



Faculty of Sciences

Department of Earth and Environmental Sciences

PhD in Environmental Science

**ASSESSMENT AND REHABILITATION OF HEAVY METAL(LOID)S
CONTAMINATED SOILS AND THE POTENTIAL OF DIFFERENT TREE
SPECIES FOR PHYTOREMEDIATION**

by

TRUST MANYIWA

Student ID number: 18100116

BSc-Environmental Science (University of Botswana)

MSc-Environmental Science (University of Botswana)

A Dissertation Submitted to the Faculty of Sciences in Partial Fulfilment of the
Requirements for the Award of the Degree of Doctor of Philosophy in Environmental
Science of BIUST

Supervisor: Prof Venecio. U. Ultra Jr.
Earth and Environmental Sciences
Faculty of Science, BIUST

September 2024



POSTGRADUATE SCHOOL

Declaration Regarding the Work and Copyright

DECLARATION REGARDING THE WORK AND COPYRIGHT

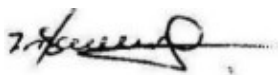
Candidate: MR. TRUST MANYIWA

Student ID: 18100116

Thesis Titled: Assessment and Rehabilitation of Heavy Metal(loid)s Contaminated Soils and the Potential of different Tree Species for Phytoremediation

I, the **Candidate**, certify that the Thesis is all my own original work and that I have not obtained a degree in this University or elsewhere on the basis of any of this work.
(If the thesis is based on a group project, then the student must indicate the extent of her / his contribution, with reference to any other theses submitted or published by each collaborator in the project, and a declaration to this effect must be included in the thesis)

This dissertation/thesis is copyright material protected under the Berne Convention, the Copyright and Neighbouring Rights Act, Act. No. 8 of 2000 and other international and national enactments, in that behalf, on intellectual property. It must not be reproduced by any means, in full or in part, except for short extracts in fair dealing; for researcher private study, critical scholarly review or discourse with an acknowledgment, without the written permission of the office of the Postgraduate School, on behalf of both the author and the BIUST.

Signed: 

Date: 4th September 2024

Primary Supervisor: Prof. Venecio Ultra

I, the Candidate's **Primary Supervisor**, hereby confirm that I have inspected the above titled thesis and, to the best of my knowledge, it is based on the original work of the candidate.

Signed: 

Date: 4th September 2024

Certification by the supervisor

Certification by the supervisor

The undersigned certifies that he has read and hereby recommends for acceptance by the Faculty of Science a dissertation titled: **Assessment and Rehabilitation of Heavy Metal(loid)s Contaminated Soils and the Potential of Different Tree Species for Phytoremediation**, in fulfillment of the requirements for the degree of Doctor of Philosophy of BIUST.

Professor: Venecio Ultra

Signature:

A handwritten signature in black ink, appearing to read 'Venecio Ultra', is written above a horizontal line.

Date: 4th September 2024

Abstract

The economy of Botswana is largely driven by the mining sector. Aside from gold, copper, nickel, and coal, Botswana is the world's largest diamond producer by value. In general, mining activities especially the waste generated such as the mine tailings often lead to a serious and widespread occurrence of environmental pollution to both soil and water bodies by heavy metal(loid)s. Increased heavy metal(loid)s pollution in the environment beyond the tolerable limit poses a threat to life forms and ecological balance. This problem is prevalent in the mining areas of Botswana since the Environmental Impact Assessment (EIA) Act of 2011 and environmental management has not been fully enforced. In some countries, the rehabilitation and ecological restoration of mine areas or the environment is mandatory. One technique that can be applied to rehabilitate mining areas is phytoremediation. Phytoremediation is a technique that uses plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues. It is considered a practical and more cost-effective remediation technology for a contaminated environment. Phytoremediation could offer an effective, environment-friendly, and economically feasible procedure to rehabilitate these mining areas. Currently, no rehabilitation efforts are being made in these mining areas, especially using phytoremediation technology. The overall aim of this study was to develop a phytoremediation technology suited to Botswana's mine tailings and the specific objectives were (i) to assess the impact of heavy metal(loid)s contamination on soil and plant communities in mine areas; (ii) to evaluate the influence of different soil amendments on heavy metal(loid)s dynamics, survival and phytoremediation efficiency of selected plants; and (iii) to evaluate the potential of different tree species for phytoremediation of mine tailings. Five tree species were evaluated on their effectiveness in

Abstract

phytoremediation of mine tailing from two mining areas. Additionally, several soil amendments like compost, fly ash, sewage sludge and mycorrhiza were also evaluated for their contribution to the survival and enhancement of the phytoremediation potential of these plants on these mine tailings. Tree species used for this phytoremediation study were *Eucalyptus globulus*, *Colophospermum mopane*, *Vachellia tortilis*, *Vachellia luederitzii* and *Faidherbia albida*. The heavy metal(loid)s pollution load in the tailings and in the vicinity of Monarch Gold mine and Bamangwato Concessions Limited (BCL) copper and nickel mine were assessed by quantifying the heavy metal(loid)s contents in soils and plants growing in the area. From this data, estimates of its possible impact to grazing cattle, goat, and sheep were performed through the daily intake of heavy metal(loid)s from plants and soils. Simultaneously, pot experiments were conducted under the screenhouse by growing some of the plants to assess their phytoremediation potential. This was done by applying different soil amendments following standard experimental designs in heavy metal(loid)s - contaminated soils or mine tailings. Results indicated that soils from the two mining areas had a pollution load index (PLI) of about 8.31 to 10.79 in BCL mine sites and between 1.84 to 2.87 in Monarch gold mine areas, indicating that there is severe pollution in the mine tailings and areas in the vicinity of the mine tailings. The plants growing in these areas had high concentrations of heavy metal(loid)s (As, Cu, Ni, Pb, Mn, Cd, Cr, and Zn) which were above the background level, indicating pollution. Different plants species were identified in mine tailings that are potential for phytoremediation agents include, *Cenchrus biflorus*, *Bolboschoenus nobilis*, *Oxycaryum cubense*, *Portulaca oleracea* *Gossypium herbaceum*, *Withania somnifera*, *Fadogia homblei*, *Lantana dinteri*, *Schinus mole*, *Colospospermum mopane*, *Boscia albitrunca*, *Senegalia mellifera* *Vachellia leuderitzii* among others. Estimation

Abstract

of the daily intake of heavy metal(loid)s by cattle, goat, and sheep grazing on plants near the BCL mine and Monarch gold mine in Selibe-Phikwe and Francistown respectively, revealed that Cu, Ni, Pb, As, Cr were above the maximum allowable limits indicating the possibility of food chain contamination. Screenhouse trials of different trees grown in mine tailings without amendments had low survival rate (0%, 50%) compared to plants grown in mine tailings with amendments (90-100% survival rate). Plants grown in tailings without amendments also had low dry matter yield compared to plants grown in tailings with amendments. Different amendments had varying effects on mine tailings as some amendments like fly ash and compost increased the pH of the mine tailings, and reduced the availability of Cu, Ni, Cr, Pb, Mn and Zn to plants. Results obtained from various experiments and analysis indicated that *Vachellia luederitzii* was the best candidate for phytoremediation of mine tailings due to its high survival rate (70-100%) and high dry matter yield. *V. luederitzii* species also accumulated high concentrations of heavy metal(loid)s compared to other species indicating that it is a hyperaccumulator. Other tree species were ranked according to the decreasing preference based on survival rate, dry matter yield and heavy metal(loid)s accumulation. The rank was as follows: *V. luederitzii* *F. albida* > *V. tortilis* > *C. mopane* > *E. globulus*. In conclusion, there is contamination of heavy metal(loid)s in abandoned sites including the vicinity. This poses a risk to human health through direct exposure, bioaccumulation, and magnification through the food chain. Mine tailings in BCL and Monarch Gold mine and the vicinity can be phytoremediated with these identified tree species and the phytoremediation potential could be improved by the addition of amendments like fly ash, compost, sewage sludge, mycorrhiza, and their combination.

Abstract

Keywords: Heavy metal(loid)s pollution, remediation, hyperaccumulator, phytostabilization, pollution load index, amendments, mine tailings

Dedication

Dedication

This thesis is dedicated to my two beloved daughters, my family, and my late father who was a resident of Monarch, Francistown and died due to respiratory-related illness in 2002 and never lived to see this moment. May your soul rest in peace Dad; you will always be in my heart Mr Edison L. Manyiwa.

Acknowledgments

Acknowledgments

To God almighty, I would like to thank you, I wouldn't have done it without your grace. A special thank you goes to my supervisor, Professor Venecio U. Ultra Jr. for his guidance and unwavering support throughout this academic journey, without a mentor like you, I wouldn't have made it Prof. Thank you for being patient with me and always believing in me. A special thanks also go to Botswana International University of Science & Technology, for granting me funds to carry out this research through a research initiation grant DVC/RDI/2/7 IV (110) and for allowing me to use their facilities and equipment for analysis and vehicles during the laborious fieldwork. The grant also allowed me to attend my first-ever international SETAC conference in Capetown, South Africa. I would also like to thank the AJ-CORE-SusMine project awarded to Prof. Ultra for assisting me with funds for field work when my grant was exhausted. A special thank you goes to my research team, K. Gajaje, S.B Taupedi, K. Sefatlhi, G. Ishmael, K. Gatlhobogwe, B.C. Ditlhakanyane, Dr. G. Rantong, K.A Opaletswe, M. Hengari, G. Gabankitse (MHSRIP), O. Monnagotla, K. July, M. Mpalo and P. Tlale, S. Kabelo and R. Kabelo. This study would not have been possible without the assistance of Mr. Modiretse Tumaletse, the environmental engineer at BCL Mine in Selibe Phikwe. My sincere gratitude also goes to my Departmental colleagues. Last but not least I would like to thank my beloved family, especially my wife Boitshepo, and my two daughters Lapelo and Lelomo, thank you for pushing me to complete my studies and for the inspiration of making sure that you lack nothing, thank you for your prayers guys. To my brother Champion, thank you for always pushing me to study for a PhD and believing in me that I can make it. This one is for you my brother. A special thank you to my mother and beloved siblings.

Table of Contents

Table of Contents

Declaration Regarding The Work And Copyright	II
Certification By The Supervisor	III
Abstract	IV
Dedication	VIII
Acknowledgments	IX
List of Figures	XIV
List of Tables	XV
CHAPTER 1	1
General Introduction	1
1.1 Statement of the Problem	6
1.2 Justification of the Study	9
1.3 Objectives of the Study	10
1.3.1 Specific Objectives	11
1.4 Structure of the thesis	11
1.5 References	12
CHAPTER 2	18
Review of Literature	18
2.1 Extent of heavy metal contamination in Botswana & non-compliance to environmental laws leading to environmental problems in Botswana	18
2.1.1 Heavy metal remediation	21
2.1.2 Phytoremediation as a rehabilitation technique	21
2.1.3 Phytoremediation potential of plant species at the study area	29

Table of Contents

2.2	Impact of heavy metal(loid)s on grazing animals	30
2.3	Impacts of stockpiling mine tailings on the physical environment	30
2.3.1	Acid mine drainage formation	31
2.3.2	Release of heavy metal(loid)s	32
2.3.3	Impacts of heavy heavy metal(loid)s on soil quality	32
2.4	Effects of Amendments on soil and heavy metal(loid)s	34
2.4.1	Arbuscular mycorrhiza	34
2.4.2	Compost	35
2.4.3	Fly ash	36
2.4.4	Cow manure	36
2.4.5	Sewage sludge	37
2.5	References	37
CHAPTER 3		48
Materials And Methods		48
3.1	Research Framework and General Methodology	48
3.2	Assessment of the impact of heavy metal contamination on soil and plant communities in mine areas	50
3.3	Effects of amendments on heavy metal(loid)s uptake by plants and availability in soil.	51
CHAPTER 4		54
Impact Of Heavy Metal(loid)s Contamination On Soil And Plant Communities In Mining Areas		54
4.1	Heavy metal(loid)s in Soils, Plants and Associated Risk on Grazing Ruminants in the Vicinity of Cu-Ni Mine in Selebi-Phikwe, Botswana	56
4.1.1	Introduction	57
4.1.2	Materials and Methods	60
4.1.3	Results	66
4.1.4	Discussion	78
4.1.5	References	84
4.2	Spatial variability of heavy metal(loid)s in soils and vegetation, and associated risk to grazing animals in the Abandoned Gold Mine in Francistown, Botswana	89
4.2.1	Introduction	90

Table of Contents

4.2.2	Materials and methods	92
4.2.3	Results	101
4.2.4	Discussion	118
4.2.5	Conclusions	126
4.2.6	General Conclusions of Chapter 4	126
4.2.7	References	127
CHAPTER 5		134
Influence of Different Soil Amendments On Heavy Metal(loid)s Uptake, Survival, And Phytoremediation Efficiency Of Selected Tree Species		134
5.1	Soil Amendments and Arbuscular Mycorrhiza Influenced the Growth and Heavy Metal Accumulation of <i>Colospospermum mopane</i> (Kirk Ex Benth.) In Heavy Metal Contaminated Soil	138
5.1.1	Introduction	139
5.1.2	Materials and Methods	142
5.1.3	Results	146
5.1.4	Discussion	153
5.1.5	References	161
5.2	Influence of mycorrhiza and fly ash on the survival, growth and Heavy metal(loid)s accumulation in three Acacia species grown in Cu-Ni mine soil	170
5.2.1	Introduction	171
5.2.2	Materials and methods	175
5.2.3	Results	179
5.2.4	Discussions	191
5.2.5	References	197
5.3	Enhancing the phytoremediation potential of <i>Eucalyptus globulus</i> in three different mine tailings of Botswana through soil amendments	202
5.3.1	Introduction	203
5.3.2	Materials and Methods	205
5.3.2.2	Plant sample processing, chemical analysis & dry matter yield	208
5.3.3	Results and Discussion	213
5.3.4	Conclusions	230
5.3.5	General Conclusions of Chapter 5	231
5.3.6	References	231

Table of Contents

CHAPTER 6	241
Potential of Different Tree Species {<i>Eucalyptus Globulus</i>, <i>Colophospermum Mopane</i> And Three <i>Vachelia (Acacia)</i> Spp.} For Phytoremediation of Mine Tailings	241
6.1 Variability of the phytoremediation potential of different tree species in BCL Cu-Ni mine tailings	243
6.1.1 Introduction	245
6.1.2 Materials and Methods	246
6.1.3 Statistical analysis	247
6.1.4 Results and Discussion	248
6.1.5 Conclusions	262
6.1.6 References	262
CHAPTER 7	268
General Discussion, Conclusions, And Recommendations	268
7.1 General Discussion and Conclusions	268
7.2 Recommendations	271
APPENDICES	273
7.3 List of Publications	273
7.4 Conference Presentations & Posters	274

List of Figures

List of Figures

Figure 1. 1 a & b, study areas. Copper Slag in BCL Mine and mine tailings at Monarch Gold mine.....	1
Figure 1. 2 The effect of heavy metal(loid)s pollution on the environments.....	7
Figure 1. 3 Copper slag residues observed in the streams near the BCL mine.....	8
Figure 1. 4 Grazing animals observed in the vicinity of BCL mine.....	9
Figure 3. 1 Research framework of this study.....	49
Figure 3. 2 Hypothetical framework after application of the research methodology.....	50
Figure 3. 3 Methodological illustration of objective one.....	51
Figure 3. 4 Methodological illustration of objective two and three.....	52
Figure 4.1. 1 Map showing the study area and the sampling sites.....	62
Figure 4.2. 1 Location of the study area and sampling sites.....	94

List of Tables

List of Tables

Table 4.1.1 Estimates of parameters used in the calculation of daily dietary intake of grazing ruminants from plants near the vicinity of the BCL mines	65
Table 4.1.2 Average total concentration of heavy metal(loid)s in soils (n=4) from different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana.	67
Table 4.1.3 Average concentration of exchangeable heavy metal(loid)s in soils (n=4) from different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana.	68
Table 4.1.4 Average ($\pm$ standard deviation) contamination factor (CF) and pollution load index (PLI) of different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana	69
Table 4.1.5 List and classification of plants collected from different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana.	71
Table 4.1.6 Heavy metal contents in the shoots of plants collected from different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana.....	73
Table 4.1.7 Average bioconcentration factor for different heavy metal(loid)s of different plant species collected from different sampling sites in the vicinity of BCL mines, Selibe-Phikwe, Botswana.	76
Table 4.1.8 Maximum and estimated daily intake of heavy metal(loid)s of cattle, goat and sheep grazing on plants near the BCL mines, Selibe-Phikwe, Botswana	77
Table 4.2.1 ICP-MS instrumental parameters employed for heavy metal determination and % recovery of concentrations of internal reference sample	97

List of Tables

Table 4.2.2 The pH, EC and total As, Mn, Cu, Cr, Zr contents and PLI of soils from abandoned Monarch Gold Mine, Francistown, Botswana ¹	100
Table 4.2.3 Available As, Mn, Cu, Cr, and Zr contents of soils from abandoned Monarch Gold Mine, Francistown, Botswana ¹	103
Table 4.2.4 Heavy metal contents in shoots of different plants growing in the tailings dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana ¹	106
Table 4.2.5 Bioaccumulation factor of heavy metal(loid)s contents in shoots of different plants growing from the tailings dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana ¹	108
Table 4.2.6 Heavy metal contents in shoots of different plants growing within 0.5 to 2km away from the tailings dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana ¹	111
Table 4.2.7 Bioaccumulation factor of heavy metal(loid)s contents in shoots of different plants growing within 0.5 to 2km away from the tailing's dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana ¹	114
Table 4.2.8 Maximum and estimated daily intake of heavy metal(loid)s of cattle, goat and sheep grazing on plants inside and near tailings dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana.....	117
Table 5.1.1 Chemical properties and heavy metal contents in soils and fly ash.....	143
Table 5.1.2 Survival, mycorrhizal root colonization and dry matter yield of Mopane in heavy metal contaminated soils as affected by soil amendments and mycorrhiza inoculation ¹ ...	147
Table 5.1.3 Concentrations of heavy metal(loid)s in <i>Colospermum mopane</i> as influenced by different amendments and mycorrhizal inoculation ¹	149

List of Tables

Table 5.1.4 Translocation factor of different heavy metal(loid)s in Mopane as affected by soil amendments and mycorrhizal inoculation ¹	151
Table 5.1.5 The pH and electrical conductivity of soil after seedling growth as influenced by different amendments and mycorrhiza ¹	152
Table 5.1.6 Concentrations of heavy metal(loid)s in <i>Colospospermum mopane</i> extracted using water as influenced by different amendments ¹	153
Table 5.2.1 Chemical properties and heavy metal contents in mine tailings and fly ash.....	176
Table 5.2.2 Percentage survival of different plant species in mine tailings from BCL mines as influenced by mycorrhiza inoculation and fly ash amendment ¹	179
Table 5.2.3. Shoots, roots and total dry weight of <i>Acacia</i> spp. grown in mine tailings as affected by mycorrhiza inoculation and fly ash amendment at 24 weeks after planting ¹ ..	180
Table 5.2.4 Root colonization of different acacia species with arbuscular mycorrhiza grown in mine tailings as affected by mycorrhiza inoculation and fly ash amendment at 24 weeks after planting ¹	181
Table 5.2.5. Heavy metal concentrations in shoot and roots of <i>A. albida</i> (n=20) grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 24 weeks after planting ¹	184
Table 5.2.6. Heavy metal concentrations in shoot and roots of <i>A. luederitzii</i> (n=20) grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 24 weeks after planting ¹	185
Table 5.2.7. Heavy metal concentrations in shoot and roots of <i>A. tortilis</i> (n=20) grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 24 weeks after planting ¹	187

List of Tables

Table 5.2.8. Translocation factor (shoot/root ratio) of heavy metal concentration of <i>A. albida</i> , <i>A. luederitzii</i> and <i>A. tortilis</i> grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 24 weeks after planting ¹	189
Table 5.2.9. Chemical properties of soils (n=60) after cultivation of plants ¹	190

CHAPTER 1

General Introduction

Mining is a large contributor to the Gross domestic product (GDP) in Botswana, contributing 19.6% (Statistics Botswana, 2023). This indicates how important mining is for the country's economy. However, it also creates adverse environmental issues including land degradation; huge stockpiles of mine tailings; and environmental pollution from elevated levels of heavy metal(loid)s (figures 1.1. a & b). Evangelou and Zang (1995) and Shu et al. (2001) have reported that the release of heavy metal(loid)s is influenced and accelerated by highly acidic conditions due to the formation of acid mine drainage (AMD). The heavy metal(loid)s are also likely to leach into the soil and reach water bodies. Additionally, they can be incorporated into the food chain where their concentrations can be increased through biological magnification (Sonone et al., 2020). Heavy metal toxicity can adversely affect the numbers, diversity, and activity of soil microorganisms, inhibiting soil organic matter decomposition and nitrogen mineralization processes which eventually restrict the growth of plants (Wong, 2003).



Figure 1. 1 a & b, study areas. Copper Slag in Bamangwato Concessions Limited (BCL) closed mine and mine tailings at Monarch Gold mine

General Introduction

Phytoremediation is a technique that involves the use of plants to accumulate elements and compounds from the environment and then metabolise various molecules in their tissues (Paz-Alberto and Singua, 2013). In other words, phytoremediation is a form of green technology that is very promising in removing environmental pollutants. (Nouri et al., 2009). This technique has gained public exposure and has been increasingly examined as a practical and cost-effective technology (Pulford and Watson, 2003). Phytoremediation offers an effective, environmentally friendly, economically feasible procedure to overcome the serious problems stemming from mining. It is a low-cost, solar-energy-driven clean-up approach that utilizes living plants for the removal of contaminants (Ali et al., 2013). Depending on the physicochemical properties of mine tailings, plants can be used to stabilize the area and/or remove the toxic components in mine tailings. This study will address the specific needs of remediation and the rehabilitation of mine tailing dumpsites as well as provide effective waste management strategies for different mines. As such, this study will provide a cost-effective and less destructive phytoremediation strategy that can stabilize the mine tailings; reduce the associated contaminants; and restore the ecosystem of the area through the use of suitable plants, with the aid of beneficial soil organisms and the re-engineering of the rhizosphere to enhance plants' capabilities to colonize the mine tailings dump site. The strategy will include the use of adaptive and tolerant plants; the cultivation of beneficial microorganisms; and the transformation of mine tailing deposits into more habitable growing media for plants. Heavy metal hyperaccumulator species for reclamation of metal-contaminated mine tailings through phytoextraction will also be included.

The efficiency and effectiveness of phytoremediation, particularly phytoextraction, is a function of the plant type and density on contaminated sites; the concentration, extent and

General Introduction

availability of pollutants; and the soil and environmental condition which varies with location (Mahar et al., 2016). Variability of the physicochemical properties of contaminated sites requires a unique approach in order for phytoremediation to be successful. With current technological advances, the following methods are now being used: chelating agents to improve heavy metal availability and plant uptake (Ultra et al., 2005); genetically modified plants with high biomass and heavy metal hyperaccumulating capabilities (Ibañez, et al., 2016); soil amendments that increase metal availability and growth of plants; and more recently, the cultivation and promotion of beneficial soil microorganisms (Kong and Glick, 2017). Castaldi et al. (2005) found that the use of amendments can enhance the growth of plants cultivated in heavy-metal(loid)s-polluted soils.

The challenge of phytoremediation of mine tailing deposits in dumpsites or in lagoon storage areas, can be attributed to the physicochemical and biological properties of mine tailings. Mine tailings are seen to be biologically inert, often containing high amounts of multiple heavy metal(loid)s. They are considered to create soil structure conditions with poor fertility, zero microbial activity, and poor physical properties. These conditions are brought about by fly ash deposit development which is characterized by “cemented” or compacted layers that are detrimental to plant growth (Yao, et al., 2017). To stabilize and successfully restore a healthy ecosystem, strategies should focus on recolonizing the barren land with plants. This will gradually improve the condition of other organisms and allow for the rebuilding of communities in the area.

To overcome these challenges, this study identified plants that could survive in these harsh environments. Candidate plants were chosen from exotic and indigenous plant communities or highly tolerant plants. These selected were *Eucalyptus globulus* (*blokomo*),

General Introduction

Colophospermum mopane, *Vachellia tortilis* (Mosu), *Vachellia luederitzii* (Mokala), and *Fairherbia albida* (Mokosho). *Vachellia* (acacia) species were chosen mainly because they are easy to cultivate and are leguminous plants and can trap nitrogen from the atmosphere and increase their chances of survival. They also contain hydrolyzable tannins that act as adsorbents of heavy metal(loid)s (Batiha et al., 2022). Other species like *C. mopane* and *E. globulus* were chosen mainly because of their potential for timber. Previous studies by Ekosse (2005) found out that *C. mopane* trees near the BCL mine in Selibe-Phikwe had high contents of metal(loid)s indicating that *C. mopane* can tolerate high levels of metal(loid)s. These trees are also not attractive to herbivory (Adesodun et al., 2010). To increase their chances of survival and colonization, ecological engineering was employed by modifying the rhizosphere. Beneficial microorganisms associated with plants and soil amendments were properly identified and introduced to mine tailing deposits to create a favorable microenvironment for plant growth and development.

This study also utilized the ability of microorganisms to produce various metabolites (siderophores, organic acids, biosurfactants, etc.) that will affect numerous biogeochemical processes in the rhizosphere (Nie et al., 2017). Soil microorganisms can enhance the supply and absorption of nutrients through cell extension; the detoxication of metals and the decrease of abiotic stress in plants; the improvement of nutrition through N₂ fixation; the production of phytohormones and siderophores; and the transformation of nutrient elements when they are either applied to seeds or incorporated into the soil. Rhizosphere microorganisms can accelerate the mobilization or immobilization of metals by releasing chelating agents; acidification; phosphate solubilization; the induction of metalloid transformation, and redox changes (Ma et al., 2016). These functions are of great importance

General Introduction

for plants' nutrient and metal absorption. Therefore, this research project will harness the plant-microbe interaction to increase the effectiveness of phytoremediation, directly affecting the plants' capability to survive while indirectly stimulating their productivity.

Previous studies indicated that mycorrhiza and rhizosphere organisms were able to transform arsenic and other heavy metal(loid)s into less toxic forms and help plants enhance absorption and tolerance (Ultra et al., 2007). Several studies also showed that rhizobacteria and mycorrhiza can alter nutrient and heavy metal bioavailability in mine tailing deposits through the release of chelating substances, through acidification of the microenvironment, and by influencing changes in redox potential (Ma et al., 2016).

To enhance the success of phytoremediation in a heavy metal-contaminated environment, some amendments are applied to the soil, usually during the plant establishment. These amendments include the application of lime in acidic sites; fly ash (FA); compost and other materials that can modify the physicochemical properties of soil (Han et al., 2012; Hussain et al. 2018; Ultra, 2020). Most of these amendments can significantly alter the growth and survival of plants as well as the accumulation and uptake of heavy metal(loid)s in them (Ultra and Manyiwa, 2021). FA is a good amendment because of its texture and the fact that it contains all essential plant nutrients except for N (Pathan et al., 2003). Using FA as an amendment improves soil's physicochemical properties and microbial activity (Singh and Pandey, 2013). However, studies by Lee et al. (2007) and Pandey and Singh (2010), have found that FA also contains heavy metal(loid)s such as Cu, Ni, Cr, Cd and others that may lead to metal toxicity in plants.

