing pressure to include the principles of sustainable mining. To keep up with the world economic forum aimed at making the mining, world sustainable by 2050 (world economic forum, 2015), a need to transition toward sustainability is necessary. This may include viewing the process of remediation not only with

NEMBA lenses but also from a wide range of factors that influence sustainable development, such as the socioeconomic benefits that can be harnessed and climate change mitigation strategies. For instance, Harrison et al. (2019) investigated the use of fiber crops such as flax, *Bambusa balcooa*, kenaf, hemp, and sisa to mitigate mine-impacted environments while providing a vast range of products. This strategy could initiate an agricultural sector that could otherwise not exist. Communities and mine workers could be skilled so that they can eventually transition to support an agricultural economy (Harrison et al., 2019).

Vetiver grass is another potential plant species where a variety of products and services can be harnessed to support sustainability without compromising biodiversity. However, the South African legal framework is clear on the use of native plant species for remediation. Although vetiver grass is not listed under invasive plant species, the consensus is that remediation should be done using indigenous plant species. This limits the innovations that could promote potential economic benefits of mine tailings (Haywood et al., 2019). Rather than viewing the thousands of square kilometers of land occupied by mine tailings as waste that needs remediating, this area could be viewed as a resource that can be used for carbon offset or for producing bioenergy plants such as vetiver grass. The shunning of some nonnative plants, such as vetiver grass, by legislation inhibits the economic potential of mine tailings, where bioenergy plants can be grown and contribute to a secondary resource economy (Haywood et al., 2019). Godfrey et al. (2007) argue that economic, social, and environmental benefits can be harnessed if waste can be viewed as a resource.

As far back as 2008, a toolkit published by the International Council on Mining and Metals (ICMM) contained recommendations that "waste materials should be viewed as a resource" (ICMM), 2008). The ICMM more recently addressed this issue of evolving from an integrated mine closure perspective (the "end-of-life" or cradle to grave perspective) to a circular economy perspective (the "restoration" or cradle-to-cradle perspective) (ICMM), 2016). South Africa's waste management legislative framework recognizes the need and opportunity for circularity, yet the existing systems make socioeconomic activity in this space burdensome, forcing activities to legal and economic perimeters. This limits the potential to expand innovations that bring multiple benefits.

2.6 EMERGING TECHNOLOGY IN THE PHYTOREMEDIATION SPACE

With the World Economic Forum aiming to make the mining world sustainable by 2050, an effort to keep pace with the growing demand for a sustainable world has been made (world economic forum, 2015). This has resulted in several technological innovations in the direction of sustainability. For example, fungicoal technology was developed as an open-cast spoil and coal discard dump rehabilitation strategy. The fungicoal strategy utilizes the mutual relationship between plants and fungi to achieve the degradation of carbonaceous pollutants, encourage the restoration of soil components and encourage grass growth to achieve long-term revegetation (Cowan et al., 2016). This process is based on a biotechnology self-cladding process whereby coal degradation by fungal inocula leads to the formation of a humic soil-like layer on the coal dump surface. Mutual interactions occur between grasses such as *Cynodon dactylon, Pennisetum clandestinum, and Eragrostis teff* root exudate; a suite of arbuscular mycorrhizal fungi, including one or more of the following: *Gigaspora gigantea, Glomus clarum, Glomus mossea, and Paraglomus occultum;* and at least one coal-degrading fungus, such as the isolates, the Aspergillus strain ECCN 84 and/or the *Neosartorya fischeri* strain ECCN 84. (Cowan et al., 2016). The objective of using fungal technology is to avoid the traditional method of obtaining cladding material from another area in the rehabilitation of coal mines, as this approach has detrimental effects on the environment.

The use of fiber crops such as flax, *Bambusa balcooa*, hemp, sisa, and kenaf is another potential technology for mitigating mine-impacted environments since these crops can bioaccumulate heavy metals in their biomass while simultaneously providing a vast range of products such as fiber for handicrafts (Harrison et al., 2019). There is potential for self-sustaining communities post mining to derive their livelihood from the agricultural sector created from fiber crop production during land remediation. Fiber crops are tolerant to low- to medium-pollution soils; however, elevated contaminant levels can affect their growth. The downside of this potential innovation is that fiber crops may accumulate high metal levels, which may affect product safety.

Other technologies that are on the rise include nanoparticles and genetic engineering for use in phytoremediation. Nanotechnology has the potential to enhance the effectiveness of phytoremediation by improving plant germination, increasing plant stress tolerance, promoting phytoenzymes production, enhancing seedling growth, and enhancing rooting and shooting characteristics. (Siddiqi & Husen, 2017). Genetic engineering has also been intensively investigated. The aim of this approach is to increase plant species tolerance through the accumulation and absorption of contaminants. The plants can be genetically modified to express recombinant proteins that help chelate, incorporate, and transport metal ions. (Vamerali et al., 2010) For example, transgenic *Arabidopsis thaliana* can remove arsenic and cadmium through the expression of AsPCSI (from garlic) and YCFI (from baker's yeast) (Guo et al., 2012), transgenic alfalfa plants can express human CYP2EI and glutathione S-transferase (Zhang & Liu, 2011), *Nicotiana tabacum* as a host for a yeast methallothioneingene for cadmium uptake through the root (Krystofova et al., 2012).

In the context of South Africa, this research aims to examine the nexus between phytoremediation and the attainment of multiple benefits, with a specific focus on vetiver grasss, compost and MLE biostimulant as a promising solution for holistic remediation, encompassing environmental, social, and economic advantages such as carbon sequestration and bioenergy.

Regarding dealing with tailings and mine footprint areas in South Africa, the focus often remains limited to revegetation with indigenous grass for pollution control while maintaining biodiversity to assume a pre-mining state. The transition to sustainability requires focus on a transformation process in which systems thinking is embraced. The existential threat of climate change, the socioeconomic realities of mining communities, and pollution levels have inspired remediation innovations with vetiver grass (*Chrysopogon zizanioides)*.

2.7 VETIVER GRASS (CHRYSOPOGON ZIZANIOIDES)

Chrysopogon zizanioides (L.) Roberty, formerly known as *Vetiveria zizanioides* (L.) Nash and commonly referred to as vetiver grass, belongs to the family Poaceae. There are two vetiver grass species in South Africa; *Vetiveria nigratana* an indigenous species and *Chrysopogon zizanioides* that was introduced to Kwazulu- Natal Province from Mauritius in the 18^{th} century. *Chrysopogon zizanioides* found all over South Africa is genetically identical to vetiver from Australia, USA, India and Mauritius (https://www.vetiver.org/SAVN_visit.htm#:~:text=There%20are%20two%20species%20of, Natal%20as%20early%20as%201860).

Vetiver grass has been extensively studied. It is a tall, fast-growing perennial grass that can reach 1–2 meters in height and has an extensive root system that can grow up to 3–4 meters deep in one year (Andra et al., 2009; Danh et al., 2009). The deep roots of vetiver grass make it extremely tolerant to drought, and vetiver grass is difficult to dislodge by strong winds or water currents. Vetiver is a sterile, nonaggressive plant; it produces neither stolons nor rhizomes and produces vegetatively by root clump subdivision (Truong, 2016).

The new shoots develop from the underground crown. This makes vetiver resistant to overgrazing, fire, frost, and traffic. Vetiver grass is tolerant to extreme temperatures and floods. It is efficient at absorbing nutrients and heavy metals. However, vetiver grass is intolerant to shade and prefer a weed-free environment (Truong, 2016). Vetiver grass is known for its ability to tolerate a wide range of pH, salinity, and metal toxicity conditions. It can accumulate high levels of toxic metals in its roots and shoots, and it contains insect-repelling aromatic oils (Andra et al., 2009; Danh et al., 2009a; Datta et al., 2011; Truong, 2016).

According to Melato et al. (2016), vetiver grass can tolerate metal-induced stress, and its metabolism and photosynthetic activities are not significantly affected. The study showed that vetiver grass can take up Zn, Cu and Ni from tailings and accumulate them in its roots, limiting translocation to the shoots, which is desirable for phytostabilization. Zhang et al. (2014) and Banerjee et al. (2016, 2019) observed a similar trend with vetiver grass exposed to Cd, and they agreed with the utilization of vetiver grass in the reclamation of Cd-contaminated soil because vetiver can take up and accumulate large concentrations of Cd. Vetiver grass can accumulate heavy metals, especially lead and zinc. The threshold levels for metals in vetiver shoots are Cu, 13–15; Zn, 880; Ni, 347; Cd, 45–48; Pb, 78; and Cr, 5–18 mg kg^{-1}(Truong, 1999). Although vetiver grass is not as effective as some other plants at accumulating heavy metals, its wide tolerance to adverse climate and soil conditions makes it a promising candidate for phytostabilization. In some cases, phytoextraction can be achieved by the addition of chelating agents (Truong, 2003). Danh et al. (2009b) showed that vetiver grass can tolerate extreme climatic variation; high levels of soil acidity and alkalinity; salinity; sodicity; and heavy metals; and still produce high amounts of biomass (>100 t/ha/year).

A typical example of where vetiver has been used in the South African mining industry is the revegetation of diamond spoils from diamond mining and processing of kimberlite. Naturally, kimberlite is high in smectite clays and may be high in sodium, making the dumps highly erodible, hostile for plant growth and difficult to revegetate. The climate where most diamond mines are located is semiarid, and these areas are subjected to extreme temperatures and unpredictable rainfall. This exacerbates the problem. Exotic plant species such as vetiver grass adapt well and establish easily under these conditions. Revegetation with indigenous species was successful only when the tailings were top-dressed with soil, making the process very expensive and at times impossible due to lack of soil. However, vetiver was successful at all the sites and was able to control runoff and erosion; withstood high and low temperatures; adapted to alkaline and high sodium; and created an ideal habitat for indigenous species (Sing, 1997).

Vetiver has the potential to be used for additional benefits after phytoremediation, such as raw material for handicrafts, industrial products (raw material for pulp and paper) and fiberboard; reduce soil erosion; and provide feedstocks for biofuels. C4 grass species, such as vetiver species, on marginal land not only remediates land but also has a high carbon dioxide sequestration capacity; therefore, this approach can be an effective mitigation strategy for climate change and its impacts. A summary of the potential products and services that can be derived from vetiver grass is shown in Figure 2-3 below.

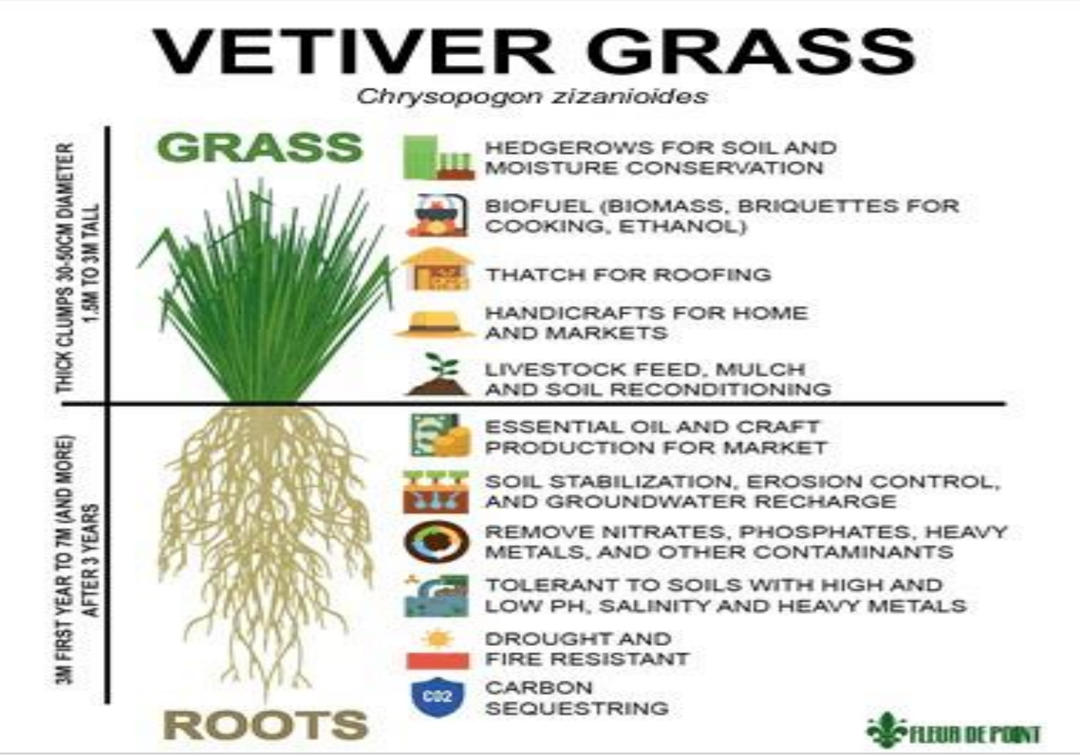

Figure 2-3: Different services and products that can be derived from vetiver grass. (Source The International Vetiver Network/Fleur de Point.)

While vetiver grass has a high tolerance for heavy metals, high levels of these metals can still have phytotoxic effects on vetiver grass, including reduced growth, photosynthetic activity, and oil yield (Singh et al., 2011). As a result, the use of amendments has been widely studied (for example Truong, 2003; Arthur et al., 2016). Over the past three decades, technological innovation has increased to promote a sustainable production system through reducing synthetic fertilizer use (Rouphael & Colla, 2020). Organic amendments are preferred over inorganic chelating agents because they reduce metal bioavailability immediately and gradually, add slow-release fertilizer, provide a microbial inoculum, and improve soil structure and cation exchange capacity. In addition, organic matter reduces erosion and increases infiltration of mine tailings (Mendez et al., 2007; Schippers et al., 2000; Schroeder et al., 2005). Organic amendments increase soil pH, reducing the activity of iron and sulfur oxidizers that cause acidification and plant death in pyritic tailings.

One promising type of organic amendment that can be used to enhance plant growth and nutrient use efficiency in mine tailings is biostimulants. Calvo et al. (2014) noted the growing scientific evidence supporting the use of biostimulants as agricultural inputs for diverse plant species. Positive outcomes, such as increased root growth, improved uptake, and tolerance to stress, have been achieved via the use of biostimulants. More recently, the new regulation (EU) 2019/1009 has defined biostimulants as a product that stimulate plant nutrient processes to improve the efficiency of nutrient use, and/ abiotic stress tolerance, soil nutrients availability and plant quality traits (Rouphael & Colla, 2020). Kauffman et al. (2007) defined biostimulants as substances other than fertilizers that support plant growth when applied in small amounts, whereas du Jardin (2015) referred to biostimulants as single compounds or groups of compounds that are not considered nutrients, pesticides, or soil improvers. There is a great diversity of biostimulants, and they may act in different ways, such as by increasing nutrient efficiency, enhancing stress tolerance, and/or improving crop quality.

Biostimulants cause stimulation in different plants (Calvo et al., 2014; Sharma et al., 2014). The primary purpose of biostimulants is to enhance plant quality and nutrient efficiency rather than to provide nutrients or target pests and pathogens (du Jardin et al., 2020). A recent global trend has seen the increased use of biostimulants, especially in the agricultural sector. However, there are few studies on biostimulants carried out in the remediation industry in South Africa. Arthur et al. (2016) investigated the effects of Kelpak® (KEL), smoke-water (SW) and vermicompost leachate (VCL) on *Brassica juncea* cultivated on Krugersdorp Goldmine

(South Africa) soils, private farmland, and nearby Game Reserve. Seedlings of *B. juncea* were planted in pots containing substrates from the places, and the biostimulant was applied. None of the seedlings survived on Krugersdorp Goldmine soils, regardless of the biostimulant treatment applied. However, the less contaminated soil collected from nearby areas and the different biostimulants applied produced better growth results than did the unsprayed plants.

One biostimulant that has been identified is *Moringa oleifera* extract, and according to (Rady & Mohamed, 2015), its discovery as a biostimulant has gained importance. The common name for Moringa is horse radish tree or drumstick. It belongs to the Moringaceae family. *Moringa oleifera* is a plant of great value that is found in tropical and subtropical countries. Moringa plants have great nutrient value and diverse medicinal uses (Mishra et al., n.d.; Moyo et al., 2011). Moringa is also called a miracle tree due to its medicinal uses and nutritional purposes.

Moringa plants are cultivated with fresh vegetables, green manure, medicine, livestock fodder, biogas, bio pesticides, and seed priming agents (Fuglie, 1999). It is a natural plant growth promoter due to its nutrient composition, phytohormones and antioxidants (Khan et al., 2021). Moringa leaf extract (MLE) is said to possess high antioxidant activity because it contains elevated levels of osmoprotectants and secondary metabolites (Rady et al., 2013). It is rich in growth-enhancing compounds such as phenolics, ascorbate, minerals (Ca, K, Zn and Fe), antioxidants (Siddhuraju and Becker, 2003), cytokinins and auxins (Barciszweski et al., 2000). As a result, this technique has attracted the interest of the scientific community (Bakhtavar et al., 2015; Yasmeen et al., 2013). Different methods have been used for the extraction of MLE from moringa biomass in the laboratory. A summary of the methods is provided in Table 2-2 below.

Table 2-2: A summary of different methods used in the extraction of MLE.

Author	**Method description**
Abou-Sreea and Matter, 2016	The 10, 20 and 30% moringa leaf extract was prepared by blending 100, 200, and 300 g of young moringa leaves with 675 ml of 80% ethanol. The obtained suspension was homogenized and filtered by wringing using a mutton cloth. Finally, the solution refiltered using No. 2 Whatman filter paper and rose to one litre.

Author	Method description
Hoque et al., 2020	10 ml of water was added to 100 g of fresh young leaves and ground with a pestle. The juice was extracted by pressing with hand and was filtered through a cheese cloth followed by refiltering using Whatman filter paper No. 2. Following the method developed by Fuglie (2000), the extract was then diluted with distilled water at a ratio of 1:32 (v/v). The extract was stored at 0°C temperature and taken out only when needed for use.
Mageed, Semida and Rady, 2017	Fresh moringa leaves were harvested at the fully matured stage and were frozen overnight and pressed in a locally fabricated machine. The extract was purified twice by filtering through filter paper (Whatman No. 1). After refining process, the extract was centrifuged at 8000 rpm for 15 min for supernatant diluted to obtain extraction at a 3% concentration to spray the plant foliage.
Fadare et al., 2018	Dry leaves of less than 4 weeks old were used to extract MLE. The leaves were plucked and air-dried under room condition for three weeks, the dry leaves were then blended and sieved to get the moringa powder. An amount of 100 g of the powder was mixed with a 1 liter of water and then sieved out using a sieving cloth, approximately 500 mls of the filtrate was then diluted with water at ratio of 1:16.
Mazrou, 2019	The fresh Moringa leaves were harvested and air-dried then grinded to fine powder. Ethyl alcohol was added to the powder to prepare four concentrations by mixing 50, 100, 200 and 300 g L^{-1} (based on fresh weight) and then the mixture was put on a shaker for 4 h for extraction preparation. MLE obtained was purified using filtering twice through filter paper (Whatman No. 1). MLE was subjected to a rotary evaporator and the extract was centrifuged at 8000 × g for 15 min for alcohol evaporation. Finally, the supernatant obtained from each concentration was diluted to 30 times using distilled water.

Author	Method description
Merwad, 2018	20 g of young *Moringa oleifera* leaves were mixed with 675 ml of 80% ethanol as suggested by Makkar and Becker (1997). The suspension was stirred using a homogenizer to help maximize the amount of extract. The solution was filtered using Whatman No.2 filter paper. MLE was used within 5 hr from extraction process, otherwise it was stored at 0^{o}C and only taken out when needed for use.
Hassanein et al., 2019	Fully expanded leaves and tender branches were collected from Moringa trees. Two hundred grams were extracted in 500 ml water using locally fabricated machine (Foidle et al., 2001). The extract was filtered through muslin cloths and centrifuged at 800xg for 15 min. The supernatant was filled up to one liter then dilutions were made (20%, 10%, 5%, 2.5%) and kept at 4^{o} C till used.

Several authors have highlighted that *Moringa oleifera* (Lam.) extracts improve plant growth in several crops because of their high protein, sugar, mineral, cytokinin, amino acid, antioxidant, proline, auxin, and gibberellin contents (Elzaawely et al., 2017; Zulfiqar et al., 2019).

Fuglie (2000) reported accelerated plant growth, improved resistance to pests and diseases, increased leaf number, increased number of roots, increased size of fruits and, generally, a 20% to 35% increase in yield due to MLE application. A study by Yasmeen et al. (2013) showed an increase in plant tolerance to abiotic stresses upon the application of MLE as a seed priming agent or on plant foliage. Several recent scientific studies have indicated that the application of moringa leaf extract as a biostimulant can improve the antioxidant defence system, provide beneficial nutrient elements, and enhance vegetative and reproductive growth, leading to yield increases and economic benefits under stressed and non-stressed conditions (Abdalla, 2013a; Aluko et al., 2017; Merwad, 2017; Mvumi et al., 2013a; Rady & Mohamed, 2015).