Alternatively, using compost as an amendment in a heavy metal contaminated environment also modifies the bio-physicochemical properties of soil and provides nutrients

for plant growth and development (Huang et al., 2016). Studies by Saum et al. (2018) and Huang et al. (2016), have shown that compost amendments can reduce heavy metal availability by providing humus as sorption sites of cationic metals and soluble organic substances that can serve as chelating agents of heavy metal(loid)s in soils. However, the application of compost can increase the concentration of heavy metal(loid)s in plants because it may contain elevated heavy metal(loid)s or other toxic substances (Petruzzelli, 1996). Therefore, caution should be exercised in applying composts in phytoremediation systems. Depending on the source and quality, compost can also cause pollution, with the severity depending on the chemical form of heavy metal(loid)s in the compost and the source of the raw materials (Castaldi et al., 2005). Other studies found that the use of compost accelerates the removal and stabilization of organic pollutants in the soil and plants by providing a favorable environment for microbial growth (Farrell and Jones, 2010).

1.1 Statement of the Problem

While the extraction of metals such as copper, nickel and gold are required for the economic and industrial growth of Botswana, the disposal of mining waste poses a considerable environmental concern. If mine waste is not properly disposed of, it can significantly contribute to heavy metal pollution in the surrounding environment, resulting in ecological landscape destruction, groundwater pollution, and a decrease in biological diversity (Sheoran et al., 2010; Rashed, 2010; Kiikkila, 2003). Copper-Nickel mines not only discharge potentially harmful metals; they also release additional particulate matter and gaseous materials into the atmosphere, such as dust, SO₂, CO, CO₂, N₂O, NO₂, and NO

(Nagajyoti et al., 2010). Heavy metal(loid)s can also lead to forest damage and reduced diversity of vascular plants (figure 1.2).



Figure 1. 2 The effect of heavy metal(loid)s pollution on the environments observed near the BCL mine

This can lead to serious impacts on the ecology of the area. Heavy metal(loid)s, because of their long-term persistence in the environment, pose a threat to human and animal health (Ekosse et al., 2005; Ekosse et al., 2007). For example, heavy metal(loid)s such as lead and cadmium are nonbiodegradable and serve no biological purpose. Others such as copper, zinc, and nickel are nutrient sources for plants at low quantities but become toxic at high concentrations. According to Gajaje et al. (2024) plants can tolerate Cu at 100 mg kg^{-1} , Zn at 200 mg kg^{-1} and Ni at 5 mg kg^{-1} , if they are above these limits they become toxic to plants. Heavy metal(loid)s can also lead to water contamination. Around the BCL mine, pollution of water sources from mine tailings was observed in the nearby water streams (Figure 1.3). These streams feed the Motloutse river supplying the Dikgathong dam which is a major source of potable drinking water for the central and southern parts of the country.

General Introduction



Figure 1. 3 Copper slag residues observed in the streams near the BCL mine

Heavy metal(loid)s tends to bioaccumulate and bio-magnify in animal tissues (Sonone et al., 2020). Since domestic animals such as cattle and goats graze in the surrounding area of the BCL mine (figure 1.4), these animals are likely to ingest soil and plant materials contaminated with heavy metal(loid)s. This, in turn, can pose a risk to people consuming animal products. Cadmium and lead are known to accumulate in animal livers and kidneys (Kirkham, 2006; Wilkinson et al., 2003) which are considered a delicacy in Southern Africa, Botswana included (Ikenaka et al., 2010; Yabe et al., 2011), and the ingestion of these organs may constitute a health risk.



Figure 1. 4 Grazing animals observed in the vicinity of the BCL mine

In Botswana, environmental problems due to mining activities largely stem from the lack of enforcement of the Environmental Impact Assessment Act of 2011. The Act states that the company or person awarded mining rights is responsible for rehabilitating the area affected by the adverse environmental impact of the implemented activity. Any company or person that fails to rehabilitate the area, is seen to have committed an offense and may be liable for a fine, or imprisonment, or both (Botswana Government Gazette, 2011). Unfortunately, this Act is not fully enforced, which leads to negligence in the rehabilitation of the mined area by some mining companies, which then results in environmental issues.

1.2 Justification of the Study

The study will develop a phytoremediation system suited for the mine tailings deposits in Botswana by enhancing the adaptation capabilities of partners of the associative symbiosis as well as the bioremediation potential of the microorganisms and different amendments. Although the use of beneficial microorganisms in phytoremediation has been explored elsewhere, no comprehensive studies have been conducted in the local

environment. Therefore, this study will use a combination of mycorrhiza inoculants and soil amendments such as: fly ash; compost; sewage sludge; and cow manure to improve the conditions of mine tailings and to support plant growth. Several studies conducted in Botswana have focused on the impacts of copper mining in the Selibe Phikwe area, on human health, air and water quality (Ekosse, 2005). However, no studies have been carried out on the phytoremediation of soils and mine tailings in Selibe Phikwe and Francistown, thus indicating a knowledge gap. According to Sherestha and Lal (2011), a full understanding of the constraints and opportunities to successfully reclaim contaminated land can be achieved through the assessment of physical, chemical, and biological factors pertaining to mine waste. Furthermore, Shrestha and Lal (2011) assert that effective reclamation will occur if these properties are conducive for plant growth. Soil properties which affect the growth of vegetation and metal uptake are therefore important factors to be considered in the rehabilitation of mine tailing dumps or stockpiles. According to Wan et al. (2024) plant metal uptake is influenced by soil factors including pH, organic matter, soil texture, clay content, cation exchange capacity (CEC), and electrical conductivity (EC). Very few studies have been conducted on the rehabilitation of heavy metal-polluted soils in Botswana. Most studies conducted in Botswana were mainly on the assessment of heavy metal(loid)s.

1.3 Objectives of the Study

The general objectives of the study are to assess the levels of heavy metal contamination in mine waste and soils impacted by mining activities and to develop phytoremediation technology using various tree species.

1.3.1 Specific Objectives

- i. To assess the impact of heavy metal contamination on soil and plant communities, plant species diversity and abundance in mine areas and nearby ecosystems through characterization of the chemical properties of soil and mine tailings from BCL Cu-Ni mine and Monarch Gold Mine; as well as associated risks on grazing animals.
- ii. To evaluate the influence of different soil amendments on selected plants with respect to heavy metal uptake; survival; and phytoremediation efficiency, by assessing the influence of different amendments (fly ash, mycorrhiza, compost) on soil chemical properties, heavy metal uptake, and availability, and the overall phytoremediation potential of these plants, by monitoring their survival, growth, and heavy metal uptake.
- iii. To evaluate the potential of different tree species {*Eucalyptus globulus (blokomo)*, *Colophospermum mopane*, *Vachellia tortilis (Mosu)*, *Vachellia luederitzii (Mokala)* and *Fairherbia albida (Mokosho)*} for phytoremediation of mine tailings by monitoring their growth, and development, as well as examining heavy metal bioaccumulation.

1.4 Structure of the thesis

This thesis is divided into chapters that have been reproduced as published material. Other Chapters are articles that have been submitted to journals for review. These have also been reproduced as submitted.

1.5 References

- Adesodun, J.K., Atayese, M.O., Agbaje, T.A., Osadiaye, B.A., Mafe, O.F. and Soretire, A.A. (2010) Phytoremediation Potentials of Sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for Metals in Soils Contaminated with Zinc and Lead Nitrates. *Water, Air, and Soil Pollution*, 207, 195-201. <http://dx.doi.org/10.1007/s11270-009-0128-3>
- Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals —concepts and applications. *Chemosphere*, 91(7), 869-881.
- Batiha, G. E. S., Akhtar, N., Alsayegh, A. A., Abusudah, W. F., Almohmadi, N. H., Shaheen, H. M. & De Waard, M. (2022). Bioactive Compounds, Pharmacological Actions, and Pharmacokinetics of Genus *Acacia*. *Molecules*, 27(21), 7340.
- Botswana Government Gazette. (2011). Environmental Assessment Act, 2011. No.10 of 2011.
- Brundrett, M.C. & Abbott, L.K. (1994). Mycorrhizal fungus propagules in the Jarrah forest: I. Seasonal study of inoculum levels. *New Phytologist* 127(3):539-46.
- Castaldi, P., Santona, L., & Melis, P. (2005). Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. *Chemosphere*, 60(3), 365-371.
- Ekosse, G., (2005). Heavy metals concentrations in the biophysical environment around the Ni-Cu mine and the smelter/concentrator plant, Selebi Phikwe, Botswana. *Global Journal of Environmental Sciences*, 4(2), pp.97-110.
- Evangelou, V. P., & Zhang, Y. L. (1995). A review: pyrite oxidation mechanisms and acid mine drainage prevention. *Critical Reviews in Environmental Science and Technology*, 25(2), 141-199.

- Farrell, M. & Jones, D.L. (2010). Use of composts in the remediation of heavy metal contaminated soil. *Journal of Hazardous Materials* 175 (1-3):575-82.
- Gajaje, K., Duelang, M., Ultra Jr, V. U., Marenga, W., & Rantong, G. (2024). Impact of fly ash deposition on plants diversity, metal content, and associated risk on grazing ruminants in the vicinity of Morupule power station, Botswana. *Arid Land Research and Management*, 1-25.
- Han, S. H., Kim, D. H., Ultra Jr, V. U., KIM, P., & Aggangan, N. S. (2012). Physiological response of Cd-and Pb-treated *Populus alba* × *glandulosa* seedlings amended with SCB liquid fertilizer. *Asia Life Sciences*, 21(2), 481-498.
- Huang, M., Zhu, Y., Li, Z., Huang, B., Luo, N., Liu, C. & Zeng, G. (2016). Compost as a soil amendment to remediate heavy metal-contaminated agricultural soil: mechanisms, efficacy, problems, and strategies. *Water Air Soil Pollution* 227(10):359.
- Hussain, F., Hussain, I., Khan, A.H., Muhammad, Y.S., Iqbal, M., Soja, G., Reichenauer, T.G. & Yousaf, S. (2018). Combined application of biochar, compost, and bacterial consortia with Italian ryegrass enhanced phytoremediation of petroleum hydrocarbon contaminated soil. *Environmental Experimental Botany* 153:80-8.
- Ibañez, S., Talano, M., Ontañón, O., Suman, J., Medina, M. I., Macek, T., & Agostini, E. (2016). Transgenic plants and hairy roots: exploiting the potential of plant species to remediate contaminants. *New biotechnology*, 33(5), 625-635.
- Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere*, 50(6), 775-780.

- Ikenaka, Y., Nakayama, S. M., Muzandu, K., Choongo, K., Teraoka, H., Mizuno, N., & Ishizuka, M. (2010). Heavy metal contamination of soil and sediment in Zambia. *African Journal of Environmental Science and Technology*, 4(11), 729-739.
- Kiikkilä, O. (2003). Heavy-metal pollution and remediation of forest soil around the Harjavalta Cu-Ni smelter, in SW Finland.
- Kirkham, M. B. (2006). Cadmium in plants on polluted soils: Effects of soil factors, hyperaccumulation, and amendments. *Geoderma*, 137(1-2), 19-32.
- Kong, Z. & Glick, B.R. (2017). The role of plant growth-promoting bacteria in metal phytoremediation. In *Advances in Microbial Physiology* (Vol. 71, 97-132). Academic Press.
- Lee, C.H., Lee, Y.B., Lee, H. & Kim, P.J. (2007). Reducing phosphorus release from paddy soils by a fly ash-gypsum mixture. *Bioresource Technology* 98(10):1980-4.
- Ma, Y., Oliveira, R. S., Freitas, H., & Zhang, C. (2016). Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. *Frontiers in plant science*, 7, 918.
- Mahar, A., Wang, P., Ali, A., Awasthi, M.K., Lahori, A.H., Wang, Q., Li, R. & Zhang, Z. (2016). Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. *Ecotoxicology and Environmental Safety*, 126, 111-121.
- Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals occurrence and toxicity for plants: a review. *Environmental chemistry letters*, 8, 199-216.
- Nie, H., Nie, M., Xiao, T., Wang, Y., & Tian, X. (2017). Hexadecane degradation of *Pseudomonas aeruginosa* NY3 promoted by glutaric acid. *Science of the Total Environment*, 575, 1423-1428.

- Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A.H, & Yusefi, N. (2009). Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential, *Environmental Earth Science* 59: 315-323.
- Pandey, V. C., & Singh, N. (2010). Impact of fly ash incorporation in soil systems. *Agriculture, ecosystems & environment*, 136(1-2), 16-27.
- Pathan, S.M., Aylmore, L.A. & Colmer, T.D. (2003). Properties of several fly ash materials in relation to use as soil amendments. *Journal of Environmental Quality* 32(2):687-93.
- Paz-Alberto, A. M., & Sigua, G. C. (2013). Phytoremediation: a green technology to remove environmental pollutants
- Pulford, I.D. & Watson, C. (2003). Phytoremediation of heavy metal contaminated land by trees- a review, *Environmental International* 29, 529-540.
- Petruzzelli, G. 1996. Heavy metals in compost and their effect on soil quality. In: de Bertoldi M., Lemmes B., Papi T. (eds.). *The science of composting*, 213–23. Dordrecht: Springer.
- Saum, L., Jiménez, M.B. & Crowley, D. (2018). Influence of biochar and compost on phytoremediation of oil-contaminated soil. *International Journal of Phytoremediation* 20(1):54-60.
- Singh, J.S. & Pandey, V.C. (2013). Fly ash application in nutrient poor agriculture soils: impact on methanotrophs population dynamics and paddy yields. *Ecotoxicology and Environmental Safety* 89:43-51.
- Shao, Y. D., Zhang, D. J., Hu, X. C., Wu, Q. S., Jiang, C. J., Xia, T. J. & Kuča, K. (2018). Mycorrhiza-induced changes in root growth and nutrient absorption of tea plants. *Plant, Soil and Environment*, 64(6), 283-289.

- Sheoran, V., Sheoran, A. S., & Poonia, P. (2010). Role of hyperaccumulators in phytoextraction of metals from contaminated mining sites: a review. *Critical Reviews in Environmental Science and Technology*, 41(2), 168-214.
- Shrestha, R. K., & Lal, R. (2011). Changes in physical and chemical properties of soil after surface mining and reclamation. *Geoderma*, 161(3-4), 168-176.
- Shu, W. S., Ye, Z. H., Lan, C. Y., Zhang, Z. Q., & Wong, M. H. (2001). Acidification of lead/zinc mine tailings and its effect on heavy metal mobility. *Environment international*, 26(5-6), 389-394.
- Sonone, S. S., Jadhav, S., Sankhla, M. S., & Kumar, R. (2020). Water contamination by heavy metals and their toxic effect on aquaculture and human health through food Chain. *Letters in Applied NanoBioScience*, 10(2), 2148-2166.
- Statistics Botswana. *Gross domestic product: first quarter of 2023*. Gaborone
- Ultra, V.U. (2020). Fly ash and compost amendments and mycorrhizal inoculation enhanced the survival and growth of *Delonix regia* in Cu-Ni mine tailings. National Science Week and STEM.
- Ultra, V. U., & Manyiwa, T. (2021). Influence of mycorrhiza and fly ash on the survival, growth and heavy metal accumulation in three *Acacia* species grown in Cu-Ni mine soil. *Environmental Geochemistry and Health*, 43, 1337-1353.
- Ultra Jr, V. U., Tanaka, S., Sakurai, K., & Iwasaki, K. (2007). Arbuscular mycorrhizal fungus (*Glomus aggregatum*) influences biotransformation of arsenic in the rhizosphere of sunflower (*Helianthus annuus* L.). *Soil Science and Plant Nutrition*, 53(4), 499-508.
- Ultra Jr, V.U., Yano, A., Iwasaki, K., Tanaka, S., Kang, Y. & Sakurai, K. (2005). Influence of chelating agent addition on copper distribution and microbial activity in soil and

copper uptake by brown mustard (*Brassica juncea*). *Soil Science & Plant Nutrition*, 51(2), 193-202.

Yabe, J., Nakayama, S. M., Ikenaka, Y., Muzandu, K., Ishizuka, M., & Umemura, T. (2011). Uptake of lead, cadmium, and other metals in the liver and kidneys of cattle near a lead-zinc mine in Kabwe, Zambia. *Environmental Toxicology and Chemistry*, 30(8), 1892-1897.

Yao, Q., Liu, J., Yu, Z., Li, Y., Jin, J., Liu, X., & Wang, G. (2017). Three years of biochar amendment alters soil physiochemical properties and fungal community composition in a black soil of northeast China. *Soil Biology and Biochemistry*, 110, 56-67.

Wan, Y., Liu, J., Zhuang, Z., Wang, Q., & Li, H. (2024). Heavy metals in agricultural soils: Sources, influencing factors, and remediation strategies. *Toxics*, 12(1), 63.

Worlanyo, A. S., & Jiangfeng, L. (2021). Evaluating the environmental and economic impact of mining for post-mined land restoration and land-use: A review. *Journal of Environmental Management*, 279, 111623.

CHAPTER 2

Review of Literature

2.1 Extent of heavy metal contamination in Botswana & non-compliance to environmental laws leading to environmental problems in Botswana

Botswana's economy is highly dependent on the exploration, processing, and export of mineral resources (Koitsiwe and Adachi, 2017). Apart from being a major source of diamonds, which accounts for about 40% of total world output; copper, nickel, gold, and soda ash also contribute significantly to the country's exports and the overall gross domestic product (GDP) (Matshediso, 2005; Yager, 2017). The mining sector accounts for about 19.6% of GDP and employs more than 5% of Botswana's residents (Imakando, 2017). With the continued expansion of the mining industry, associated environmental problems have also increased because some mining operations were not compliant with the provisions of environmental safety (Wiston, 2017). The BCL Cu-Ni mine in Selebi Phikwe was reported to have generated serious environmental problems that are directly related to poor mine waste management (Motswaiso, 2019). Based on the report by Ekosse (2008), the high environmental and health hazards due to mining were related to an unusually high occurrence of ailments and illnesses, coupled with associated diseases among workers at Selebi Phikwe. The high frequency of ailments and illnesses among the residents was attributed to environmental factors such as exposure to contaminated particulate air matter (PAM) rich in sulfur and heavy metal(loid)s that are directly linked to mining and smelting

of Ni-Cu. It was also found that, residents near the mining facilities had persistently complained of chest pains (Ekosse, 2007).

Assessment of the physical environment of Selebi Phikwe found severe deterioration of the soil, air, and groundwater quality which is traceable to the BCL mining operation (Likuku et al., 2013). The windward side of the BCL mines has high concentrations of Pb, Ni, Cu, and Zn which are associated with the deposition of particulate air matter from smelter emissions. The soils at the site are considered to be severely contaminated with Cu and Ni, and moderately contaminated with Fe, Mn, Pb, and Zn, but showed signs of low contamination of Co. The surface soils around the Selebi Phikwe Ni-Cu plant contained high amounts of cobalt oxide and silicon sulfide, which are indicative of mineralogical alteration from mining waste deposited on the soils, and the heavy minerals fraction of soil samples are higher on sites close to the mines and the smelter plant (Ekosse, 2005).

The tailings which are produced from the processing of ores are a point source of contaminants in the area. Schwartz and Kgomanyane (2008) elucidated that highly acidic seepage water (1.7-2.8 pH) which is strongly enriched in SO_4^{2-} (5,680 g L⁻¹) and heavy metal(loid)s (6,230 mg L⁻¹ Ni, 1,860 mg L⁻¹ Cu and 410 mg L⁻¹ Co) have slipped through the Selebi Phikwe fractured aquifer. They are considered to be the major source of groundwater pollution in the Selebi Phikwe area. The tailing dams also release high concentrations of heavy metal(loid)s into the surrounding environment through surface erosion and the deposition of particulate air matter (PAM) (Ekosse, 2005; Schippers et al., 2007). Analysis of PAM collected near the Selebi Phikwe Ni-Cu plant contained quartz, pyrrhotite, chalcopyrite, and albite, which formed from the mineralization of chalcocite and the gases H₂S and SO₂ which were released from the concentration/smeltering processes (Ekosse et al.,

2005). These minerals in PAM are very reactive with H_2S , and SO_2 , and atmospheric moisture, releasing H_2SO_4 , to form acid rain. The very strong acidity of PAM damages property, plants, and the entire ecosystem of the surrounding area. Evidence has shown that PAM can damage crops, trees, and shrubs by blocking gas exchange and reducing photosynthesis, thus resulting in the occurrence of dead spots near the BCL mines.

In Francistown, heavy metal pollution and contamination from Monarch Gold mine pose a great risk to human health and the ecosystem at large. If ignored, it could pose an even greater danger for future generations because heavy metal pollution can cause chronic diseases like cancer (Ekosse et al., 2005). In this regard, Maleki et al. (2014) found that humans are being exposed to heavy metal(loid)s through air, water, and soil. People living near mines or smelters like Monarch could be exposed to high metal levels by breathing air and touching contaminated soil (Ekosse et al., 2005; Vogel and Kasper, 2002). Previous studies on tailings in the Monarch gold mine found total levels of As to be between 200 to 1700 ppm, and Sb to be 40-400 ppm (Vogel and Kasper, 2002). These values for As and Sb exceed the Intervention Action values set by Albanese et al. (2007). These environmental problems continue to rise because some mining operations are not compliant with environmental health and safety regulations (Ekosse et al., 2005). These regulations include the Botswana Environmental Impact Assessment Act of 2011, Waste Management Act 2020, Botswana Bureau of Standards 93:2012 on Waste water – Physical, microbiological and chemical requirements – Specification and, the Atmospheric Pollution (Prevention) Act of 1971.

2.1.1 Heavy metal remediation

Various physical and chemical methods are available to restore soils contaminated with heavy metal(loid)s. By using physical procedures, contaminated dirt is removed to be buried elsewhere, and treated on or off-site. The treated soil is then returned to the location where it was originally sorted (Ghosh and Singh, 2005). However, this method is not desirable, because the excavation merely shifts the contamination problem elsewhere; and because of the high costs and hazards associated with transportation of contaminated soils. The alternative approach to contain the waste involves covering the soil with an inert substance that will compound the contamination and lessen its solubility (Wuana and Okieimen, 2011). However, containment methods only reduce the risk of potential exposure to plants whilst their concentration remains unchanged. Because of the environmental, destructive nature and the high costs associated with the above-mentioned methods, there is a need for inexpensive and environmentally friendly clean-up technologies, and phytoremediation can be used instead as a convenient alternative.

2.1.2 Phytoremediation as a rehabilitation technique

Phytoremediation is a form of green technology that is very promising in its ability to remove environmental pollutants through the use of plants. (Nouri et al., 2009). Since most of the plant roots are in the soil, they play an important role in the removal of metals through different methods like filtration, adsorption, and cation ion exchange capacity. The research community widely views it as the ecologically responsible alternative to the environmentally destructive, physical remediation methods currently used (Meagher, 2000). Phytoremediation is attractive as it offers site restoration, partial decontamination, maintenance of the biological activities and improvement of the physical structure of the soil.

Review of Literature

It is relatively inexpensive, visually unobtrusive, and it also offers the possibility of bio-recovery of metals. (Pulford and Watson, 2003). The advantages of phytoremediation have been outlined by Wuana and Okiemen (2011) as being: less disruptive to the environment; economically viable; an agent of reduction in the dispersal of dust and contaminants by wind; and an instrument in reduction of surface runoff. It is also more likely to be accepted by the public as it is more aesthetically pleasing than traditional methods, while also reducing the risk of spreading contaminants because it avoids excavation and the transport of polluted media. It also eliminates the potential of treating the polluted site with more than one type of pollutant.

There are different techniques of phytoremediation, including: phytodegradation; phytovolatilization; phytofiltration; phytostabilisation; phytoextraction; and phytostimulation (Figure 1.2.1). These techniques are selected for use depending on the composition, and the nature and properties of the contaminant, i.e. stable or inert, volatile. This is also dependent on the plant characteristics (Favas et al., 2014).

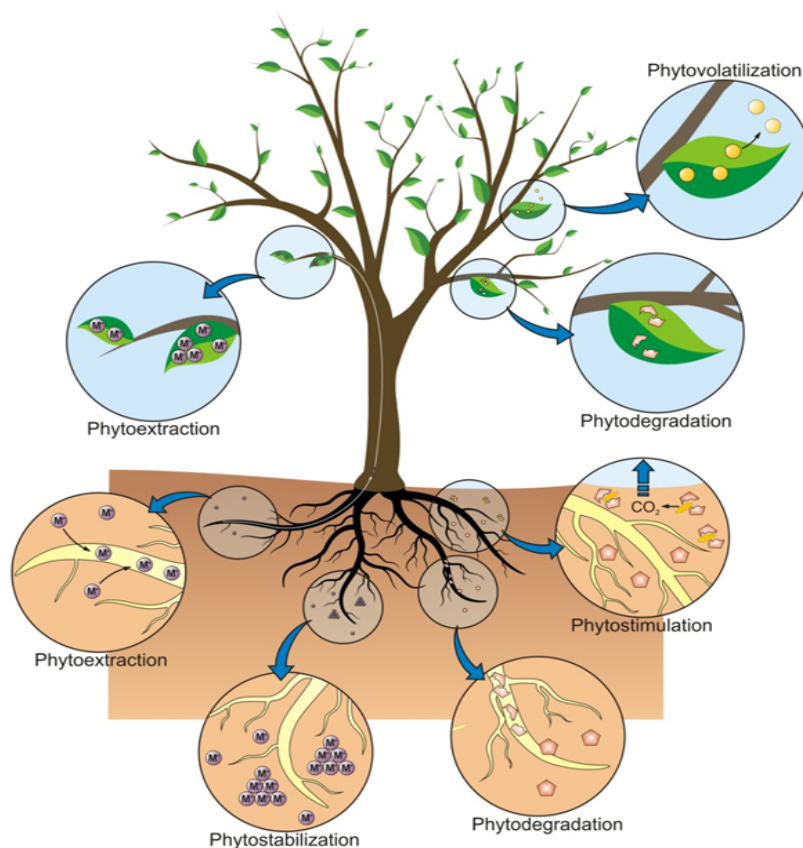


Figure 2.1.1. Schematic representation of phytoremediation techniques (Favas et al., 2014)

2.1.2.1 Phytostabilisation

Phytostabilisation involves using plants to reduce the movement and bioavailability of pollutants in the environment by preventing their migration (Pulford and Watson, 2003). It creates a vegetative cap with the plant canopy, serving to reduce aeolian dispersion and plant roots, thus preventing water erosion, immobilizing metals by adsorption or accumulation, and thereby providing a rhizosphere wherein metals precipitate and stabilize. The techniques focus on the sequestration of metals within the rhizosphere but not in the plant tissue. Consequently, metals become less available, leading to a reduction in exposure to livestock, wildlife, and humans (Mendez and Maier, 2008).

The plants used to stabilize contaminated soils and waste should be easily propagated and established; they should be fast growing; they should have extensive roots systems; and they should be tolerant or adaptive to adverse substrate conditions that include elevated contaminant levels and poor physical and fertility characteristics (Titshall et al., 2013). The use of annual indigenous species is often more advantageous than using introduced species as they are better adapted to environmental conditions at the site and require less maintenance (Titshall et al., 2013). The use of native plants will also avoid the introduction of non-native and potentially invasive species that may result in decreasing the regional diversity, ultimately resulting in poor plant colonization and soil conditions. Conesa et al. (2008) has suggested the use of annual species as they tend to have a higher rate of adaptation because of their shorter life cycle, thus promoting a larger variety of genotypes in a short timeframe. Therefore, the study will explore the use of native plants for phytoremediation.

2.1.2.2 Phytoextraction

Phytoextraction, also known as phyto-accumulation, is a technique that involves the uptake and translocation of heavy metal(loid)s by the roots into the upper tissues of plants (Ghori et al., 2016). Some plants have quite an extraordinary ability to accumulate heavy metal(loid)s. This ability is termed “phyto-accumulation” (Pulford and Watson, 2003; McGrath et al., 2006). The plants absorb contaminants through the root system and store them in the root biomass, and then transport them up to the stems and/or the leaves. A living plant may continue to absorb the contaminant until it is harvested, after which a lower concentration of the contaminant will remain in the soil. These plants must therefore be

grown repeatedly to achieve a significant cleanup of the soil which can support other vegetation species. More than 400 hundred species including trees, vegetable crops, grasses, and weeds are known to be hyper accumulators of metals. Phytoextraction applies to metals including Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb and Zn; as well as metalloids such as As and Se; and to radio nucleotides such as ^{90}Sr , ^{137}Cs , ^{234}U ; and non-metals such as B (Salt et al., 1995; Kumar et al., 1995). Successful phytoextraction depends on the bioavailability of the contaminant in the environment; root uptake and translocation of the plant; and the plant tolerance to the contaminant (Cunningham and Berti, 2020). Plant productivity and the accumulation factor (ratio of metal in the plant to that in the soil) are also very important parameters (Schonoor, 1997) In general, Garbisu and Alkorta (2001), Marchiol et al. (2004), have suggested that the ideal plants to be used in phytoextraction should: be tolerant to high levels of metal; be able to accumulate high levels of metals in harvestable parts; have a rapid growth rate; have the potential to produce high biomass in the field; and have a profuse root system. Hyperaccumulators generally have a shallow root zone which limits metal extraction since those far below the rooting depth will not be efficiently extracted (Mandez and Maier, 2008).

Biomass production has also been observed for plants growing in tailings. This has been correlated to adverse conditions such as low pH, high metal levels, lack of soil structure and lack of normal heterotrophic microbial community. This implies that, with respect to heavily contaminated sites, low biomass production will affect the concentration of metals that can be extracted and sequestered to the shoots. Furthermore, remedial outcomes generally take longer than conventional approaches as several cropping cycles are required.

Hence, highly contaminated sites may require hundreds of cropping cycles to reduce metals to a non-hazardous level (Mandez and Maier, 2008).

2.1.2.3 Phytodegradation (Phytotransformation)

According to Newman and Reynolds (2004), organic contaminants are degraded (metabolized) or mineralized inside plant cells by enzymes such as nitroreductase (nitroaromatic compound degradation), dehalogenases and laccases (aniline degradation). Plants with these enzymatic systems include *Populus* species and *Myriophyllum spicatum* (Schnoor et al, 1995). Organic or inorganic pollutants are absorbed into the lignin of the cell wall of root cells or into humus during phytostabilization (phytoimmobilization). Metals are precipitated as insoluble forms in the soil matrix by the direct action of root exudates. The primary goal is to prevent pollutants from being mobilized and to minimize their dispersion in the soil (Ali et al., 2013; Pilon-Smits, 2000).

2.1.2.4 Phytofiltration

Phytofiltration is a technique that involves the use of plants to absorb, concentrate, and/or precipitate pollutants from an aqueous media, (typically heavy metal(loid)s or radioactive elements), via their root system or other submerged organs. The plants are grown in a hydroponic environment, which allows effluents to pass through and be "filtered" by the roots (Rhizofiltration) or other organs that collect and concentrate toxins (Pilon-Smits, 2000, Frers, 2009, Dhote, 2009). Plants having a high root biomass or absorption surface, greater accumulation capacity (aquatic hyperaccumulators), and resistance to pollutants, will perform well. *Helianthus annuus*, *Brassica juncea*, *Phragmites australis*,

Fontinalis antipyretica, and various Salix, Populus, Lemna, and Callitriche species are promising examples (Prasad, 2004; Poschenrieder et al., 2004, Dushenkov et al., 2000, Favas et al., 2012).

2.1.2.5 Rhizodegradation (Phytostimulation)

In this form of phytoremediation, the growing roots of plants support the expansion of rhizosphere bacteria that degrade plant, exudates and metabolites for carbon and energy. The microbial population in the rhizosphere is varied due to the uneven spatial distribution of nutrients. However, the Pseudomonas species are the most common organisms associated with roots (Ali et al., 2013; Khan, 2009). Some studies indicated that the use of phytostimulation is restricted to organic pollutants (Prasad, 2004; Frers, 2009).

2.1.2.6 Disadvantages/challenges of Phytoremediation

A frequently cited disadvantage of phytoremediation compared to strategies such as excavation and *ex situ* treatment, is that the rate of remediation is slower and can take many years. In part, this is because plant growth is hard to achieve in heavily impacted soils, thereby limiting catabolism of contaminants (Gerhardt et al., 2009). There are also challenges when the contaminated soil is deeper than the rooting zone. Another challenge is that there are stressors that affect phytoremediation. These include variations in temperature; nutrients and precipitation; herbivores (insects and/or animals); plant pathogens; and competition by weed species that are better adapted to the site. Any of these abiotic or biotic stressors can diminish or prevent plant growth, and this will negatively impact phytoremediation (Gerhardt et al., 2009). In addition, factors such as root structure;

organic composition of the soil; soil pH; moisture content; and microbial activity; often exhibit spatial variability at sites and can change over time (Gerhardt et al., 2009).

2.1.2.7 Phytoremediation potential of vegetation naturally growing on contaminated sites

Some plants are able to grow and thrive on contaminated sites despite all the substrate properties that often-become limitations to plant establishment and growth. It is important to use native plants for phytoremediation because these plants are often better in terms of survival, growth and reproduction under stress than plants that are introduced from other environments (Solomou et al., 2022). Vegetation growing naturally or spontaneously on heavy metal-contaminated sites has been assessed and evaluated for use in phytoremediation, according to a study by Nouri et al. (2011) and Marchiol et al. (2013). In these cases, it was found that some plants species are able to concentrate considerable amounts of one or more metals from the soil into either their roots and or shoots, suggesting they are better used as phytoextractors. In contrast, others would grow well in the soil but accumulate very little or no metals in their tissues. These types of plants were often found to concentrate metals into the soils surrounding their roots suggesting that they would do better for phytostabilisation purposes.

Nouri et al. (2011) studied the phytoremediation potential of native plants grown in the vicinity of the Ahangaran Pb-Zn mine. They concluded that none of the plants studied were suitable for phytoextraction but were instead suitable for phytostabilisation. A study by Maiti and Jaiswal et al. (2008) which collected dominant vegetation species from a contaminated site, revealed that the plants removed only Mn by phytoextraction while other metals like Zn, Cu, Pb and Ni were removed by rhizofiltration from plants growing on fly ash

lagoons. Marchiol et al. (2013) also conducted a study to evaluate the phytoremediation potential of native plants growing on a contaminated site of the former zinc smelter where byproducts and industrial waste were dumped. They found that the metal uptake ranges and toxic concentrations in some of the plants made them potentially useful in phytoremediation. The potential for phytoextraction was demonstrated in *P australis* for Sb and in *E. camaldulensis* and *G lelegans* for Ti. *S holoschoenus* behaved as a potential stabilizer species.

2.1.3 Phytoremediation potential of plant species at the study area

Few plants species grow naturally in the two mining areas. The dominant species in the Monarch Gold Mine were *Cenchrus bioflorus* grass, *Dichrostacys cineria*, *Colospospermum mopane*, and *Boscia albitrunca* tree species. In Selibe Phikwe, the most dominant plant species were *Boscia albitrunca* and *Colophospermum mopane*. These plants, by observation, appeared to be doing very well in this environment. They had a well-established root system and a large biomass making them ideal candidates for phytoremediation. This is also supported by Usman et al. (2018). This could also be an indication that they have mechanisms that enable them to survive, including heavy metal tolerance and/or exclusion. Their potential in phytoremediation can be estimated by measuring the heavy metal contents from the soils they are growing in.

Phytoremediation potentials of plant species have been evaluated. One study by Titshall et al (2013), which investigated the potential of five grass species to revegetate mine tailings, revealed that *Eragrostis superba* absorbed some lead and zinc from the tailings. It was also found to be a fast-growing species that establishes readily and has a high seed-producing potential. According to Titshall et al. (2013), these are favorable traits suggesting

that it can be suitable for the revegetation of mine tailings. Therefore, this study will explore the use of different tree species for the phytoremediation of mine tailings.

2.2 Impact of heavy metal(loid)s on grazing animals

Heavy metal pollution and contamination pose a great risk to human health and the ecosystem at large. Studies conducted by Ekosse (2005) and Letshwenyo (2016), indicated that soils and plants in the vicinity of Cu-Ni Mine (BCL) in Selibe Phikwe had elevated levels of heavy heavy metal(loid)s mainly due to erosion from mine tailings. Plants that accumulate these metals become a part of the food chain when they are consumed by animals. Consumption of vegetation with elevated metal concentrations can be a major exposure pathway for the animals or humans who consume them. Although some of these metals are not phytotoxic, they may pose an extreme risk to some humans who consume them (Meliker et al., 2007). As a result, further research is needed about the availability of heavy metal(loid)s in soils, the uptake and accumulation of pollutants by plants, and the possible harm that heavy metal buildup in plants and soil poses to nearby grazing animals.

2.3 Impacts of stockpiling mine tailings on the physical environment

Stockpiling mine tailings exposes minerals that are sulphide minerals which are unstable in oxidising conditions, these include chalcopyrite, a sulfide found in the form of impurities in Cu-Ni mine tailings. Oxidation of such minerals will result in the formation of AMD and the release of heavy metal(loid)s into the soils. The AMD which is formed often contaminates the soil and water bodies in and around the mine site (Galhardi & Bonotto, 2016).

2.3.1 Acid mine drainage formation

Acid mine drainage is a widespread environmental concern associated with both operational and abandoned mines. According to Bhuiyan et al. (2010), AMD, which usually occurs at mining sites, poses serious environmental problems for the global community. It can occur during the extraction of minerals such as copper, nickel and gold, as well as ore bodies containing acid, which form metal sulfides such as chalcopyrite (Masloboev et al., 2014). Metal sulfides, especially chalcopyrite (CuFeS_2), chalcocite (Cu_2S) and iron (II) sulfide (FeS), are common minerals in mine tailings (Dang et al., 2002). AMD results from a series of complex geochemical reactions that occur when mineral sulfides are exposed to the atmosphere (Knight, 2018). It is produced when the sulfides, contained in exposed mine tailings, are decomposed by a series of oxidation and hydrolysis reactions that culminate in the production of iron and aluminum-rich sulphuric acid (Brake et al., 2001). The metal sulfides can also be oxidized easily during the natural weathering process of mine tailings. The process, however, may be slow as tailings are often compacted. It has been observed that open pit and underground mining activities may greatly accelerate AMD production as they expose fresh sulfides and iron to the surface.

AMD formed is further released as the leachate from the mine tailings enters the natural stream systems. There, its acidity and high concentrations of dissolved constituents adversely impact aquatic life. The discharge of AMD into streams, rivers and lakes causes an instant threat to the biota and ecological balance (Evangelou and Zhang, 1995). A study carried out by Simati and Ndlovu (2014) showed that AMD was a multi factor pollutant affecting aquatic ecosystems through chemical, physical, biological and ecological means, among others.

2.3.2 Release of heavy metal(loid)s

Heavy metal(loid)s are also released when mine tailings are exposed to natural weathering conditions, allowing them to migrate into surrounding water, soil and sediments (Dang et al., 2002). Shu et al (2001), reported that the release of heavy metal(loid)s is influenced and accelerated by high acidic conditions due to the formation of AMD. If the pH drops below 4, the hydrogen ions resulting from the process can expedite the weathering of mine tailings and speed up the release of toxic elements (Dang et al., 2002; Bhuiyan et al., 2010). Most of the elements of environmental concern in Monarch Gold mine and BCL mine tailings are, As, Cd, Hg, Pb, Zn, Cr, Cu and Se (Ekosse, 2005; Vogel and Kasper, 2002). Their occurrence in plants and water bodies adversely affect the lives of local people in many ways, as they can be incorporated into food chains and their levels will increase through biological magnification (Ali and Khan, 2018).

2.3.3 Impacts of heavy metal(loid)s on soil quality

Heavy metal pollution of soils is increasingly becoming a global problem. Soil pollution by heavy metal(loid)s differs from air or water pollution, because heavy metal(loid)s persist in soils much longer than in other compartments of the biosphere (Singh and Steinnes, 2020). Heavy metal contaminated mine lands and tailings are often characterized by sparse or no vegetation cover which is attributable to poor soil quality. They are extremely difficult to revegetate due to the chemical and physical limitations for plant growth.

During the various processes of mining activities, nutrients are leached due to an accelerated erosion rate. The productive soil profile then becomes totally devastated,

containing no supportive and productive properties for plants. Among the possible chemical limitations are the absence of Nitrogen (N), minimally available phosphorus (P) and the presence of potentially toxic concentrations of heavy metal(loid)s. Plant growth is also hampered by poor physical properties such as unfavorable porosity, aeration, and water infiltration along with bulk density (Juwarkar and Jambhulkar, 2008). Conesa et al., 2005; Mendez and Maier, 2008; Pérez-Esteban et al., 2013), have also pointed out that high levels of acidity, toxic concentrations of metals, low levels of major plant nutrients, low CEC, poor physical structure and excess salinity, are the major problems for plant growth.

The accumulation of heavy metal(loid)s in soils may affect nutrient cycling throughout the whole ecosystem by inhibiting decomposition and subsequently reducing the supply of nutrients released by mineralization (Zhao et al., 2021). The mineralization of organic matter is carried out by a large community of organisms and involves a wide range of microbial processes. These processes (organic matter decomposition and mineralization of nitrogen) are often affected as the number, diversity and activity of soil microorganisms are inhibited by toxic metals. This decreases organic matter and its associated nutrients in most degraded land (Wong, 2003). Organic matter provides a continuous source of nutrients and it provides most of the N reserves in soils. An immediate effect of acidity on plants is the high concentration of hydrogen ions which deactivate most enzyme systems, restrict respiration, and prevent root uptake of mineral salts and water.

Heavy metal(loid)s are also known to affect the growth, morphology and metabolism of microorganisms in soils. In a study to investigate the influence of heavy metal(loid)s on the functional diversity of the soil microbial community, the effect of heavy metal contamination on such things as microbial biomass, respiration and N-mineralisation, as

well as various enzymes involved in the cycling of Carbon (C), Nitrogen (N), phosphorus (P) and Sulphur (S), were evaluated. It was found that the enzyme activities which are related to the cycling of N, P and S, show a considerable decrease in activities (Kandeler et al., 1996). Changes in the numbers, composition, diversity, growth, or activity of the microbial community due to pollution will therefore have lasting effects on the overall functioning of the ecosystem.

Heavy metal(loid)s in soils also affect soil base saturation and cation exchange capacity (CEC). Some heavy metal(loid)s such as copper are able to occupy the CEC and are bound more strongly to the soil than other cations thus affecting other chemical processes in the soil. A study by Derome and Nieminen (1998), found that Cu and Ni displaced base cations from the exchange capacity which has resulted in the deficient base cation (exchangeable Ca, Mg, K) and base saturation. Studies by Ekosse et al. (2005) and Vogel and Kasper (2002) found that soils in Selibe Phikwe and Francistown Monarch Gold mine contain heavy metal(loid)s such as Cr, Ni, Cu, As, Zn, Mn and their concentrations are much higher in soils closest to the mining areas compared to areas distant from the mine.

2.4 Effects of Amendments on soil and heavy metal(loid)s

2.4.1 Arbuscular mycorrhiza

Arbuscular mycorrhizal fungi is an amendment that has been utilized by several phytoremediation studies in heavy metal-contaminated soils. However, the inoculation effects on heavy metal concentration and alleviation of heavy metal toxicity in plants associated with its use are contradictory and not clear. Mycorrhiza improves the roots morphology in plants (Shao et al., 2018). A study by Riaz et al. (2020) reports that mycorrhiza may not affect, decrease or increase the uptake of toxic metals by plants. Dhalaria

et al. (2020) and Riaz et al. (2020), discovered that mycorrhizal fungi protect plants from the toxic effect of highly concentrated heavy metal(loid)s by possibly binding the metals in their hyphae or by reducing the translocation of metals to the plant tops (Krishnamoorthy et al., 2019). Therefore, this study will evaluate the effects of mycorrhiza on the uptake of heavy metal(loid)s by plants.

2.4.2 Compost

Compost is another amendment that can be used in the heavy metal contaminated environment to modify the bio-physicochemical properties of soils and provides nutrients for plant growth and development (Huang et al., 2016). Studies by Saum et al. (2018) and Huang et al. (2016), have shown that compost amendments can reduce heavy metal availability by providing humus as sorption sites of cationic metals, as well as by providing soluble organic substances that could serve as chelating agents of heavy metal(loid)s in soils. However, application of compost can also increase the concentrations of heavy metal(loid)s in plants because it may also contain elevated heavy metal(loid)s, as well as toxic substances (Petruzzelli, 1996). Therefore, caution should be exercised in applying composts in phytoremediation systems. Depending on the source and quality, compost can also cause pollution. However, the severity of pollution depends on the chemical form of heavy metal(loid)s present in compost and the source of the raw materials (Castaldi et al., 2005). Other studies found that the use of compost accelerates the removal and stabilization of organic pollutants present in the soil and plants by providing a favorable environment for microbial growth (Farrell and Jones, 2010).

2.4.3 Fly ash

Fly ash (FA) is a good amendment because of its texture and alkalinity. It also contains almost all the essential plant nutrients except for N (Pathan et al., 2003). Using FA as an amendment improves soil physicochemical properties and microbial activity (Singh and Pandey, 2013). However, studies by Lee et al. (2007) and Pandey and Singh (2010), have found that FA contains heavy metal(loid)s such as Cu, Ni, Cr, Cd and others that may exhibit metal toxicity in plants.

2.4.4 Cow manure

Cow manure (CM) as a soil amendment is beneficial since it can lower heavy metal availability by providing humus as sorption sites of cationic metals and soluble substances that could act as chelating agents of heavy metal(loid)s in soils. Although cow manure is beneficial and can improve microbial activity (Farrell and Jones, 2010), its use has also been found to elevate heavy metal(loid)s in plants (Petruzzelli 1996). Therefore, proper application of cow manure should be followed in phytoremediation studies. Several researchers have proposed that organic residues (such as animal manures) might be used as an alternative to liming materials and that their application can increase plant growth in acidic soils by ameliorating aluminum (Al) toxicity. Recent findings have also indicated that animal manures could serve as substitutes in the liming of acidic soils (Pocknee and Sumner, 1997; Wong et al., 1998; Mokolobate and Haynes, 2002).

2.4.5 Sewage sludge

Sewage sludge is a good soil amendment because it is highly nutritious and contains high levels of nitrogen, organic matter, and other nutrients essential to plant growth (Cao et al., 2013). The high microbial load of sewage sludge also supplies the necessary microbes that will help in the transformation of nutrients and improve nutrient availability, and consequently improve absorption by plants (Akmal et al., 2019). Sewage sludge generally has high organic matter content (Zbytniewski and Buszewski, 2005).

There is a need to evaluate the effect of these amendments on the potential of different tree species to be used for the phytoremediation of heavy metal contaminated soils to determine their suitability.

2.5 References

- Akmal, M., Gondal, T. A., Khan, K. S., Hussain, Q., Ahmad, M., Abbas, M. S., ... & Khosa, S. A. (2019). Impact of biochar prepared from leaves of *Populus euphratica* on soil microbial activity and mung bean (*Vigna radiata*) growth. *Arabian Journal of Geosciences*, 12, 1-8.
- Albanese S., De Vivo, B., Lima, A., & Cicchella, D. (2007). Geochemical background and baseline values of toxic elements in stream sediments of Campania region (Italy). *Journal of Geochemical Exploration*, 93(1), 21-34.
- Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere*, 91(7), 869-881.

Review of Literature

- Bhuiyan, M. A., Parvez, L., Islam, M. A., Dampare, S. B., & Suzuki, S. (2010). Heavy metal pollution of coal mine-affected agricultural soils in the northern part of Bangladesh. *Journal of hazardous materials*, 173(1-3), 384-392.
- Brake, S., Connors, K., & Romberger, S. (2001). A river runs through it: impact of acid mine drainage on the geochemistry of West Little Sugar Creek pre-and post-reclamation at the Green Valley coal mine, Indiana, USA. *Environmental Geology*, 40, 1471-1481.
- Cao, J. P., Li, L. Y., Morishita, K., Xiao, X. B., Zhao, X. Y., Wei, X. Y., & Takarada, T. (2013). Nitrogen transformations during fast pyrolysis of sewage sludge. *Fuel*, 104, 1-6.
- Castaldi, P., Santona, L., & Melis, P. (2005). Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. *Chemosphere*, 60(3), 365-371.
- Conesa, H.M., Robinson, B.H., Schulin, R. & Nowack B. (2007). Growth of *Lygeum spartum* in acid mine tailings: response of plants developed from seedlings, rhizomes and at field conditions, *Environmental Pollution* 145 700-707.
- Cunningham, S. D., & Berti, W. R. (2020). Phytoextraction and phytostabilization: technical, economic, and regulatory considerations of the soil-lead issue. In *Phytoremediation of contaminated soil and water* (pp. 359-376). CRC Press.
- Dang, Z., Liu, C., & Haigh, M. J. (2002). Mobility of heavy metals associated with the natural weathering of coal mine spoils. *Environmental Pollution*, 118(3), 419-426.
- Derome, J., & Nieminen, T. (1998). Metal and macronutrient fluxes in heavy-metal polluted Scots pine ecosystems in SW Finland. *Environmental Pollution*, 103(2-3), 219-228.

Review of Literature

- Dhalaria, R., D. Kumar, H. Kumar, E. Nepovimova, K. Kuča, M. Torequl Islam, & R. Verma. (2020). Arbuscular mycorrhizal fungi as potential agents in ameliorating heavy metal stress in plants. *Agronomy* 10 (6):815.
- Dhote S, Dixit S. (2009). Water quality improvement through macrophytes – a review. *Environmental Monitoring and Assessment*, 152 149-153.
- Ekosse, G.I.E. (2008). Environmental Effects of Nickel—Copper Exploitation. *Journal of Applied Sciences*, 8(13), pp.2344-2356.
- Ekosse, G.I.E. (2005). Heavy metals concentrations in the biophysical environment around the Ni-Cu mine and the smelter/concentrator plant, Selebi Phikwe, Botswana. *Global Journal of Environmental Sciences*, 4(2), pp.97-110.
- Ekosse, G.I.E. (2007). Influenza/common cold within the Selebi Phikwe nickel-copper exploitation environment, Botswana: Health. *African Journal for Physical Health Education, Recreation and Dance*, 13(4), pp.473-490.
- Ekosse, G.I.E., Ngila, J.C. & Forchheh, N. (2005). Multivariate analyses of heavy metals in soils and *Colophospermum mopane* leaves around the Selebi Phikwe nickel-copper mine and smelter/concentrator plant area, Botswana. *Journal of Applied Sciences and Environmental Management*, 9(1), pp.177-185.
- Evangelou, V. P., & Zhang, Y. L. (1995). A review: pyrite oxidation mechanisms and acid mine drainage prevention. *Critical Reviews in Environmental Science and Technology*, 25(2), 141-199.
- Farrell, M. and Jones, D.L. (2010). Use of composts in the remediation of heavy metal contaminated soil. *Journal of Hazardous Materials* 175 (1-3):575-82.

Review of Literature

- Favas PJC., Pratas, J., Prasad, M.N.V. (2012). Accumulation of arsenic by aquatic plants in large-scale field conditions: Opportunities for phytoremediation and bioindication. *Science of the Total Environment*; 433 390-397.
- Frers C. El uso de plantas acuáticas en el tratamiento de aguas residuales. Carmen de Areco, Argentina: *El Planeta Azul*; 2009.
- Galhardi, J. A., & Bonotto, D. M. (2016). Hydrogeochemical features of surface water and groundwater contaminated with acid mine drainage (AMD) in coal mining areas: a case study in southern Brazil. *Environmental Science and Pollution Research*, 23, 18911-18927.
- Garbisu, C. & Alkorta, I. (2001). Phytoextraction: A cost-effective plant-based technology for the removal of metals from the environment, *Bioresources Technology* 77: 229-236
- Gerhards K.E, Huang X, Click B.R & Greenberg B.M. (2009). Phytoremediation and Rhizoremediation of organic soils contaminants: Potential and challenges *Plant Science* 176: 20-30.
- Ghori, Z., Iftikhar, H., Bhatti, M. F., Sharma, I., Kazi, A. G., & Ahmad, P. (2016). Phytoextraction: the use of plants to remove heavy metals from soil. *Plant metal interaction* (pp. 385-409). Elsevier.
- Ghosh, M., & Singh, S. P. (2005). A review on phytoremediation of heavy metals and utilization of it's by products. *Asian Journal of Energy & Environment*, 6(4), 18.
- Huang, M., Zhu, Y., Li, Z., Huang, B., Luo, N., Liu, C. and Zeng, G. (2016). Compost as a soil amendment to remediate heavy metal-contaminated agricultural soil: mechanisms, efficacy, problems, and strategies. *Water Air Soil Pollution* 227(10):359.

- Imakando, S. (2017). The socio-economic impact of mining: a comparative study of Botswana and Zambia (Doctoral dissertation).
- Juwarkar, A. A., & Jambhulkar, H. P. (2008). Phytoremediation of coal mine spoil dump through integrated biotechnological approach. *Bioresource Technology*, 99(11), 4732-474.
- Kandeler, F., Kampichler, C., & Horak, O. (1996). Influence of heavy metals on the functional diversity of soil microbial communities. *Biology and fertility of soils*, 23, 299-306.1.
- Knight, R. D., Roberts, S., & Cooper, M. J. (2018). Investigating monomineralic and polymineralic reactions during the oxidation of sulphide minerals in seawater: Implications for mining seafloor massive sulphide deposits. *Applied Geochemistry*, 90, 63-74.
- Koitsiwe, K., & T. Adachi. (2017). Linkages between mining and non-mining sectors in Botswana. *Mineral Economy*. 30 (2):95–105.
- Krishnamoorthy, R., Venkatramanan, V., Senthilkumar, M., Anandham, R., Kumutha, K., & Sa, T. (2019). Management of heavy metal polluted soils: perspective of arbuscular mycorrhizal fungi. *Sustainable green technologies for environmental management*, 67-85.
- Lee, C.H., Lee, Y.B., Lee, H. & Kim, P.J. (2007). Reducing phosphorus release from paddy soils by a fly ash-gypsum mixture. *Bioresource Technology* 98(10):1980-4.
- Likuku, A.S., Mmolawa, K.B. & Gaboutloeloe, G.K. (2013). Assessment of heavy metal enrichment and degree of contamination around the copper-nickel mine in the Selebi Phikwe Region, Eastern Botswana. *Environmental Ecology Research* 1(2):32-40.

Review of Literature

- Maleki, A., Amini, H., Nazmara, S., Zandi, S., & Mahvi, A. H. (2014). Spatial distribution of heavy metals in soil, water, and vegetables of farms in Sanandaj, Kurdistan, Iran. *Journal of Environmental Health Science and Engineering*, 12, 1-10.
- Marchiol, L., Sacco, P., Assolari, S. & Zerbi G. (2004). Reclamation of polluted soils: phytoremediation potential of crop related Brassica species, *water air and soil Pollution* 158: 354-356
- Masloboev, V. A., Seleznev, S. G., Makarov, D. V., & Svetlov, A. V. (2014). Assessment of eco-hazard of copper-nickel ore mining and processing waste. *Journal of Mining Science*, 50, 559-572.
- Matshediso, I.B. (2005). A review of mineral development and investment policies of Botswana. *Resources Policy*, 30(3), 203-207.
- Maiti, S. K., & Jaiswal, S. (2008). Bioaccumulation and translocation of metals in the natural vegetation growing on fly ash lagoons: a field study from Santaldih thermal power plant, West Bengal, India. *Environmental monitoring and assessment*, 136, 355-370.
- McGrath, S. P., Lombi, E., Gray, C. W., Caille, N., Dunham, S. J., & Zhao, F. J. (2006). Field evaluation of Cd and Zn phytoextraction potential by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*. *Environmental Pollution*, 141(1), 115-125.
- Maleki, Mendez, M.O. & Maier, R.M. (2008). Phytoremediation of mine tailings in temperate and arid environments. *Reviews in Environmental Science and Biotechnology*, 7, 47-59.
- Meliker, J. R., Wahl, R. L., Cameron, L. L., & Nriagu, J. O. (2007). Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: a standardized mortality ratio analysis. *Environmental Health*, 6(1), 1-11.