Abdalla, (2013b) reported an increase in *Eruca vesicaria* (rocket plant) weight; height; photosynthetic pigments; and nitrogen (N), phosphorus (P), potassium (K), iron (Fe), calcium

(Ca), and magnesium (Mg) accumulation with 2% MLE foliar spray. MLE also increased the plant weight, root dry matter, plant height and yield of maize and common bean (Mvumi et al., 2013b). Recent studies have shown that Moringa extract is effective at improving the growth performance of squash (*Cucurbita pepo* L.) plants under drought stress (Abd El-Mageed et al., 2017; Zulfiqar et al., 2019).

In a study by Abou-Sreea and Matter (2016), MLE was sprayed on Foeniculum vulgare, and gradual increases in plant height, number of branches, fresh and dry weight were noted. It was revealed that spraying the plants with a 30% MLE concentration had the highest effect in both seasons. Hoque et al. (2020) used MLE to improve the growth, yield, and nutritional status of cabbage. A significant difference in these parameters was observed between the cabbage sprayed and the unsprayed cabbage. The sprayed cabbage treatment had superior results. MLE improves the volatile oil content, radical scavenging activity and total phenolic content of coriander (Mazrou, 2019). According to Merwad (2017), increases in plant height, pod length, number of pods per plant, root length, chlorophyll a, b, and carotenoids, which ranged from 7.8 to 49%, 38 to 103%, 39 to 132%, 9 to 85%, 17 to 48%, 25 to 71% and 35 to 104%, respectively, were observed in the 2014 and 2015 seasons.

The present knowledge makes certain that Moringa leaves, seeds, and pods are rich in antioxidants, including carotenoids such as lutein, alpha-carotene, beta-carotene, and xanthin, as well as chlorophyll (Fuglie 2005; Merwad 2015). Nasir et al., (2016); Brockman & Brennan, (2017). For example, Merwad, (2017) justified the consideration of MLE as an alternative to synthetic agrochemicals due to its ability to enhance growth, yield, and plant physiology under abiotic stresses (e.g., drought and salinity). However, no study has investigated the potential effectiveness of MLE for enhancing the growth of plants in stressful environments such as tailings. Therefore, investigating the use of MLE in enhancing the growth of vetiver grass on compost-amended tailings and footprint areas is novel.

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CHAPTER THREE

METHODOLOGY

3.1 INTRODUCTION

This chapter highlights the research design that governs the data collection and analysis. The methods employed and their relevance, such as the sampling methods used, sample analysis techniques and methods, as well as data collection and analysis, are described and discussed. However, specific details of the experimental designs are presented in the Materials and Methods sections in chapter 4 of this thesis.

This study was conducted in two phases. Phase 1 was the greenhouse study, during which the study parameters were optimized. These experiments included testing combinations of different compost percentages (0%, 30% and 60%) and biostimulant types (laboratory extracted MLE and commercial MLE) to determine the parameters that yielded optimal results in terms of biomass production and metal accumulation, as these are the important determining factors for successful remediation of both tailings and footprint substrates. However, there were some challenges that were overlooked at project inception. These limitations included the impracticability of conducting field studies on historic orphaned tailings and footprint areas due to the safety risk of accessing those sites for planting and monitoring of grass due to illegal mining activity at abandoned sites. Additionally, the grass could be uprooted from the site. As a result, safer and protected new footprint and tailing sites were sought in phase 2 (the field study).

3.2 STUDY SITES

The Witwatersrand is the world’s largest gold mining region. It was dominated by gold production throughout the twentieth century. A third of the total gold produced worldwide can be traced back to the Witwatersrand goldfields (Tucker et al., 2016). Over 52 000 tons of gold have been mined from the Witwatersrand since 1886 (Tucker et al., 2016). The Witwatersrand landscape is therefore characterized by tailings dams, which, according to GDARD (2012), are said to cover 321 km^2 of the area. Figure 3-1 below shows the three study sites where the research was conducted.

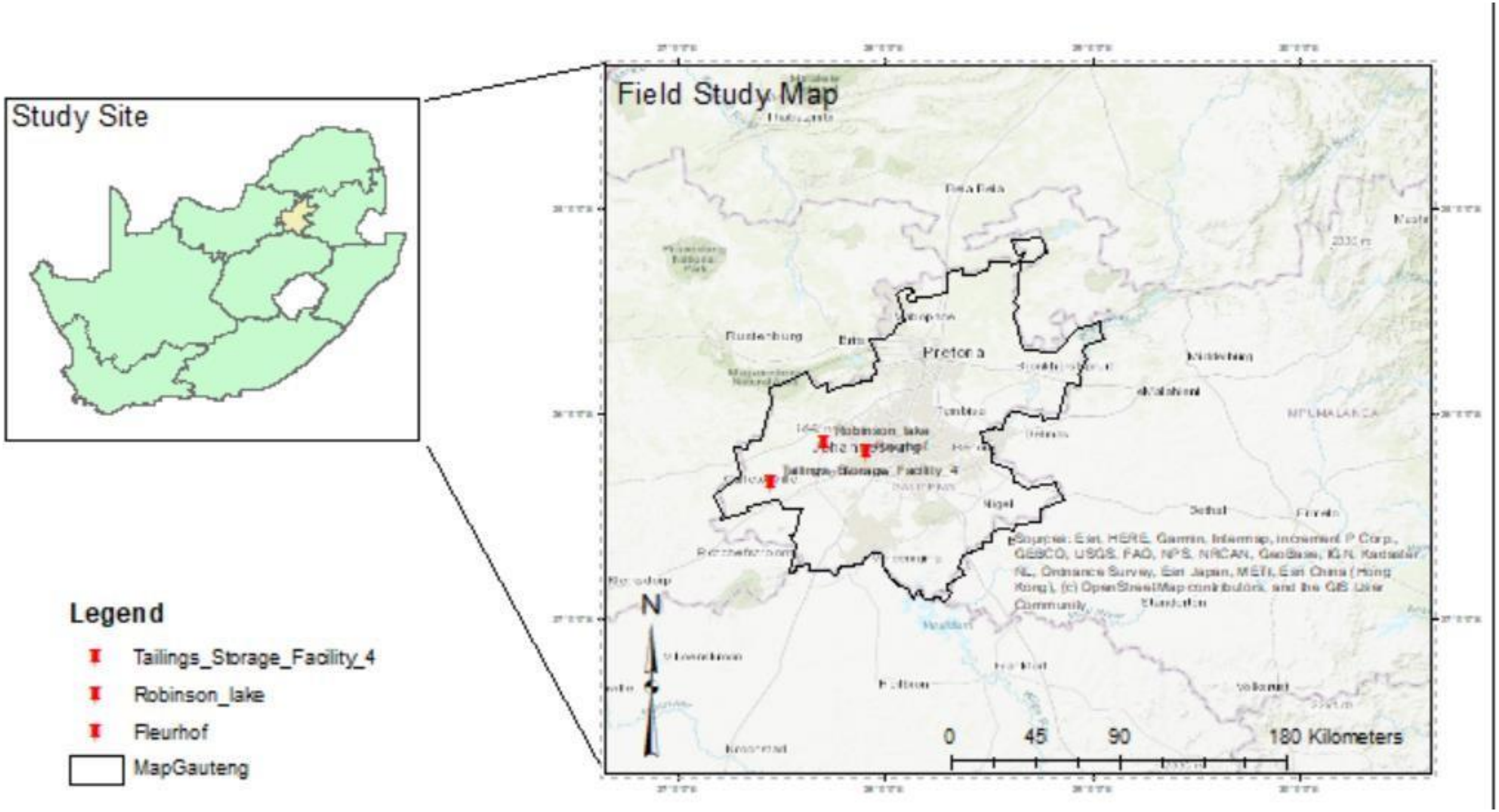

Figure 3-1: The map showing the three study sites: The tailings storage facility 4; the Robinson lake (DRD 6), and the historic tailings dump near Fleurhof Dam

The Witwatersrand is also known for its vast agricultural and industrial activities. However, the metal toxicity levels in this region are not suitable for agricultural activities. Elevated concentration levels in tailings dam samples have been reported. For example, the concentrations of Cd, Pb, and Cu have been reported to range between 0.001 mg/kg and 22.890 mg/kg; 0.001 mg/kg and 184.200 mg/kg; and 0.001 mg/kg and 929.000 mg/kg, respectively (Abiye et al. 2018a). These metals can be leached from tailings dams to the land underneath and surrounding areas. In addition, high metal concentrations were recorded in the reclaimed sites (footprint areas). Against this background, the Witwatersrand goldfield was selected as the study site.

3.2.1 Greenhouse study

The greenhouse experiment is designed such that the extrinsic variability is reduced, and the effect of the variable factor can be scrutinized while other factors are held constant. Plant interactions in natural plant communities are met by logistic and analytical constraints. For instance, in natural environments, large numbers of different species are highly likely to exist, and varying environmental factors can lead to spatiotemporal heterogeneity. In contrast, greenhouse environments are artificially manipulated to minimize variability. Environmental parameters are controlled; hence, they are uniform, with plants of specific ages being used (Tilman, 1987; Stiling, 1992). The precision, repeatability and experimental control of these methods make greenhouse experiments favourable (Harper, 1982; Hairston, 1989). Hairston (1989) argues that the use of greenhouse study is appealing due to the high degree of experimental control, repeatability, precision, and amenability to rigorous statistical design. However, scholars such as Diamond (1986) have noted undoubted limitations, which include a lack of realism, which limits the ability to apply the results to complex natural systems. Moreover, long-term experiments involving perennial plant species such as vetiver grass are especially unrealistic because pot experiments affect root growth. Recently, the continuous change in climatic conditions has become another issue of concern with greenhouse results. However, greenhouse studies allow for relative efficiency determination (Connolly et al., 1990) as well as separation of species interaction components (Goldberg 1990). In support of the greenhouse concept, Gibson et al. (1999) argue that interactions under controlled environments are important. Often, the aim of greenhouses is to generate predictions that can then be tested under field conditions. Therefore, a preliminary assessment of this study was conducted under greenhouse conditions.

3.2.1.1 Substrate collection

The samples that were used in the greenhouse were collected from the tailings dam next to the Fleurhof Dam. An auger was used to extract the soil samples from the surface to a depth of approximately 25 cm. The soil samples from the tailings dam were then transported to a Hoffmeyer greenhouse at the University of the Witwatersrand. Representative samples of the tailings were taken to the laboratory for analysis to determine the physical and chemical properties of the tailings prior to planting (Figure 3-2).

Figure 3-2: Samples that were collected from the study sites and brought to the Laboratory for analysis

3.2.1.2 Vetiver grass preparation

Vetiver grass tillers, which were donated by Hydromulch, were transported to the greenhouse. In the greenhouse, the grass was removed from the original potting bags and trimmed to a height of 25 cm. This procedure was used to discount the initial size differences to adequately assess the growth parameters over the experimental period and to make a fair comparison of the different treatments. Figure 3-3 shows the vetiver grass before transportation to the greenhouse.

Figure 3-3: Four weeks old vetiver grass delivered to the greenhouse in Johannesburg from the supplier (Hydromulch pty ltd) for the experiment

3.2.1.3 Compost

The compost was purchased from a local nursery and was used in the amelioration of the tailings. The type of compost used is shown in Figure 3-4 below.

Figure 3-4: The Culterra organic compost used in the experiment

3.2.1.4 Moringa leaf extracts

Two types of MLE were investigated for their effectiveness in enhancing vetiver grass growth and metal uptake in the phytoremediation of compost-amended gold mine tailings. The laboratory extracted MLE was considered ideal in this study due to its potential to create an economy through the MLE production value chain, unlike commercial MLE. Producing Moringa at the local level could be a vehicle for creating employment and skilling communities. Communities can be trained to grow Moringa and sell it to the mines and other industries where it can be utilized. This will promote agricultural and economic activity, which could otherwise not exist. However, further considerations of laboratory MLE was benchmarked against that of commercial MLE.

Laboratory Extracted moringa leaf extract

Moringa leaf extract was prepared from dry Moringa leaves. The extraction procedure used for MLE preparation in the laboratory is shown in Figure 3-5 below. The details of the extraction methodology are presented in the materials and method section 4.2 of chapter 4.

Figure 3-5: Picture showing the filtration of MLE (left) and evaporation of alcohol during the extraction process of MLE in the laboratory

Commercially available Moringa leaf extract

Commercially available MLE was obtained from the market for comparison with the laboratory extracted MLE. The composition of the commercially available MLE used in the study is shown in Figure 3-6. Further details of the methodology presented in chapter 4, Materials and Methods section.

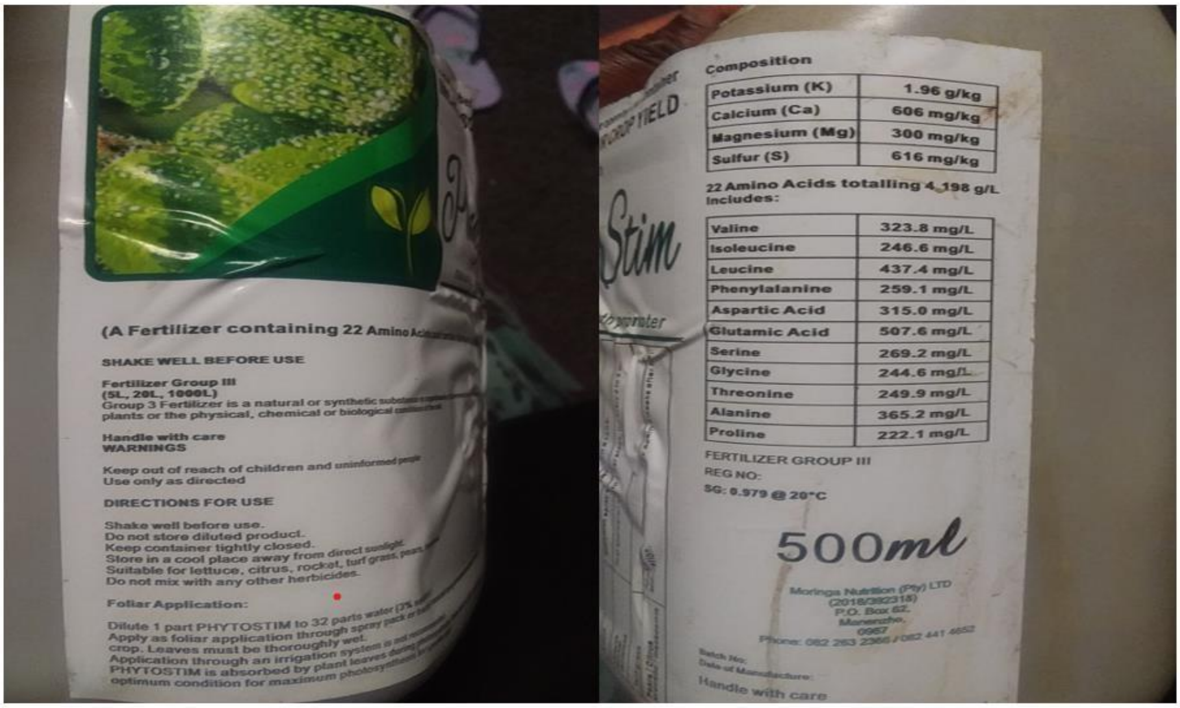

Figure 3-6: Labels showing the composition of the commercially available MLE that was used in the study

3.2.1.5 Greenhouse experiment setup

The tailings material from the abandoned mining sites was amended with 0%, 30% or 60% compost amendments, and the 2.5 L pots were labelled and set up in a greenhouse, as shown in Figure 3-7 below. The detailed experimental design is presented in chapter 4, Experimental Design section, of paper 1.

Figure 3-7: Preparation of material for the greenhouse experiment, each treatment with three replicates

3.2.2 Field experiment

The field experiments were conducted in two environments: the tailings footprint area and the tailings storage facilities. The footprint area was utilized as a rock dump site, and for many years, this land was compacted by the rocks that were removed from the surrounding mine shafts. The two study sites are shown in Figure 3-8: the footprint (left) and tailings storage facilities (right). The detailed experimental design is presented in chapter 4, Experimental Design section, of paper 2.

Figure 1-8: The image of vetiver grass in the second week after planting on the footprint and tailings in November 2021 at the Witwatersrand Goldfields

The vetiver grass was monitored for 16 weeks prior to harvesting (Figure 3-9).

Figure 3-9: Harvesting of vetiver grass after the end of the 16-week period at the Driefontein Rock Dump (left) and the tailings storage facility (right)

3.2.3 Analysis

This section provides a general overview of the analysis methods. The detailed methodologies for both the greenhouse and field studies are presented in Chapter 4 of this thesis (paper 1 and paper 2, respectively).

3.2.3.1 Soil and Plant sample preparation, measurement, and method validation

Prior to the analysis of both the soil samples and plant samples, the soil and washed plant samples were dried, ground to powder and sieved to obtain the desired particle sizes. Before analysis, accurate measurement of the sample is critical for quantifying the compounds in the sample. In this study, a balance was used to measure the mass of the samples used in the analysis. A 5% error in the sample volume leads to a 5% change in the calculated amount. The method validation parameters included blanks, calibration curves and running standards between sample analyses. The experiments were conducted using high-purity chemicals and glassware that were washed with soap and rinsed with acidified deionized water and deionized water before being dried in an oven at 40°C. This procedure was used to ensure that the experiments were as accurate and precise as possible, with minimal risk of contamination. A balance (Figure3-10) was used to measure the samples prior to analysis.

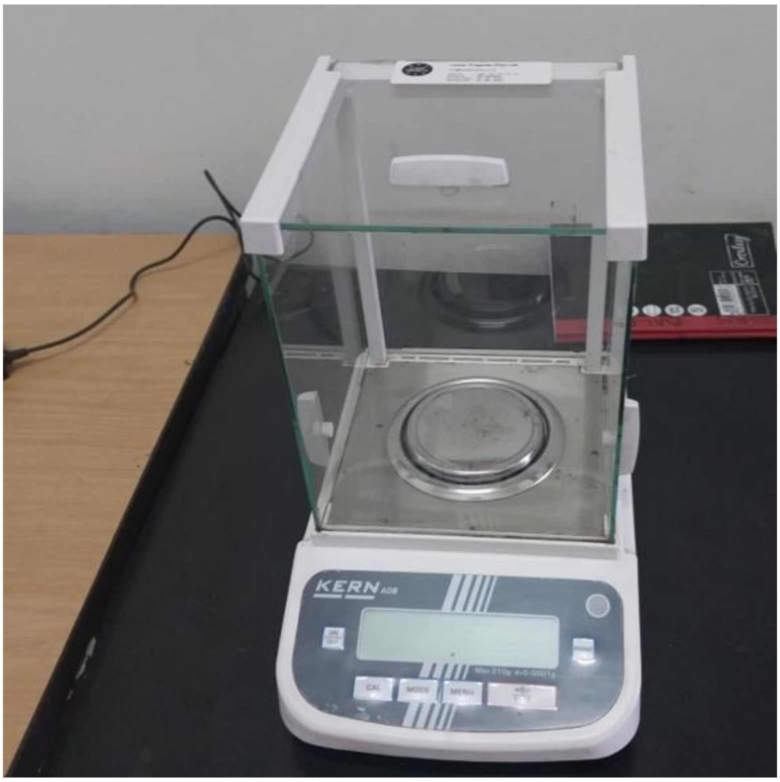

Figure 3-10: The balance used for weighing the materials in preparation for analysis

3.2.3.2 pH and electrical conductivity

The EC was measured in units called "seimens per unit area" (e.g., mS/cm or MiliSeimens per centimetre). Prior to measuring the pH and EC, the pH meter was calibrated using standard buffer solutions with a pH of 4, 7 or 10. The standard calibration fluid supplied by the manufacturer was used to calibrate the EC meter. The prepared samples and the pH and EC meter used are shown in Figure 3-11 below.

Figure 3-11: Picture of samples of known mass prepared for pH and conductivity analysis

3.2.3.3 Element concentration measurement

Multianalyte procedures have become relevant tools in analytical chemistry because they allow the analysis of several compounds or elements with a single sample pretreatment, thus saving time and resources. After acid digestion of the sample material, the samples are subjected to inductively coupled plasma optical emission spectroscopy (ICP–OES), where nebulized particles form fine aerosols that flow into the plasma where desiccated, vaporize into gases, and finally dissociate into atoms that can be ionized. In the plasma, atoms and ions become excited, reverting to their ground state with the emission of light, which is measured using an optical spectrometer. All the elements present in the radiation source emit their characteristic spectra at the same time. The advantages of ICP–OES include that it can be used to measure almost all the elements in a periodic table with detection limits in the range of micrograms per litre to milligrams per litre, that ICP–OES has a wide dynamic concentration range, and that quantitative analysis of multiple elements can be performed within a minute. However, ICP–OES is tremendously sensitive and thus highly appropriate for trace element analysis. Figure 3-12 shows an ICP–OES image and a schematic diagram.

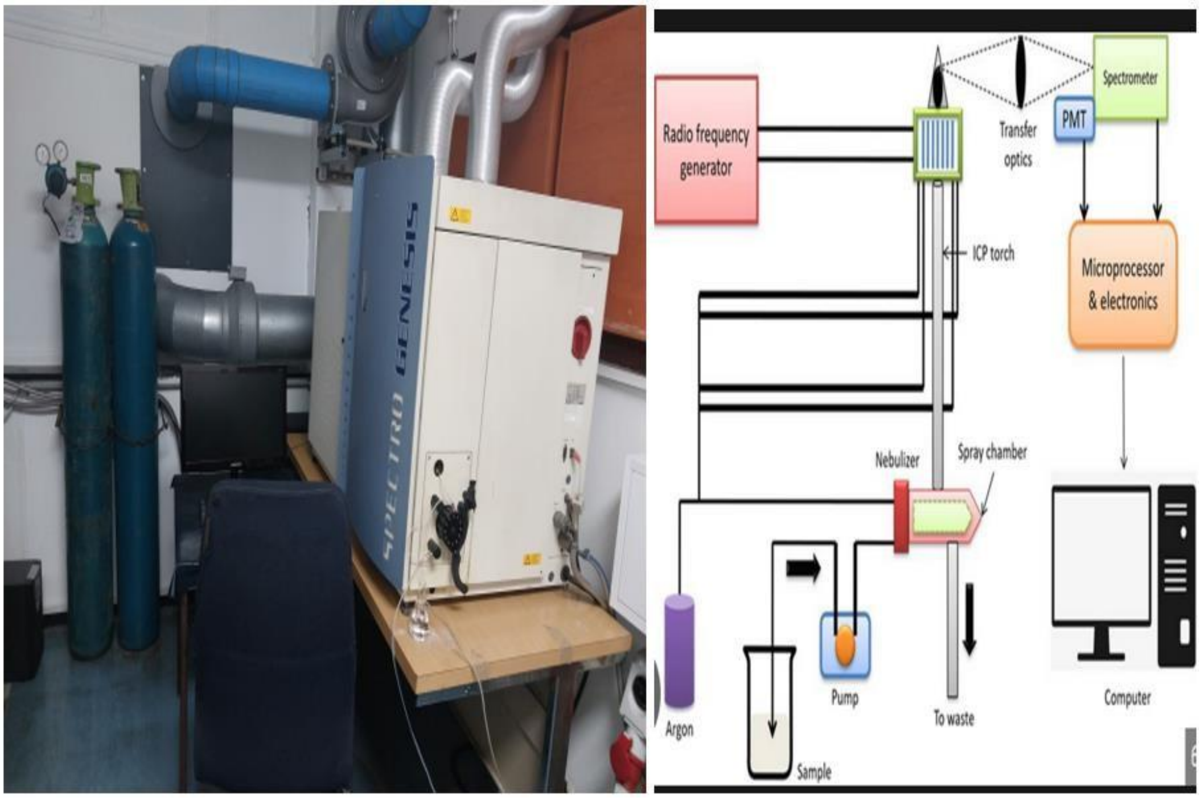

Figure 3-12: The picture of the ICP–OES used in the analysis of element concentrations (left) and a schematic diagram of the ICP-OES (Khan et al., 2022)

At the end of the experiments, all the digested material was poured in the waste container marked inorganic waste and the solid waste, both grass, soil and tailings that were brought in the laboratory for analysis was put in plastic bags and marked as hazardous waste for the waste collection company to dispose of accordingly.

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CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 OVERVIEW OF THE CHAPTER

In this chapter, the empirical evidence gathered through the research is presented and analysed to answer the research questions posed in chapter 1. The evidence is presented in a clear and concise manner and is accompanied by appropriate statistical analysis to ensure that the results are reliable and valid. The findings of this study are presented in two papers that are currently under review. The key data presented in these papers is critical for determining whether the process used for phytoremediation can be applied in the future. This evidence provides valuable insight into the efficacy of the approach and its potential for application on a larger scale.

The first paper explored the optimal compost percentage, biostimulant type, and biostimulant application frequency for phytoremediation using vetiver grass. Through a greenhouse experiment, these parameters were optimized to identify the best conditions for phytoremediation. The greenhouse experiment provided valuable insights that guided the field study and improved the likelihood of successful phytoremediation. In addition to phytoremediation efficiency, cost-effectiveness, the production of useful byproducts, and other potential benefits were also considered when selecting the optimal treatment for the field study. This comprehensive approach ensures that the chosen treatment is not only effective but also sustainable and provides additional benefits. By considering all of these factors, treatments can be selected based on their overall impact and benefits rather than just their phytoremediation efficiency. This approach ensures that the chosen treatment is the most beneficial for the environment and society.

The second paper presents the real-world results of phytoremediation treatment, which was applied to the footprint and tailings storage facilities. This study provides valuable insights into the effectiveness of treatment in a real-world setting rather than a controlled environment. Unexpected results and challenges that were encountered during the field study are also highlighted. The results from both papers are analyzed and discussed in detail, and conclusions and recommendations are made based on the findings. This study highlights the importance of considering a wide range of factors when selecting a treatment for phytoremediation to ensure the most effective and sustainable solution.

4.1 SYNERGISTIC EFFECT OF COMPOST AND MORINGA LEAF EXTRACT BIOSTIMULANTS ON THE REMEDIATION OF GOLD MINE TAILINGSUSING CHRYSOPOGON ZIZANIOIDES

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ABSTRACT

Phytoremediation is a cost-effective and environmentally friendly method for restoring degraded land. However, optimizing phytoremediation conditions and using locally sourced materials can further improve the effectiveness and sustainability of this approach. This paper investigated the synergistic effect of compost and two types of moringa leaf extract (MLE). A 3x2x2 fully crossed factorial design was used to optimize the phytoremediation parameters. The treatments consisted of three compost concentrations (0%, 30% and 60%), two biostimulant types (laboratory extracted MLE and commercial MLE) and two application regimens (once a week and twice a week). Each treatment had three replicates, and a randomized complete block design was used. The experiment was performed over a sixteen-week period. Thereafter, the biomass and metal concentrations in the vetiver grass were measured. Two-way ANOVA was used to analyse the data to determine the significance of the differences between the treatments. All the vetiver that was planted in the unamended tailings died within four weeks despite the MLE treatment. Vetiver grass plants that received 60% compost and laboratory-extracted MLE twice a week (60% L2) had the highest biomass production, followed by vetiver grass that received 60% compost and laboratory-extracted MLE once a week (60% L1). There was no significant difference in biomass production due to the application frequency in plants grown in 30% compost amendments and sprayed with commercial biostimulant (30% C1 and 30% C2). The 30%C1 and 30%C2 treatments had lower biomass production than did the 60%L1 and 60%L2 treatments but still had significantly greater biomass production than did the other treatments that were investigated. The metal accumulation data was inconclusive, as most of the toxic metals were not accumulated by vetiver grass. As a result, the optimal treatment was chosen based on biomass production and cost effectiveness. The 30% C1 treatment provides a cost-effective method and results in significant plant growth improvement.

Keywords: sustainable phytoremediation, commercial moringa leaf extract, biostimulant, moringa leaf extract, vetiver grass, compost, mine tailings, mining

4.1.1 Introduction

Mining and quarrying operations have led to the disruption of 0.4 million square kilometres of global land area (Hooke et al., 2012). This problem is compounded by the annual deposition of approximately five billion tons of mining waste, or tailings, into the environment (Lu & Wang, 2012). In South Africa, for example, a total of 200,000 hectares of land were transformed for mining in 1997, with an additional 47,000 hectares used to store more than 471 million metric tons of mine waste (Twerefou, 2009). In addition, the large-scale disruption of land for mining and quarrying operations has been linked to chronic health risks and hazards (McSwane et al., 2015); in particular, the surrounding communities are at risk due to the environmental impacts of mining (Durand, 2012). To mitigate these impacts, many countries have enacted laws that require mining companies to take responsibility for remediating the environmental damage caused by their operations.

Phytoremediation, a low-cost, eco-friendly approach that harnesses the power of plants to remove contaminants from the environment, has been widely recognized as a viable alternative to more expensive and potentially harmful traditional remediation methods (Shah & Daverey, 2020). Phytoremediation has also been praised for its ability to improve food safety, promote the development of renewable energy sources, and sequester carbon (Claveria et al., 2010). Phytostabilization and phytoextraction are the two most common phytoremediation technologies (Yan et al., 2020). Phytoextraction is a phytoremediation technique in which plants absorb contaminants from the soil or water and accumulate them in their aboveground biomass (Antonkiewicz et al. 2022; Rafati et al. 2011). Phytostabilization is a phytoremediation technique that involves establishing vegetation to concentrate metals in the roots or change their chemical state in the rhizosphere (Mahajan & Kaushal, 2018).

In recent years, there has been a shift in phytoremediation research toward more sustainable approaches (Hou & Al-Tabbaa, 2014; Montanarella & Vargas, 2012). The concept of 'sustainable remediation' has been a turning point for the remediation field, establishing new norms and standards for practitioners (Hou & Al-Tabbaa, 2014). Sustainable remediation not only addresses environmental concerns but also provides value-added benefits and economic opportunities for communities in the areas being remediated. The use of locally extracted MLE biostimulants is a promising approach for phytoremediation. This method combines the advantages of cost-effectiveness and value-added benefits of locally sourced materials. For example, communities may be able to produce MLE that can be used in phytoremediation while simultaneously creating new employment opportunities and revitalizing communities. As such, sustainable remediation offers a win–win solution for both the environment and local economies.

du Jardin, (2015) defines biostimulants as “a group of substances from natural origin that contribute to boosting plant yield and nutrient uptake, while reducing the dependency on chemical fertilizers”. The discovery of moringa leaf extract (MLE) as a biostimulant has gained importance in recent years (Rady & Mohamed, 2015). *Moringa oleifera* leaves contain a unique combination of antioxidants, nutrients, and possibly hormones that can enhance plant growth. Its balanced composition and natural properties make it an excellent alternative to synthetic growth promoters (Yasmeen, 2011). MLE has been extensively studied as a biostimulant for horticultural crops, but its potential for enhancing growth in mine-impacted soils has not been fully explored. This untapped potential presents an opportunity for further research into the use of MLE for phytoremediation purposes. The goal of this study was to compare the efficacy of a laboratory extracted MLE as a biostimulant to that of a commercially available biostimulant under different compost amendments on vetiver grass planted on compost amended gold mine tailings. The ultimate goal is to determine the economic, environmental, and social benefits of using biostimulants for the remediation of mine-impacted soils.

Vetiver grass is known ability to tolerate a range of environmental conditions, including those found in mine-impacted soils, and due to its potential for biomass valorization, a process of extracting value from plant biomass in the form of products or services. This includes carbon sequestration, the production of goods such as paper and textiles, and the generation of biofuels. The concept of biomass valorization is supported by several studies, including Danh et al. (2009a) , who showed that vetiver grass can produce high amounts of biomass (>100 t/ha/year) and can potentially be utilized as a raw material for handicrafts, essential oils, raw material for pulp, paper, and fiberboard (Darajeh et al., 2019). While Vetiver grass is not native to South Africa, it has been found to be non-invasive and does not pose a threat to native flora or fauna (Truong, 2003). In a greenhouse study conducted by (Melato et al., 2016), Vetiver grass was found to thrive and accumulate more metals in its roots than in its leaves when grown on tailings from the AngloGold Ashanti West Wits site in Gauteng, South Africa. This finding suggested that vetiver grass could be an effective phytostabilization agent for this type of contaminated site. However, the use of remedies, such as compost, is important for ensuring that plants can thrive in tailings. Without amelioration, plants may be stunted and unable to achieve the desired results. Organic amendments such as compost are preferred over inorganic chelating agents because they reduce metal bioavailability immediately and gradually, add slow-release fertilizer, provide a microbial inoculum, and improve soil structure and cation exchange capacity. In addition, organic matter reduces erosion and increases infiltration of mine tailings (Mendez et al., 2007; Schippers et al., 2000; Schroeder et al., 2005).

4.1.2 Materials and Methods

4.1.2.1 Study Site and Design

Fleurhof is a settlement located in the Witwatersrand Basin, Gauteng Province, South Africa. This area is characterized by historic tailings dumps and are the main source of pollution, which they release into the surrounding environment through acid mine drainage and erosion of tailings deposits (Salomons, 1995). Tibane & Mamba (2022) found that surrounding communities are prone to health risks and that due to their economic status, they cannot relocate; therefore, mitigation measures are ethical. The study site climate was described by Humphries et al. (2017) as having winter and summer temperatures averaging 15°C and 20°C, respectively. The annual rainfall is between 600 mm and 700 mm (Humphries et al., 2017). Figure 4-1 below shows the Google Earth image of the study site.

Figure 4-1: Google map image of the sampled area, a historic tailing storage facility near Fleurhof Dam in Johannesburg, South Africa

4.1.2.2 Design and preparation of trials

The gold mine tailings were collected using a soil auger at depths between 5 cm and 30 cm on tailings adjacent to the Fleurhof Dam (26°11′53.4"S 27°54′33.5"E). The samples were stored in polypropylene bags and transported to the greenhouse, where they were amended with Culterra organic compost manufactured from dead decaying plant material. The compost was purchased from Schäffler's Garden Nursery at 28 Johannesburg Road, Lyndhurst, Johannesburg. The experimental design is shown in figure 4-2 below.

Replicate 1	Replicate 2	Replicate 3
0%0	0%L1	30% 0
0%C1	0%C1	0%0
30% C2	30%L2	0%C2
60% C1	0%L2	30% C2
60% C2	30% L1	0%L1
30% 0	60% L1	0%C1
30%L2	60% C1	60% C2
60% L1	60% L2	30% C1
0%L1	0%C2	60% L1
60% 0	30% 0	60% C1
60% L2	30% C1	30% L1
0%C2	60% 0	60% 0
30% C1	30% C2	60% L2
30% L1	60% C2	30%L2
0%L2	0%0	0%L2

Figure 4-2: Experimental design for the greenhouse showing three replicates of each experimental pots randomly arranged in the greenhouse. 0%, 30% and 60% is the percentage compost amendments, C and L is the commercial and laboratory Moringa leaf extract

Forty-five 2.5 L pots containing 2.0 L of substrate material were used in this experiment. Fifteen pots contained nonamended tailings, another fifteen contained tailings and compost homogeneously mixed at a ratio of 7:3 (v/v), and the last set of 15 pots contained tailings and compost homogeneously mixed at a ratio of 4:6 (v/v). One -month -old vetiver tillers donated by Hydromulch (Bapsfontein, Gauteng Province, South Africa) were removed from the soil that came with them and pruned to a height of 20 cm to establish the baseline height. The tillers were subsequently transplanted into the prepared potting pots. Within each compost treatment (0%, 30% and 60% compost amendments), the pots were randomly assigned to one of the 5 biostimulant treatments: commercial biostimulant sprayed once a week (C1), commercial biostimulant sprayed twice a week (C2), laboratory-extracted biostimulant sprayed once a week (L1), laboratory-extracted biostimulant sprayed twice a week (L2), and no biostimulant (O). Three replicates were used for each treatment to validate the empirical data. The 45 pots were arranged in accordance with a completely randomized block design in a glass greenhouse in Johannesburg, South Africa. The greenhouse humidity ranged from 55–65%. The daytime temperature was 30 °C, and the nighttime temperature was 20 °C. Table 4-1 provides a description of the different treatments used in the experiment.

Table 4-1: Description of different treatments under which vetiver grass was exposed to for 16 weeks

Biostimulant	0% compost	30% Compost	60% Compost
No Biostimulant	0%O	30% O	60% O
Lab Extracted Biostimulant once a week	0%L1	30% L1	60% L1
Lab Extracted Biostimulant twice a week	0%L2	30%L2	60% L2
Commercial Biostimulant once a week	0%C1	30% C1	60% L1
Commercial Biostimulant twice a week	0%C2	30% C2	60% L2

Samples of compost and tailings (before mixing) were taken to the laboratory for analysis.

4.1.2.3 Biostimulant preparation and application

The commercial biostimulant was purchased from a commercial supplier in Pretoria, South Africa. The solution was diluted at a ratio of 1:33 (v/v) with tap water to make a 3% (v/v) solution that was used in the experiment. The dry moringa leaves for the laboratory MLE were obtained from Moringa Community Farm in Hammanskraal, South Africa. The MLE was extracted according to the methods of Rady & Mohamed (2015). The moringa leaves were ground to powder using a blender. MLE was obtained by blending 300 g of moringa powder with 675 ml of 80% ethanol as suggested by Makkar and Becker (1997). The mixture was incubated for 4 hours on an orbital digital shaker, after which the volume was increased to 1000 ml. The extract was then centrifuged at 4000 rpm for 15 minutes, followed by filtering using No. 2 Whatman filter paper and vortexing on a rotary evaporator. The extract was used to spray vetiver grass during the experiment.

4.1.2.4 Chemicals and reagents

Analytical grade chemicals, solvents and reagents were used. Chemicals such as hydrochloric acid (HCl), nitric acid (HNO_3), hydrofluoric acid (HF) and hydrogen peroxide (H_2O_2) were purchased from Fisher Scientific (London, UK). A direct-Q 3 UV Millipore system (Massachusetts, USA) was used to prepare ultrapure water that was used to dilute stock solutions and a range of working standard solutions.

4.1.2.5 Instrumentation

An analytical balance (Precisa 180A, Switzerland) was used to measure the mass of the sample. The digestion of plant and soil samples was performed using a Multiwave 3000 microwave digester (Anton Paar, Switzerland). Metals were analysed using inductively coupled plasma optical emission spectroscopy (ICP–OES) (Spectro, Kleve, German). A Bante 900P multiparameter water quality meter (Bante Instruments) was used to measure electrical conductivity (EC) and pH.

4.1.2.6 pH and Conductivity of the tailings

A mass of 2,000 g of the material was weighed into a 50 ml plastic centrifuge tube. A volume of 40 ml of deionized water was added, and the mixture was subsequently placed on an end-to-end rotator for 10 minutes prior to measuring the pH and EC.

4.1.2.7 Vetiver Grass Preparation for analysis

At harvest, the entire plant was removed from the pot. The number of tillers and leaves and the length of the leaves were measured. The plants were then washed under running tap water, rinsed in 0.10% HNO_3 to remove any metal on the surface and finally rinsed with distilled water. The roots and shoots were separated and air-dried in the laboratory at room temperature until they reached a constant weight. The dry masses of the roots and leaves were recorded.

4.1.2.8 Element content: tailings

The tailings material was dried in the laboratory at room temperature for 10 days, homogenized and sieved. To evaluate the total metal concentration, a mass of approximately 0,250 g of < 25 µm particles was weighed and placed into acid-washed digestion tubes (PTFE-TFM liners), into which 9 ml of HCl, 3 ml of HNO_3, 1 ml of HF and 6 drops of H_2O_2 were added before being placed into the microwave digester. Extraction was performed in 60 minutes. The solution was transferred to 50 ml centrifuge tubes, which were filled with deionized water. All the samples were filtered through 22 µm Whatman Puradisc syringe filters prior to analysis.

The metal concentrations in 30% and 60% of the compost amendments were obtained by using proportions. The formula $Cmix = (C_1 x P_1) + (C_2 x P_2) + \ldots$, where C_1, C_2, etc., are the concentrations of each individual metal and P_1, P_2, etc., are the proportions of each metal in the mixture was used to determine the metal concentration in the amended tailings.

4.1.2.9 Element content: vetiver grass

The dried leaves and roots were ground to powder using a blender. A mass of approximately 0,1000 g of ground material was placed in a microwave digestion vessel. To this mixture, 8 mL of HNO_3 and 2 mL of H_2O_2 were added. Digestion was carried out for 30 minutes in a microwave digester. The digested samples were then transferred to 50 ml centrifuge tubes, which were filled with deionized water. All the samples were filtered through 22 µm pore filters prior to analysis.