Review of Literature

- Mokolobate, M. S., & R. J. Haynes. (2002). Comparative liming effect of four organic residues applied to an acid soil. *Biology and Fertility of Soils* 35:79–85.
- Motswaiso, F. S. (2019). Geochemical investigation of metals and trace elements around the abandoned Cu-Ni mine site in Selibe Phikwe, Botswana. *Journal of Geosciences Environmental Protection*, 7 (5):275.
- Newman, H.R. (2009). the Mineral industry of Botswana, Minerals Yearbook 3
- Nouri, M., & Haddioui, A. (2016). Human and animal health risk assessment of metal contamination in soil and plants from Ait Ammar abandoned iron mine, Morocco. *Environmental monitoring and assessment*, 188, 1-12.
- Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A.H, & Yusefi, N. (2009). Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential, *Environmental Earth Science* 59: 315-323.
- Nouri, J., Lorestani, B., Yousefi, N., Khorasani, N, Hassani, A.H, Seif F, & Cheragi, M. (2011). Phytoremediation potential of native plants grown in the vicinity of Ahangaran lead-zinc mine (Hamedan, Iran) *environmental Earth Science* 62:639-644.
- Newman, L. A., & Reynolds, C. M. (2004). Phytodegradation of organic compounds. *Current opinion in Biotechnology*, 15(3), 225-230.
- Pandey, V. C., & Singh, N. (2010). Impact of fly ash incorporation in soil systems. *Agriculture, ecosystems & environment*, 136(1-2), 16-27.
- Pathan, S.M., Aylmore, L.A. & Colmer, T.D. (2003). Properties of several fly ash materials in relation to use as soil amendments. *Journal of Environmental Quality* 32(2):687-93.

Review of Literature

- Pérez-Esteban, J., Escolástico, C., Moliner, A., & Masaguer, A. (2013). Chemical speciation and mobilization of copper and zinc in naturally contaminated mine soils with citric and tartaric acids. *Chemosphere*, 90(2), 276-283.
- Petruzzelli, G. (1996). Heavy metals in compost and their effect on soil quality. In: de Bertoldi M., Lemmes B., Papi T. (eds.). *The science of composting*, 213–23. Dordrecht: Springer.
- Pilon-Smits, E. & Pilon, M. (2000). Breeding mercury-breathing plants for environmental cleanup. *Trends in Plant Science*, 5(6) 235-236.
- Pocknee, S., & M. E. Sumner. (1997). Cation and nitrogen contents of organic matter determine its liming potential. *Soil Science Society of America Journal* 61:86–92.
- Prasad, M.N.V. (2004). *Phytoremediation of metals and radionuclides in the environment: the case for natural hyperaccumulators, metal transporters, soil-amending chelators and transgenic plants*. In: Prasad MNV. (ed.) *Heavy metal stress in plants: from biomolecules to ecosystems*, Second Edition. Berlin: Springer, p345-391.
- Pulford, I.D. & Watson, C. (2003). Phytoremediation of heavy metal contaminated land by trees- a review, *Environmental International* 29, 529-540.
- Riaz, M., Kamran, M., Fang, Y., Wang, Q., Cao, H., Yang, G., ... & Wang, X. (2021). Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: A critical review. *Journal of Hazardous Materials*, 402, 123919.
- Salt, D.E., Smith, R., & Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Physiology Plant Molecular Biology*. 49:643–668.

Review of Literature

- Saum, L., Jiménez, M.B. & Crowley, D. (2018). Influence of biochar and compost on phytoremediation of oil-contaminated soil. *International Journal of Phytoremediation* 20(1):54-60.
- Schnoor, J.L. (1997). *Phytoremediation, Technology Overview Report, Ground-Water Remediation Technologies Analysis Center, Series E, Vol. 1, October.*
- Schnoor, J.L., Licht, L.A., McCutcheon, S.C., Wolfe, N.L. & Carreira, L.H. (1995). Phytoremediation of organic and nutrient contaminants. *Environmental Science & Technology* 1995; 29 318A-323A.
- Schwartz, M.O. and Kgomanyane, J. (2008). Modelling natural attenuation of heavy-metal groundwater contamination in the Selebi-Phikwe mining area, Botswana. *Environmental geology*, 54(4), pp.819-830.
- Shao, Y. D., Zhang, D. J., Hu, X. C., Wu, Q. S., Jiang, C. J., Xia, T. J. & Kuča, K. (2018). Mycorrhiza-induced changes in root growth and nutrient absorption of tea plants. *Plant, Soil and Environment*, 64(6), 283-289.
- Simate, G. S., & Ndlovu, S. (2014). Acid mine drainage: Challenges and opportunities. *Journal of environmental chemical engineering*, 2(3), 1785-1803.
- Singh, J.S. & Pandey, V.C. (2013). Fly ash application in nutrient poor agriculture soils: impact on methanotrophs population dynamics and paddy yields. *Ecotoxicology and Environmental Safety* 89:43-51.
- Solomou, A.D., Germani, R., Proutsos, N., Petropoulou, M., Koutroumpilas, P., Galanis, C., & Kolimenakis, A. (2022). Utilizing mediterranean plants to remove contaminants from the soil environment: A short review. *Agriculture*, 12(2), 238.

Review of Literature

- Rylott, E.L. & Bruce, N.C. (2008). Plants disarm soil: engineering plants for the phytoremediation of explosives. *Trends in Biotechnology*; 27(2) 73-81.
- Titshall, L.W., Hughes, J.C., & Bester, H.C. (2013). Characterisation of alkaline tailings from Pb/Zn mine in South Africa and evaluation of their revegetation potential using 5 indigenous grass species, *South Africa journal of plant and soil* 30;2 97-105
- Vogel, H., & Kasper, B. (2002). Mine soils on abandoned gold mine tailings in Francistown. *Report by the Bundesanstalt für Geowissenschaften und Rohstoffe and Department of Geological Survey (Environmental Geology Division)*. Lobatse, Botswana.
- Wiston, M. (2017). Status of air pollution in Botswana and significance to air quality and human health. *Journal of Health and Pollution*, 7(15), pp.8-17.
- Wuana, R. A., & Okieimen, F. E. (2011). Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Notices*, 2011.
- Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere*, 50(6), 775-780.
- Wong, M. T. F., S. Nortcliff, & R. S. Swift. (1998). Method for determining the acid ameliorating capacity of plant residue compost, urban waste compost, farmyard manure, and peat applied to tropical soils. *Communications in Soil Science and Plant Analysis* 29:2927–2937.
- Yager, T. R. (2017). *The mineral industry of Botswana. Minerals yearbook: Area reports: International review 2013 Africa and the Middle East* (Vol. 3, p. 1).

Review of Literature

- Zbytniewski, R., & Buszewski, B. (2005). Characterization of natural organic matter (NOM) derived from sewage sludge compost. Part 1: chemical and spectroscopic properties. *Bioresource technology*, 96(4), 471-478.
- Zhao, H., Lin, J., Wang, X., Shi, J., Dahlgren, R. A., & Xu, J. (2021). Dynamics of soil microbial N-cycling strategies in response to cadmium stress. *Environmental science & technology*, 55(20), 14305-14315

CHAPTER 3

Materials and Methods

3.1 Research Framework and General Methodology

This chapter describes the research framework of this study (figure 3.1) and methods used in evaluating different soil amendments and the potential of different tree species for phytoremediation. A comprehensive assessment of heavy metal concentration in plants, heavy metal concentration and availability in soils, and the estimates of risk associated with grazing animals in the study areas, was conducted at Monarch Gold Mine, BCL Mine and the areas around these mines. This in turn served as a guide for selecting the trees species to use for pot experiments. Identified tree species were cultivated using mine tailings from the two mines. Different amendments were then applied: fly ash: compost; cow manure; arbuscular mycorrhiza; and sewage sludge. The amendments on mine tailings and tree species were evaluated to assess these trees for their phytoremediation potential.

Assessing the levels of heavy metal contamination in mine waste and soils impacted by mining activities and to develop phytoremediation technology using various tree species.

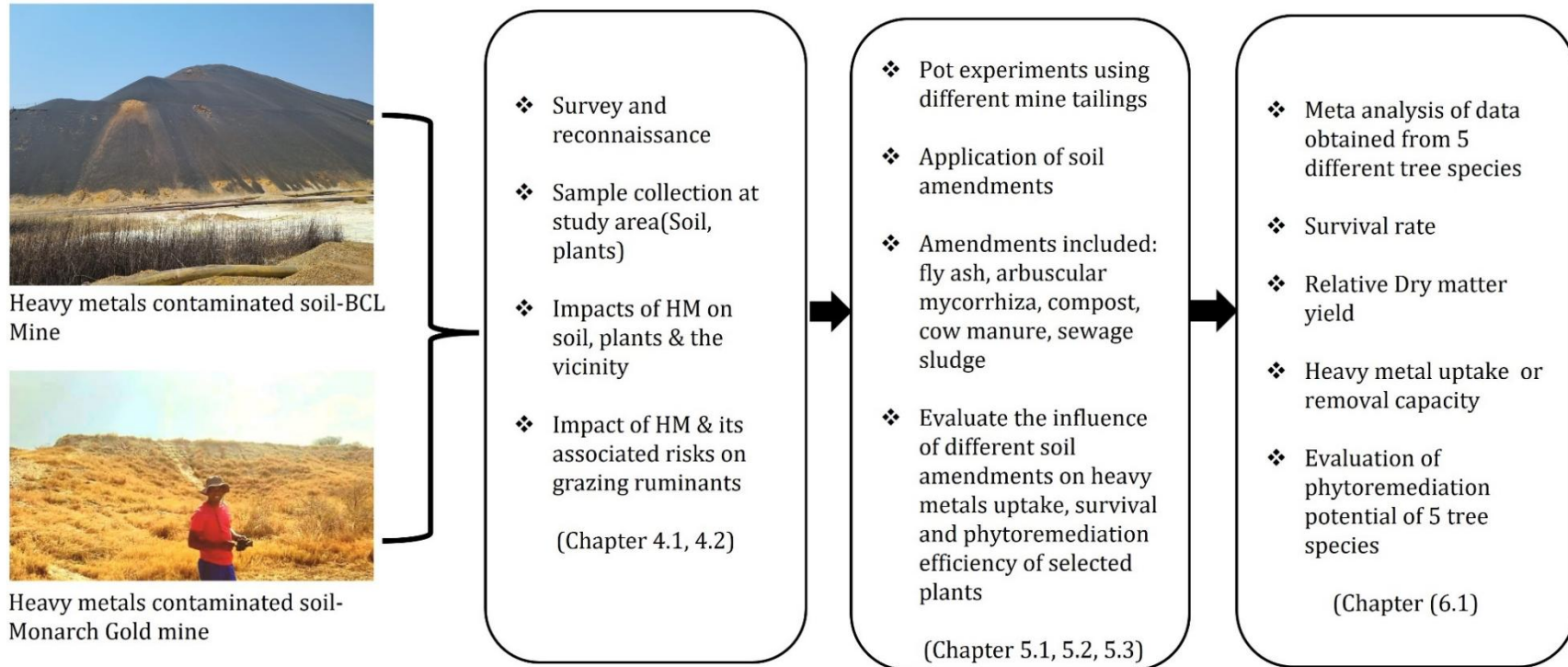


Figure 3. 1 The research framework of this study

With this framework, the study aims to achieve certain objectives. The framework on Figure 3.2 illustrates the overall hypothesis of the study, indicating that mining activities lead to environmental problems that affect the soil quality i.e. poor physicochemical and biological properties. Therefore, the study utilized microorganisms (arbuscular mycorrhiza) and different amendments such as compost, fly ash, and sewage sludge to improve the condition of the mine tailings (potting soil) to optimize plant growth.

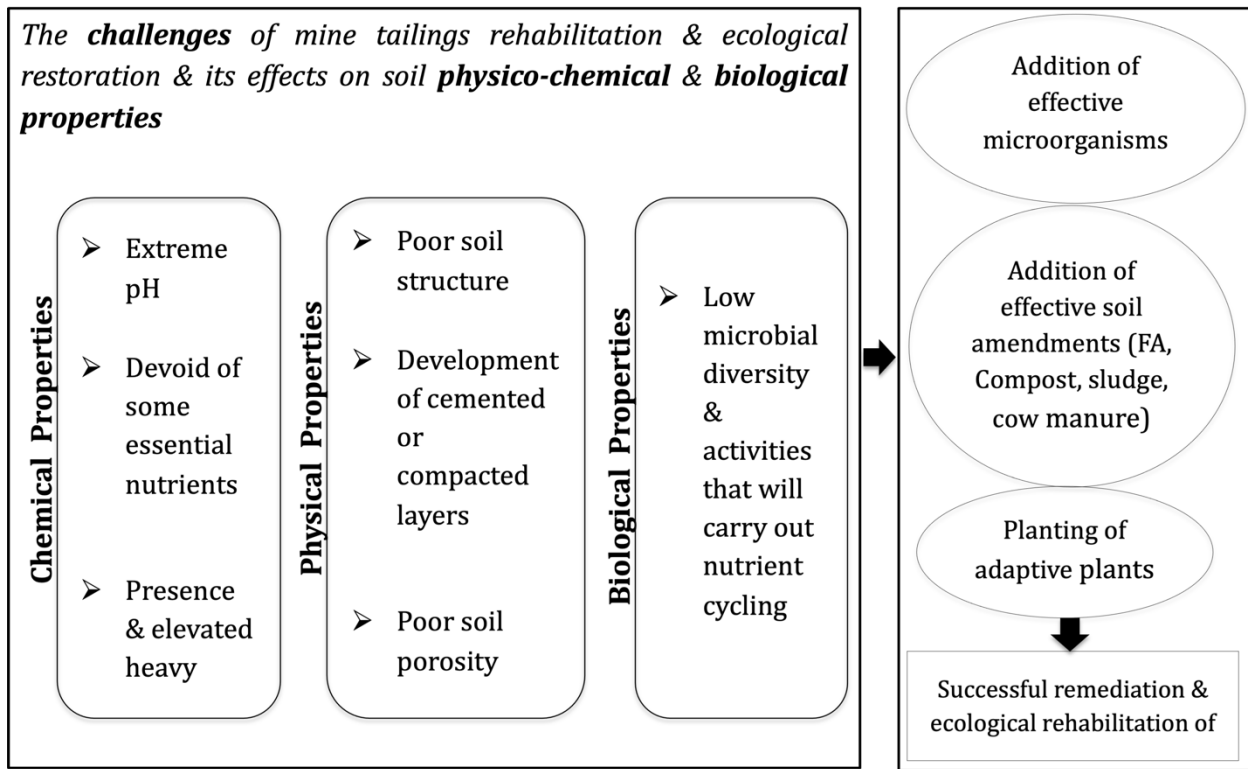


Figure 3. 2 Hypothetical framework after application of the research methodology

3.2 Assessment of the impact of heavy metal contamination on soil and plant communities in mine areas

Soil and plant samples were collected in the tailings dumpsite of Monarch Gold Mine and the vicinity of the BCL mine. Species diversity was carried out in the field. Collected samples were analyzed for total and exchangeable heavy metal content; pH; EC;

translocation factor; bioaccumulation factor; and pollution load index. The maximum and estimated daily heavy metal intake of grazing animals based on the concentration of heavy metal(loid)s from the different plant groups, as well as the average daily intake of forage and soils, were estimated. Figure 3.3) illustrates how objective 1 was achieved.

WP1: Assessment of the impact of heavy metals contamination on soil and plant communities in mine areas and the vicinity

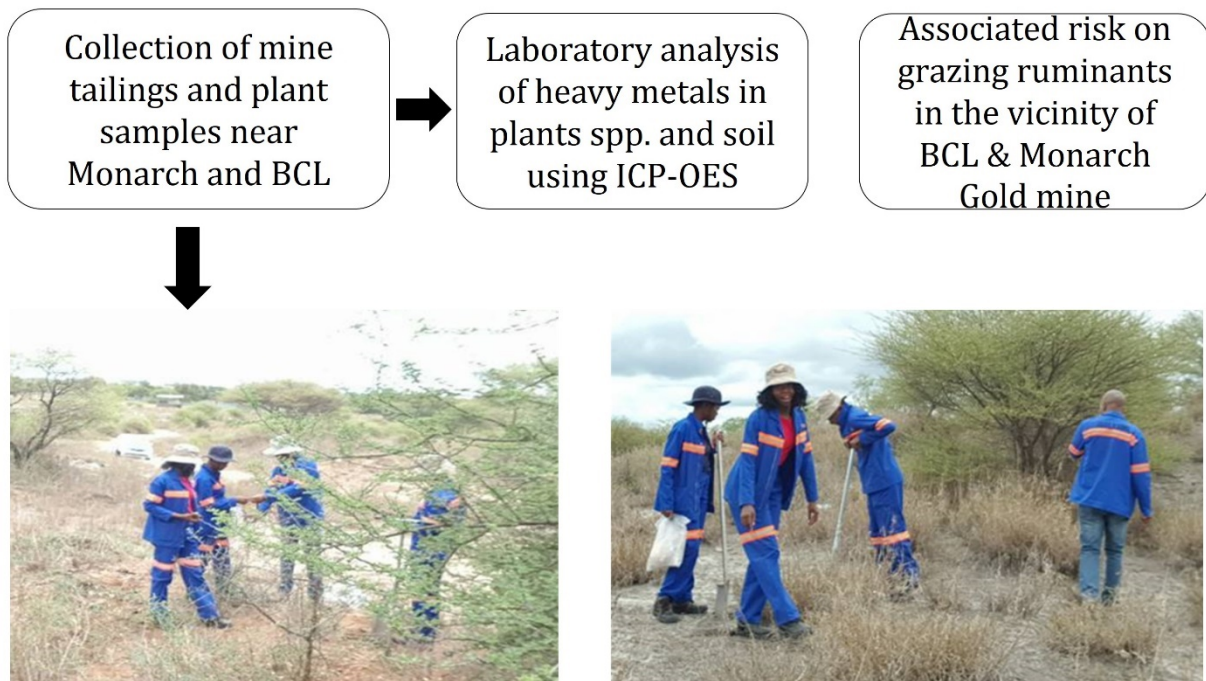


Figure 3. 3 Methodological illustration of objective one {NB: ICP-OES stand for Inductively Coupled Plasma Optical Emission Spectroscopy}

3.3 Effects of amendments on heavy metal(loid)s uptake by plants and availability in soil.

Figure 3.4 illustrates the methodology for objectives two and three. In order to test the efficacy of amendments on mine tailings using pot experiments, different tree species from BCL and the Monarch Gold Mine were cultivated on mine tailings. Amendments like fly

ash, compost, sewage sludge, cow manure and arbuscular mycorrhiza were applied at different rates as indicated in figure 3.4. These plants were cultivated for 52 weeks (*E. globulus*), 40 weeks (*C. Mopane*) and 24 weeks for 3 *Vachellia* (*Acacia*) species. Next both soil and plants were harvested and analysed for various parameters to evaluate the effect of the amendments.

WP2: Evaluate the influence of different soil amendments on heavy metals uptake, survival and phytoremediation efficiency of selected plants;

Pot Experiments-to determine the impact of amendments on heavy metals uptake
(Rates of Amendments Application, Applied singly or in combination)

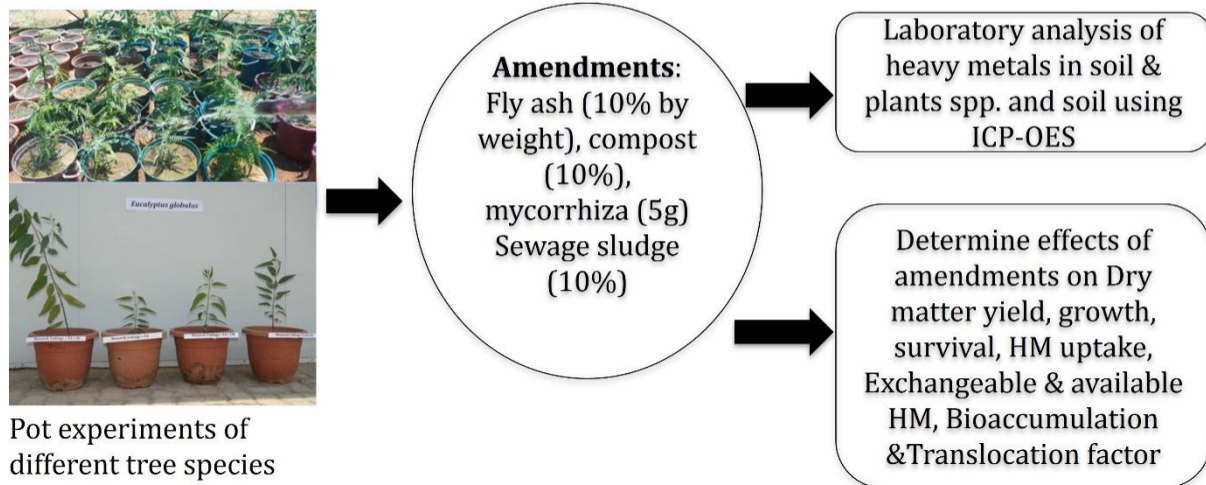


Figure 3. 4 Methodological illustration of objectives two and three.

The study was developmental research, therefore depending on the availability of materials and resources, the experiment was conducted based on what was available. Therefore, experiments were conducted for 40 weeks (*C. mopane*), 24 weeks for *Acacia* (*Vachellia*) species and 52 weeks for *E. globulus*. Synchronization of all experiments to evaluate the different species at the same time was not possible because of limited facilities. In essence the experiment to evaluate the effect and assessment of amendments on these

tree species was conducted on a staggered basis. Ideally the planting period should have been equal.

CHAPTER 4

Impact of Heavy metal(loid)s Contamination on Soil and Plant Communities In Mining Areas

This Chapter addresses objective one which is to assess the impact of heavy metal contamination on soil and plant communities, plant species diversity and abundance in mine areas and nearby ecosystems through characterization of the chemical properties of soil and mine tailings from BCL Cu-Ni mine in Selibe Phikwe and Monarch Gold Mine in Francistown; as well as associated risks on grazing animals. The objective was achieved by sampling both plants and soil in the vicinity of the mines and the tailings dumpsite in Monarch Gold mine. Overall, the results found out that the pollution load index was above one, indicating that there is pollution in soils and plants growing in the two study areas. The data on the heavy metal accumulation of the different plants was used to identify potential plants or combinations of plants that could be utilized for the rehabilitation of the study area through phytoremediation. The data provided preliminary estimates on the possible risk associated with heavy metal contamination among grazing animals in the Selibe-Phikwe BCL mine and Monarch Gold mine. The estimates of the daily intake of the minerals due to the consumption of plants in the vicinity of the mine warrant evaluation of actual levels of heavy metal(loid)s in grazing animals near the study area and in other mining areas in Botswana. This Chapter presents two publications (4.1 and 4.2) that have been reproduced as published.

Publication 4.1;

Manyiwa, T., Ultra, V. U., Rantong, G., Opaletswe, K. A., Gabankitse, G., Taupedi, S. B., & Gajaje, K. (2021). Heavy metals in soil, plants, and associated risk on grazing ruminants in the vicinity of Cu–Ni mine in Selebi-Phikwe, Botswana. *Environmental Geochemistry and Health*, 1-16. <https://doi.org/10.1007/s10653-021-00918-x>

Publication 4.2;

Manyiwa, T., Ultra Jr, V. U., Hengari, M., & Rantong, G. (2023). Spatial variability of Heavy metals in soils and vegetation and associated risk to grazing animals in the abandoned gold mine in Francistown, Botswana. *Environmental Pollutants and Bioavailability*, 35(1), 2254493.

Authors Contribution:

Trust Manyiwa is the main author and my supervisor, Prof. Venecio Ultra is the co-author.

Manyiwa T- conceptualization and planning, data gathering and analysis, validation of methodology, interpretation of results, writing and revision of manuscript. **Ultra VU-** project administration, conceptualization and planning; supervision of fieldwork and laboratory analyses; interpretation of results; and review and editing of manuscript. **Rantong G-** conceptualization, planning of vegetation sampling and analysis, data gathering and analysis, editing and reviewing of the article. **Opaletswe, K. A., Gabankitse, G., Taupedi, S. B., & Gajaje, K., Hengari, M-** Fieldwork and sample collection; sample preparation and analysis, data interpretation and analysis, writing and review of different sections of the manuscript.

4.1 Heavy metal(loid)s in Soils, Plants and Associated Risk on Grazing Ruminants in the Vicinity of Cu-Ni Mine in Selebi-Phikwe, Botswana

Abstract

The impact of BCL Cu-Ni mines on the surrounding environment has indicated high levels of heavy metal contamination in soil and some plant species. A comprehensive assessment of heavy metal concentration in plants, heavy metal concentration and availability in soils, and the estimates of risk associated with grazing animals in the area are presented. A comprehensive quantification of heavy metal contents in 82 plant samples revealed that some plants such as *B. albitrunca* and *B. foetida* are suitable for multi-metal phytoextraction while others like *V. tortilis*, *C. glandilosa* and *M. angolensis* can accumulate one or two of the metals in soils or tolerate high levels of contamination. Current levels of soil contamination were manifested by acidification and high electrical conductivity, high contamination factors, and a pollution index between 8.31 to 10.79. The amount of exchangeable fractions of metals was higher than in soils in the control, which is attributed to the high solubility of deposited materials on the soil surfaces. Daily intake estimates showed a possible risk associated with Pb and Cu contamination among grazing animals in the study area. Overall, the information has identified potential plants or a combination of plants that could be utilized for the rehabilitation of the study area through phytoremediation. In addition, the estimates of the daily intake of the minerals due to the consumption of plants in the vicinity of the BCL mines warrant for evaluation of the actual levels of heavy metal(loid)s in grazing animals near the study area and in other mining areas in Botswana.

Keywords: Animal dietary intake, Heavy metal availability, Plant uptake, BCL mines

4.1.1 Introduction

Botswana's economy is highly dependent on the exploration, processing, and export of mineral resources (Koitsiwe and Adachi, 2017). Apart from being the major source of diamonds (which is about 40% of the total world input), copper, nickel, gold, and soda ash also contribute significantly to the country's exports and the overall gross domestic product (GDP) (Matshediso, 2005; Yager, 2017). The mining sector accounts for about 35% of GDP and provides employment to more than 5% of Botswana (Imakando, 2017). With the continued expansion of the mining industry, associated environmental problems also increased because some mining operations were not compliant with the provisions of environmental safety (Wiston, 2017). The case of BCL Cu-Ni mine in Selebi Phikwe was reported to have generated serious environmental problems that are directly related to poor mine waste management (Motswaiso, 2019; Ultra and Manyiwa, 2020). Based on the report by Ekosse (2008), higher environmental and health hazards due to mining was indicated by an unusually high occurrence of ailments and illnesses coupled with associated diseases among workers at Selebi Phikwe. This high frequency of ailments and illnesses was attributed to environmental factors such as exposure of the residents to contaminated particulate air matter (PAM) rich in sulfur and heavy metal(loid)s that are directly linked to mining and smelting of Ni-Cu. Previously, residents near the mining facilities had persistently complained of chest pains (Ekosse, 2007).