4.1.2.10 Chemical analysis

ICP–OES was used to determine the concentrations of the elements in the tailings, compost, vetiver roots, and leaves. A total of 16 elements consisted of trace metals such as cadmium (Cd), cobalt (Co), chromium (Cr), arsenic (As), nickel (Ni), lead (Pb), and copper (Cu); essential macro- and micronutrients, including phosphorus (P), magnesium (Mg), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), sulphur (S), zinc (Zn) and aluminum (Al) were determined. The method for determining the concentrations of the metals in the plant parts and soils was validated using standard methods. The concentrations of the calibration standards were 0,100, 0,200, 0,500, 1,00 and 2,00, and the average coefficient correlation (r) was 0,8997.

4.1.2.11 Data Processing and Statistical Analysis

The data were processed and statistically analyzed using the Data Analysis Tool Pack in Microsoft Excel. The presented results are an average of triplicate samples. The values for the plant height are in cm, the mass was measured in grams (g), and the concentrations are expressed in mg/kg of plant or tailings dry weight. Two-way ANOVA with replication was used to determine statistically significant differences between the treatments.

4.1.3 Results and discussion

4.1.3.1 Conductivity and pH

The pH and EC of the tailings material and compost were measured prior to mixing. The average pH and EC of the unamended tailings were 3.10 and 2280 µS/cm, respectively. The compost had an average pH of 8.57 and an average EC of 2788 µS/cm. The initial (after compost amendment) and 16-week (final) EC and pH were measured, and the results are presented in Figure 4-3 and 4-4 below. The 30% and 60% compost-amended tailings both showed slight increases in EC and pH at the initial measurement. At week 16, the pH increased by at least 1 log unit for the 30% compost-amended tailings and an average of 2 log units for the 60% compost-amended tailings. The immediate slight increase in pH and EC noted during the initial measurement may be due to the addition of compost, which had a basic pH. The slight increase in EC noted during the initial measurement was transient, as demonstrated by a reduction in EC at 16 weeks. Initial measurements of different amended tailings revealed an increase in EC followed by a decrease in EC at week 16 to an average of 1130 µS/cm and 1304 µS/cm for the 60% and 30% compost-amended tailings, respectively. This trend was also noted in studies by Gil-Loaiza et al. (2016) and Melato et al. (2016). Gil-Loaiza et al. (2016) reported a significant positive relationship in response to compost amendment ($R^2 = 0{,}959$ $P < 0{,}205$). The decrease in EC could be due to the uptake of cations from the tailings by vetiver grass, leading to a decrease in the number of metal ions (Melato et al., 2016). Biostimulant application did not result in any differences in the measured pH or conductivity among the various treatments.

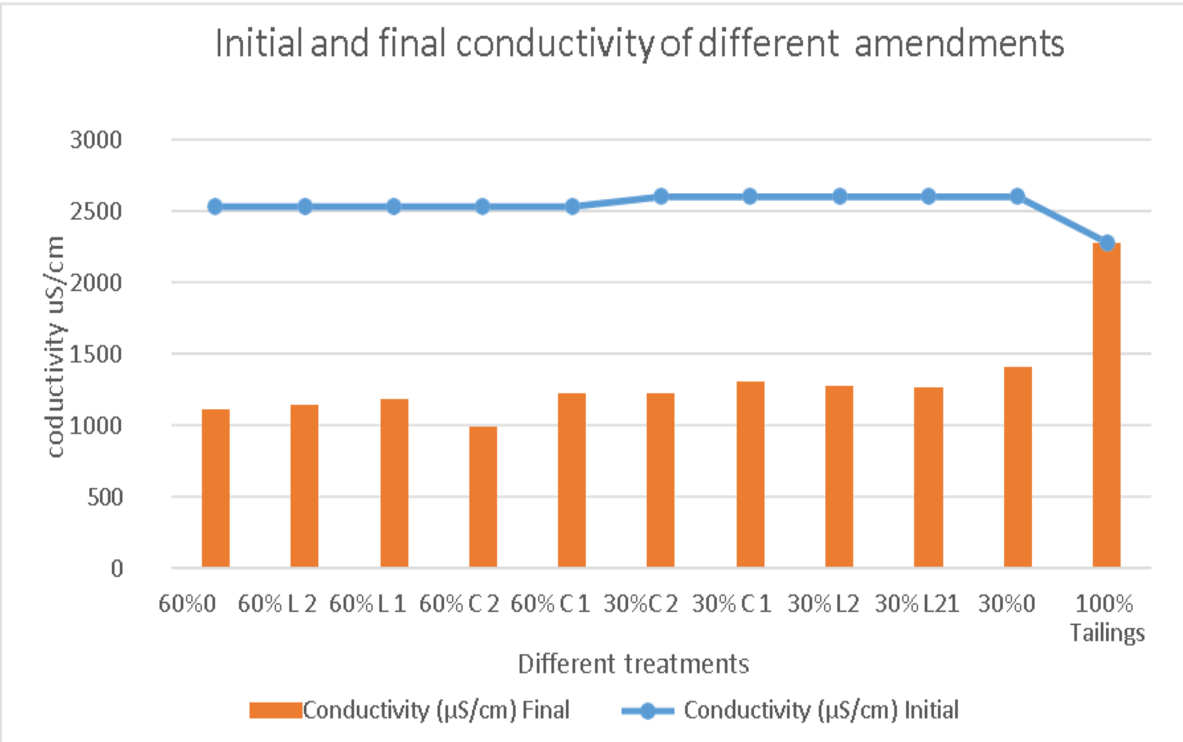

Figure 4-3: Initial and final EC of different compost amendments

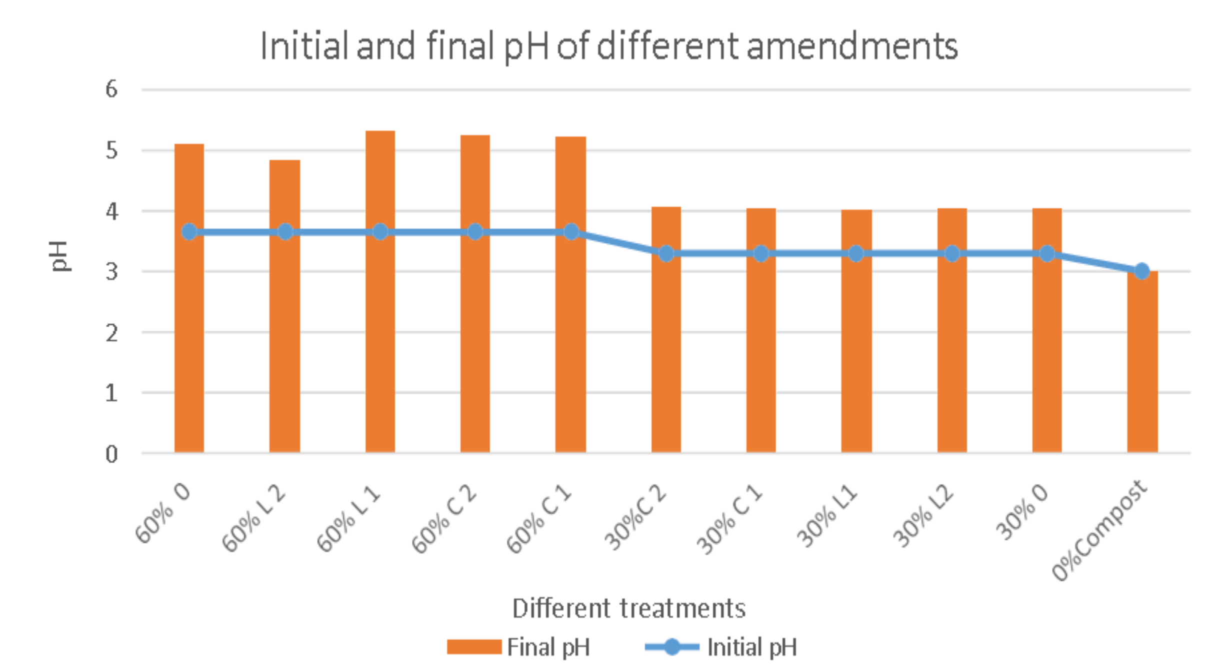

Figure 4-4: Initial and final pH of the different compost amendments

4.1.3.2 Growth parameters

All the grass that was planted in the pots containing unamended tailings died within four weeks, regardless of whether they were sprayed with MLE. This indicates that the unamended tailings were not suitable for plant growth, and that MLE alone was not sufficient to promote plant survival in this environment, likely due to metal toxicity. Toxicity can interfere with important cellular processes, damage membrane integrity, and ultimately cause plant death (Manara 2012). These findings align with the results of Arthur et al. (2016), who also reported that *B. juncea* seedlings could not survive in unamended Krugersdorp Goldmine soils, regardless of biostimulant treatments used. These findings support the use of compost to improve the suitability of mine- impacted environments for plant growth.

Previous research has shown that biostimulants can improve plant growth and tolerance to abiotic stresses in agricultural soils for example, (Yasmeen et al., 2013; Elzaawely et al., 2017; Zulfiqar et al., 2019; Abdalla, 2013a; Aluko et al., 2017; Merwad, 2017; Mvumi et al., 2013a; Rady & Mohamed, 2015). In this study, the performance of the biostimulant was dependent on the presence of compost. The effects of biostimulants on tailings could not be achieved without compost. This novel finding that compost is a critical factor in the effectiveness of biostimulants in tailings may need further investigation.

However, it is important to note that Melato et al. (2016) found that vetiver grass did not exhibit any signs of stress or death 16 weeks after planting in unamended acidic tailings, which contrasts with the findings of this study. The difference in these study findings could be due to differences in the composition of the tailings or the growth conditions.

The grass that were planted on the 30% and 60% compost amendments survived, and images of the vetiver grass plants from the different treatments are shown in Figure 4-5 below.

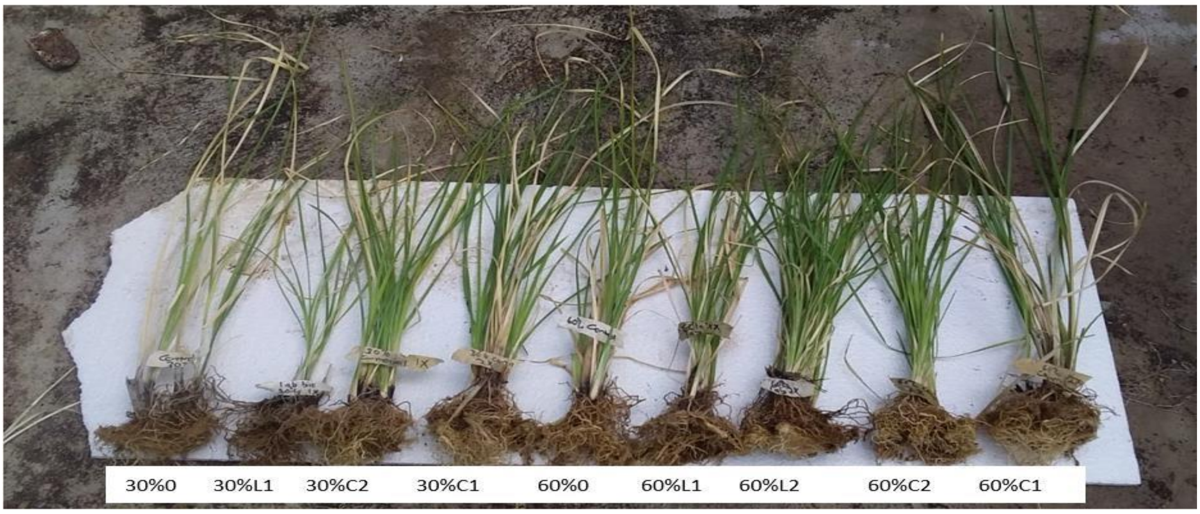

Figure 4-5: Images of vetiver grass harvested from different treatment group after 16 weeks

It is evident from the presented image that 60%L2 had the most biomass, while 30%L1 had the least biomass. A few leaves in the 30%0, 30%L1 and 30%L2 amendments showed a light-yellow color appearing on the edge of some older leaves. A summary of the growth parameters from the different treatments is provided in Table 4-2 below.

Table 4-2: Average number of leaves, number of tillers, vetiver leaf length, root dry mass and leaf dry mass (n=3) at week 16 measured on vetiver harvested from different treatments

Parameters	**Leaf length (cm)**	**No. of leaves**	**No. of Tillers**	**Root dry mass (g)**	**Leaf dry mass (g)**
60% 0	47.95±5.81	9.88±4.94	3.25±0.96	8.82±3.34	5.22±2.03
60% L2	34.18±3.16	16.63±8.21	6.40±2.22	29.28±7.32	17.93±5.24
60% L 1	44.68±6.76	12.25±6.02	4.50±1.73	19.62±6.45	11.64±4.54
60% C 2	40,13±4.09	8.63±4.34	3.25±1.50	15.23±5.32	8.01±3.07
60% C 1	51.55±10.07	8.63±5.78	3.25±1.26	15.29±6.01	7.21±2.75
30% 0	48.65±3.21	9.63±3.89	3.50±0.58	7.54±3.24	3.44±1.21
30% L 2	33.99±5.44	9.13±2.17	3.25±0.54	7.01±3.96	2.34±0.76

30% L 1	33.63±7.23	9.00±2.27	3.25±0.50	6.57±2.31	2.02±0.95
30% C 2	44.05±6.61	9.50±3.16	4.25±0.96	16.03±6.02	8.91±2.77
30% C 1	49.40±2.54	9.38±4.41	3.50±1.29	15.44±6.29	7.74±2.99

The compost amendment increased the soil pH and organic carbon, which likely reduced the activity of chemoautotrophic sulphur oxidizers. This in turn promotes the development of the carbon and nitrogen cycles, which are indicative of healthy soils. Compost amendment can influence the diversification of the microbial community, which are beneficial to plant growth (Solís-guez et al., 2012; Johnson & Hallberg, 2008; Zornoza et al., 2015). However, there was no significant difference ($p = 0.97$) in growth parameters between the 30% and 60% compost amendments (without biostimulant) (Figure 4-6).

These findings suggested that both amendments had similar effects on plant growth. The effects of compost may reach a saturation point at a certain concentration. Increasing the amount of compost beyond that point may not lead to further improvements in plant growth. Excessive amount of compost can cause problems such as nutrient imbalances, waterlogging, and reduced oxygen levels in the soil. These conditions can stunt plant growth and can even lead to plant death. This could explain why there was no significant difference ($p = 0.99$) in growth parameters between the 30% and 60% compost amendments.

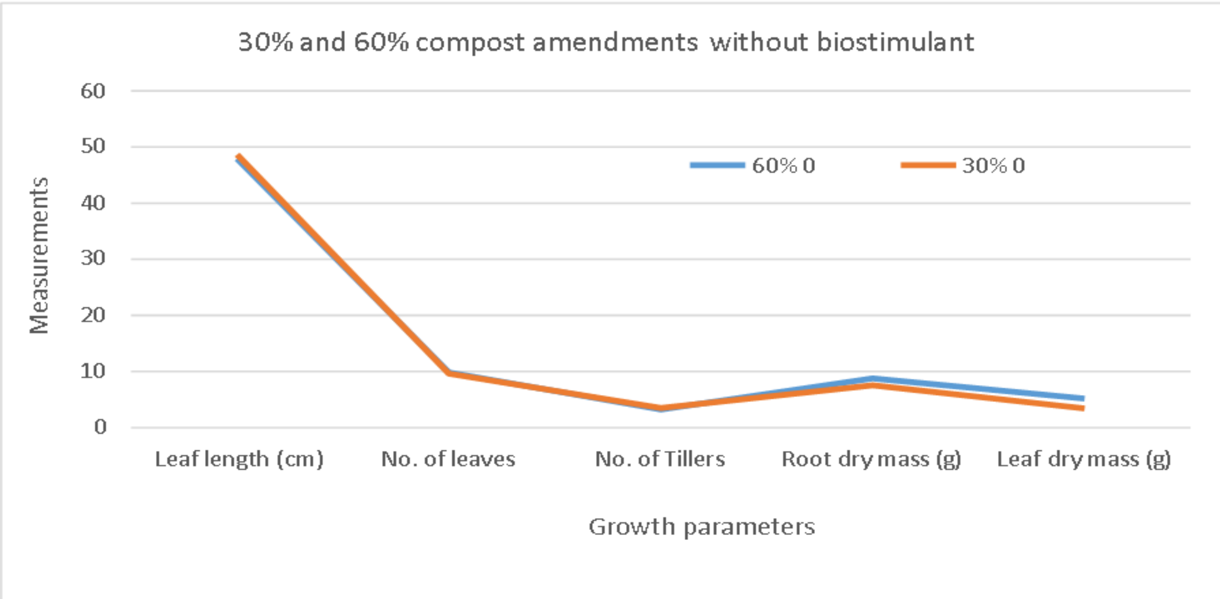

Figure 4- 2: Effect of 30% and 60% compost on the length, number of leaves, root and leaf dry mass of vetiver grass planted in the footprint and tailings material in the Witwatersrand Goldfields in South Africa

The application of laboratory extracted MLE twice a week produced vetiver with significantly (p = 0.02) improved length, root and leaf dry mass results than the commercial MLE when applied to vetiver grown on 60% compost otherwise there was no significant difference between the application of commercial or laboratory MLE when the application is done once a week. (Figure 4-7).

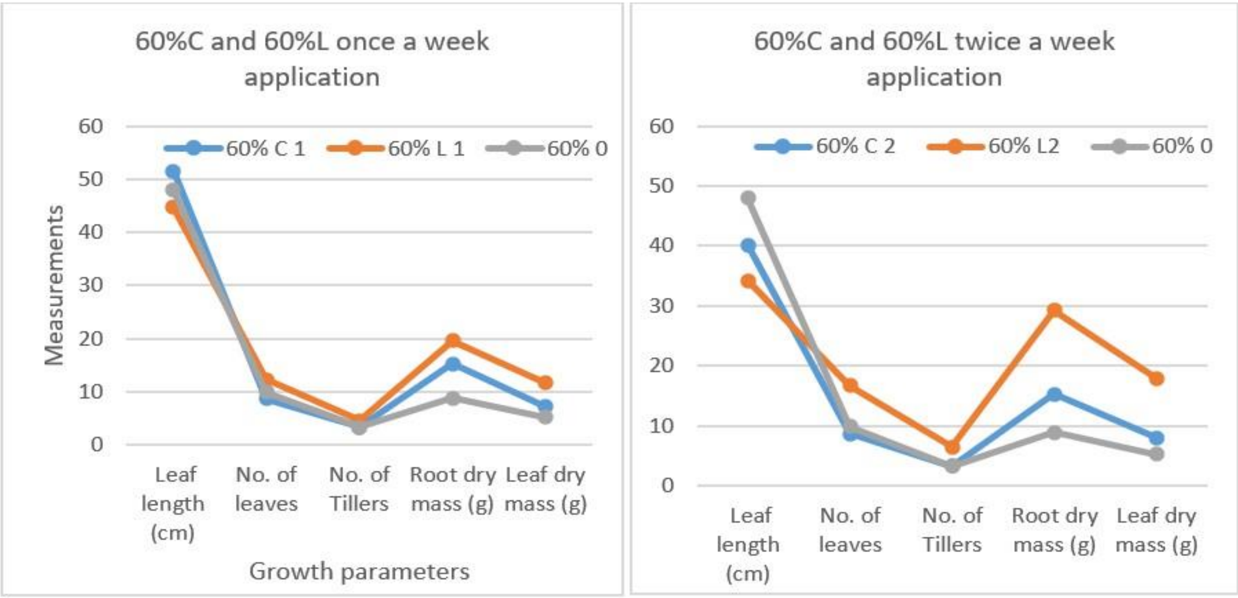

Figure 4-7: Effect of laboratory and commercial Moringa leaf extract on vetiver grass planted on tailings amended with 60% compost

The effect of spraying frequency was investigated on vetiver grass grown in 60% compost amendment. There was no significant difference (p = 0.95) between the two application frequencies (once or twice a week) spraying with commercial MLE biostimulant except on the leaf length where a significant difference was observed (p =0.03) (Figure 4-8). On the contrary, a significant difference (p = 0.02) amongst leaf length, root and leaf mass on vetiver grass sprayed once a week and twice a week with laboratory-extracted MLE was observed.