Assessment of the physical environment of Selebi Phikwe indicated severe deterioration of soil, air, and groundwater quality which is traceable to the BCL mining operation (Likuku et al., 2013). The windward side of BCL mines has high concentrations of Pb, Ni, Cu, and Zn which are associated with deposition of particulate air matter from smelter

emissions. The soils at the site are considered as severely contaminated with Cu and Ni, moderately contaminated with Fe, Mn, Pb, and Zn, but showed signs of low contamination with Co. The surface soils around the Selebi Phikwe Ni–Cu plant contained high amounts of cobalt oxide and silicon sulfide, which are indicative of mineralogical alteration of mining waste deposited on soils and the heavy minerals fraction of soil samples are higher on sites close to either the mines or the smelter plant (Ekosse, 2005).

The tailings which are produced from the processing of ores are a point source of contaminants in the area. Schwartz and Kgomanyane (2008) elucidated that highly acidic seepage water (1.7-2.8 pH) which is strongly enriched in SO_4^{2-} (5,680 g/L) and heavy metal(loid)s (6,230 mg L⁻¹ Ni, 1,860 mg L⁻¹ Cu and 410 mg L⁻¹ Co) seeped through the fractured aquifer and is considered as the major source of groundwater pollution in the Selebi Phikwe. The tailing dams also release high concentrations of heavy metal(loid)s into the surrounding environment through surface erosion and deposition of particulate air matter (Ekosse, 2005; Schippers et al., 2007). Analysis of PAM collected near the Selebi Phikwe Ni–Cu plant contained quartz, pyrrhotite, chalcopyrite, albite, and a secondary pollutant djurleite polymorphs formed from mineralization of chalcocite and the gases H₂S and SO₂ released from the concentration/smelting processes (Ekosse et al., 2005). These minerals in PAM are very reactive with H₂S, SO₂ and atmospheric moisture to release H₂SO₄, to form acid rain. The very strong acidity of PAM damages property, plants, and the whole ecosystem of the surrounding area. Evidence showed that PAM can damage crops, trees, and shrubs by blocking gas exchange and reducing photosynthesis, thus the occurrence of dead spots near the BCL mines. Plants growing or grown in soils near the BCL mines showed elevated levels of heavy metal(loid)s. Ekosse et al. (2005) reported that the leaves *C. mopane*

growing in the vicinity of BCL Mines contained Cd between 0.01 and 0.05 ppm, Co ranged from 1.0 to 28 ppm; Cr from 0.03 to 11 ppm; Cu from 4 to 116 ppm; Fe from 31 to 430 ppm; Ni from 19 to 120 ppm; Se was between 0.01 and 0.03 ppm, and Zn between 17 and 79 ppm. The concentrations of heavy metal(loid)s in soils and leaves of *C. mopane* decreased with an increase in distance from the mine and smelter/concentration plant (Ekosse et al., 2005).

So far, studies conducted on the impacts of BCL mining operations have focused on human health, pollution migration from mine waste to groundwater, atmosphere, and soil in the form of acid mine drainage, suspended particulate air matter and deposited heavy metal contaminants on surface soil, respectively. Some reports also elucidated impacts on vegetation and other organisms such as the heavy metal accumulation in *C. mopane* and the phane caterpillar (*Imbrasia belina*). The current situation of the study area demands updated additional information on the impacts of mine operations on the surrounding ecosystems such that effective remediation strategies will be identified and implemented. To bridge the gap of information, additional data on heavy metal availability in soils and the accumulations of pollutants in plants should be secured. Understanding the heavy metal bioavailability in the soil is crucial in understanding the subsequent risks of pollutants to crops, animals, and consumers through food safety and food security.

The environmental risk assessment associated with heavy metal in soils, total metal concentration is used commonly but considered as a poor variable because it is not sensitive to the mobility, bioavailability, and fate of contaminants in the environment (Bai et al., 2011). Instead, quantifying the available forms is considered a better indicator in estimating the potential risk of toxic metals entering the biosphere because it is shown to have higher correlations to bioaccumulation in plants and other organisms. In addition, it is a more

sensitive parameter in predicting mobility between different environmental compartments. The available fractions of heavy metal(loid)s in soils are estimated using milder extractants which can dissolve metals from the soil matrix comparable to the strength of the soil solution (Chojnacka et al., 2005). These extractants can quantify the fractions of heavy metal(loid)s that can be mobilized in response to plant uptake and percolating water in the profile. Therefore, quantifying the available fractions of heavy metal contaminants in soil could be more useful in risk assessment and identifying remediation strategies.

This study highlights the importance of determining the availability of heavy metal(loid)s in soils, the uptake of heavy metal(loid)s by all plants in the area, and the potential risk associated with heavy metal accumulation in plants and soils to ruminants grazing in the area. Exhaustive quantification of heavy metal content in plants was conducted to identify potential agents for phytoremediation. The data presented here is necessary for understanding the impacts of heavy metal contamination from Cu-Ni (BCL) mines, the processes involved that control the fate and migration of pollutants, their potentially toxic effects on living organisms, and provides valuable input in identifying effective phytoremediation strategies.

4.1.2 Materials and Methods

4.1.2.1 Description of the study area

The assessment was conducted in the vicinity of the BCL copper-nickel mine located in Selebi Phikwe (longitudes 27.8° and 27.9° East; latitudes 22.0° and 22.9° South). The BCL mine extracted and processed Cu-Ni-bearing minerals from 1973 to 2014. The climatic condition of the study area is a hot and rainy season with sub-tropical thunderstorms from

November to March; a cool dry season from April to August; and a hot season with minimum precipitation from September to November. The dominant soils of the study area are classified as Eutric Regosols and Haplic Luvisols (Likuku et al., 2013).

Currently, the BCL mine is under liquidation but its 40 years operation had generated a huge amount of mine tailings, slags, and smelter stack. Waste management is considered to be extremely poor and this mine waste serves as a source of pollutants in the adjacent areas. The landscape of the study area is covered with grasses, shrubs, and trees where ruminants are grazing and browsing.

4.1.2.2 Sampling site and sampling procedure

Seven sampling points were established within a 1 km distance from the BCL mine site (figure 4.1.1). All these sites were outside of the perimeter fence of the BCL mine. Each sampling point had an area of 100 m² where representative soil samples were collected, and plants were sampled. Four samples for each site were gathered by collecting the surface soils up to 15 cm deep. The shoots of representative plants growing in each sampling point were gathered. Onsite identification of plants was performed, and additional samples were gathered for verification of the plant's identity.

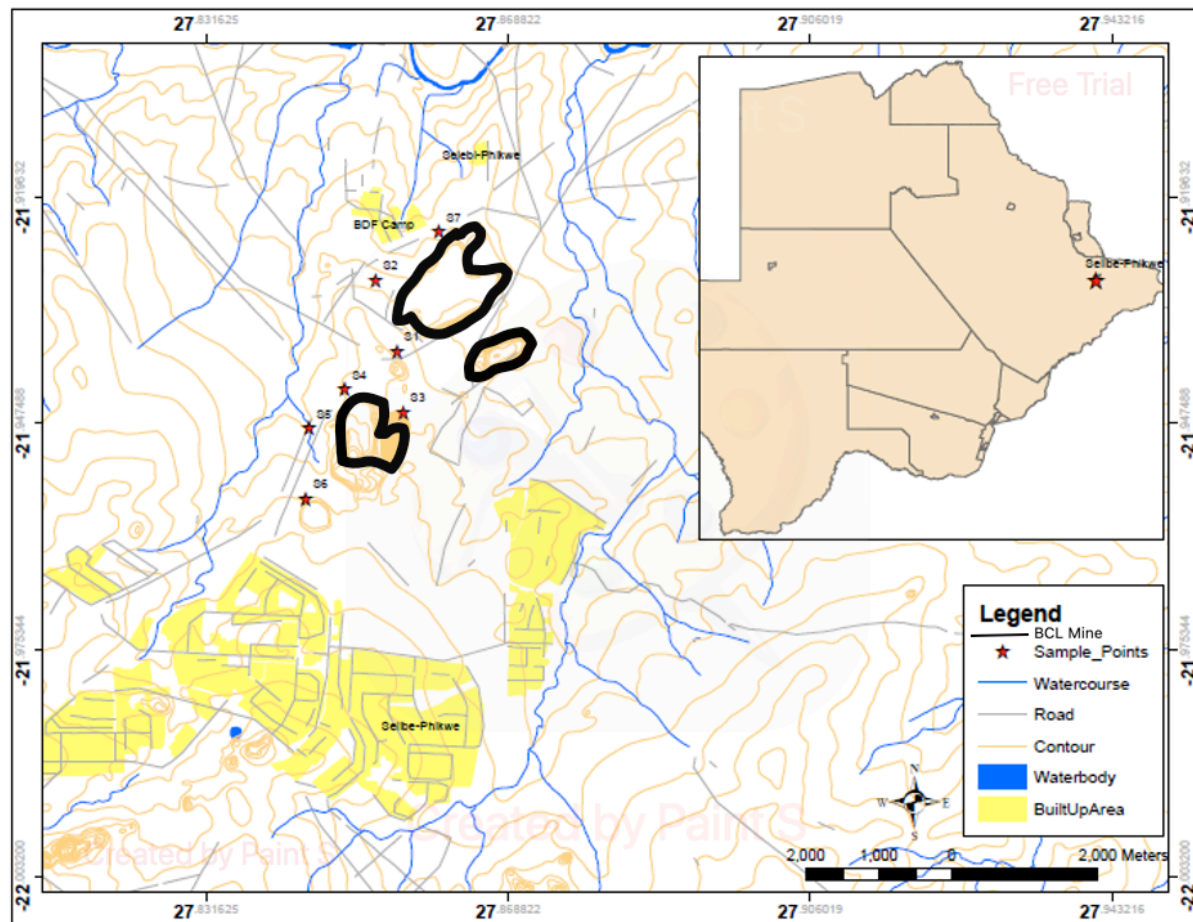


Figure 4.1. 1 Map showing the study area and the sampling sites.

4.1.2.3 Soil sample processing and chemical characterization

Soil samples were air-dried for 3 days and then sieved through a 2 mm stainless steel mesh wire prior to chemical characterization. The chemical characterization of soil followed the same procedure outlined in Ultra (2020a, 2020b). Briefly, the soil pH and electric conductivity (EC) were measured in water suspension (1:2, soil: water) after shaking for 1 hour using a portable pH/EC meter. The total contents of Zn, Pb, Mn, Cu, Ni, and As were measured after wet acid digestion in an open vessel at 120 °C for 6 hours using HNO₃: HCl mixture (1:3) in a hot plate followed by quantification using, Inductively coupled plasma mass spectrometry (ICP-MS) (Ultra, 2020a, 2020b). The available fractions of these heavy

metal(loid)s in soil were assessed by determining the ammonium acetate exchangeable. Samples were shaken with 1N ammonium acetate (pH 7) at a 1:100 ratio for 2 hours at room temperature. The exchangeable fraction (exch. fraction) was analyzed for different metal(loid)s using Thermo Scientific™ iCAP Q ICP-MS, Illinois 60060 USA). Exchangeable metal(loid)s were analysed to assess the availability of the metals to plants. As a reference, the Ultra (2020a, 2020b) reported that the BCL mine tailings contain high amounts of As (154.31 mg kg⁻¹), Cu (5311.62 mg kg⁻¹), Mn (1137.53 mg kg⁻¹), Ni (2025.22 mg kg⁻¹), Pb (552.81 mg kg⁻¹), and Zn (220.27 mg kg⁻¹), while the BCL smelter slags contained As (28.47 mg kg⁻¹), Cu (3328.13 mg kg⁻¹), Mn (928.55 mg kg⁻¹), Ni (1003.62 mg kg⁻¹), Pb (275.48 mg kg⁻¹), and Zn (115.38 mg kg⁻¹) based on analyses using portable X-ray fluorescence (Olympus delta 50kv PXRF, Tokyo 163-0914, Japan) spectrometry.

4.1.2.4 Estimation of geoaccumulation index, contamination factors and pollution load index in soil

Using the total concentrations in soils, the contamination factors (CF) of metal(loid)s in the soil samples were determined as indicated in Eq.1.

$$CF = \frac{C_m \text{ Sample}}{C_m \text{ Background}} \quad [\text{Eq.1}]$$

where C_m is the measured concentration of heavy metal in the soil; B_m is the local background concentration value of the metal(loid)s.

Pollution Load Index, (PLI), proposed by Tomlinson et al. (1980) was calculated using Eq 2.

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \dots \times CF_N)^{1/N} \quad [\text{Eq.2}]$$

where N is the number of metals studied and CF is the contamination factor calculated as described in (Eq.1).

The geoaccumulation index allows the assessment of contamination by comparing the current levels of metal concentrations and the original pre-industrial concentrations in the soils. The effects of mining activities were assessed following the geoaccumulation index approach proposed by Müller [28], as given in (3). Factor 1.5 is introduced in (3) to minimize the effect of possible variations in the background values, C_m Background, which may be attributed to lithogenic variations in soils.

$$I_{geo} = \log_2 \left[\frac{C_m \text{ Sample}}{(1.5 \times C_m \text{ Background})} \right] \quad [\text{Eq.3}]$$

4.1.2.5 Plant sample processing and chemical analysis

The air-dried shoots were oven-dried at 70 °C for 3 days, and ground using a food processor to homogenize prior to chemical analysis. To assess the heavy metal contents, the powdered tissues were digested using a nitric acid and hydrogen peroxide mixture in an open vessel at 120 °C for a minimum of 4 hours and analyzed for heavy heavy heavy metal(loid)s using ICP-MS. Samples were analyzed in duplicates.

4.1.2.6 Risk assessment of ruminants grazing in the area

Nouri and Haddioui (2015) estimated the daily intake (DI) of metals for free-range ruminants based on the amount of biomass consumed and soil ingestion. This could be estimated on a daily basis by:

$$DI_{\text{animal}} = [\text{Metal}]_{\text{feed}} \times I_{\text{feed}} + [\text{Metal}]_{\text{soil}} \times I_{\text{soil}} \quad [\text{Eq.4}]$$

Where:

DI_{animal} is the daily intake of metal for animals (mg d^{-1}); $[\text{Metal}]_{\text{feed}}$ and $[\text{Metal}]_{\text{soil}}$ are the concentration of metals in feeds and soil, respectively, (mg kg^{-1}); and I_{feed} and I_{soil} are the daily intake of feed and soil, respectively, (kg d^{-1}).

Based on the results of Mphinyane et al. (2015) on the analysis of diet preference of cattle, sheep, and goats grazing in rangeland of Botswana, the amount of grasses, herbs (forbs) and shrubs/woody plants differed between these groups of ruminants (Table 4.1.1).

Table 4.1.1 Estimates of parameters used in the calculation of daily dietary intake of grazing ruminants from plants near the vicinity of the BCL mines

Parameters	Cattle	Goat	Sheep	Reference
Diet Composition				
Grass	75%	20%	74%	Mphinyane et al. (2015)
Woody Species	23%	78%	21%	Mphinyane et al. (2015)
Forbs	2%	2%	5%	Mphinyane et al. (2015)
Average daily dry matter consumption (kg/day)	16.9	0.90	2.50	de Vries et al. (2007)
Average daily soil intake (kg/day)	0.41	0.10	0.10	de Vries et al. (2007)

Cattle diets consist of 75% grasses, 23% woody plants, and 2% forbs; goat diet consists of 20% grasses, 78% woody plants, and 2% forbs; while sheep diet consists of 74% grasses, 21% woody plants and 5% forbs. Incorporating the proportion of these diets and their equivalent average metal concentration in plants collected from the study site, the daily intake of metals of different ruminants was calculated as:

$$DI_{\text{animal}} = [\text{Metal}]_{\text{grass}} \times I_{\text{grass}} + [\text{Metal}]_{\text{shrubs}} \times I_{\text{shrubs}} + [\text{Metal}]_{\text{forbs}} \times I_{\text{forbs}} + [\text{Metal}]_{\text{soil}} \times I_{\text{soil}}$$

[Eq. 5]

$[\text{Metal}]_{\text{Grass}}$, $[\text{Metal}]_{\text{Shrubs}}$, $[\text{Metal}]_{\text{Forbs}}$ are the average concentrations of metals for grasses, shrubs, and forbs, respectively (mg kg^{-1}) presented in Table 4.1.2; and I_{grass} , I_{shrubs} , I_{forbs} are the equivalent intake of grasses, shrubs, and forbs, respectively and it is calculated as the daily dietary intake multiplied by the percentage intake of grasses, shrubs, and forbs for each ruminant.

4.1.3 Results

4.1.3.1 Heavy metal concentration and availability

The pH, electrical conductivity, and total concentration of Zn, Pb, Mn, Cu, Ni, and As in soils from different sampling sites in the vicinity of BCL mines are presented in Table 4.1.2.

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

Table 4.1.2 Average total concentration of heavy metal(loid)s in soils (n=4) from different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana.

Sampling Site	Coordinates	pH	EC ($\mu\text{S m}^{-1}$)	Zn	Pb	Mn	Cu (mg kg^{-1})	Ni	As
Site 1	-21.9386, 27.85499	5.20±0.08	2165.33±237.21	66.39±2.17	244.33±3.87	375.29±6.14	927.04±23.41	884.04±32.14	19.88±0.24
Site 2	-21.9298, 27.85239	4.22±0.06	2122.85±175.24	55.65±3.42	256.17±4.72	443.64±3.47	1442.60±32.16	855.79±24.47	11.01±0.16
Site 3	-21.9461, 27.85583	4.46±0.03	1425.00±135.24	67.43±2.12	247.51±3.89	570.32±16.47	732.57±21.43	1257.97±36.68	8.25±0.28
Site 4	-21.9433, 27.84861	4.82±0.10	1414.00±186.35	76.50±3.87	245.15±6.48	495.15±8.72	470.87±9.68	233.01±22.41	12.86±0.37
Site 5	-21.9481, 27.84417	4.49±0.06	1993.00±165.42	47.19±5.47	240.67±8.47	259.33±11.74	826.13±11.45	1209.23±38.75	10.46±1.04
Site 6	-21.9567, 27.84390	4.36±0.09	2590.00±2590.00	65.96±6.23	247.58±6.49	276.11±9.12	1474.85±36.42	601.55±20.14	11.22±0.16
Site 7	-21.9239, 27.86032	4.89±0.03	4870.00±269.73	77.75±7.12	253.84±8.41	553.07±12.42	1246.64±16.72	173.90±16.73	7.63±0.27
Control (Background concentration)		6.42		25.50	49.70	364.35	41.40	60.60	3.93

The soil pH is acidic with values ranging from 4.22 to 5.2 and an average of 4.63. The average EC was 2368.60 $\mu\text{S m}^{-1}$ and a range of 1414 to 4870 $\mu\text{S m}^{-1}$. The average total Zn concentration in soils was 65.27 mg kg^{-1} with a concentration range from 47.19 to 77.75 mg kg^{-1} ; average total Pb concentration was 247.89 with narrow range concentrations of 240.67 to 256.17 mg kg^{-1} , Mn had an average concentration of 424.7 mg kg^{-1} and a standard deviation of 125.75 mg kg^{-1} ; Cu concentration ranged from 470.87 mg kg^{-1} to 1474.85 mg kg^{-1} and an average of 1017.24; Ni has an average of 745.07 mg kg^{-1} and a range of 173.9 to 1257.97 mg kg^{-1} . The As concentration ranged from 7.63 to 19.88 mg kg^{-1} and an average of 11.62 mg kg^{-1} .

The available fraction of heavy metal in soils is presented in Table 4.1.3. The concentrations of exchangeable Zn was about 3.34% of the total concentration ranging from 1.21 to 3.44 mg kg^{-1} ; exch-Pb was about 2% of the total concentration ranging from 1.24 to 3.64 mg kg^{-1} ; exch-Mn was 2.25% of the total Mn in soil and a range from 0.38 to 23.3 mg kg^{-1} ; exch-Cu ranged from 9.52 to 24.46 mg kg^{-1} and about 1.66% of the average total Cu in the soil; exch-Ni ranged from 12.12 to 26.28 mg kg^{-1} which is about 3.92% of the total Ni in the soil, and exch-As ranged 0.76 to 1.15 mg kg^{-1} and an average of 0.90 mg kg^{-1} .

Table 4.1.3 Average concentration of exchangeable heavy metal(loid)s in soils (n=4) from different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana.

Sampling Site	Concentration of Exchangeable Fractions (mg kg^{-1})					
	Zn	Pb	Mn	Cu	Ni	As
Site 1	1.21±0.02	5.73±0.08	5.35±0.03	10.42±0.05	18.27±0.04	1.05±0.01
Site 2	2.40±0.05	4.00±0.07	3.96±0.02	20.64±0.08	16.90±0.03	1.15±0.03
Site 3	3.44±0.01	3.08±0.11	0.38±0.01	17.16±0.06	12.12±0.16	0.78±0.03
Site 4	1.26±0.03	3.56±0.08	1.72±0.01	9.52±0.11	15.04±0.11	0.80±0.01
Site 5	2.62±0.06	8.76±0.09	23.30±0.08	16.12±0.09	26.28±0.09	0.78±0.01
Site 6	1.70±0.08	5.82±0.04	12.20±0.04	24.46±0.13	25.84±0.13	0.76±0.00
Site 7	2.34±0.04	3.52±0.06	8.50±0.03	13.34±0.07	16.50±0.07	0.97±0.02
Control	0.22	0.28	5.42	1.32	0.85	0.08

4.1.3.2 Concentration factor and pollution load index

Based on the background concentration of different metals in the study area, the concentration factors in the surface soils were greater than 1 for all metals in all sites except for Mn (Table 4.1.4). The CF of the different metals ranged from 2.13 to 3.5 for Zn, 4.94 to 5.15 for Pb, 0.71 to 1.52 for Mn, 11.37 to 35.62 for Cu, 2.87 to 20.76 for Ni and 1.94 to 5.06 for As. The pollution index ranged from 8.31 to 10.79, in which site 2 had the highest while site 4 had the lowest PLI.

Table 4.1.4 Average ($\pm$ standard deviation) contamination factor (CF) and pollution load index (PLI) of different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana

Sampling Site	Contamination Factor (CF)						PLI
	Zn	Pb	Mn	Cu	Ni	As	
Site 1	2.99 $\pm$ 0.08	4.92 $\pm$ 0.10	1.03 $\pm$ 0.02	22.39 $\pm$ 0.06	14.59 $\pm$ 0.11	5.06 $\pm$ 0.04	10.14 $\pm$ 0.05
Site 2	2.51 $\pm$ 0.06	5.15 $\pm$ 0.08	1.22 $\pm$ 0.01	34.85 $\pm$ 0.01	14.12 $\pm$ 0.09	2.80 $\pm$ 0.01	10.79 $\pm$ 0.06
Site 3	3.04 $\pm$ 0.80	4.98 $\pm$ 0.03	1.57 $\pm$ 0.00	17.69 $\pm$ 0.03	20.76 $\pm$ 0.13	2.10 $\pm$ 0.02	10.08 $\pm$ 0.04
Site 4	3.45 $\pm$ 0.02	4.93 $\pm$ 0.04	1.36 $\pm$ 0.01	11.37 $\pm$ 0.04	3.85 $\pm$ 0.02	3.27 $\pm$ 0.1	8.31 $\pm$ 0.02
Site 5	2.13 $\pm$ 0.03	4.84 $\pm$ 0.07	0.71 $\pm$ 0.02	19.95 $\pm$ 0.02	19.95 $\pm$ 0.06	2.66 $\pm$ 0.01	10.09 $\pm$ 0.05
Site 6	2.97 $\pm$ 0.04	4.98 $\pm$ 0.08	0.76 $\pm$ 0.02	35.62 $\pm$ 0.06	9.93 $\pm$ 0.07	2.85 $\pm$ 0.02	10.56 $\pm$ 0.06
Site 7	3.50 $\pm$ 0.04	5.11 $\pm$ 0.06	1.52 $\pm$ 0.01	30.11 $\pm$ 0.06	2.87 $\pm$ 0.02	1.94 $\pm$ 0.06	9.71 $\pm$ 0.04

4.1.3.3 Composition and classification of plants in the vicinity of BCL Mines

There were 82 plant shoot samples collected in the study area. Forty-nine samples were classified into fourteen tree species, fifteen samples classified as five forb species, and 18 samples classified as six grass or sedge species (Table 4.1.5). In site 1, twenty plant species were collected and identified, fifteen species in site 7, twelve species in site 3, eleven species in site 6, nine species in site 5, eight species in site 4, and seven species in site 2. Except for *Boscia albitrunca*, *Boscia foetida*, *C. mopane*, and *Combretum apiculatum* which appeared five times in different sites, all other plant species appeared three times in one of

the different sites. All of the tree species are native and non-invasive except for *Dichrostacy cinerea* which is considered as an invasive tree. Most of the trees have either edible fruits or some of their parts are used as part of traditional medicine. All of these tree species are utilized as forage by livestock and wildlife. The five forb species consist of *Senna italica*, *Solanum panduriforme*, *Aloe marlothii*, *Limeum viscosum*, and *Ximenia caffra* are native species which are being utilized by humans as food or as part of traditional medicine. All of these species are commonly browsed by livestock and wild animals under normal conditions except for *S. panduriforme* and *A. marlothii* which are only utilized by some livestock and wild animals under extreme drought conditions or in overgrazed areas.

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

Table 4.1.5 List and classification of plants collected from different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana.

Scientific Name	Site of Collection	Classification (Type; Native/non-native, invasive; local uses)
<i>Faidherbia albida</i>	Sites 3,6,7	Tree; Native; Forage for livestock and wildlife
<i>Vachellia luederitzii</i>	Sites 1, 2,7	Tree; Native; Edible gum, Forage livestock and wildlife
<i>Senegalia nigrescens</i>	Sites 6,2,7	Tree; Native; Forage for livestock and wildlife
<i>Vachellia tortilis</i>	Sites 5, 1, 4	Tree; Native; Edible gum; Forage for livestock and wildlife
<i>Azanza garkeana</i>	Sites 3,5,6	Tree; Native; Edible fruits; Medicinal plant; Forage for livestock and wildlife
<i>Boscia albitrunca</i>	Sites 1, 3, 4, 5, 6	Tree; Native; Edible roots and fruits; Forage for livestock and wildlife
<i>Boscia foetida</i>	Sites 1, 3, 4, 5, 6	Tree; Native; Edible fruits; Medicinal; Forage for livestock and wildlife
<i>Colophospermum mopane</i>	Sites 1, 3, 4, 5, 6	Tree; Native; Host for mopane worm; Medicinal; Forage for livestock and wildlife
<i>Combretum apiculatum</i>	Sites 1, 3, 5, 6	Tree; Native; Medicinal; Forage for livestock and wildlife
<i>Commiphora glandulosa</i>	Sites 1, 3, 6	Tree; Native; Medicinal; Fruits and young leaves as forage by wildlife
<i>Dichrostacy cinerea</i>	Sites 1, 4, 6	Tree; Native and invasive; Medicinal; Forage livestock and wildlife
<i>Grevia bicolor</i>	Sites 1, 4, 7	Tree; Native; Edible fruits; Medicinal; Forage for livestock and wildlife
<i>Lonchocarpus nelsii</i>	Sites 1, 3, 5	Tree; Native; Medicinal; Forage for livestock and wildlife
<i>Maerua angolensis</i>	sites 1, 3, 7	Tree; Native; Edible leaves; Medicinal; Forage for livestock and wildlife
<i>Senna italica</i>	Sites 4, 5, 7	Fords; Native; Medicinal; Forage for livestock and wildlife
<i>Solanum panduriforme</i>	Sites 1, 5, 7	Fords; Native; Medicinal; Pesticidal, Forage for livestock and wildlife during drought or overgrazed areas.
<i>Aloe marlothii</i>	Sites 1, 3, 7	Fords; Native; Medicinal; Forage for livestock and wildlife during extreme drought
<i>Limeum viscosum</i>	Sites 1,3,6	Fords; Native; Edible seeds, Forage for livestock and wildlife
<i>Ximenia caffra</i>	Sites 1, 2, 7	Fords; Native; Edible; Medicinal; Forage for livestock and wildlife
<i>Cyperus difformis</i>	Site 4, 6, 7	Sedges; Native and invasive; Forage for livestock and wildlife
<i>Aristida spiciformis congesta</i>	Sites 1,3, 7	Grass; Native, Forage for livestock and wildlife
<i>Cynodon dactylon</i>	Sites 1, 2, 7	Grass; Native and invasive; Medicinal; Forage for livestock and wildlife
<i>Dactyloctenium aegyptium</i>	Sites 1, 2, 7	Grass; Native; Edible seeds; Medicinal; Forage for livestock and wildlife
<i>Pennisetum setaceum</i>	Sites 1, 2, 7	Grass; Non-native and invasive; Ornamental plant; Forage for livestock and wildlife
<i>Stipagrostis uniplumis</i>	Sites 1, 2, 7	Grass; Native; Forage for livestock and wildlife

There were five grasses, three of which are native and non-invasive: *Aristida spiciformis congesta*, *Dactyloctenium aegyptium*, and *Stipagrostis uniplumis*; while *Cynodon dactylon* is native and invasive, and *Pennisetum setaceum* is a non-native and invasive grass. *Cyperus difformis* is a native and invasive sedge. All these grasses and sedges are utilized by livestock and wild animals as forage. In addition, seeds of *D. aegyptium* are consumed by humans.

4.1.3.4 Heavy metal accumulation and bioaccumulation in plants

The heavy metal concentration in the shoots of plants growing in the vicinity of BCL mines is presented in Table 4.1.6. Based on the plant type, the total Zn concentration in trees ranged from 13.42 to 244.75 mg kg⁻¹ in which *B. albitrunca* and *B. Foetida* had Zn concentration above 200 mg kg⁻¹ and *C. apicuclatum* had 113.14 mg kg⁻¹ Zn in shoots. Forb species had Zn content ranging from 10.81 to 26.43 mg kg⁻¹ while the grasses had Zn content range of 33.54 to 87.57 mg kg⁻¹. The Pb concentration in tree species average and range were 170.37 mg kg⁻¹ and 74.25 to 247.19 mg kg⁻¹, respectively; 155.74 mg kg⁻¹ was the average concentration of forbs and they ranged from 54.6 to 144.39 mg kg⁻¹; and the average concentration in grasses was 130.48 mg kg⁻¹ and ranged from 94.22 to 161.98 mg kg⁻¹. The tree species that had Pb concentrations above 200 mg kg⁻¹ are *Vachellia tortilis*, *B. Foetida*, *Commiphora glandilosa*, and *Maerua angolensis*, while all other tree species had more than 100 ppm Pb except for *Azanza garkeana*. High Pb concentration above 100 ppm in forbs was found on *A. Marlothii*, *S. panduriforme*, and *X. caffra*; and grasses on *A. sapiciformis*, *C. dactylon*, *C. difformis*/*Cyperus haspan*, *D. aegyptium*, and *P. setaceum*.

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

Table 4.1.6 Heavy metal contents in the shoots of plants collected from different sampling site in the vicinity of BCL mines, Selibe-Phikwe, Botswana.

Plants Name	Concentration in Shoots (mg kg ⁻¹)					
	Zn	Pb	Mn	Cu	Ni	As
Trees						
<i>Faidherbia albida</i> (n=3)	32.78±2.14	133.79±12.46	69.88±10.22	35.76±6.42	44.31±12.18	4.49±1.40
<i>Vachellia luederitzii</i> (n=3)	39.21±3.46	146.88±10.12	92.06±8.17	68.06±10.14	136.57±16.24	6.67±1.78
<i>Senegalia nigrescens</i> (n=3)	64.16±5.14	171.24±9.48	114.59±9.16	28.32±5.14	112.87±6.48	9.74±0.98
<i>Vachellia tortilis</i> (n=3)	37.42±3.28	233.51±13.75	41.82±22.47	55.44±6.89	38.47±3.42	8.43±1.10
<i>Azanza garkeana</i> (n=3)	28.83±2.13	74.25±10.42	68.14±14.36	60.71±7.81	44.99±5.46	11.36±0.76
<i>Boscia albitrunca</i> (n=5)	244.75±9.47	154.03±16.72	305.10±13.78	335.27±16.47	158.79±10.44	6.79±0.83
<i>Boscia foetida</i> (n=5)	216.84±12.16	247.19±32.14	172.25±10.44	227.47±21.43	115.03±9.87	12.40±1.14
<i>Colophospermum mopane</i> (n=5)	63.18±11.42	106.68±13.42	81.38±11.43	140.07±11.48	165.75±8.42	6.81±0.82
<i>Combretum apiculatum</i> (n=4)	113.14±14.73	155.24±3.71	124.31±9.45	69.42±9.42	77.00±8.76	6.78±0.24
<i>Commiphora glandulosa</i> (n=3)	13.95±1.77	214.12±12.14	137.80±3.78	64.98±11.46	23.11±2.43	9.68±0.38
<i>Dichrostacys cinereal</i> (n=3)	13.42±3.21	151.00±16.43	42.66±5.46	333.15±16.42	113.42±6.45	13.42±0.28
<i>Grevia bicolor</i> (n=3)	16.87±2.46	199.01±23.11	53.84±5.71	152.28±8.33	98.95±8.76	44.28±0.34
<i>Lonchocarpus nelsii</i> (n=3)	14.72±3.78	162.77±34.15	91.42±6.47	48.28±9.12	16.05±2.10	17.84±0.86
<i>Maerua angolensis</i> (n=3)	17.09±2.77	235.46±22.41	66.73±5.79	48.25±4.65	46.82±3.44	72.31±1.42
Average	65.45	170.37	104.43	119.10	85.15	16.50
SD	75.38	50.36	68.81	106.16	50.26	18.86
Forbs						
<i>Senna italica</i> (n=3)	11.09±2.44	54.60±6.23	43.51±3.14	135.83±13.42	54.50±6.42	7.25±0.56
<i>Solanum panduriforme</i> (n=3)	16.70±3.56	143.98±11.18	46.38±6.49	263.97±14.72	35.96±4.76	25.38±1.10
<i>Aloe marlothii</i> (n=3)	26.43±3.78	144.39±9.43	12.52±2.78	244.52±21.43	69.48±4.52	7.42±0.34
<i>Limeum viscosum</i> (n=3)	10.81±2.48	95.38±8.72	281.39±12.48	179.82±8.23	82.13±8.77	8.21±0.27
<i>Ximenia caffra</i> (n=3)	14.07±3.89	140.34±15.48	91.06±6.41	144.19±13.42	51.90±6.73	14.48±0.87
Average	15.82	115.74	94.97	193.67	58.79	12.55
SD	6.40	39.93	107.91	58.12	17.65	7.77
Grasses and Sedges						
<i>Aristida spiciformis congesta</i> (n=3)	34.88±3.76	133.05±9.17	95.11±6.17	160.40±9.72	145.98±12.42	25.36±1.43
<i>Cynodon dactylon</i> (n=3)	63.91±4.77	107.21±23.47	149.83±8.12	275.36±14.68	188.37±9.47	11.21±0.46
<i>Cyperus difformis</i> (n=3)	45.02±6.14	161.98±13.56	274.73±12.44	201.75±21.34	153.16±16.42	18.73±0.77
<i>Dactyloctenium aegyptium</i> (n=3)	49.61±5.48	128.86±12.14	44.70±6.43	429.36±67.44	83.99±7.44	39.81±1.73
<i>Pennisetum setaceum</i> (n=3)	33.54±9.42	157.59±18.24	69.40±6.79	391.91±25.46	320.96±24.88	42.66±1.66
<i>Stipagrostis uniplumis</i> (n=3)	87.57±3.87	94.22±16.47	140.58±10.46	319.42±16.48	411.10±30.17	20.81±0.93
Average	52.42	130.49	129.06	296.37	217.26	26.43
SD	20.47	26.81	81.99	105.10	123.38	12.38

The Mn concentration in tree species ranged from 28.32 to 335.27 mg kg⁻¹, forbs ranged from 135.83 to 263.97 mg kg⁻¹; and grasses range from 135.83 to 274.7263.97 mg kg⁻¹. The plant species that had Mn concentration above 100 mg kg⁻¹ are *Senegalia nigrescens*, *B. foetida*, *C. apiculatum*, *L. viscosum*, *Cdactylon*, *C. difformis/C. haspan*, and *S. uniplumis*. The Cu concentration in tree species ranged from 41.82 to 305.27 mg kg⁻¹, forbs ranged from 12.52 to 28139 mg kg⁻¹; and grasses range from 44.70 to 274.73 mg kg⁻¹. The plant species that had Cu concentrations above 200 mg kg⁻¹ are *B. albitrunca*, *B. foetida*, *D. cinereal*, *S. panduriforme*, *A. Marlothii*, *C. dactylon*, *C. difformis/C. haspan*, *D. aegyptium*, *P. setaceum* and *S. uniplumis*.

Ni concentration in the shoots of trees ranged from 16.05 to 165, 35.96 to 82.13 in forbs, and 83.99 to 411.0 in grasses. The Highest Ni concentration was found in *S. uniplumis* (411.10) followed by *P. setaceum* (320.96). Tree species with Ni concentration greater than 100 ppm are *Vachellia luederitzii*, *S. nigrescens*, *B.albitrunca*, *B. foetida*, *C. mopane* and *D. cinereal*; while *A. spiciformis*, *C. dactylon* and *C. difformis* were for grasses. The arsenic concentration in the shoots of trees ranged from 4.49 to 72.31, 7.25 to 26.38 in forbs, and 11.21 to 42.66 in grasses. The highest concentration among the tree species was obtained from *M. anglonsis*; *S. panderiforme* in forbs and *P. setaceum* in grasses. Overall average and standard deviation in the concentrations among the plant shoot samples were: 54.41 ± 65.19 for Zn; 146.88 ± 52.44 for Pb; 114.56 ± 86.92 for Mn; 177.10 ± 116.77 for Cu; 104.31 ± 85.90 for Ni; and 19.06 ± 17.83 for As.

Bioconcentration factor (BF) is the concentration of heavy metal(loid)s in trees divided by the concentration of heavy metal(loid)s in soil (Takarina and Pin, 2017). The bioconcentration factor indicates the ability of plants to remove metal compounds from the

soil/substrate. (Takarina and Pin, 2017). The average bioconcentration for Zn, Pb, Mn, Cu, Ni, and As of different plant species collected from different sampling sites are presented in Table 4.1.7. Based on the plant type, the BF of Zn in trees ranged from 0.19 to 3.88 in which *B. albitrunca*, *B. foetida*, *C. mopane* and *C. apicuclatum* have $BF > 1$. The forb species had Zn-BF ranging from 0.014 to 0.4 while the grasses had Zn-BF in the range of 0.53 to 1.32. Among the grasses, *C. dactylon* and *S. uniplumis* had Zn-BF greater than one. The BF for Pb among tree species ranged from 0.3 to 1.01 with *B. foetida* having Pb-BF greater than one. The range of Pb-Bf among the forbs and grasses was 0.22 to 0.59 and 0.39 to 0.66, respectively. All the Pb-BF in forbs and grasses are lower than one. The BF of Mn in tree species ranged from 0.11 to 0.85, forbs ranged from 0.03-0.75, and grasses ranged from 0.11 to 0.55. All the plant species have Mn-BF lesser than one. Similar to Mn, the BF of Cu and Ni were all less than one among the plant samples. The overall range of BF-Cu was from 0.02 to 0.43 while BF-Ni was 0.04 to 0.66. In contrast, some of the plant species in the vicinity of BCL mines have BF of As higher than one. The As-BF among tree species ranged from 0.47 to 3.64, the forbs ranged from 0.37 to 1.28 and the grasses ranged from 0.79 to 3.08. The tree species with $As-BF > 1$ were *A. garkeana*, *B. foetida*, *Grewia bicolor*, *Lonchocarpus nelsii*, and *M. angolensis*. In forbs, *S. panduriforme* and in grasses *A. spiciformis*, *C. difformis/C. haspan*, *D. aegyptium*, *P. setaceum*, and *S. uniplumis* had BF-As greater than one.

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

Table 4.1.7 Average bioconcentration factor for different heavy metal(loid)s of different plant species collected from different sampling sites in the vicinity of BCL mines, Selibe-Phikwe, Botswana.

Scientific Name	Bioconcentration factor					
	Zn	Pb	Mn	Cu	Ni	As
Trees						
<i>Faidherbia albida</i> (n=3)	0.49±0.08	0.54±0.08	0.12±0.06	0.05±0.00	0.04±0.00	0.54±0.02
<i>Vachellia luederitzii</i> (n=3)	0.65±0.10	0.59±0.06	0.23±0.04	0.06±0.00	0.16±0.00	0.47±0.03
<i>Senegalia nigrescens</i> (n=3)	0.97±0.07	0.69±0.02	0.41±0.06	0.02±0.00	0.19±0.02	0.87±0.02
<i>Vachellia tortilis</i> (n=3)	0.68±0.07	0.96±0.04	0.14±0.02	0.06±0.00	0.04±0.00	0.61±0.04
<i>Azanza garkeana</i> (n=3)	0.43±0.03	0.30±0.01	0.12±0.02	0.08±0.01	0.04±0.00	1.38±0.06
<i>Boscia albitrunca</i> (n=5)	3.88±0.06	0.63±0.06	0.85±0.04	0.43±0.02	0.28±0.01	0.59±0.03
<i>Boscia foetida</i> (n=5)	3.44±0.02	1.01±0.02	0.48±0.03	0.29±0.04	0.20±0.02	1.08±0.03
<i>Colophospermum mopane</i> (n=5)	1.05±0.08	0.44±0.00	0.24±0.02	0.15±0.00	0.18±0.00	0.61±0.01
<i>Combretum apiculatum</i> (n=4)	1.87±0.02	0.63±0.04	0.37±0.04	0.08±0.00	0.09±0.00	0.60±0.00
<i>Commiphora glandulosa</i> (n=3)	0.21±0.00	0.88±0.14	0.37±0.06	0.07±0.00	0.03±0.00	0.49±0.02
<i>Dichrostacy cinereal</i> (n=3)	0.20±0.01	0.62±0.03	0.11±0.07	0.36±0.02	0.13±0.00	0.68±0.08
<i>Grevia bicolor</i> (n=3)	0.25±0.00	0.81±0.08	0.14±0.03	0.16±0.02	0.11±0.02	2.23±0.08
<i>Lonchocarpus nelsii</i> (n=3)	0.19±0.01	0.66±0.04	0.18±0.03	0.10±0.00	0.07±0.00	1.39±0.06
<i>Maerua angolensis</i> (n=3)	0.26±0.02	0.96±0.06	0.18±0.06	0.05±0.00	0.05±0.00	3.64±0.06
Average	1.10	0.67	0.29	0.15	0.12	0.89
SD	1.20	0.21	0.20	0.13	0.08	0.89
Forbs						
<i>Senna italica</i> (n=3)	0.14±0.02	0.22±0.04	0.09±0.03	0.29±0.01	0.23±0.02	0.56±0.02
<i>Solanum panduriforme</i> (n=3)	0.25±0.04	0.59±0.06	0.12±0.04	0.28±0.02	0.04±0.00	1.28±0.08
<i>Aloe marlothii</i> (n=3)	0.40±0.02	0.59±0.08	0.03±0.00	0.26±0.00	0.08±0.00	0.37±0.04
<i>Limeum viscosum</i> (n=3)	0.16±0.06	0.39±0.02	0.75±0.01	0.19±0.00	0.09±0.00	0.41±0.03
<i>Ximenia caffra</i> (n=3)	0.21±0.04	0.57±0.03	0.24±0.02	0.16±0.02	0.06±0.00	0.73±0.04
Average	0.23	0.47	0.25	0.24	0.10	0.67
SD	0.10	0.16	0.29	0.06	0.08	0.37
Grasses and Sedges						
<i>Aristida spiciformis congesta</i> (n=3)	0.53±0.12	0.54±0.06	0.25±0.00	0.17±0.02	0.17±0.02	1.28±0.04
<i>Cynodon dactylon</i> (n=3)	1.06±0.10	0.43±0.02	0.37±0.03	0.24±0.02	0.22±0.02	0.79±0.02
<i>Cyperus difformis</i> (n=3)	0.59±0.08	0.66±0.08	0.55±0.02	0.43±0.03	0.66±0.03	1.46±0.02
<i>Dactyloctenium aegyptium</i> (n=3)	0.84±0.06	0.51±0.06	0.11±0.01	0.35±0.02	0.10±0.00	3.08±0.06
<i>Pennisetum setaceum</i> (n=3)	0.55±0.08	0.63±0.04	0.17±0.01	0.35±0.04	0.37±0.01	3.01±0.08
<i>Stipagrostis uniplumis</i> (n=3)	1.32±0.04	0.39±0.07	0.37±0.01	0.34±0.02	0.47±0.02	1.05±0.03
Average	0.82	0.53	0.30	0.31	0.33	1.78
SD	0.32	0.11	0.16	0.09	0.21	1.01

4.1.3.5 Metal Intake by Grazing Animals

The heavy metal intake of grazing animals estimated based on the concentration of the heavy metal(loid)s from different plant groups and the average daily intake of forage and soils are presented in Table 4.1.8. Assuming that cattle will consume 16.9 kg per day which is composed of 75% grasses and sedges, 23% of shrubs and woody species, and 2% forbs from the vicinity of BCL mines, the daily intake of heavy metal(loid)s would be 950.97 mg for Zn, 2456.86 mg day⁻¹ for Pb, 2247.97 mg day⁻¹ for Mn, 4701.95 mg day⁻¹ for Cu, 3410.10 mg day⁻¹ for Ni and 408.16 mg day⁻¹ for As. For goats having a daily feed consumption of 0.90 kg/day, consisting of 20% grasses, 78% of shrubs and woody species, and 2% forbs, the daily intake of heavy metal(loid)s would be 56.01 mg day⁻¹ for Zn, 15383 mg day⁻¹ for Pb, 129.80 mg day⁻¹ for Mn, 226.56 mg day⁻¹ for Cu, 163.34 mg day⁻¹ for Ni and 15.89 mg day⁻¹ for As. With an average daily forage intake of 2.50kg for sheep which consists of 74% grasses, 21% of shrubs and woody species, and 5% forbs, the daily intake of heavy metal(loid)s would be 139.85 mg day⁻¹ for Zn, 370.09 mg day⁻¹ for Pb, 347.93 mg day⁻¹ for Mn, 736.74 mg day⁻¹ for Cu, 528.49 mg day⁻¹ for Ni and 60.29 mg day⁻¹ for As.

Table 4.1.8 Maximum and estimated daily intake of heavy metal(loid)s of cattle, goat and sheep grazing on plants near the BCL mines, Selibe-Phikwe, Botswana

Type of Ruminant	Daily Intake of Metals (mg day ⁻¹)					
	Zn	Pb	Mn	Cu	Ni	As
Cattle	950.97	2456.86	2247.97	4701.95	3410.10	408.16
Goat	56.01	153.83	129.80	226.56	163.34	15.89
Sheep	139.85	370.09	347.93	736.74	528.49	60.29
Maximum Daily Intake (mg day ⁻¹)						
Cattle	11200 ^a	480 ^b	2000 ^c	1000-2000 ^d	3500 ^e	32-640 ^c
Goat	1000 ^a	30-80 ^b	150 ^c	150-180 ^d	700 ^e	2-40 ^c
Sheep	2500 ^a	75-100 ^b	375-400 ^c	230-250 ^d	1000 ^e	5-100 ^c

^aBased on 700 mg Zn/kg of diet in cattle's and 1000 mg Zn/kg of diet in sheep and goat which result to reduced body weight gain (Radostits et al., 2002). ^bBased on the 30ppm maximum acceptable limit for mineral mixture (Marçal et al. 2005) ^cBased on Nouri & Haddioui (2016). ^dBased on the 1-2g Cu/day to cause chronic poisoning in calves and daily ingestion of 3.5 mg Cu/kg BW of sheep and goat. ^eBased on Outridge and Scheuhammer (1993)

4.1.4 Discussion

After more than 40 years of operation of BCL Cu-Ni mine in Selebi Phikwe, the chemical properties and the heavy metal contents of the surrounding area manifested serious environmental pollution which emanated from the weak and inefficient mine waste management and environmental protection programs. The deterioration of the soils at the vicinity of the BCL mine is reflected in the severe acidification of the surface soil and high EC ranging from 1414 to 4870 $\mu\text{S m}^{-1}$. The concentrations of Zn, Pb, Cu, Ni, and As also increased based on the background levels and from previous studies. Soil samples taken from the same area in April 2017 have Cu, Ni, Cr, As, Co and Zn amounting to as high as 921.1 ppm, 543.7 ppm, 253.81 ppm, 7.0 ppm, 23.6 ppm, and 83.5 ppm, respectively (Motswaiso et al., 2019) while Ekosse (2005) reported the concentration of Cu between 11 ppm to 116 ppm, Ni between 19 ppm to 120 ppm, Zn between 17 and 68 ppm taken from January to July 2000. It could also be observed that the concentrations of heavy metal(loid)s in this area are comparable to the concentrations in mine tailing and in particulate matter. The concentrations of some heavy metal(loid)s in PAM were 87 ppm Cu, 41 ppm Ni, and 4 ppm Zn (Ekosse, 2005) while in the mine tailings were 779-896 ppm for Cu, 328-389 ppm for Ni and 46-77 ppm for Zn (Ekosse, 2005). Based on the contamination factor, the highest enhancement was recorded on the following order: $\text{Cu} > \text{Ni} > \text{Pb} > \text{As} > \text{Zn} > \text{Mn}$. The CF of Cu in all sites is considered to be under a very high concentration factor category ($\text{CF} > 6$) based on the proposed scheme of Likuku et al. (2013). It could be noted that the CF for Cu obtained in this current study is more than 1.79x to as high as 5.6x the previous value for the same site obtained in 2013. For Ni, the CF in some site increased up to 5.7x in site 3 while for Pb, the CF increased within the range of 2.24x to 2.38x; while for Zn, the CF increased from 1.76x to 2.89x based on the CFs in the previous study (Likuku et al., 2013). Consequently, the PLI in our current evaluation is comparable between

the different sampling sites ranging from 8.31 to 10.79 which is more than 5.1x higher than the previous value for the same site. It appeared that the rate of increase of contamination in recent years is very much higher compared to the previous years when BCL was operating. Since 2014, BCL was under liquidation and practically ceased its operation. The temporary closure resulted in the unregulated and loosening of pollution prevention measures in the mining facilities. Actual observations indicate heaps of mine tailings and slags scattered outside the perimeter fence aside from visible indication of erosion and sediment deposition coming from the mine tailings dump site. This current data underscores the immediate need to control pollution dispersal from the BCL mine facilities. Delaying the rehabilitation process and pollution control measures would further deteriorate the adjacent areas and pose a very high risk to the community and ecosystem.

Continued deposition of heavy metal(loid)s in the surface soil around the vicinity of the mining area has a profound effect on soil acidity and EC. Acidification is attributed to the deposition of sulphidic mine tailings and the associated increase of heavy metal(loid)s. The high EC of the surface soils could indicate a high concentration of soluble salts which could be associated with the deposited particles from the waste of BCL mine. The previous evaluation showed that the mine tailings have high soluble salts in the form of sulfates and sulfur-rich minerals contained in tailings dump (Ekosse 2005; Motswaiso, 2019). Hydrolysis of metals will release protons that will acidify the soil (Motuzova and Makarychev 2014) and will result in increased solubility of heavy metal(loid)s. Based on the concentration of exchangeable metals in soils, exch Zn accounts for 3.43% of the total concentration which are higher than the fraction of exch Zn from the control soil. The exchangeable fractions were about 2.00% for Pb, 2.25% for Mn, 1.66 for Cu, 3.92% for Ni, and 8.34% for As. These high concentrations of exchangeable fractions of metals are attributed to the high solubility of deposited materials on the soil surfaces. As pointed out by Ekosse et al. (2003),

the slopes of the tailings dump serve as migratory pathways for ions of SO_4^{2-} , CO_3^{2-} , Cl^- , NO_3^- , PO_4^{2-} , Cd, Co, Cr, Cu, Fe, Ni, Se, and Zn which are leached through labile corridors to the subsurface environments. In addition, the higher concentrations of the available fraction are due to the fine particles windblown from the tailings dump which could be easily solubilized due to their small size and high surface area. Similarly, Schwartz et al (2006) indicated that the tailings in the BCL mines are made up of 88% pyrrhotite, 6% pyrite, 4% pentlandite, and 2% chalcopyrite; with goethite and sulfate minerals as the oxidation products. When exposed to the surface environment, oxidizable heavy metal(loid)s associated with mineral sulfides will be dissolved and will contribute to the high exchangeable fraction in soils (Ekosse et al., 2003). These results provide the current state of contamination on surface soil around the BCL mine and its relative availability for plant uptake or mobility for migration into vulnerable environmental compartments. The information on the amount of exchangeable fraction of heavy metal(loid)s also provides better assessment of their potential risk and the potential toxicity of heavy metal(loid)s to the ecosystem and human health which are primarily dependent on their mobility and bioaccessibility (Broadway et al., 2010; Lee et al., 2015).

Previous assessment of the impacts of BCL mining on plant communities had reported the heavy metal concentration in mopane (Ekosse et al., 2005), and the dominance of *Aristida conjesta*, and *G. flava* in the study area (Likuku et al., 2013). By far, this is the first systematic study to account for the heavy metal concentration in all plant species growing in the area in February 2018. It should be noted that the different sites have different plant composition, and that the variation of species composition could be the result of the prevailing environmental condition which includes the severity of the heavy metal concentration. However, a detailed analysis of how this variation occurred with respect to the micro environmental conditions has to be explored in the future.

The 25 plant species growing in the area could be considered as potential plants for phytoremediation. These plants can accumulate heavy metal(loid)s or tolerate high concentrations of metal(loid)s without interference of their growth and development. Some of these plants have the ability to accumulate high concentrations of several heavy metal(loid)s while others can either accumulate one particular metal and maintain a low concentration of other metals to survive in an environment with multi-metal contamination. Among the tree species, *B. albitrunca* and *B. foetida* exhibited high concentrations and BF for Zn, Pb, Cu, and are potential phytoremediation agents for these multi-metal contaminants. Other species are potential agents for phytoremediation of single metal such as *C. apiculatum* for Zn; *V. tortilis*, *C. glandilosa*, and *M. Angolensis* for Pb; *B. albitrunca*, *D. cinereal*, *S. panduriforme*, *A. Marlothii*, *C. dactylon*, *C. haspan* and *D. aegyptium* for Cu. Species that are potential agents for Ni phytoremediation include *V. luederitzii*, *S. nigrescens*, *C. mopane*, *D. cinereal*, and *S. panduriforme*; while *M. anglonsis*, *G. bicolor*, and *D. aegyptium* have the potential for As phytoremediation. Some of these plants have been reported by other authors as potential phytoremediation agents. For instance, in the report of Auret (2018), several plant species were found to have concentration anomalies for several elements which were suggested as suitable for biogeochemical prospecting of gold ore deposits. These include *B. albitrunca* which can absorb significant amounts of Ag, Pb, As, W, and Hg (Mshumi, 2006), and recently, Auret (2018) indicated for the high accumulation of Mn, Zn, Cd, and Sb; *B. foetida* subsp. *rehmanniana* (B, Mn, Fe, Cu, Zn, As and Se); *C. apiculatum* (B, Mn, Fe, Co, Zn); *G. bicolor* (Fe, Cu, Sb, Hg, and Pb), *C. apiculatum* (B, Mn, Fe, Co, Zn, and Ag), and *P. nelsii* (B, Mn, Cu, Zn, As and Mo). Our data showed the consistency of these plants in accumulating multi-metal from soil (Table 4.1.6).