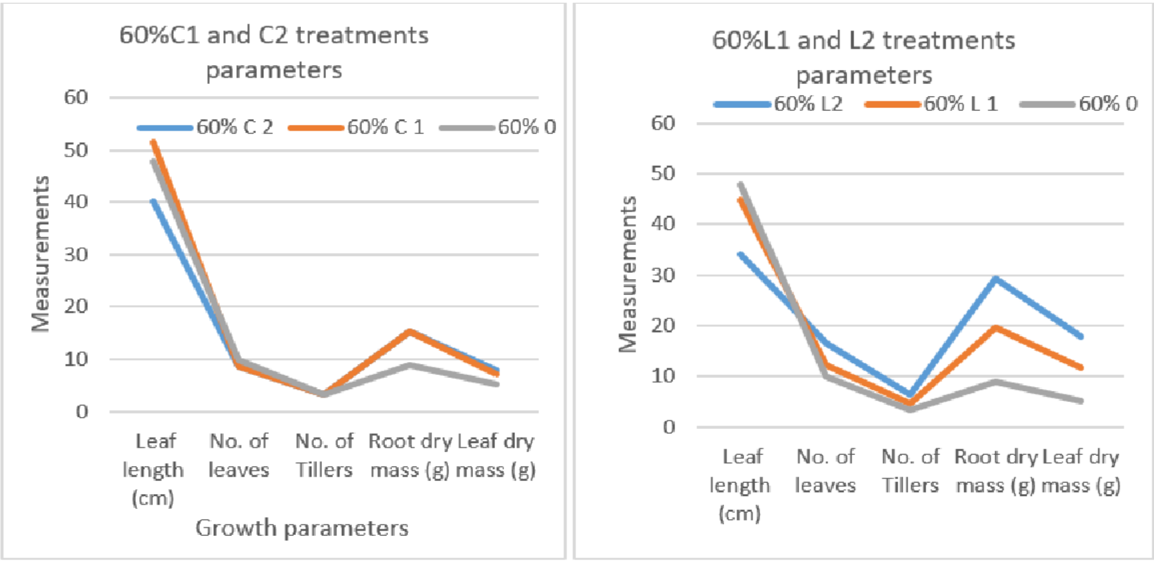

Figure4-8: Effect of spraying frequency on vetiver grass grown on 60% compost amendment

In the 30% compost amendment, the commercial MLE treatment led to a significant increase ($p = 0.01$) in root and leaf dry masses when applied once or twice a week, whereas the application of laboratory extracted MLE once or twice a week had no effect on all the measured growth parameters. Surprisingly, a significant decrease ($p = 0.01$) in the length of the leaves sprayed once or twice a week with laboratory extracted MLE was observed (Figure 4-9).

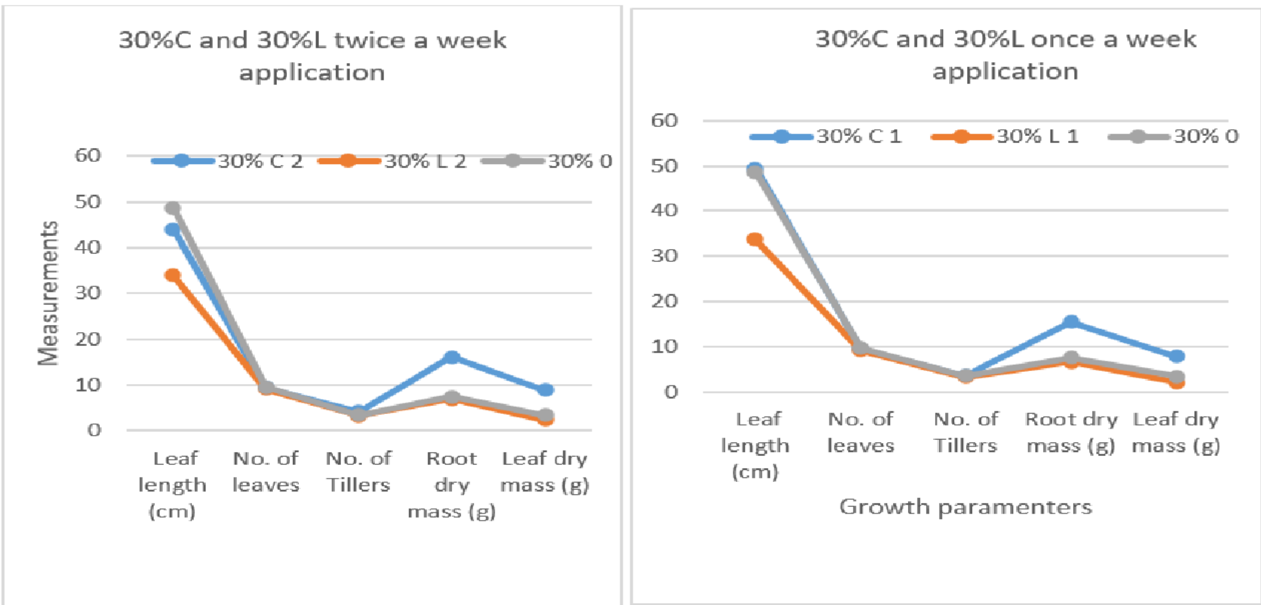

Figure 4-9: Comparison of the effects of the laboratory extracted biostimulant and commercial biostimulants on vetiver grass grown on 30% compost amendments

For both biostimulant types in the 30% compost treatment, the application frequency did not lead to any significant difference ($p = 0.98$) in growth parameters. The results are presented in Figure 4- 10.

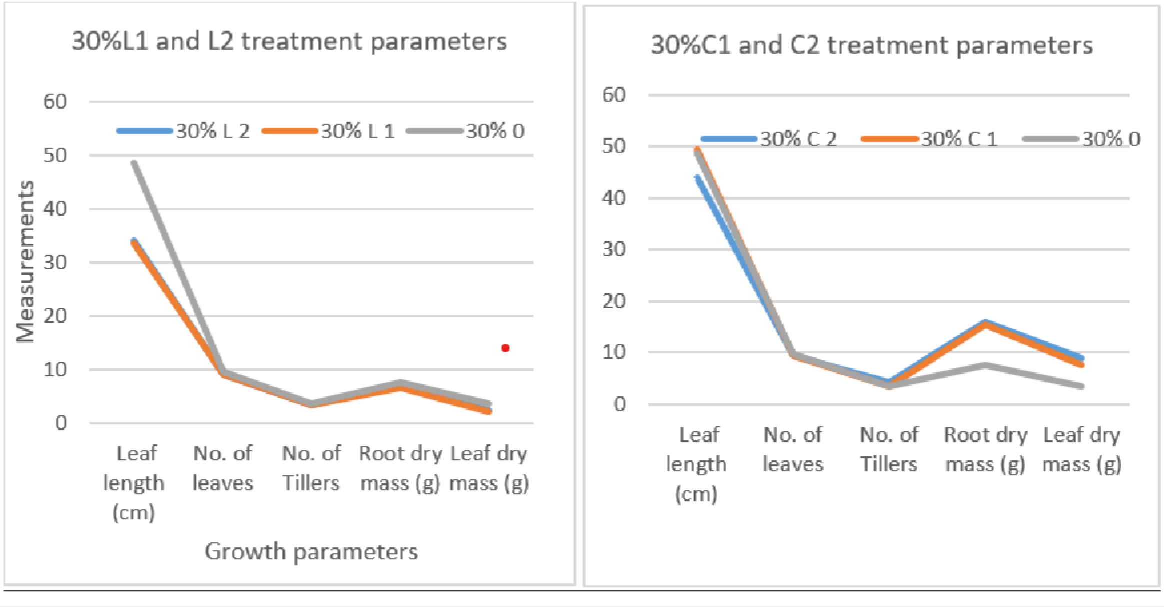

Figure 4-10: Effect of spraying frequency on vetiver plants grown on 30% compost amendments

Biostimulants are known to contain cytokinins and auxins (Aremu et al., 2015), which are expected to stimulate shoot initiation and plant growth (Bulak et al., 2014). This has proven to be true, as shown by the high number of tillers and leaves in treatments such as 60%L1, 60%L2, 30%C1 and 30%C2. However, the reason why laboratory extracted MLE biostimulants perform better on vetiver cultivated on tailings amended with high amounts of compost and why commercial MLE biostimulants perform better on vetiver amended with low amounts of compost are unknown but can be attributed to a possible difference in chemical composition between the two types of MLE. The variation in the chemical composition of the biostimulants may be linked to the difference in the extraction methods used.

4.1.3.3 Initial element concentrations of the unamended tailings and compost

The baseline concentrations of elements in the unamended tailings material and compost were measured and are presented in Table 4-3 below.

Table 4-3: Baseline metal concentrations in unamended tailings and compost

Sample	100% Tailings	Compost	30% compost amendment	60% compost amendment
As	90.73	62.69	82.34	73.91
Cd	19.70	61.69	32.30	44.89
Co	38.85	51.24	42.53	46.26
Cr	84.36	345.77	162.78	241.21
Cu	90.53	127.36	101.58	112.63
Ni	52.73	121.89	73.48	94.23
Pb	58.17	57.21	57.83	57.57
Zn	149.42	198.01	163.99	178.57
Al	10756.07	17684.58	12834.62	14913.18
Ca	10139.28	11583.08	10572.42	11005.56
Fe	55045.76	40850.75	50787.26	46528.75
K	795.46	6714.43	2571.15	4346.84
Mg	1769.63	2758.71	2066.33	2363.07
Mn	257.46	431.84	309.77	362.09
P	1092.32	14717.41	5179.85	9267.37
S	21997.61	3886.57	16564.35	11130.98

The levels of As, Cd and Cr, in the tailings were above the South African soil screening values (SSV) 1 and SSV 2 (As, Cd, Cr) and Cu, and Pb exceeded SSV1 only. SSV1 are soil quality values that are protective of both human health and eco-toxicological risk for multi-exposure pathway including watercourse. SSV2 means soil quality values that are protective of risk to human health in the absence of a water resource. Only the SSV2 informal residence values have been provided in this study. As and Cd are classified as group 1 carcinogens, while Cr and Pb are classified as group 2 carcinogens by the International Agency for Research on Cancer. The health risks associated with these metals include various types of cancer, cardiovascular disease, respiratory problems, neurological disorders, and developmental delays in children. Therefore, corrective actions are inevitable.

4.1.3.4 Element concentrations at week 16

The concentrations of the elements measured at week 16 in the vetiver grass leaves, roots and amended tailings are presented in Figure 4-11 to 4-13 below. Of the 16 elements investigated, 14 were detected in the amended tailings. Cd and Co concentrations were below the detection limits in the tailings and Cd, Co, Cr, As, Ni and Pb were below the detection limits in leaves and roots. P, Mg, K, Ca, and Mn were greater in the leaves than in the roots of the grasses across all the treatments. The Fe and Al concentrations were greatest in the roots, while the Zn concentration in the roots and leaves varied from treatment to treatment. The 60% L2 treatment had the highest concentrations of Zn and Mg in its leaves compared to those in the other treatments. The second highest Zn concentration in the leaves was recorded in the 30% C2 treatment group. The highest P concentration was recorded in vetiver grown in 60%0 treatment, while the roots of plants in this treatment had the lowest P concentration. The 30%C1 and 30%C2 treatments presented higher P concentrations in the leaves than in the roots. The roots presented higher concentrations of Al and Fe than the leaves across all the treatments. The concentration of K in the leaves was highest in the 60%0 treatment group.

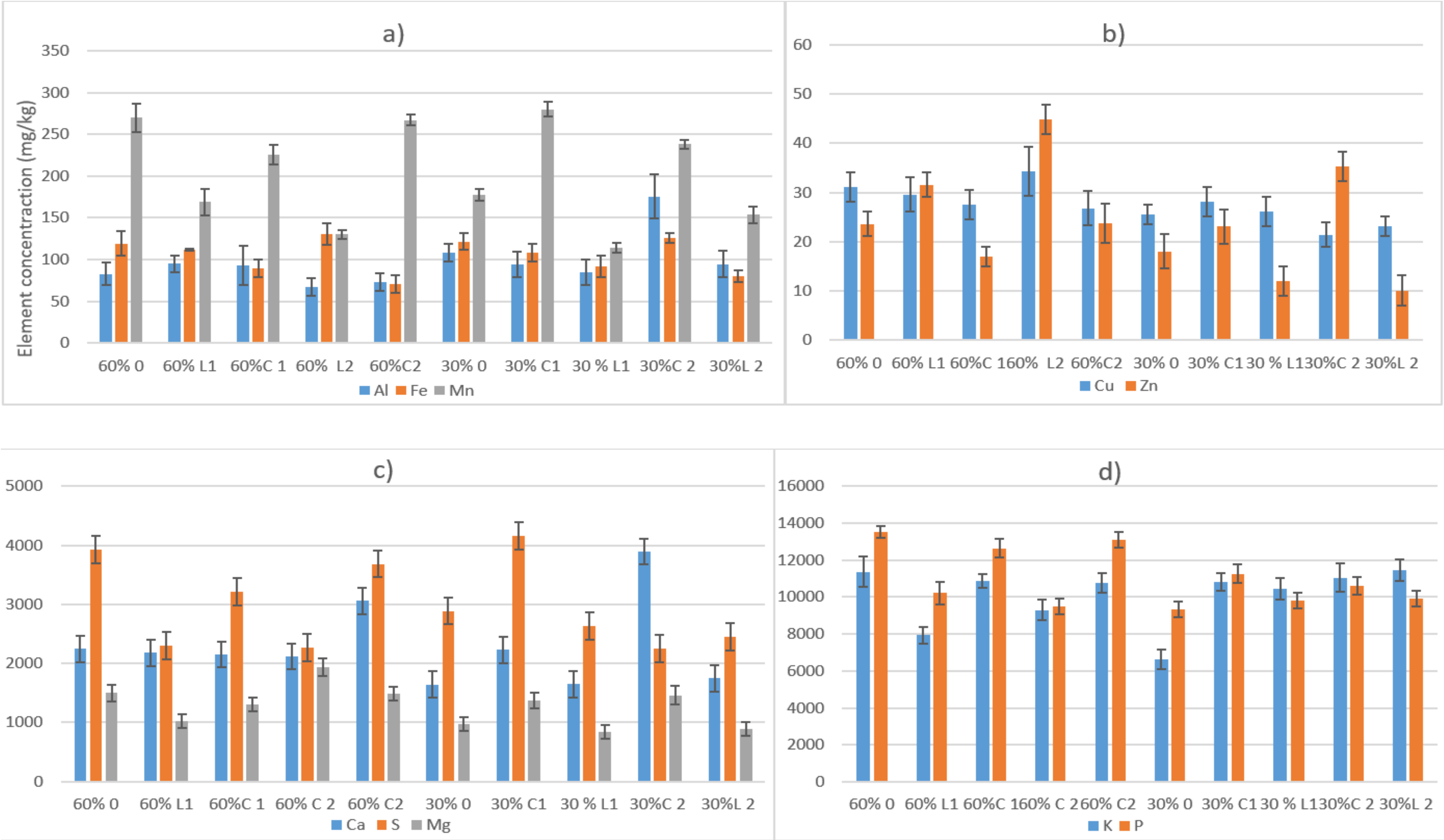

Figure 4-11: a) Al, Fe and Mn; b) Cu and Zn; c) Ca, S and Mg; d) K and P concentrations in the vetiver grass leaves from different treatments

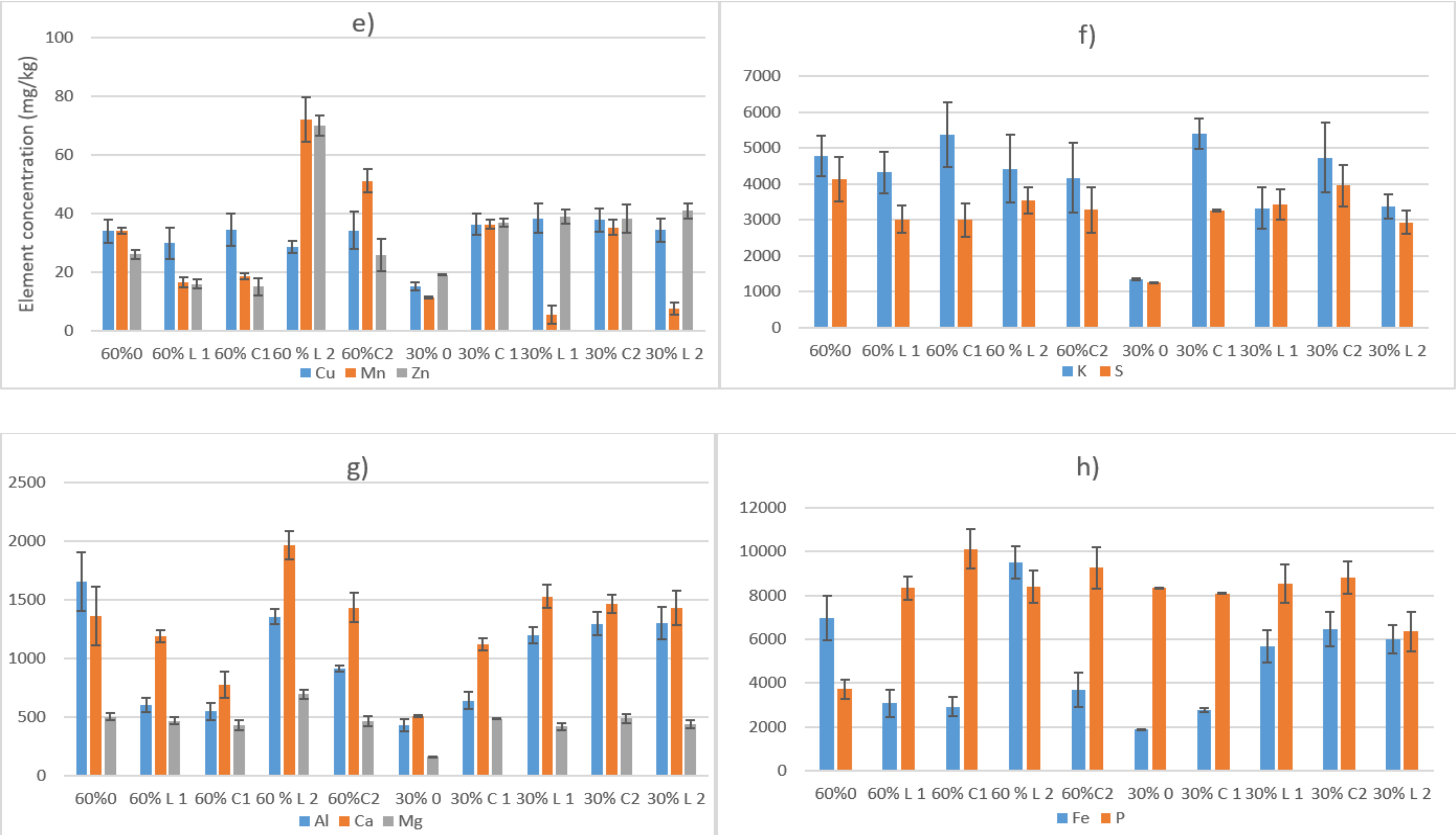

Figure 4-12: e) Cu, Mn, and Zn; f) K and S; g) Al, Ca and Mg; h) Fe and P concentrations in the roots of vetiver grass from different treatments

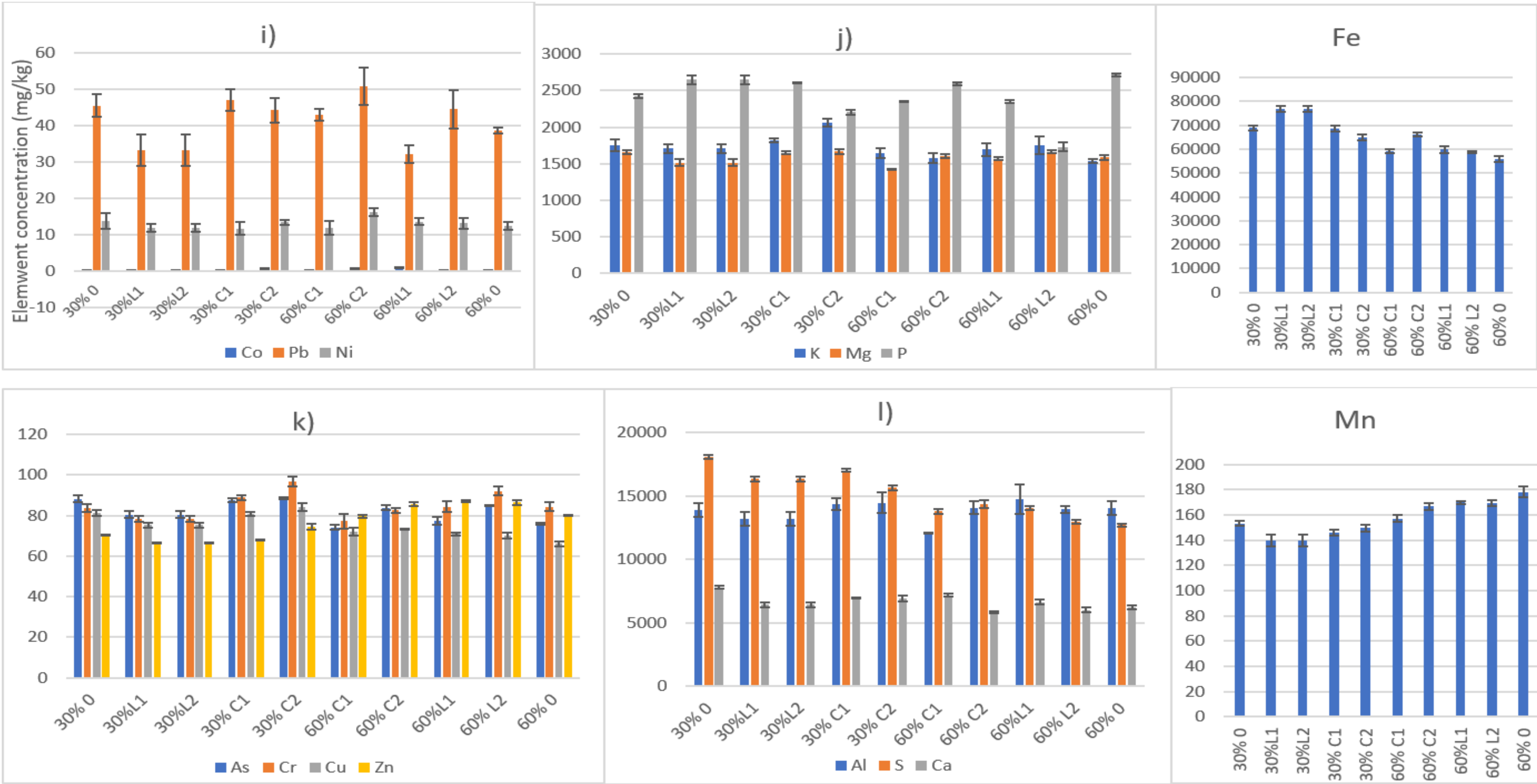

Figure 4-13: Concentrations of Mn, Fe, i) Co, Pb and Ni; j) K, Mg and P; k) As, Cr, Cu and Zn; and l) Al, S and Ca in amended tailings measured at week 16

The undetected levels of Cr, As, Ni, and Pb in the leaves and roots of vetiver grass grown in both 30% and 60% compost-amended tailings are likely the result of metal adsorption by the compost (Li et al., 2021; Brown et al., 2004), which led to low bioavailability of metals in compost-amended tailings, as suggested by (Bennett et al., 2003). In addition to adsorption, the increase in pH caused by compost amendment can also lower the bioavailability of metals. For example, (Bech et al., 1997) reported that the available fraction of As is largely determined by soil pH, even in tailings containing high As concentrations.

Although the results of this study did not demonstrate the uptake of metals of concern by vetiver grass, the observed increase in root and leaf biomass in the compost-amended tailings in the 30% C1, 30% C2, 60% L1 and 60% L2 treatment groups indicates that this technique may be effective at stabilizing contaminants in the rhizosphere. Singh (2012) argues that the objective of phytostabilization is to establish plants in contaminated soils and trap metals in the rhizosphere instead of extracting them from the soil (Ernst, 2005). The vegetative cap, plant–root exudates, organic matter, and established microbial community promote the stabilization of inorganic contaminants in the root zone (Mendez & Maier, 2008;Santibañez et al., 2012). While Wuana & Okieimen (2011) suggested that vegetation cover can complex or reduce the valence of metals in the rhizosphere, they also noted that the accumulation of metals in the roots by vegetation cover also reduces metal mobility, thereby improving the phytostabilization process.

However, the results of this study differ from the findings of Andra et al. (2009); Danh et al. (2009a); Datta et al. (2011); Truong, (2016), who reported that vetiver can accumulate high levels of toxic metals in its roots and shoots. According to Melato et al. (2016), vetiver grass can take up toxic metals from tailings and accumulate them in its roots, limiting translocation to the shoots, which is desirable for phytostabilization. Zhang et al. (2014) observed high accumulation of Cd in vetiver grass exposed to Cd. Banerjee et al., (2016, 2019) confirmed the findings that vetiver uptake and accumulate large amounts of Cd and can be utilized in the reclamation of Cd-contaminated soil. The findings of this study were unexpected and can be attributed to the physical-chemical properties of the tailings being strongly influenced by compost amendments.

Vetiver grass is highly likely to absorb nutrients such as P, K, Mg, Mn, Cu and Ca at elevated concentrations. This could also explain the ability of vetiver to grow under the experimental conditions, as these elements are essential for plant growth. Overall, the least nutrient accumulation in the roots and leaves of vetiver grass was recorded in the 30%0, 30%L1 and 30%L2 treatments. This explains the poor growth of vetiver grass under these treatments and the signs of chlorosis noted in vetiver grass subjected to these treatments. Although vetiver grass is not as effective as some other plants at accumulating heavy metals, its wide tolerance to adverse climate and soil conditions makes it a promising candidate for phytostabilization. Its high biomass has the potential for carbon offsetting and bioenergy production while also providing a valuable source of material for phytoproducts. These benefits can improve the economic activities and livelihoods of local communities.

4.1.4 Conclusions

The results of the study showed that there was no significant difference between the once a week and twice a week MLE application frequency except between the 60%L1 and 60%L2 treatments, where the vetiver biomass in the 60%L2 treatment was significantly greater. Therefore, the application frequency is generally not a significant factor in determining plant biomass.

Among the 60% compost amendment treatments, the laboratory-extracted MLE-treated plants produced the highest plant biomass. In the 30% treatment group, the plants treated with commercial MLE produced the highest biomass compared to plants treated with laboratory extracted MLE. Generally, the order of increase in growth parameters was 60% L treatments >30% C treatments > 60% C treatments > 60% 0 treatments = 30% 0 =30% L treatments. The results suggest that the interaction between the biostimulant and compost has the most significant influence on plant biomass. This conclusion is supported by the fact that the effect of the biostimulants alone did not prevent plants from dying when they were grown in unamended tailings. These findings suggested that it is important to obtain right ratios of the compost and biostimulants to achieve the desired results.

The results showed that vetiver grass could not accumulate some of the metals of concern (As, Cd, Cr and Pb) in its biomass. The lack of significant accumulation of metals in vetiver grass does not negate the phytostabilization of mine tailings using compost, vetiver, and MLE. Although metal accumulation in plant tissue is one of the measures of phytostabilization, vegetation establishment is the most important factor in the phytostabilization of mine tailings because it improves soil stability, prevents erosion, provides aesthetic benefits and provides other phytoproducts and services. Future studies should, however, focus on finding the lowest effective compost percentage when combined with laboratory extracted MLE, which can maximize biomass production and the phytoavailability of metals. Consequently, the laboratory moringa leaf extraction method must be optimized. The ultimate goal is to create a cost-effective, sustainable environmental cleanup strategy that reduces contaminants while providing social, economic, and other environmental benefits.

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Conflict of interest statement

The authors report that there are no competing interests to declare.

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4.2 THE EFFECT OF ORGANIC COMPOST AND MORINGA LEAF EXTRACT BIOSTIMULANTS ON THE PHYTOREMEDIATION OF MINE FOOTPRINTS AND TAILINGS STORAGE FACILITIES IN SOUTH AFRICA USING *CHRYSOPOGON ZIZANIOIDES*

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ABSTRACT

Mining has occurred for more than one hundred years in the Witwatersrand and has left many tailings storage facilities, and some have been successfully re-mined leaving behind contaminated footprint areas. This study aimed to assess the potential of compost and moringa leaf extract in enhancing the stabilization of tailings and the cleanup of the footprint areas. A 3×1×2 factorial split-plot design with 3 levels of compost treatment (0%, 15%, and 30%) was set up over a 16-week period at the footprint area and tailings storage facility. Vetiver grass planted on 0% compost amended tailings died within 4 weeks of planting yet on 0% footprint, vetiver grass survived, although its growth was significantly lower compared to growth in amended footprint soils. Generally, spraying with MLE did not result to any significant difference in vetiver grass biomass. A significant increase in the uptake of As, Cu, and Mn by the leaves and Cr, Cu, Mn, and Ni by the roots of vetiver grass planted on 0% compost-amended footprint soils was observed in the plants treated with the MLE biostimulant, but no significant increase in metal uptake by vetiver grass grown on 15% and 30% compost amended soil. On the tailings, moringa leaf extract application decreased the concentration of all metals in vetiver leaves, except for Mn. The results suggest that compost and moringa leaf extract application is essential for the phytostabilization of gold mine tailings but only moringa leaf extract is essential for the cleanup of the footprint area.

Key words

Sustainable phytoremediation, moringa leaf extract, biostimulant, , vetiver grass, compost, mine tailings, mining, mine footprint

4.2.1 Introduction

Mining is an absolute necessity for the economy in most countries worldwide (Walser, 2002); however, it is marred by deleterious effects on the environment and health. For instance, in the Witwatersrand, 400 km^2 of land is used for tailings storage facilities (TSFs) (Bambas-Nolen et al., 2013) and metals continue to be released from these facilities decades after mining has ended (Tibane & Mamba, 2022; Tutu et al., 2008). While efforts have been made in recent years to reclaim mine waste dumps (Bambas-Nolen et al., 2013), the resulting footprint areas still have high concentrations of toxic metals. These metals pose significant risks to human health and the environment (Kamunda et al., 2016).

According to the World Health Organization (WHO), chromium (Cr), copper (Cu), zinc (Zn), iron (Fe), cadmium (Cd) and lead (Pb) are metals of immediate concern (Hisfa et al., 2010). Arsenic (As), Cr, Cu, manganese (Mn), nickel (Ni) and Pb are classified as toxic metals, they are neither destroyed nor degraded (Okereafor et al., 2017). As, Cd and Pb are widely displaced but are of no biological importance to plants or animals (Ebenebe et al., 2017). Arsenic is a well-known toxin and carcinogen. Pb affects and damages several body organs and systems (Mahurpawar 2015). Cr, Cu, Mn, and Ni are micronutrients, but at relatively high concentrations, these metals are toxic to human, animal, and plant health (Ebenebe et al., 2017).

Concerns about the failure to remediate footprint areas have been discussed by Liefferink and Liefferink (2014). A major concern is the use of contaminated areas for informal settlements (Fourie et al., 2008). An estimated 1.6 million people have been reported to live next to mine residue areas in informal settlements (Tang and Watkins, 2011). These communities are at high risk of chronic health problems and hazards posed by pollution in soil and water bodies (McSwane et al., 2015; Swart, 2003). As a result, polluters are compelled to remediate the impacted areas.

Several remediation techniques have been developed and applied (Liu et al., 2018). They range from chemical, physical, to biological methods. Biological techniques such as phytoremediation are advantageous over other techniques because they are cost-effective, eco-friendly, and carbon neutral (Megharaj & Naidu, 2017). Phytoremediation occurs when plants remove or contain contaminants within their system to minimize their spread (Jensen & Gujarathi, 2016).

However, Guidi Nissim et al. (2023) argue that the decision-making process around mine waste dump remediation should consider a broader range of factors, such as ecosystem services, resilience to global climate change, biodiversity, carbon sequestration, and value-added services. Therefore, to create a more sustainable and resilient future, innovative approaches and practices must be designed and implemented in a way that considers multiple perspectives and addresses global challenges in a holistic manner (Sans et al., 2017). The potential benefits of innovative solutions, such as the use of vetiver for land remediation and value-added products and services, are not well-researched in South Africa. Vetiver grass is known for its tolerance of harsh conditions, including high metal content, low nutrient levels, wide pH ranges, and drought (Truong, 2016; Danh et al., 2009).

However, conditions like low nutrients levels and high metal content may limit the development of plants. As a result, amendments such as compost, biosolids, lime, and/or inorganic fertilizers are often applied to improve plant growth (Lee et al., 2014; Li & Huang, 2015). The use of inorganic fertilizers may contribute to surface water eutrophication and water quality degradation (Schoumans et al., 2014). In contrast, the application of organic amendments helps to immediately decrease metal bioavailability, serve as a microbial inoculum, improve soil structure, reduce erosion, and increase infiltration (do Carmo et al., 2016)

Because organic composts contain low levels of nutrients, which are usually complexed in organic chemical structures (Angin et al., 2008), biostimulants can be integrated into phytoremediation technology to complement organic compost. Biostimulants as materials other than fertilizers that promote plant growth when applied in low quantities (Grammenou et al., 2023). *Moringa oleifera* leaf extract use as a biostimulant has attracted the interest of the scientific community because its leaves are a rich source of growth hormones, antioxidants, vitamins, and mineral nutrients (Bakhtavar et al., 2015). This study was conducted using vetiver grass under MLE and compost amendments. The biomass production and metal accumulation were measured over time to assess the impact of these conditions on the plant's ability to phytostabilize the tailings and phytoextract metals from the footprint area.

4.2.2 Materials and Methods

4.2.2.1 Study sites

Footprint area

The footprint area is known as Sibanye Robinson Lake or Driefontein rock dump 6 (DRD 6) (26°23'05"S 27°26'13"E). It is located approximately 3 kilometres southeast of Carletonville, Gauteng Province. The footprint area was utilized as a rock storage facility. The soils within the study area were significantly disturbed due to the placement and removal of a rock disposal area, the creation and spill from an in-stream dirty water dam and subsequent partial removal of the spill. The land is earmarked for agricultural, pasture or residential purposes.

Tailings

Driefontein tailings storage facility 4 (TSF 4) (26°21'9.90"S 27°27'29.01"E) is located approximately 3 kilometres east of Carletonville, Gauteng Province. The TSF4 was first used in the 1990s, and it is still operational. Tailings deposition is performed via the cyclone method. The current maximum height and volume are 40 m and 55 million cubic meters, respectively (https://thevault.exchange/?get_group_doc=245/1559906270-sibanye-stillwater-tailings-storage-facilities-07jun2019.pdf). TSF 4 is surrounded by farmland and human settlements. The location of the study site are shown on Google Maps in Figure 4-14 below.

Figure 4-14: Google Earth imagery of the tailings (TSF 4) and the footprint area (DRD 6) where vetiver grass was planted for field study

The mean annual precipitation (MAP) at the study sites is estimated to range between 601 and 800 mm per annum. In summer, average daily temperatures (ADT) range from 17°C to 27°C, with a maximum of 38°C, and winter (ADT) ranges from 0 to 13°C (Compaan, 2011).

4.2.2.2 Design and preparation of small-scale field trials

The study was conducted during the rainy season of 2021. The experiments at the DRD 6 and TSF 4 were carried out concurrently. A split-plot design was used in the study. The experiment at each site had a 3×1×2 factorial design, with 3 levels of compost treatment (0%, 15% and 30%), 1 level of vetiver cultivar (*Chrysopogon zizanioides*), and 2 levels of MLE treatment (MLE and no MLE). Treatment details and their descriptions are presented in Table 4-4 below.

Table 4-4: Description of the six treatments at each study sites

Treatment	**Description**
0%C 0%B	No amendment, no MLE biostimulant (control)
0%C 3%B	No amendment, 3% MLE biostimulant
15%C 0%B	15% compost amendment, no MLE biostimulant
15%C 3%B	15% compost amendment, 3% MLE biostimulant
3O%C 0%B	30% compost amendment, no MLE biostimulant
30%C 3%B	30% compost amendment, 3% MLE biostimulant

Three blocks measuring 1 m × 2 m were prepared by ripping the compacted land to a depth of approximately 25 cm using a wood handle pick. Twenty holes were dug into each block, which were filled with an equal amount of soil amended with different levels of compost. A single vetiver grass slip was randomly picked from the batch and was planted in each hole. The blocks were split into 2 sections each with 10 holes, and 3% (v/v) commercial MLE was applied to one section, while water was applied to the other section once a week.

4.2.2.3 Biostimulant preparation

The biostimulant was purchased from a local commercial supplier in Pretoria (South Africa) and was diluted with tap water to form a 3% MLE (v/v) as per the supplier recommendations for dilution. The resultant solution was then sprayed once a week on vetiver grass using a 5 l Garden Pressure Sprayer.

4.2.2.4 Vetiver grass preparation for planting

One-month-old vetiver tillers donated by Hydromulch (Bapsfontein, Gauteng Province, South Africa) were removed from the soil that came with them and pruned to a height of 25 cm to establish the baseline height. The tillers were subsequently transplanted into the amended soils. Vetiver grass was watered every day during the first week, after which the plants were not watered unless it rained. The grass was foliar sprayed at one-week intervals with either MLE biostimulant or water until week 16. Figure 4-15 below shows a block of the experiment in the footprint area.

Figure 4-15: Image of vetiver grass plot (left sprayed with Moringa leaf extract and on the right sprayed with only water) at Sibanye rock dump 6 footprint area 6 weeks after planting

4.2.2.5 Chemicals and reagents

The chemicals, reagents and solvents used were of analytical grade. Hydrochloric acid (HCl), nitric acid (HNO_3), hydrofluoric acid (HF) and hydrogen peroxide (H_2O_2) were purchased from Fisher Scientific (London, UK). The ultrapure water used throughout the experiment was prepared using a direct-Q 3 UV Millipore system (Massachusetts, USA). The stock solutions and a range of working standard solutions were diluted with ultrapure water.

4.2.2.6 Instrumentation

The mass of the samples was measured using an analytical balance (Precisa 180A, Switzerland). The soil and plant samples were digested using a Multiwave 3000 microwave digester (Anton Paar, Switzerland). Element analysis was performed using inductively coupled plasma optical emission spectroscopy (ICP–OES) (Spectro, Kleve, German), and the pH and electrical conductivity (EC) were measured using a Bante 900P multiparameter water quality meter (Bante Instruments).

4.2.2.7 Vetiver grass preparation for analysis

At 16 weeks, three plants from each section were uprooted from the ground. The number of tillers and leaves and the length of the leaves were measured, averaged, and recorded. The plants were then washed under running tap water, rinsed in 0.10% HNO_3 to remove any metal on the surface and finally rinsed with distilled water. The roots and shoots were separated and air-dried in the laboratory at room temperature until they reached a constant weight. The dry masses of the roots and leaves were recorded.

4.2.2.8 Vetiver preparation for microwave extraction

The vetiver leaves and roots were ground to powder using a Mellerware blender. A mass of approximately 0,1000 g of ground material was placed in a microwave digestion vessel. To this mixture, 8 mL of HNO_3 and 2 mL of H_2O_2 were added. Digestion was carried out for 30 minutes in a microwave digester. The digested samples were then transferred to 50 mL centrifuge tubes, which were filled with deionized water. All the samples were filtered through 22 µm pore syringe filters prior to analysis.

4.2.2.9 Preparation of tailings and footprint soils for physico-chemical measurements

A mass of 2,000 g of < 125 µm substrate material was weighed into a 50 ml plastic centrifuge tube. A volume of 40 ml of deionized water was added, and the mixture was subsequently placed on an end-to-end rotator for 10 minutes prior to measuring the pH and EC.

4.2.2.10 Soil preparation for microwave digestion and analysis

Footprint soils and tailings were dried in the laboratory at room temperature for 10 days, homogenized and sieved. To evaluate the total element concentration, a mass of approximately 0,250 g of < 25 µm particles was weighed and placed into acid-washed digestion tubes (PTFE-TFM liners), into which 9 ml of HCl, 3 ml of HNO_3, 1 ml of HF and 6 drops of H_2O_2 were added before being placed into the microwave digester. The extraction was performed for 60 minutes. The solution was transferred to 50 mL centrifuge tubes, which were filled with deionized water. The solution was then filtered through 0.22 µm Millex PVDF membrane syringe filters prior to analysis in the ICP–OES.

4.2.2.11 Chemical analysis

ICP–OES was used to determine the concentrations of the elements. A total of 15 elements, consisting of toxic metals and macro- and microelements, were quantified in the footprint and tailings material to determine the baseline element content prior to planting. After the experiment, six metals (As, Cr, Cu, Mn, Ni and Pb) were measured in the roots and leaves of vetiver grass to quantify these metals in the plant material. The method for determining the concentrations of the metals in the plant parts and soils was validated using standard methods. The correlation coefficients of the standards for tailings/soil and plant material were 0,9993 and 0,9997, respectively.

4.2.2.12 Statistical analyses

The SAS Enterprise Guide 7.1 was used to compute the two-way ANOVA test and Fisher's least significant difference test to test for significant differences in values with changes in treatments.

4.2.3 Results and discussion

4.2.3.1 Physical and chemical assessment of the study sites.

The physicochemical properties (pH and conductivity) of the footprint and tailings were measured, and the results are presented in Table 4-5. The EC was far below the threshold value of 400 mS/m (Deuel and Holliday, 1994). The pH was alkaline at TSF4 and slightly acidic to neutral in the footprint area.