Based on the BF, trees have higher BF-Zn than grasses and forbs. Plants in the study area had a moderate concentration of Zn in shoots which could be attributed to inherently low Zn

concentration in surface soils. Normally, Zn concentration in plants is in the range of 20-200 ppm and a relatively low bioaccumulation factor (<0.1) is attributed to low Zn availability in the rhizosphere as it is readily precipitated as insoluble sulfate, thereby minimizing absorption and transport to the leaves, so that hyperaccumulation of Zn is exceedingly rare (Ghaderian and Ravandi, 2012). Once it is taken-up, Zn is very mobile and easily bio-accumulated and moderately translocated from the roots to above-ground parts of the plant (Kabata-Pendias, 2011). In this study, only *B. albitrunca* and *B. foetida* had Zn concentrations above 200 ppm with $BF > 3$. Our data showed that all plants from the study area have $Zn-BF > 0.1$ which implied that these plants have the ability to accumulate Zn compared to other plants. The high Zn-BF of plants in the study area could be attributed to high Zn availability (Table 4.1.3).

Cu is more mobile than Pb in soil solution and readily taken up by plants with a plant/soil transfer factor of 0.1, while Pb would have a much lower plant/soil transfer factor (Kabata-Pendias, 2011). However, in the study site, the Cu-BF of plants was lower compared to the Pb-BF. Such discrepancy could be attributed to the very high concentrations of Cu in soils in which the BF of an element tends to decrease with an increased concentration in soil. Alternatively, most of these plants appeared to accumulate more Pb than Cu (Table 4.1.6). Ordinarily, Cu concentrations in plants are within 5-25 ppm and concentrations above 100 ppm are considered high (Ghaderian & Ravandi, 2012). In the case of Ni, a plant/soil transfer factor of 0.01 was commonly observed and favorably accumulated in the roots (Kabata-Pendias, 2011). Plants in the study area had Ni-BF of more than 0.1 which could be attributed to the high availability of Ni in the soil ranging from 0.96 to 9.49% of the total concentrations.

The general trends in As absorption in plants can be expressed by a plant/soil transfer factor of 0.01 (Kabata-Pendias, 2011) and it appeared that all the plants in the study area had BF

from 0.37 to 3.08 (Table 4.1.7). This high As-BF in plants from the study area could be attributed to the high concentration of exchangeable As in soil which is around 5 to 12% of the total concentration (Table 4.1.3). As in plants correlates positively with the available and total concentrations in soils and is taken up passively with the water flow (Kabata-Pendias, 2011). With increasing soil As, high As concentrations were recorded in both old leaves and roots, but at a low content of As, a higher accumulation in leaves than in roots was reported (Kabata-Pendias, 2011).

Currently, the area outside the perimeter fence of the BCL mine where the plant samples were collected is accessible to grazing animals. Based on estimated heavy metal(loid)s intake, grazing cattle could have a daily intake of Pb and Cu by more than 5x and 4x, respectively of the maximum daily intake while the estimated DI for Zn, Mn, Ni, and As were below the maximum DI. Similarly, goat and sheep grazing in the area will exceed the Pb and Cu allowable daily intake. Goats that will feed mostly on shrubs and woody species in the area will intake 3.8x and 1.5x of the maximum allowable intake for Pb and Cu, respectively; whereas sheep will intake more than 3.7x and 2.9x of Pb and Cu, respectively. The intake of goat and sheep for other metals in this study is within the allowable maximum daily intake. Elevated levels of Pb in animal diets contribute to risk to public health since there is an accumulation of this mineral in meat and milk (Miranda et al., 2005). On the other hand, cattle are relatively tolerant of copper and support up to 100 mg of Cu/Kg of food but consumption of plants high in Cu can lead to chronic copper poisoning and cause significant liver damage and haemolysis (Radostits et al., 2002).

Overall, the data presented provide updated information on the levels of contamination in soil and plants in the vicinity of BCL mines and provided preliminary estimates on the possible risk associated with heavy metal (loid)s contamination among grazing animals in the study area. The information on the heavy metal accumulation of the different plants was able to identify potential

plants or combinations of plants that could be utilized for the rehabilitation of the study area through phytoremediation. The estimates of the daily intake of the minerals due to consumption of plants at the vicinity of the BCL mines warrants for evaluation of actual levels of heavy metal(loid)s in grazing animals near the study area and in other mining areas in Botswana.

4.1.5 References

- Auret, N. (2018). *Biogeochemical exploration on the Kalahari Copperbelt* (Doctoral dissertation, North-West University, Potchefstroom Campus).
- Bai, J., Cui, B., Chen, B., Zhang, K., Deng, W., Gao, H. & Xiao, R. (2011). Spatial distribution and ecological risk assessment of Heavy metals in surface sediments from a typical plateau lake wetland, China. *Ecological Modelling*, 222(2), pp.301-306.
- Broadway, A., Cave, M. R., Wragg, J., Fordyce, F. M., Bewley, R. J., Graham, M. C., Ngwenya, B. T., & Farmer, J. G. (2010). Determination of the bioaccessibility of chromium in Glasgow soil and the implications for human health risk assessment. *Science of the Total Environment*, 409(2), 267–277.
- Chojnacka, K., Chojnacki, A., Gorecka, H. & Górecki, H. (2005). Bioavailability of heavy metals from polluted soils to plants. *Science of the Total Environment*, 337(1-3), pp.175-182.
- de Vries, W., Romkens, P. F., & Schutze, G. (2007). Critical soil concentrations of cadmium, lead, and mercury in view of health effects on humans and animals. In D. M. Whitacre (Ed.), *Reviews of environmental contamination and toxicology*. (pp. 91–130). Springer.
- Ekosse, G., (2005). Heavy metals concentrations in the biophysical environment around the Ni-Cu mine and the smelter/concentrator plant, Selebi Phikwe, Botswana. *Global Journal of Environmental Sciences*, 4(2), pp.97-110.

- Ekosse, G., Van Den Heever, D., De Jager, L. & Totolo, O. (2003). Environmental mineralogy of soils around Selebi Phikwe nickel-copper plant, Botswana. *International journal of environmental studies*, 60(3), pp.251-262.
- Ekosse, G.I.E. (2007). Influenza/common cold within the Selebi Phikwe nickel-copper exploitation environment, Botswana: Health. *African Journal for Physical Health Education, Recreation and Dance*, 13(4), pp.473-490.
- Ekosse, G.I.E. (2008). Environmental Effects of Nickel—Copper Exploitation. *Journal of Applied Sciences*, 8(13), pp.2344-2356.
- Ekosse, G.I.E., Ngila, J.C. & Forchheh, N. (2005). Multivariate analyses of Heavy metals in soils and *Colophospermum mopane* leaves around the Selebi Phikwe nickel-copper mine and smelter/concentrator plant area, Botswana. *Journal of Applied Sciences and Environmental Management*, 9(1), pp.177-185.
- Ghaderian, S. M., & Ravandi, A. A. G. (2012). Accumulation of copper and other Heavy metals by plants growing on Sarcheshmeh copper mining area, Iran. *Journal of Geochemical Exploration*, 123, 25–32.
- Headley, S. A., dos Reis, A. C. F., Nakagawa, T. L. D. R., & Bracarense, A. P. F. R. L. (2008). Chronic copper poisoning in sheep from Northern Parana', Brazil: *Case report.Semina: Ciencias Agrarias*, 29(1), 179–184.
- Imakando, S. (2017). The socio-economic impact of mining: a comparative study of Botswana and Zambia (Doctoral dissertation).
- Kabata-Pendias, A. (2011). *Trace elements in soils and plants*. (4th ed.). CRC Press.
- Koitsiwe, K. & Adachi, T. (2017). Linkages between mining and non-mining sectors in Botswana. *Mineral Economics*, 30(2), pp.95-105.

- Likuku, A.S., Mmolawa, K.B. & Gaboutloeloe, G.K. (2013). Assessment of heavy metal enrichment and degree of contamination around the copper-nickel mine in the Selebi Phikwe Region, Eastern Botswana. *Environment and Ecology Research*, 1(2), pp.15-17.
- Marcal, W. S., Pardo, P. E., Nascimento, M. R. L., Veras, E. B., & Moreno, A. D. (2003). Levels of lead in mineral salt commercial mixtures for beef cattle. *Journal of Veterinary Science*, 4, 235–238.
- Matshediso, I.B. (2005). A review of mineral development and investment policies of Botswana. *Resources Policy*, 30(3), 203-207.
- Menzies, N. W., Donn, M. J., & Kopittke, P. M. (2007). Evaluation of extractants for estimation of the phytoavailable trace metals in soils. *Environmental Pollution*, 145(1), 121–130.
- Miranda, M., Lopez-Alonso, M., Castillo, C., Herná'ndez, J., & Benedito, J. L. (2005). Effects of moderate pollution on toxic and trace metal levels in calves from a polluted area of northern Spain. *Environment International*, 31(4), 543–548.
- Motswaiso, F.S. (2019). Geochemical Investigation of Metals and Trace Elements around the Abandoned Cu-Ni Mine Site in Selibe Phikwe, Botswana. *Journal of Geoscience and Environment Protection*, 7(05), p.275.
- Motuzova, G., & Makarychev, I. (2014). Heavy metals pollution as a factor of soil acidification. *Environmental Research Journal*, 8(2), 187–193.
- Mphinyane, W. N., Tacheba, G., & Makore, J. (2015). Seasonal diet preference of cattle, sheep and goats grazing on the communal grazing rangeland in the Central District of Botswana. *African Journal of Agricultural Research*, 10(29), 2791–2803.
- Mshumi, U. (2006). *Can Acacia karroo and Boscia albitrunca be used in the biogeochemical prospecting for gold: A case study at the Blue Dot Mine, Amalia, Northwest province, South Africa* (Doctoral dissertation, University of the Western Cape).

- Nouri, M., & Haddioui, A. (2016). Human and animal health risk assessment of metal contamination in soil and plants from Ait Ammar abandoned iron mine, Morocco. *Environmental monitoring and assessment*, 188(1), 6.
- Outridge, P.M. & Scheuhammer, A.M., (1993). Bioaccumulation and toxicology of nickel: implications for wild mammals and birds. *Environmental Reviews*, 1(2), pp.172-197.
- Schippers, A., Kock, D., Schwartz, M., Böttcher, M.E., Vogel, H. and Hagger, M. (2007). Geomicrobiological and geochemical investigation of a pyrrhotite-containing mine waste tailings dam near Selebi-Phikwe in Botswana. *Journal of Geochemical Exploration*, 92(2-3), pp.151-158.
- Schwartz, M.O. & Kgomanyane, J. (2008). Modelling natural attenuation of heavy-metal groundwater contamination in the Selebi-Phikwe mining area, Botswana. *Environmental geology*, 54(4), pp.819-830.
- Takarina, N. D., & Pin, T. G. (2017). Bioconcentration factor (BCF) and translocation factor (TF) of Heavy metals in mangrove trees of Blanakan fish farm. *Makara Journal of Science*, 77-81.
- Tomlinson, D. L., Wilson, J. G., Harris, C. R., & Jeffrey, D. W. (1980). Problems in the assessment of heavy-metal levels in estuaries and the formation of a pollution index. *Helgolander Meeresuntersuchungen*, 33(1), 566–575.
- Ultra, V. U. (2020a). Fly ash and compost amendments and mycorrhizal inoculation enhanced the survival and growth of *Delonix regia* in Cu–Ni mine tailings. *Philippine Journal of Science*, 149(3), 479–489.
- Ultra, V. U. (2020b). Growth and yield of lemongrass (*Cymbopogon citratus*) in fly ash with nutrient amendments and mycorrhiza for three-ratoon period. *International Journal of Phytoremediation*, 22(14), 1551–1561.

- Ultra, V.U., & Manyiwa, T. (2020). Influence of mycorrhiza and fly ash on the survival, growth and heavy metal accumulation in three *Acacia* species grown in Cu–Ni mine soil. *Environmental Geochemistry and Health*, pp.1-17.
- Wiston, M. (2017). Status of air pollution in Botswana and significance to air quality and human health. *Journal of Health and Pollution*, 7(15), pp.8-17.
- Yager, T. R. (2017). *The mineral industry of Botswana. Minerals yearbook: Area reports: International review 2013 Africa and the Middle East* (Vol. 3, p. 1).
- Yutong, Z., Qing, X. & Shenggao, L. (2016). Chemical fraction, leachability, and bioaccessibility of Heavy metals in contaminated soils, Northeast China. *Environmental Science and Pollution Research*, 23(23), pp.24107-24114.

4.2 Spatial variability of heavy metal(loid)s in soils and vegetation, and associated risk to grazing animals in the Abandoned Gold Mine in Francistown, Botswana

Abstract

The environmental impact of the abandoned Monarch Gold Mine in Botswana was analysed based on the concentrations of As, Mn, Cu, Cr and Zr in tailings and its vicinity, and the plants growing in the area. Results showed that the soil in the tailings dam (TD) and in the vicinity of the tailings dam (VTD), and the river sediments (RS) were severely contaminated with pollution load index (PLI) ranging from 1.89 to 2.86 in decreasing magnitude from TD>VTD> RS. The main contaminant is As but Cu, Cr and Zr are all also slightly above the critical values for soil. The TD has fewer plant species than VTD and accumulated elevated levels of these heavy metal(loid)s (HM). The livestock grazing on these plants also consume the soil which could result to HM bioaccumulation. Therefore, proper management of the site is recommended to prevent the spread of pollutants and exposure to HM by animals and humans.

Keywords: Contaminant, pollution load index, bioaccumulation, pollutant

4.2.1 Introduction

Botswana's economy is based on natural resources and the extraction of these resources leads to environmental pollution, contamination and health risks to humans (Ekosse et al., 2005). The most common problems are heavy metal contamination due to mining activities and water pollution from industries, which threaten both human health and the ecosystem (Ultra and Manyiwa 2020). A study by Manyiwa and Ultra (2021); using daily intake estimates for ruminants like cattle, sheep and goats, and found that these animals were at risk due to heavy metal pollution from the BCL Copper and Nickel mine which is associated with lead (Pb) and copper (Cu) contamination in the surrounding areas. These environmental problems continue to rise because some mining operations are not compliant with environmental health and safety regulations (Ekosse et al., 2005).

Soil contamination from heavy metal(loid)s continues to be one of the most devastating environmental problems worldwide. The cause is mainly mining activities and poor environmental management (Motswaiso, 2019). Heavy metal pollution and contamination pose a great risk to human health and the ecosystem at large. If ignored, it could pose an even greater danger for future generations because heavy metal pollution can cause chronic diseases like cancer (Ekosse et al., 2005). In this regard, Maleki et al. (2014) found that humans are being exposed to heavy metal(loid)s through air, water and soil. People living near mines or smelters like Monarch could be exposed to high metal levels by breathing air and touching contaminated soil. Previous analysis there has found total levels of As are 200 to 1700 ppm and Sb is 40 to 400 ppm (Vogel and Kasper, 2000). These values for As and Sb exceed the intervention action values set by Albanese et al. (2007).

Heavy metal(loid)s generally occurs naturally in the soil, but mining activities increase the exposure potential through excavation, crushing, treatment, and disposal of mine tailings in the area (Vogel and Kasper, 2002). Furthermore, Vogel and Kasper (2002) also indicated that Monarch gold mine tailings have high levels of Cu which range from 865 to 2125 ppm. The intervention value for copper in non-agricultural areas is 200 ppm. Even though copper is an essential trace element for animals, plants and humans, high concentrations are considered to be phytotoxic and may be detrimental to human health. Other heavy metal(loid)s that were found to have high concentrations include Ni, Zn, and Ba. This indicates the danger these tailings pose to people residing near the mine and the water sources around the mine. High levels of arsenic in water have been found to cause several types of cancer (Meliker et al., 2007). Arsenic could also lead to cardiovascular diseases, neurological diseases, and skin hyperpigmentation and depigmentation (Vogel and Kaper, 2002).

Worldwide, mine tailings release vast amounts of heavy metal(loid)s into the surrounding areas through surface erosion and deposition of Particulate Air Matter (PAM) (Schipper et al., 2007). Studies conducted in mine tailings at the Selibe-Phikwe Cu-Ni Mine (BCL) indicated that PAM near the mine contained quartz, pyrrhotite, chalcopyrite, albite, as well as djurleite polymorphs, a secondary pollutant which is formed from the mineralization of chalcocite and the H_2S and SO_2 gases which are released through the concentration and smelting processes (Ekosse et al., 2005). These gases react with atmospheric moisture and form H_2SO_4 to create acid rain which damages plants and affects the ecosystem (Ultra and Manyiwa, 2020). Studies conducted by both Ekosse (2005) and Ultra and Manyiwa (2020); indicated that soil and plants in the vicinity of Cu-Ni Mine (BCL) in Selibe Phikwe had elevated levels of heavy metal(loid)s mainly due to erosion from mine tailings. Plants that accumulate these metals become a part of the food chain when they

are consumed by animals. Consumption of vegetation with elevated metal concentrations can be a major exposure pathway for the animals or humans who consume them. Although some of these metals are not phytotoxic, they may pose extreme risk to some humans who consume them (Meliker et al., 2007). With respect to selenium and molybdenum, their uptake into the edible portion of plants is generally not sufficient to cause plant toxicities, but it has created toxicities in the animals who consume them (Manyiwa et al., 2021). Thus, measuring plant uptake of metals from the soil is a means to evaluate metal exposure to higher organisms. Information regarding the degree of bioaccumulation in plants is essential for ecological risk assessment because of the compounding effect it has on wildlife.

To date, studies conducted on Monarch Gold mine tailings have focused on the presence of heavy metal(loid)s in mine tailings, but they have not analyzed the effects and accumulation of these heavy metal(loid)s on plants and their potential risk to grazing animals. As a result, more knowledge is needed about the availability of heavy metal(loid)s in soils, the uptake and accumulation of pollutants by plants, and the possible harm that heavy metal buildup in plants and soil poses to nearby grazing animals.

4.2.2 Materials and methods

4.2.2.1 Description of the study area

Francistown, the oldest city in Botswana, is the site of the Monarch Gold Mine, which is located at the confluence of the Tati and Ntshe rivers. It commenced gold mining in the 1890s and continued until mid-1998 when it was permanently closed (Vogel and Kasper, 2002). In Francistown, where the study was conducted, the abandoned Monarch mine with a latitude of 21°S and longitude of 27°E and an average elevation of 988 meters above sea level, is situated near the

Ntshe River which leads to Tati and Shashe rivers that supply the Shashe dam. Water from the Shashe dam is used for domestic consumption by residents in the local and surrounding areas. Therefore, if not properly monitored, heavy metal(loid)s from the abandoned mine could lead to contamination of water sources and the environment. Monarch Gold mine tailings pose a huge health hazard, primarily due the mine's proximity to built-up areas. For example, children use the site as a playground, despite the high levels of As and Sb that have been recorded there.

4.2.2.2 Sampling and Sampling Points Description

A total of twenty-two soil samples were collected from topsoil at a depth of up to 15 cm, in and around the abandoned mine tailings. These samples were stored in well-labeled zip-lock bags. A Garmin GPS was used to record the coordinates of each sample collection point. The samples were placed into three groups based on their location. The groups included (1) tailings dumps (TD) which comprised five samples; (2) vicinity of tailings dump (VTD) consisting of twelve samples; and (3) river sediment (RS) from the river with five samples collected at intervals of 500 meters along the Ntshe river. The shoots of representative plants growing in each sampling point were collected and the identification of the plants was conducted onsite. In each sampling area, the samples were replicated three times and duplicate samples were gathered for verification of the plant's identity at the Botany laboratory. Figure 4.2.1 shows a map of the study area with sampling points.

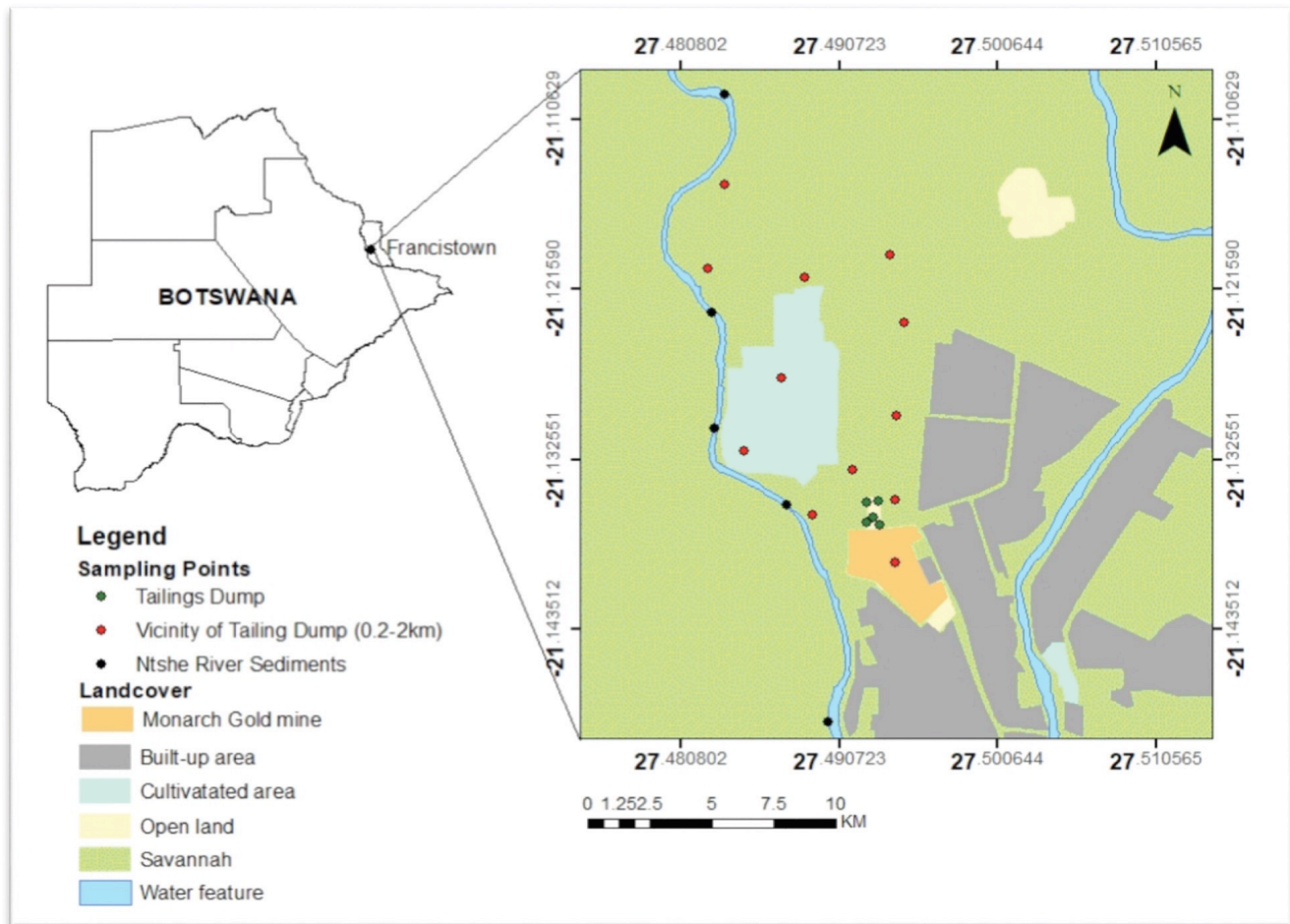


Figure 4.2. 1 Location of the study area and sampling sites

4.2.2.3 Soil sample processing and chemical characterization

Soil samples were air-dried for 3 days in the laboratory and then sieved through a 2 mm stainless steel mesh wire prior to chemical characterization (Dick et al., 1997). The chemical characterization of the soil followed the same procedures outlined by Ultra (2020). Briefly, the soil pH and electric conductivity (EC) were measured in water suspension (1:2, soil: water) after mechanically shaking for 2 hours using a portable Hanna pH/EC Multiparameter. 5 g of the plant sample was weighed in a 250 mL conical flask and 5 mL of nitric acid and 15 mL of hydrochloric acid (HNO_3 : HCl mixture (1:3)) were added to the sample and covered with a watch glass. The sample was placed on a hot hotplate at 120°C for 6 hours. and the total contents of As, Mn, Cu, Cr

and Zr were quantified using ICP-MS (Ultra, 2020). The available fractions of these heavy metal(loid)s in soil were assessed by determining the ammonium acetate exchangeable. 10g of the sample was weighed and 100 mL of ammonium acetate was added to the samples. Samples were mechanically shaken with 1N ammonium acetate (pH 7) at a 1:100 ratio for 2 hours at room temperature. After shaking the samples were filtered with Whatman filter paper No. 42 and 0.45 μm syringe filters. The Exchangeable fraction was analyzed for different heavy metal concentrations using ICP-MS (Thermo Scientific™ iCAP Q ICP-MS, Illinois 60060 USA).

4.2.2.3 Plant sample processing and chemical analysis

The air-dried shoots were oven-dried at 70 °C for 72 hours and ground into powder using a Philips home blender to homogenize samples prior to chemical analysis. To assess the heavy metal content, the powdered plant tissues were digested using a nitric acid and hydrogen peroxide mixture in an open vessel covered with a watch glass at 120 °C for a minimum of 4 hours and then analyzed for heavy metal(loid)s using ICP-MS (Manyiwa et al., 2021).

4.2.2.4 Validation of the analytical method (ICP-MS)

The method was validated for linearity, limits of detection (LODs), limits of quantification (LOQs), accuracy, and precision. For each analyte, a calibration curve was generated by plotting peak area versus concentration at eight different concentrations between 10 and 1000 pmol/mL under optimum extraction conditions. Table 4.2.1 indicates ICP-MS instrumental parameters employed for heavy metal determination. For all chemical analyses of soil and plant samples, precision and accuracy were assured through batch inclusion of internal reference samples (soil

and corn tissue) whose concentrations were cross verified by three laboratories in Botswana and by repeat analysis of samples when the coefficients of variation of replicate analysis (n=2) were more than 10%. The recovery of the internal standard sample was more than 95% for all elements measured (Table 4.2.1). All the chemical analyses were conducted at the Soil Science Laboratory of the Department of Earth and Environment Sciences, BIUST.