Table 4-5: pH and conductivity results of the DRD6 footprint soils and TSF4 tailings material

Site	**pH**	**Conductivity/µS/cm**
Tailings storage facility	9,33± 1,22	557 ± 59,2
Footprint soil	6,79 ± 1,67	132,2 ± 23,5

The low EC could have been due to high rainfall during the sampling period, which may have washed away the salts. According to Li et al. (2022), soil pH affects the bioavailability, solubility, and translocation of metals in plants. Metals tend to form less soluble phosphates and carbonates at high pH (Olaniran et al., 2013). This reduces their availability for uptake by plants. Several studies have shown that only biologically available forms of metals in the soil can significantly affect plants. The remaining fraction is bound by the soil matrix irreversibly and therefore cannot interact with plants. These metals can be transformed from one form to the other by pH changes (Lu et al., 2022).

4.2.3.2 Results for growth parameters

The growth of vetiver grass was monitored after planting. Figure 4-16 and Figure 4-17 show the changes in the appearance of vetiver grass during the different time periods on the footprint and tailings, respectively.

Figure 4-16: Vetiver grass growing at the footprint site during the study period from the 5th of November 2021 to the 14th of March 2022

Figure 4-17: Vetiver grass growth on the tailings over different weeks from the 5th[th] of November 2021 to the 14[th] of March 2022

It is apparent that vetiver grass growth on the footprint was greater than that on the tailings. The tailings contained higher concentrations of Cr, Mn, and Ni than did the footprint, and the slow death rate at the tailings could be attributed to high metal concentrations and low nutrient contents. The toxicity of different metals is influenced by the total concentration and the type and concentration of various ionic species (Sarathchandra et al., 2022).

Images of roots from plants subjected to different treatments are shown in Figure 4-18 below. The vetiver grass plants grown on the amended footprint were generally longer and more abundant than the vetiver roots on the tailings.

Figure 4-18: Images of the roots of vetiver grass harvested from different treatments (tailings and footprint soils amended with different compost amendments(C) and different biostimulant amendments (B) in the tailings and footprint substrate at 16 weeks

The quantitative results of the different parameters measured under the different treatments in both the tailings and footprint areas are presented in Figure 4-19 below. The unamended footprint soils supported the growth of vetiver grass even though the least growth was noted in this treatment. There was no significant difference ($p = 0.99$) in growth parameters between vetiver grass grown in 15% and that grown in 30% compost amendment. Spraying with MLE in amended footprint soils resulted in no significant difference in biomass yet in unamended soils, a significant difference in the leaf mass and number of leaves was observed.

In the tailings, the vetiver planted on the unamended tailings died within the first 4 weeks of planting, regardless of spraying with MLE. The 15% and 30% compost amended tailings supported vetiver growth and there was no significant difference in vetiver growth parameters between vetiver grown in 15% and 30% compost amendment. Application of MLE on vetiver grown on the amended tailings led to no statistically significant differences on tailings.

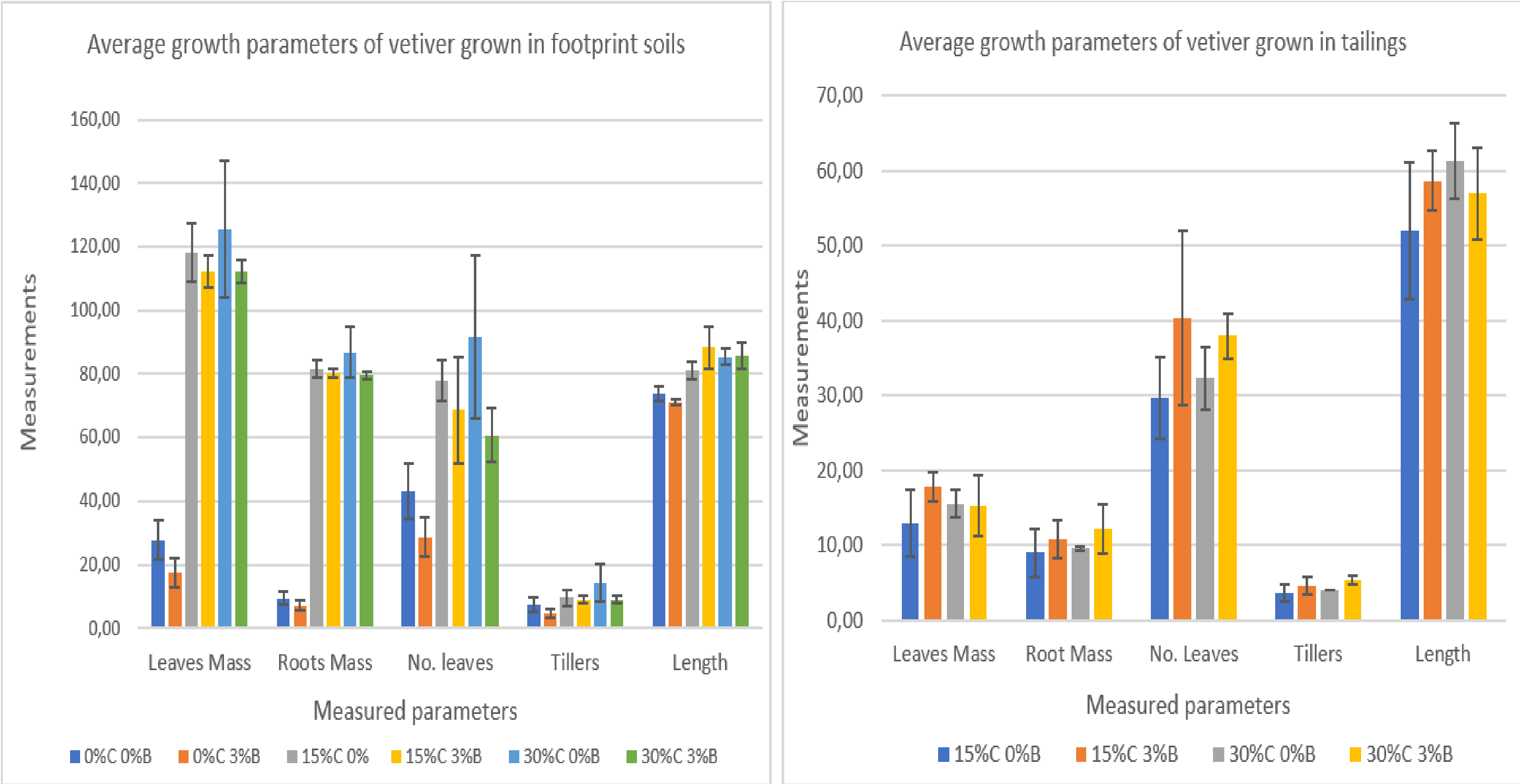

Figure 4-19: The graph showing different measured growth parameters from vetiver grass harvested from the footprint (left) and tailings (right) at week 16 from different treatments: unamended footprint and tailings without biostimulant (0%C0%B), unamended footprint and tailings with 3% biostimulant, 15% compost amendment with no biostimulant (15%C0%B), 15% compost amendment and 3% biostimulant (15%C3%B), 30% compost amendment without biostimulant (30%C0%B) and 30% compost amendment with biostimulant (30%C3%B)

The growth of vetiver at the footprint was significant ($p = 0.01$) than the growth of vetiver grass at the tailings despite the similarity in treatment regime. The difference in biomass production noted is probably a result of the specific growing conditions (nutrients and water availability). According to Saleh et al (2020), plants can suffer when heavy metal exceeds a certain threshold by disturbing its metabolism and plant physiology.

Compost application led to improved vetiver growth in both the footprint and tailings. This is due to improved organic matter and plant nutrients (Sanchez-Monedero et al., 2004; Tejada et al., 2009). These findings are consistent with the findings of Wu et al. (2011) who noted an increase in vetiver biomass due to the addition of compost, and Dominguez et al. (2020) noted an increase in the growth of *Silybum* species in response to compost addition. This was because of an increase in total organic carbon. Compost amendments not only provide essential nutrients (which are normally limited in tailings) but also improve the soil structure, total organic carbon, CEC, and water holding capacity (Alvarez-Lopez et al., 2016). As a result, the retention of soil moisture and improvement in nutrients increase plant dry weight (Malakouti and Sepehr, 2004). Moreover, there was no significant difference between the 15% and 30% compost applications. While the rate of amendment application is expected to yield variable results, the application of high levels of fertilizer can lead to plant toxicity due to an increase in nitrogen through proline production. This could explain why there was no significant effect on the root biomass, leaf biomass, height, or number of tillers on vetiver growing on 15% and 30% compost amendments.

There was no significant difference ($p = 0.97$) on vetiver grass biomass that resulted due to foliar application of MLE to vetiver grass on compost amended footprint and tailings material. These findings are however different from the findings on several studies; for example, Abdalla, (2014) found that spraying of rocket plants with Moringa leaf and twigs extract resulted in increased growth parameters. El Sheikha et al. (2022) investigating MLE on snap beans also proved that MLE application positively affects the growth, yield, and quality parameters of snap beans. Consistent results were also obtained by Culver et al. (2012), who showed that greenhouse and field tomato growth and yield were significantly increased by foliar spraying of tomato plants with MLE. The reasons for no difference due to MLE application on vetiver grown on compost amended substrates could be due to the interaction of the effects of compost with that of the biostimulant.

4.2.3.3 Baseline element concentrations in the footprint and tailings

Table 4-6 provides the baseline concentrations of the selected elements, and the South African soil screening values (SSVs) as listed in the norms and standards for the remediation of contaminated land and soil quality, are also included in the table. SSV1 represents 'soil quality values that are protective of both human health and eco-toxicological risk for multi-exposure pathways, inclusive of contaminant migration to the water resource'. SSV1 is conservative, and its concentration is the lowest among the potential source-pathway-receptor models. SSV2 represents 'soil quality values that are protective of risk to human health in the absence of a water resource'. The SSV1 and SSV2 (informal residence) values were used as the remediation target values because no site-specific risk assessments were conducted.

The concentrations of As, Cr, and Mn in the footprint and of As and Cr in the tailings were above the SSV1 and SSV2 (informal residence) limits, whereas the concentrations of Cu, Pb Ni in both the tailings and footprint exceeded the SSV1 concentration limits. Cd was below detection in both the footprint and tailings.

Table 4-6: South African soil screening values and concentrations of elements in DRD6 footprint soils and tailings (TSF4)

Element	**Concentration mg/kg**		**Soil screening values (SSV) for metals**	
	Measured concentrations in the footprint	Measured concentrations in tailings	SSV 1 All land uses Protective of the water resources	SSV 2 Informal Residence
Al	39878,50	16806,12	----	----
As	31,53	151,93	5,80	23,00
Ca	5702,36	5093,07	----	----
Cd	BD	BD	7,50	15,00
Co	37,92	61,92	300,00	300,00
Cr	149,20	112,22	6,50	6,50
Cu	85,05	99,31	16,00	1100,00
Fe	38796,50	37065,63	----	----
Mg	3861,87	3711,60	----	----
Mn	1418,98	607,32	740,00	740,00
Ni	149,36	141,52	91,00	620,00

Pb	25,99	28,11	20,00	110,00
Na	1237,32	1288,77	----	----
P	1850,29	976,52	----	----
Zn	123,14	142,02	240,00	9200,00

∗BD- below detection,------------------ No values provided, exceeds SSV1 and SSV2, exceeds SSV1 only

A decreased amount of nutrients and high metal concentration in the substrates could have significantly affected vetiver growth, leading to death in unamended tailings. These findings are consistent with the findings of Roongtanakiat et al. (2003), who noted a reduction in vetiver growth due to high landfill leachate of organic and inorganic pollutant concentrations. The low P levels in the tailings further justify the suppressed growth of vetiver grass on the tailings. Wagner et al. (2003) argued that the growth of vetiver grass is largely influenced by the availability of soil P. Slow growth and even death of vetiver may occur due to low P.

4.2.3.4 Metal concentration in vetiver grown at the footprint area

The effect of compost amendment and the MLE biostimulant on metal accumulation in the leaves and roots are shown in Figure 4-20 and 4-21, respectively. Table 4-7 provides a summary of the effects of compost and MLE on the metal accumulation in leaves and roots of vetiver grass.

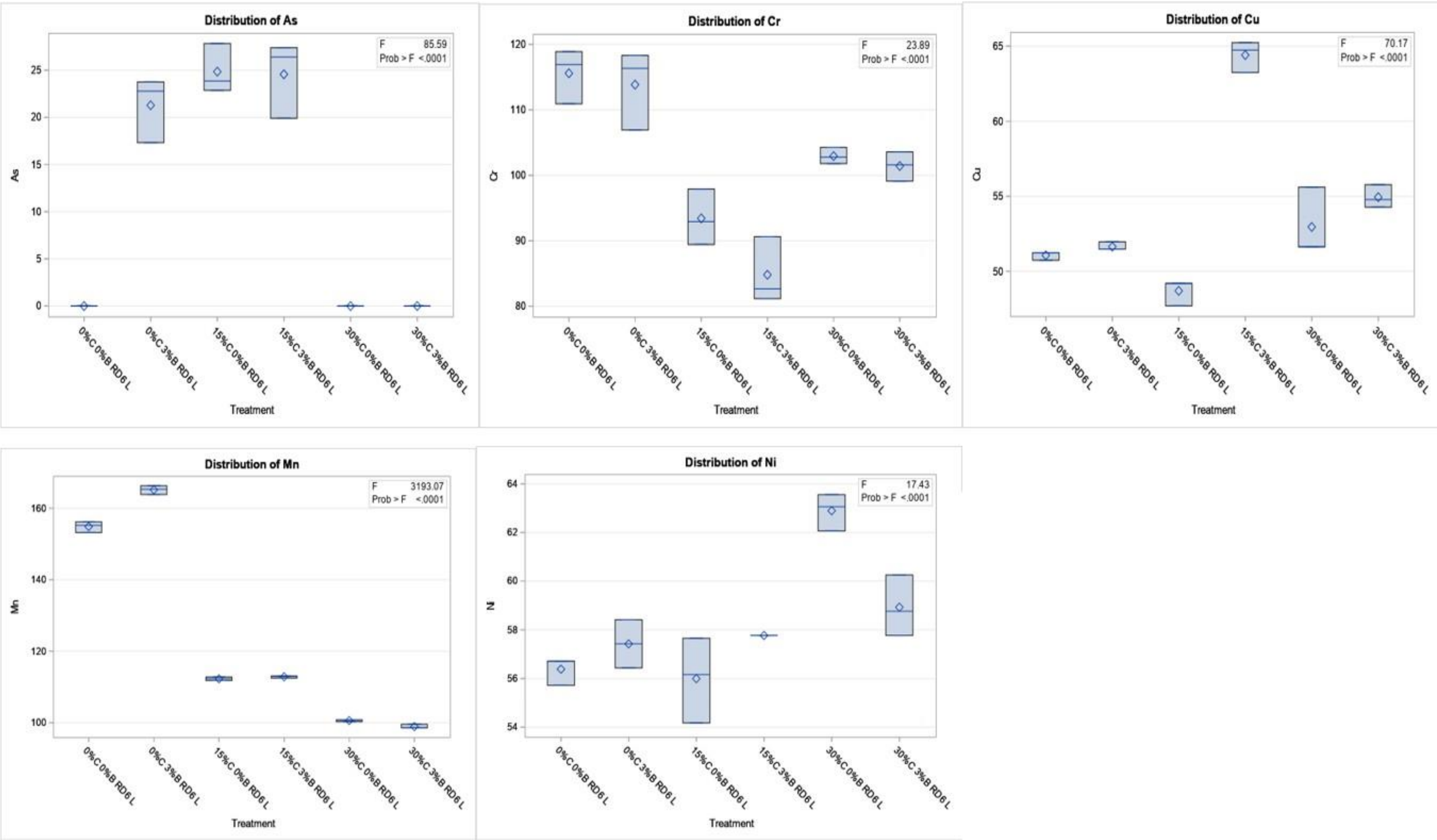

Figure 4-20: Metal concentration in the leaves of vetiver grass grown in different compost and biostimulant amendments at Sibanye Stillwater DRD6 footprint area

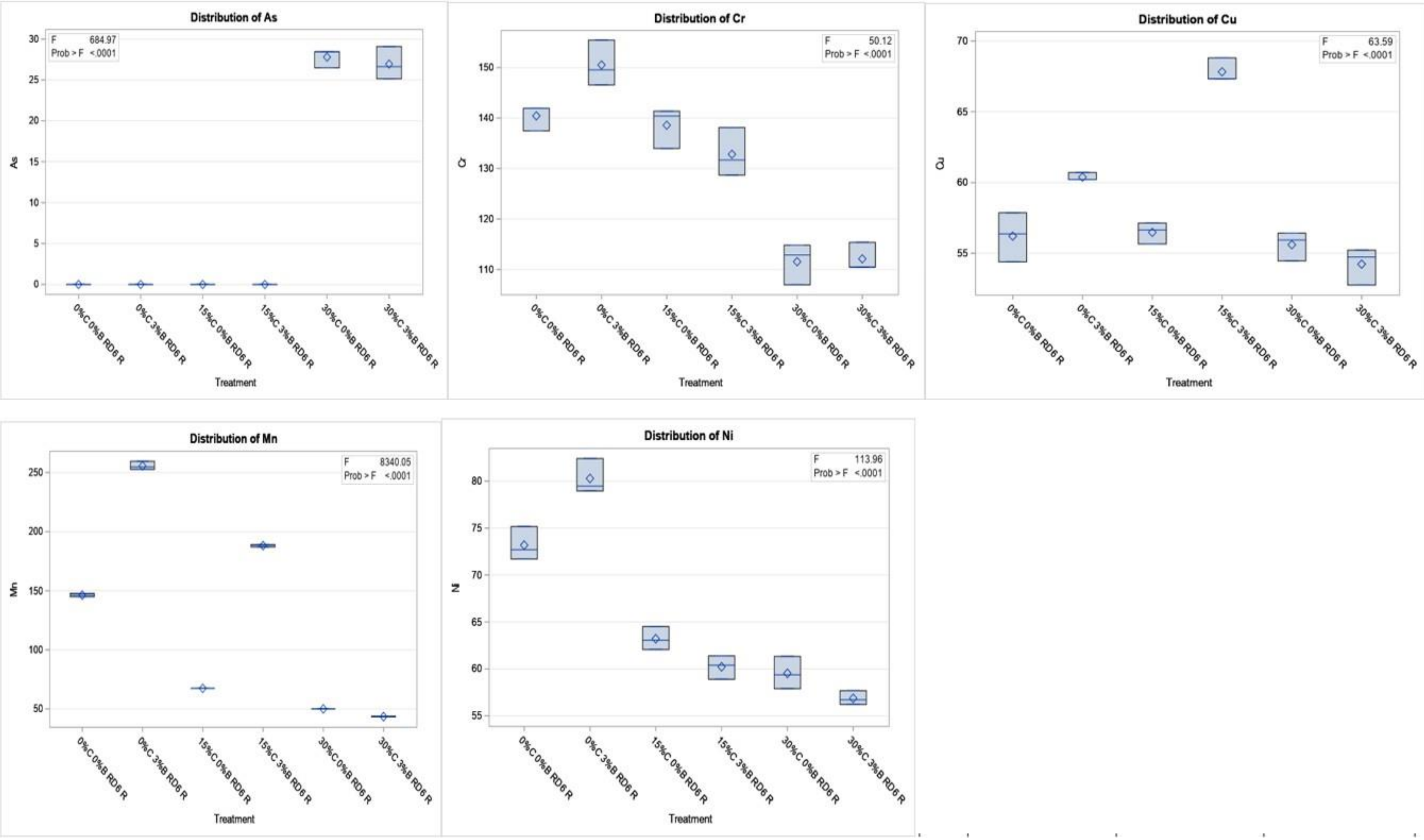

Figure 4-21: Concentration of As, Cr, Cu, Mn and Ni in the roots of vetiver grass grown in different compost and biostimulant amendments from Sibanye Stillwater DRD6 footprint area in the Witwatersrand Goldfields

Table 4-7: Summary of the effects of biostimulant and compost on metals in vetiver leaves and roots grown on footprint soil

	RD6 VETIVER LEAVES				
	Effect of applying 3% MLE biostimulant			**Effect of compost**	
	0%	**15%**	**30%**	**15%**	**30%**
As	**Increase**	**No difference**	**No difference**	**Increase**	**No difference**
Cr	**No difference**	**No difference**	**No difference**	**Decrease**	**Decrease**
Cu	**Increase**	**Increase**	**No difference**	**Decrease**	**Increase**
Mn	**Increase**	**No difference**	**Decrease**	**Decrease**	**Decrease**
Ni	**No difference**	**No difference**	**Decrease**	**No difference**	**Increase**
Pb	**BD**	**BD**	**BD**	**BD**	**BD**
	RD6 VETIVER ROOTS				
	Effect of applying 3% MLE biostimulant			**Effect of compost**	
	0%	**15%**	**30%**	**15%**	**30%**
As	**No difference**	**No difference**	**No difference**	**No difference**	**Increase**
Cr	**Increase**	**No difference**	**No difference**	**Decrease**	**Decrease**
Cu	**Increase**	**Increase**	**No difference**	**No difference**	**No difference**
Mn	**Increase**	**Increase**	**Decrease**	**Decrease**	**Decrease**
Ni	**Increase**	**Decrease**	**Decrease**	**Decrease**	**Decrease**
Pb	**BD**	**BD**	**BD**	**BD**	**BD**

Lead was below detection in the leaves and roots of vetiver grass across all the treatments. A decrease in the accumulation of Cr, Cu and Mn was noted in vetiver leaves grown in 15% compost amendment; Cr and Mn, in 30% compost amendment; and Cr, Mn, and Ni, in roots of vetiver grass grown in 15% and 30% compost amendment. A decrease could be due to a decrease in heavy metal bioavailability as heavy metals change from available forms to organic matter associated carbonates or metal oxides in the presence of organic matter (Walker et al., 2004). The reduction in metal accumulation due to compost application can also be ascribed to an increase in pH, which also leads to a decrease in metal availability (van Herwijnen et al. 2007). An increase in soil fertility due to amendment addition is another factor that leads to increased nutrient availability, thus reducing metal uptake due to competition for transporters (Farrell et al., 2010). This alleviates metal toxicity and growth deficiency (Brown et al., 2003). As a result, the growth of plants on amended soils was greater than that on soils without amendments.

The application of the MLE biostimulant led to a distinct increase in metal uptake in unamended soils. These findings are consistent with the findings of Posmyk & Szafrańska (2016) who reported that biostimulants can improve plant physiological processes such as nutrient uptake. Boyd and Rajakaruna (2013) noted the relationships of elements such as Mn, Cu, Ni, Cd, and As with plant performance (Boyd and Rajakaruna, 2013) and that nonessential elements and micronutrients can be toxic to plant growth (Bandara et al., 2020). The noted decrease in leaf biomass and length on 0%C 3%B treatment could be attributed to the increase in the uptake of elements such as As, Cd and Mn.

Generally, the application of MLE to vetiver grass grown on 15% and 30% compost-amended soils led to a decrease in the uptake of metals by vetiver grass compared to that in unamended soils. These findings suggest that using compost for remediation of footprint areas may not be ideal, as vetiver grass, in the presence of compost is unable to accumulate metals. MLE may be useful in the remediation of the footprint area using vetiver grass because it increases the phytoextraction of the metal. This will lead to a shorter turnaround time for the cleanup of the footprint area, making the land available for other land uses.

4.2.3.5 Metal concentration in tailings

The accumulation of As, Cr, Cu, Mn and Ni in the leaves and roots of vetiver grass grown in the tailings are presented in Figure 4-22 and 4-23 below. A summary of the metal accumulation in vetiver grass leaves and roots from the tailings is presented in Table 4-8 below.

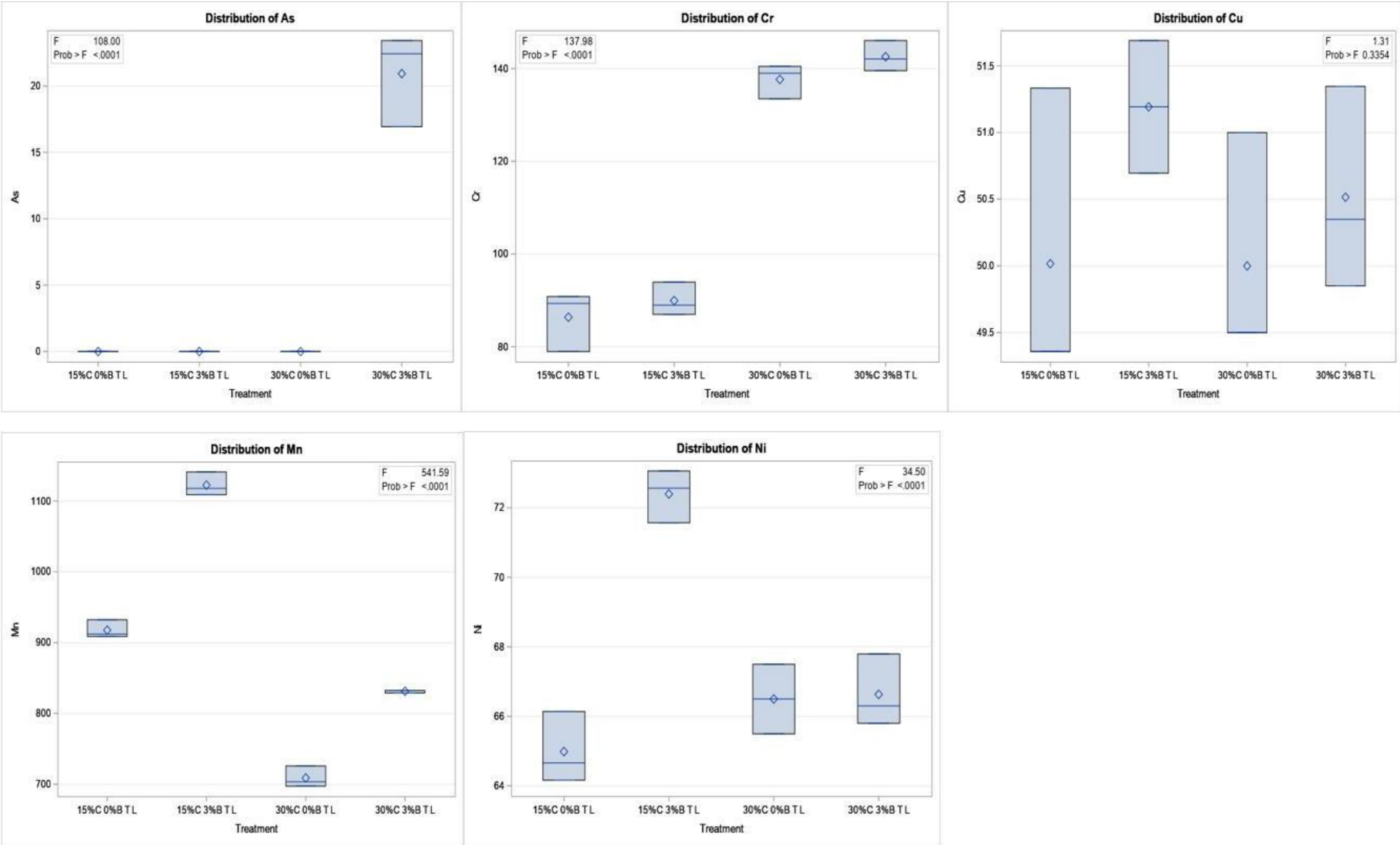

Figure 4-22: Concentration of As, Cr, Cu, Mn and Ni in the leaves of vetiver grass grown in different compost and biostimulant amendment at Sibanye Stillwater tailings in the Witwatersrand Goldfields, South Africa

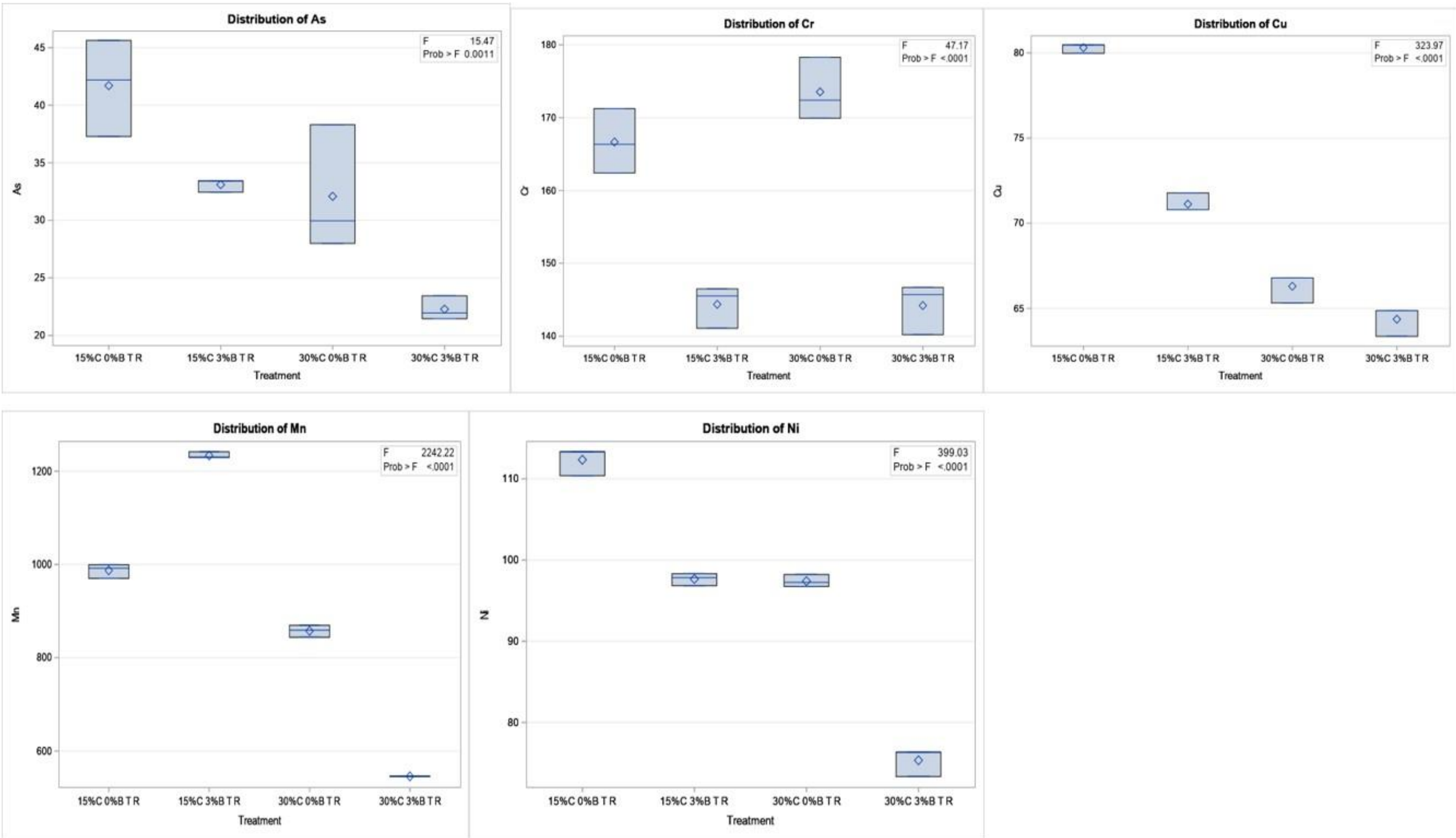

Figure 4-23: Concentrations of As, Cr, Cu, Mn and Ni in vetiver roots grown in different compost and biostimulant amendments at Sibanye Stillwater tailings in the Witwatersrand Goldfields in South Africa

Table 4-8: Summary of the effect of biostimulants on the metals in the leaves and roots of vetiver grass grown on amended tailings

	TAILINGS LEAVES		TAILINGS ROOTS	
	Effect of biostimulant		Effect of biostimulant	
	15% Compost	30%Compost	15% Compost	30% Compost
As	decrease	decrease	No difference	increase
Cr	decrease	decrease	No difference	No difference
Cu	decrease	decrease	No difference	No difference
Mn	increase	decrease	increase	increase
Ni	decrease	decrease	increase	No difference
Pb	BD	BD	BD	BD

The application of MLE led to an increase in biomass under both the 15% and 30% amendments, even though the difference was not significant. The slight improvement in biomass due to the application of MLE could be because of the decrease in the uptake of these metals by vetiver grass, whose effects are detrimental to plant growth. The decline in the concentration of metals in response to MLE application noted above could be the reason why an improvement in biomass was observed in the sprayed vetiver grass treatment group. The essence of remediating tailings is largely for stabilizing contaminants so that they do not spread to other environments. Therefore, both compost and MLE improved the phytostabilization of metals on the tailings and are recommended for use in tailing remediation. However, a longer cycle is needed.

4.2.4 Conclusion

The overarching principle of the current study was to develop a proof of concept for a strategy that integrates ecosystem services, carbon sequestration, bioenergy, and value-added benefits into decisions pertaining to remediation. While the use of compost improves plant growth, compost reduces the accumulation of metals on vetiver grass; therefore, the use of compost is not recommended for footprint area remediation. The findings suggest that only MLE biostimulants may be used in the remediation of footprint areas because their application significantly improves the uptake of most metals. This approach will shorten the turnaround time required in the cleanup of the footprint area so that it can be repurposed for other land uses.

On the other hand, the grass on unamended tailings could not survive beyond 4 weeks, and only vetiver grown in 15% and 30% amendments survived, thus making compost essential for phytoremediation of the tailings. Unlike in the footprint area, MLE application on vetiver grown on the tailings led to a reduction on metal uptake into leaves. Both MLE and compost can be used in the effective phytoremediation of mine tailings, by promoting the production of large amount of biomass that could improve phytostabilisation, carbon sequestration and bioenergy production. Improving carbon sequestration by vetiver can reduce the polluters' carbon liability. This is also true if bioenergy can be produced from vetiver growing on tailings, as this will contribute to the energy transition from coal to clean energy. However, long-term studies spanning seasons should be conducted, and further studies to prove the potential of vetiver for carbon sequestration and bioenergy need to be pursued.

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CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1 INTRODUCTION

Chapter 5 brings together the key findings and lessons learnt from the phytoremediation of compost amended gold mine tailings and footprint soils using vetiver grass enhanced with MLE. The ultimate aim was a solution that does not focus on addressing environmental contamination only but adopts a multidimensional and holistic approach which emphasizes integrating ecosystem services, resilience to global climate change, carbon sequestration, bioenergy, phytoproducts, and cultural benefits into the decision pertaining to remediation to achieve a secure, sustainable, and resilient future. This chapter is fragmented into three sections. The first section reviews the key findings as they relate to the research aims. Recommendations are proposed in the second section of the chapter, and future research focus areas are provided on the third and last sections.

5.2 RECAPPING KEY FINDINGS

This study sought to establish the synergistic effect of MLE and compost in improving the phytoremediation of footprint areas and TSFs using vetiver grass. Other than vetiver's phytoremediation potential, vetiver's potential to provide feedstocks for biofuels and bioproducts; as an effective mitigation strategy to address climate change and its impacts; tolerance to adverse physical, chemical and climatic conditions without the need to use any environmentally degrading substances, such as inorganic fertilizer and/or cladding material, and potential for social and economic sustainability during and after mine closure were considered in this phytoremediation study.

The initial step in this study was the optimization of the parameters in the greenhouse. The efficacy of the laboratory extracted and commercial MLE were assessed, and the rate of their application (once or twice a week) was assessed for their ability to enhance the growth and metal remediation of vetiver grass on 0%, 30% and 60% compost amendments. Experimenting with laboratory-extracted MLE was done to determine whether laboratory-extracted MLE could be an alternative for enhancing the growth and metal accumulation of vetiver grass if effective. In addition, production of the biostimulant can be initiated as a community project to upskill and cross-skill the mining community on another possible venture to sustain them as mining curtails. The results showed that vetiver grown on 60% compost amendment and sprayed twice a week with laboratory extracted MLE biostimulant yielded the best results in terms biomass production. However, the 30% compost amendment coupled with once a week spraying with commercial MLE biostimulant was the preferred treatment for use in the field experiment. The 60%L2 treatment was not adopted due to the high cost associated with higher compost amendments. The difference in efficacy of the commercial and laboratory extracted MLE was attributed to the extraction processes of the two types which may have resulted in different chemical composition of these two types of biostimulants. Recommendations for optimizing the laboratory MLE method were made, as was conducting the study over a longer period.

For the field study, the 30%C1 treatment was recommended. A new lower level of compost amendment (15%) was also introduced. The field study commenced on the mine footprint area, which was utilized as a rock dump (DRD6) and an active tailings storage facility (TSF4). Compost amendments (0%, 15% and 30%) were used to ameliorate the tailings and the footprint area. Vetiver grass was planted and sprayed once a week with either commercial MLE or water for sixteen weeks. Surprisingly, the influence of compost and MLE biostimulants on the footprint differed from the influence on the tailings. Vetiver grass grown on 0% compost on tailings did not survive beyond 4 weeks, but the vetiver on the footprint survived, although its growth parameters were significantly lower than those from amended soils. The application of compost led to significant growth. However, in both the tailings and footprint area, there were no significant differences in most growth parameters (length, number of tillers, number of leaves, dry roots, or leaf mass) between vetiver plants grown in 15% and 30% compost-amendment. Unexpectedly, the MLE biostimulant led to a decrease in biomass from the footprint soil, but an increase was observed in the tailings even though neither difference was significant.

MLE biostimulant application generally led to a decrease in metal uptake in the leaves of vetiver grass grown in the tailings and either no difference or a decrease in the metal concentration in the roots and shoots of vetiver grass grown in 15% and 30% footprint soil amendments was observed. An increase in metal uptake was observed on the leaves and roots of vetiver grass grown on unamended footprint soils. The relationship between metal uptake and the growth of vetiver grass was inversely proportional.

Footprint remediation is needed for cleanup purposes so that the land may be re-purposed for postmining land use. Therefore, to clean up such land, the ideal phytoremediation technology is phytoextraction. The use of biostimulants only is therefore commendable in the remediation of footprint soils. However, without compost, the biomass produced is limited; therefore, value-added benefits such as biomass for bioenergy, high carbon sequestration potential, and biomass for handcrafted material cannot be attained.

The use of both compost and MLE for tailings is commendable because phytostabilization of the tailings is the main objective of tailings remediation. Both MLE and compost promoted the growth of vetiver grass on tailings. This approach is important for controlling erosion and stabilizing contaminants. In terms of improving carbon sequestration, bioenergy production and biomass for bioproducts, vetiver from tailings could be utilized for such purposes. However, for bioproducts such as handicraft material, risk assessments may need to be conducted first.

5.3 RECOMMENDATIONS AND POLICY

While this study offers high potential for a holistic solution in terms of considering other crises (carbon sequestration, energy production, and poverty alleviation) in the decision pertaining to phytoremediation, the first drawback is the South African legal framework, which discourages the utilization of exotic plant species in phytoremediation but also seeks to adopt holistic sustainability, which could be attained with the use of such species. This presents challenges in terms of adopting potential innovative solutions that may require exotic plant species. As such, goals and realities are at odds as long as the legal framework remains rigid. With the corporations just seeking to stay within the legal parameters and willing to do as much as attaining only the bare minimum of the prescribed remediation requirements, innovations with the potential to attain sustainable development goals may not materialize outside academic research because of lack of will power and the discomfort in adopting different or new ways of doing things. The gap between research and implementation will continue to widen. Therefore, a reform of the legal framework to accommodate upcoming technology may be necessary.

Secondly, there may be need for a reform in phytoremediation process. The current phytoremediation practice in South Africa is largely guided by the principles developed over 2 decades, before the intensification of the current challenges, as witnessed through consistent heatwaves, flush floods, and a general change in weather patterns. In light of increased environmental awareness, research should focus on innovations that can address multiple crises rather than one crisis at a time.

5.4 FUTURE RESEARCH FOCUS

In relation to the current project, further work on the following:

- Optimization of the laboratory MLE method may be necessary to determine the method that produces the best results under low compost amendments. The results from several studies where laboratory MLE was used to improve element accumulation and biomass produced positive results.

- Land cleanup using Phyto technologies is a lengthy process; therefore, a longer research cycle may be necessary for a better assessment.
- Other environmentally friendly ameliorants that improve the growth and phytoextraction potential of vetiver grass may be used in addition to MLE in the footprint area to improve the results.
- To promote economic growth within the mining industry, community members may be trained in the process of composting using different kinds of materials (e.g., food waste) that can be used in the remediation of tailing storage facilities.
- It is important to assess whether vetiver grass has high carbon sequestration potential, as claimed in the literature. The high carbon sequestration potential of vetiver grass would be advantageous to the climate, as would the mining company, as this would reduce the company's carbon footprint.
- Investigating how much biomass can vetiver grass on tailings and footprint can produce to feed into a bioenergy project.
- Conduct a risk assessment prior to using vetiver grass for phytoproducts.