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

Table 4.2.1 ICP-MS instrumental parameters employed for heavy metal determination and % recovery of concentrations of internal reference sample

Element	ICP-MS instrumental parameters			Concentration of internal reference sample (mg kg ⁻¹)		Mean Recovery Concentrations (mg kg ⁻¹)		% Recovery	
	Wavelength (nm)	LOD (µg/L)	LOQ (µg/L)	Soil	Plant	Soil	Plant	Soil	Plant
As	189.042	0.074	0.28	62.25	10.15	60.03±1.32	9.7±0.39	96.43	95.57
Mn	257.610	0.033	0.11	720.50	42.05	715.10±5.50	40.0±1.90	99.25	95.12
Cu	224.700	0.12	0.39	260.11	35.50	254.32±3.25	33.9±1.68	97.77	95.49
Cr	283.563	0.19	0.65	80.30	10.22	77.65±2.81	9.95±0.28	96.70	97.34
Zr	339.198	0.21	0.57	95.70	5.06	93.40±6.88	4.89±0.22	97.60	96.64

4.2.2.5 Risk assessment of ruminants grazing in the area

The daily intake of metals from the different ruminants was estimated based on the formula by Manyiwa and Ultra (2021) and calculated using the formula below as:

$$DI_{animal} = [Metal]_{grass} \times I_{grass} + [Metal]_{shrubs} \times I_{shrubs} + [Metal]_{forbs} \times I_{forbs} + [Metal]_{soil} \times I_{soil}$$

[Eq. 1]

$[Metal]_{Grass}$, $[Metal]_{Shrubs}$, $[Metal]_{herbs}$ are the average concentrations of metals for grasses, shrubs, and herbs, respectively ($mg\ kg^{-1}$) presented in Table 4.2.8; and I_{grass} , I_{shrubs} , I_{herbs} are the equivalent intake of grasses, shrubs, and herbs, respectively, and daily intake of metals was calculated as the daily dietary intake multiplied by the percentage intake of grasses, shrubs, and herbs for each ruminant. Cattle diets consist of 75% grasses, 23% woody plants, and 2% herbs; goat diets consist of 20% grasses, 78% woody plants, and 2% herbs; while sheep diets consist of 74% grasses, 21% woody plants and 5% herbs. Incorporating the proportion of these diets and their equivalent average metal concentration in plants collected from the study site was used in the estimation of the daily intake.

4.2.2.6 Determination of contamination factors, pollution load index and bioaccumulation factor in soil

Using the total concentrations in soils and plants, contamination factors (CF), pollution load index (PLI) and bioaccumulation factor (BAF) of heavy metal(loid)s in the soil samples were determined as indicated in Equation 2, 3, 4 (Manyiwa et al., 2021; Aladesanmi et al., 2019).

$$CF = \frac{C_m \text{ Sample}}{C_m \text{ Background}} \quad [\text{Eq. 2}]$$

Where C_m is the measured concentration of heavy metal in the soil; and B_m is the local background concentration value of the heavy metal(loid)s (Table 4.2.2). The background data was obtained from soil samples collected at about 1.5 km along the leeward side of the Cu-Ni smelter area (therefore assumed to mimic pre-industrial data) which has similar texture and mineralogy to the study sites. Pollution Load Index (PLI), proposed by Tomlinson et al. (1980), was calculated using Eq 3.

$$PLI = (CF_1 \times CF_2 \times CF_3 \times \dots \times CF_N)^{\frac{1}{N}} \quad [\text{Eq. 3}]$$

where N is the number of metals studied and CF is the contamination factor calculated as described in (Eq 2).

The bioaccumulation factor is the ratio of the concentration of heavy metal(loid)s in plants and in soils. It is an indicator of a plant's capacity to accumulate heavy metal(loid)s. The BAF was calculated using Eq 4 proposed by (Aladesanmi et al., 2019).

$$BAF_i = \frac{P_i}{S_i} \quad [\text{Eq. 4}]$$

Where P_i is the concentration of a heavy metal in plants (mg kg^{-1}); and S_i is the concentration of the same heavy metal in the soil where the plant was collected (mg kg^{-1})

Table 4.2.2 The pH, EC and total As, Mn, Cu, Cr, Zr contents and PLI of soils from abandoned Monarch Gold Mine, Francistown, Botswana¹.

Sample Code		pH	EC (µs/cm)	As	Mn	Cu	Cr	Zr	PLI
				mg kg ⁻¹					
TD1	-21.136267, 27.492877	8.11±0.65	1849.50±164.61	1429.02±122.09	520.69±49.47	195.45±16.61	125.21±9.02	262.19±11.80	2.79±0.14
TD2	-21.136759, 27.493280	7.18±0.54	1236.75±111.31	3326.73±229.54	507.70±45.19	310.98±24.56	88.53±5.75	363.93±14.19	3.25±0.17
TD3	-21.136606, 27.492467	8.76±0.51	3540.00±300.90	1158.80±96.18	710.10±28.40	238.83±15.76	122.42±9.79	113.83±6.26	2.67±0.13
TD4	-21.135304, 27.492468	8.18±0.52	3312.00±261.65	1710.96±157.41	703.92±64.94	321.65±19.23	132.84±11.82	149.65±8.98	2.87±0.08
TD5	-21.135210, 27.493174	6.49±0.57	2600.00±223.60	1298.85±105.21	446.21±32.13	455.28±24.95	112.82±9.59	110.70±4.70	2.74±0.15
Mean of TD		7.74±0.90a	2507.65±970.77a	1784.87±885.66a	577.72±121.35b	304.44±99.09a	116.36±17.13a	200.06±110.31a	2.86±0.23a
VTD1	-21.139177, 27.494202	8.38±0.52	724.38±54.33	194.21±8.25	630.44±36.54	269.96±14.80	106.30±5.02	160.98±8.00	2.11±0.19
VTD2	-21.135145, 27.494260	8.34±0.62	680.80±38.12	193.72±8.02	692.46±38.33	258.74±15.11	192.40±6.78	153.07±7.08	2.15±0.16
VTD3	-21.133199, 27.491574	7.94±0.59	871.50±56.65	193.47±8.01	661.45±35.25	213.02±13.58	110.46±5.61	157.03±7.68	2.09±0.15
VTD4	-21.136152, 27.489049	8.26±0.66	694.68±31.26	193.78±8.11	756.72±41.51	179.23±12.02	196.31±7.89	164.97±8.27	2.14±0.17
VTD5	-21.129681, 27.494341	8.19±0.61	493.95±24.65	198.59±8.55	741.02±39.08	196.12±11.57	151.56±6.33	153.72±7.55	2.12±0.21
VTD6	-21.131959, 27.484725	8.72±0.58	496.65±29.64	193.66±8.03	748.87±43.55	194.57±13.22	177.24±6.02	161.64±7.95	2.13±0.22
VTD7	-21.123736, 27.494760	6.84±0.63	605.28±33.58	197.83±8.66	926.23±60.19	238.81±14.09	148.42±6.11	163.31±8.21	2.14±0.18
VTD8	-21.120834, 27.488545	7.38±0.55	258.80±12.90	181.84±7.98	886.99±55.45	216.69±14.23	162.83±6.77	139.29±7.06	2.11±0.17
VTD9	-21.120226, 27.482439	7.64±0.62	404.60±22.21	102.34±5.87	906.61±62.44	224.32±12.54	148.75±7.37	131.23±6.88	2.01±0.13
VTD10	-21.119366, 27.493880	8.30±0.67	388.30±20.02	168.27±7.08	958.93±68.54	311.85±16.33	175.63±6.52	135.26±7.02	2.13±0.14
VTD11	-21.114814, 27.483501	8.01±0.68	450.00±23.84	111.92±7.52	927.98±65.22	268.09±14.27	182.42±6.07	151.63±7.36	2.07±0.13
VTD12	-21.127273, 27.487099	8.25±0.63	606.20±33.42	173.78±7.88	923.78±64.08	284.88±15.45	157.46±6.74	142.71±7.22	2.12±0.15
Mean of VTD		8.02±0.51a	556.04±172.14b	175.28±33.29b	813.46±119.52a	238.02±40.86a	159.15±28.74a	151.24±11.49a	2.11±0.04b
RS1	-21.156367, 27.492226	8.08±0.53	55.5±2.28	21.28±1.09	756.24±41.53	135.36±7.01	138.42±7.12	128.47±6.95	1.84±0.11
RS2	-21.149502, 27.490040	8.11±0.58	39.0±2.15	31.37±1.15	687.38±39.55	132.20±6.95	163.41±9.08	137.26±7.28	1.88±0.13
RS3	-21.135484, 27.487433	8.38±0.61	26.4±1.56	21.45±1.10	852.14±53.21	240.21±14.67	124.18±6.88	168.43±9.03	1.91±0.15
RS4	-21.130512, 27.482886	8.25±0.60	33.7±2.10	19.61±1.06	763.85±40.18	198.20±13.08	137.48±7.02	142.78±7.39	1.88±0.13
RS5	-21.108979, 27.483533	8.24±0.57	28.9±1.63	26.71±1.13	681.80±38.13	168.21±11.24	162.42±8.91	169.44±9.11	1.91±0.15
Mean of RS		8.21±0.12a	36.70±11.56c	24.08±4.87c	748.28±69.32a	174.84±45.39a	145.18±17.14a	149.28±18.66a	1.89±0.03c
Background Data				10.0	300.0	40.0	20.0	20.0	nd
P-Value		0.062 ns	0.038*	0.000***	0.008**	0.085 ns	0.072 ns	0.078 ns	0.045*

¹The mean concentration per site followed by the same letter in each column are not significantly different from each other based on 5% level of significance.

ns: not significant; *significant; ** highly significant; *** very highly significant.

TD: Tailings Dump VTD: Vicinity of Tailing Dump (0.2-2km), RS: Ntshe River Sediments nd: no data

4.2.2.7 Statistical analysis

The data was analyzed using a single-factor ANOVA in CRD to determine the differences of heavy metal concentration between sampling sites (TD, VTD and RS). When significant differences were observed, Tukey's test was performed for treatment mean (sampling site) comparison at 5% level of significance. Each parameter was analyzed separately per plant and soil samples.

4.2.3 Results

4.2.3.1 Soil chemical properties and heavy metal concentration and availability

The pH, electrical conductivity, and total concentration of As, Mn, Cu, Cr, and Zr in soils from the three different sampling sites (TD, VTD (0.2-2km) and RS) are shown in Table 4.2.2. The pH of soil was alkaline, ranging from 7.18 to 8.76 except for one sample in the TD and one sample in the VTD which had a slightly acidic pH of 6.49 and 6.84 respectively. The average electrical conductivity (EC) for TD was $2507.65 \mu\text{S m}^{-1}$ with a range of 1236.75 to $3540 \mu\text{S m}^{-1}$. The average EC for soils in VTD was $556.04 \mu\text{S m}^{-1}$ with a range of 258.80 to $871.50 \mu\text{S m}^{-1}$; whereas the average EC for RS was $36.70 \mu\text{S m}^{-1}$, with a range of 26.4 to $55.5 \mu\text{S m}^{-1}$.

The average concentration of the total As in TD was $1784.87 \text{ mg kg}^{-1}$ which is more than 10 times the average of the total As in soils in VTD ($175.28 \text{ mg kg}^{-1}$), while the average of the total As in RS was 24.08 mg kg^{-1} . The average concentration of Mn in the TD was $577.72 \text{ mg kg}^{-1}$, whereas in VTD and RS they were 813.46 and $748.28 \text{ mg kg}^{-1}$, respectively. The Cu concentration in TD was $304.44 \text{ mg kg}^{-1}$, $238.02 \text{ mg kg}^{-1}$ in VTD and $174.84 \text{ mg kg}^{-1}$ for RS. Total Cr in TD was $116.36 \text{ mg kg}^{-1}$, while in VTD it was $159.15 \text{ mg kg}^{-1}$, and 145.18

mg kg⁻¹ in RS. The Zr in TD was 200.06 mg kg⁻¹ whereas in VTD and in RS they were 151.24 and 149.28 mg kg⁻¹, respectively.

The available fraction of heavy metal in soils is shown in Table 4.2.3. The average concentration of exchangeable As (exch-As) in TD was 2.44 mg kg⁻¹ with a range of 1.75 to 3.77 mg kg⁻¹. This was about 0.137% of the total As in TD. The average As concentration for VTD was 0.72 mg kg⁻¹ (0.41%) and it was 1.21 mg kg⁻¹ (5%) for RS. The Exch-Mn was about 3.70% of the total concentration ranging from 13.98 to 25.47 mg kg⁻¹ TD, whereas for VTD the exch-Mn ranged from 4.59 to 16.17 mg kg⁻¹ making up 1% of the total Mn, and for RS the exch-Mn ranged from 9.60 to 14.07 mg kg⁻¹ making up 1.6% of the total Mn. The Exch-Cu in TD was about 0.9% of the total Cu concentration with a range of 2.12-3.46 mg kg⁻¹. In VTD the exch-Cu was about 1% of total Cu concentration with a range of 1.19 to 4.26 mg kg⁻¹ and in RS, the exch-Cu was 0.67% of total Cu, with a range concentration of 0.89 to 1.61 mg kg⁻¹. In TD, the exch-Cr was about 1.9% of the total Cr, with a range of 1.66 to 2.97 mg kg⁻¹. In VTD the exch-Cr was 1.1% of the total Cr concentration with a range of 0.62 to 2.03 mg kg⁻¹ and in RS, the exch-Cr was 0.9% of the total Cr, with a range concentration of 1.07 to 1.61 mg kg⁻¹. The exch-Zr was 0.43% of the total concentration ranging from 0.63 to 1.35 mg kg⁻¹ for TD, whereas for VTD, the exch-Zr ranged from 0.49 to 0.82 mg kg⁻¹ making up 0.42% of the total Zr, and for RS the exch-Zr ranged from 0.59 to 0.88 mg kg⁻¹ making up 0.5% of the total Zr.

Table 4.2.3 Available As, Mn, Cu, Cr, and Zr contents of soils from abandoned Monarch Gold Mine, Francistown, Botswana¹.

Sample Code	As	Mn	Cu	Cr	Zr
Concentration (mg kg ⁻¹)					
Tailings Dump					
TD1	1.75±0.10	24.38±1.66	2.12±0.13	1.72±0.09	1.15±0.07
TD2	1.88±0.11	13.98±0.98	3.28±0.19	1.66±0.08	1.35±0.08
TD3	3.77±0.21	25.47±1.73	2.39±0.11	1.83±0.12	0.46±0.03
TD4	2.99±0.18	19.24±1.08	2.87±0.15	2.69±0.13	0.72±0.05
TD5	1.81±0.14	23.79±1.42	3.46±0.16	2.97±0.16	0.63±0.04
Mean of TD (% of the total concentration)	2.44±0.90a (5.3%)	21.37±4.77a (10.4%)	2.82±0.57a (8.9%)	2.17±0.61a (4.6%)	0.86±0.37a (1.2%)
Vicinity of Tailings Dump					
VTD1	0.50±0.03	7.48±0.34	2.07±0.11	1.02±0.05	0.70±0.05
VTD2	0.54±0.04	6.65±0.29	1.47±0.08	1.66±0.08	0.51±0.03
VTD3	0.53±0.03	5.82±0.23	1.19±0.07	0.62±0.04	0.62±0.04
VTD4	0.68±0.04	12.56±0.68	2.99±0.17	1.30±0.07	0.68±0.05
VTD5	0.67±0.04	5.82±0.23	2.48±0.14	1.16±0.06	0.82±0.06
VTD6	0.74±0.05	5.81±0.28	1.84±0.09	1.53±0.08	0.75±0.06
VTD7	0.79±0.05	4.59±0.23	2.08±0.11	1.24±0.07	0.71±0.05
VTD8	0.77±0.05	8.30±0.50	1.88±0.12	1.89±0.09	0.55±0.03
VTD9	0.78±0.05	10.75±0.63	2.10±0.11	2.03±0.10	0.49±0.02
VTD10	0.95±0.07	16.17±0.72	4.26±0.23	1.69±0.08	0.63±0.04
VTD11	0.85±0.07	8.91±0.45	1.80±0.08	1.27±0.07	0.73±0.05
VTD12	0.86±0.06	7.26±0.30	1.70±0.11	1.87±0.09	0.76±0.05
Mean of VTD (% of the total concentration)	0.72±0.14c (1.4%)	8.34±3.35b (14.6%)	2.35±0.81a (0.4%)	1.75±0.41a (1.1%)	0.63±0.10a (3.2%)
River Sediments					
RS1	1.40±0.07	14.07±0.84	0.91±0.04	1.61±0.11	0.68±0.04
RS2	1.15±0.04	9.60±0.61	0.89±0.04	1.57±0.10	0.59±0.04
RS3	1.15±0.04	10.96±0.68	1.61±0.10	1.07±0.06	0.88±0.06
RS4	1.15±0.04	12.88±0.76	1.33±0.07	1.27±0.07	0.80±0.05
RS5	1.20±0.05	12.68±0.73	1.13±0.04	1.29±0.07	0.78±0.05
Mean of RS (% of the total concentration)	1.21±0.11b (0.8%)	12.04±1.76b (13.5%)	1.17±0.30b (0.26%)	1.36±0.23a (0.15%)	0.75±0.11a (0.03%)
<i>P-Value</i>	0.036*	0.009**	0.040*	0.080 ns	0.079 ns

¹The mean concentration per site followed by the same letter in each column is not significantly different from each other based on 5% level of significance

ns: not significant: *Significant: ** highly significant

4.2.3.2 Pollution Load Index

The contamination factor was used to determine the pollution load index (PLI) of the three different sites (Table 4.2.2). In all the sites, the pollution index was greater than 1 based on the combined CF of the metals under consideration. TD had the highest pollution

index ranging from 2.67 to 3.25, followed by the VTD dump with a PLI ranging from 2.01 to 2.15, and then the RS with 1.84 to 1.91 PLI.

4.2.3.3 Composition, classification, heavy metal concentrations and bioaccumulation factor of plants in the tailings dump of Monarch gold mine.

A total of 94 plant samples which were collected from Monarch tailings dump belonged to grass, herbs, trees, and shrubs. A total of 24 samples of *Cenchrus biflorus* (Roxb.) were collected from the TD indicating a dominance of this grass in the area. Other plant species growing in the TD included three herb species: *Portulaca oleracea* (L. Portulacaceae) (13 samples) *Gossypium herbaceum* (Linn) (6 samples), and *Withania somnifera* (L.) Dunal (4 samples); three species of shrubs: *Lantana dinteri* (Moldenke), *Maytenus senegalensis* (Lam.) Exell., and *Monechma leucoderme* (Schinz) C.B.Clark; eight tree species: *Dichrostacyx cineria* L. Wight & Arn, *Schinus molle* L. var. *areira* DC, *Colospospermum mopane* (Kirk Ex Benth.), *Melia azedarach* L., *Boscia albitrunca* (Burch.) Gilg & Gilg-Ben., *Acacia mellifera* (M.Vahl) Benth, *Acacia dudgeonii* (Craib ex Holl), and *Acacia schweinfurthii* (Brenan & Exell) were identified with the most dominant species being *D. cineria*, *C. mopane* and *B. albitrunca*. All these species are commonly grazed and browsed by livestock and wild animals.

Heavy metal content in the shoots of these different plants growing in the tailing's dumpsite of the abandoned Monarch Gold Mine is presented in Table 4.2.4. The only grass growing in the TD is *C. biflorus* with an As concentration of 47.14 mg kg⁻¹ and a range of 15.11 -135.03 mg kg⁻¹, Mn at 56.68 mg kg⁻¹, Cu at 115.94 mg kg⁻¹, Cr at 77.38 mg kg⁻¹ and Zr at 37.39 mg kg⁻¹. Among the different plant groups, herbs had the highest average Mn concentration at 108.48 mg kg⁻¹, while the grass had the highest average Cu concentration.

Grasses and herbs have higher average Cr concentrations than trees and shrubs while for Zn, there were no significant differences between plant groups. Among the different species, the As concentration was highest in one of the *C. bicolor* samples with an amount of 135.03 mg kg⁻¹, while for Mn, the highest value was recorded from *P. oleracea* at 240.14 mg kg⁻¹. The highest concentrations of Cu and Zr were recorded in *C. biflorus* at 300.25 and 112.90 mg kg⁻¹, respectively, while for Cr, the highest concentration was recorded in *Gossypium herbaceum*.

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

Table 4.2.4 Heavy metal contents in shoots of different plants growing in the tailings dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana¹.

Plant Species	Classification	As	Mn	Cu	Cr	Zr
(mg kg ⁻¹)						
<i>Cenchrus biflorus</i>	Grass (n=24)	47.14 (15.11 -135.03)	56.68 (198.06- 7.90)	115.94 (300.25-36.94)	77.38 (109.08-26.97)	37.39 (112.90-8.23)
<i>Portulaca oleracea</i>	Herb (n=13)	45.06 (135.03-10.61)	138.56 (240.14-47.28)	45.9 (143.23-14.14)	69.15 (113.71-18.40)	34.08 (111.30-11.93)
<i>Gossypium herbaceum</i>	Herb (n=6)	5.97 (7.95-3.80)	50.7 (100.41-18.93)	65.59 (172.38-16.10)	77.13 (190.06-23.92)	36.62 (51.51-32.11)
<i>Withania somnifera</i>	Herb (n=4)	5.76 (9.55-1.98)	97.41 (100.79-94.04)	45.4 (46.36-42.35)	63.7 (69.17-57.26)	32.43 (40.52-24.34)
Mean for Herbs		25.98±23.25b	85.84±40.82a	68.21±33.18b	71.84±6.64a	35.13±2.29a
<i>Lantana dinteri</i>	Shrubs (n=3)	4.17 (4.89-3.44)	40.4 (46.85 – 36.91)	21.3 (24.83-17.78)	53.05 (55.99-46.45)	27.08 (30.60-21.47)
<i>Maytenus senegalensis</i>	Shrubs (n=4)	8.62 (11.38-5.86)	33.9 (53.38-14.42)	31.79 (49.57-14.65)	44.19 (46.85-42.38)	61.29 (64.25-58.36)
<i>Monechma leucoderme</i>	Shrubs (n=3)	6.87 (8.45-4.39)	33.9 (53.38-24.67)	48.27 (76.65-29.88)	34.3 (43.61-20.08)	53.56 (69.16-41.09)
<i>Dichrostacyx cineria</i>	tree (n=6)	26.37 (32.42-20.32)	33.15 (38.12-28.17)	59.08 (62.81-55.36)	25.63 (33.13-18.14)	10.02 (11.55-8.49)
<i>Schinus molle</i>	Tree (n=3)	0.92 (1.41-0.44)	50.02 (70.03-30.00)	55.19 (91.88-36.47)	91.14 (112.82-59.35)	27.21 (36.42-22.57)
<i>Colospospermum mopane</i>	Tree (n=6)	0.65 (1.26-4.35)	30.6 (33.45-28.74)	75.85 (142.58-64.23)	55.01 (94.07-43.28)	41.93 (46.58-38.47)
<i>Melia azedarach</i>	Tree (n=5)	3.4 (6.48-2.84)	30.61 (37.98-26.44)	31.98 (36.66-26.49)	32.21 (46.25-24.58)	53.42 (67.25-42.12)
<i>Boscia albitrunca</i>	Tree (n=6)	1.68 (2.14-1.05)	48.19 (88.67-38.65)	43.17 (65.02-36.42)	36.88 (61.29-21.85)	32.28 (41.38-29.48)
<i>Acacia mellifera</i>	Tree (n=4)	1.87 (2.49-1.32)	29.7 (40.72-26.24)	69.44 (72.54-65.32)	52.46 (58.34-36.41)	37.41 (39.42-35.41)
<i>Acacia dudgeonii</i>	Tree (n=3)	2.54 (3.16-2.13)	33.33 (47.42-21.46)	34.75 (44.25-22.34)	44.57 (55.43-32.46)	27.48 (34.85-22.34)
<i>Acacia schweinfurthii</i>	Tree (n=4)	3.19 (5.88-2.68)	27.44 (39.20-18.47)	44.31 (54.88-32.47)	38.42 (41.68-32.19)	29.86 (34.16-23.41)
Mean for Trees & Shrubs		5.48±7.35c	35.57±7.47c	46.83±16.85b	46.17±17.60b	34.71±14.97a
<i>P-Value</i>		0.000***	0.000***	0.043*	0.004**	0.165ns
Critical Concentration in Plants ¹		5.00a	200.00b	10.00a	1.30a	No data

¹Source: Ogundele et al. (2015), Shah et al. (2013), Otte et al. (1990). The mean concentration per plant group followed by the same letter in each column are not significantly different from each other based on 5% level of significance. ns: not significant; *significant; ** highly significant; *** very highly significant

The bioaccumulation factor (BAF) for heavy metal(loid)s in plants growing in the TD of the Monarch Gold mine is presented in Table 4.2.5. Among all the plant species, the BAF for As was in the range of 0.001 to 0.076 wherein a higher BAF value was recorded in *Cenchrus bioflorus*. For Mn the BAF ranged from 0.047 to 0.24 with the highest BAF recorded for *Portulaca oleracea*. For Cu, the BAF ranged from 0.07 to 0.381 while for Cr, the range was 0.28 to 0.783 with the highest BAF in *Schinus mole*. For Zr, the BAF ranged from 0.05 to 0.27.

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

Table 4.2.5 Bioaccumulation factor of heavy metal(loid)s contents in shoots of different plants growing from the tailings dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana¹.

Plant Species	Classification	As	Mn	Cu	Cr	Zr
		Concentration in shoots/Concentration in soil (mg kg ⁻¹)				
<i>C. biflorus</i>	Grass (n=24)	0.026a (0.076-0.008)	0.098a (0.343-0.014)	0.381a (0.986-0.121)	0.665a (0.937-0.232)	0.187a (0.546-0.041)
<i>P. oleracea</i>	Herb/Succulent (n=13)	0.025 (0.076-0.006)	0.240 (0.416-0.082)	0.151 (0.470-0.046)	0.594 (0.977-0.158)	0.170 (0.556-0.060)
<i>G. herbaceum</i>	Herb (n=6)	0.003 (0.004-0.011)	0.088 (0.174-0.033)	0.215 (0.566-0.053)	0.663 (1.633-0.206)	0.183 (0.257-0.161)
<i>W. somnifera</i>	Herb (n=4)	0.003 (0.005-0.001)	0.169 (0.174-0.163)	0.149 (0.152-0.139)	0.547 (0.594-0.492)	0.162 (0.203-0.102)
Mean for herbs		0.01±0.01a	0.15±0.07a	0.22±0.11a	0.62±0.06a	0.18±0.01a
<i>L. dinteri</i>	Shrubs (n=3)	0.002 (0.003-0.002)	0.070 (0.081-0.064)	0.07 (0.082-0.058)	0.46 (0.481-0.399)	0.14 (0.153-0.107)
<i>M. senegalensis</i>	Shrubs (n=4)	0.005 (0.006-0.003)	0.059 (0.092-0.025)	0.104 (0.163-0.048)	0.380 (0.403-0.364)	0.306 (0.321-0.292)
<i>M. leucoderme</i>	Shrubs (n=3)	0.004 (0.005-0.002)	0.059 (0.092-0.043)	0.159 (0.252-0.098)	0.295 (0.375-0.173)	0.268 (0.346-0.205)
<i>D. cineria</i>	tree (n=6)	0.015 (0.018-0.011)	0.057 (0.066-0.049)	0.194 (0.206-0.182)	0.220 (0.285-0.156)	0.050 (0.058-0.042)
<i>S. mole</i>	Tree (n=3)	0.001 (0.001-0.0002)	0.087 (0.121-0.052)	0.181 (0.302-0.120)	0.783 (0.970-0.510)	0.136 (0.182-0.113)
<i>C. mopane</i>	Tree (n=6)	0.000 (0.007-0.001)	0.053 (0.058-0.050)	0.249 (0.468-0.211)	0.473 (0.808-0.372)	0.210 (0.233-0.192)
<i>M. azedarach</i>	Tree (n=5)	0.002 (0.004-0.002)	0.053 (0.066-0.046)	0.105 (0.120-0.087)	0.28 (0.397-0.211)	0.270 (0.336-0.211)
<i>B. albitrunca</i>	Tree (n=6)	0.001 (0.001-0.0006)	0.083 (0.153-0.067)	0.142 (0.214-0.120)	0.317 (0.527-0.188)	0.161 (0.207-0.147)
<i>A. mellifera</i>	Tree (n=4)	0.001 (0.001-0.0007)	0.051 (0.070-0.045)	0.228 (0.238-0.215)	0.451 (0.501-0.313)	0.187 (0.197-0.177)
<i>A. dudgeonii</i>	Tree (n=3)	0.001 (0.002-0.001)	0.058 (0.082-0.037)	0.114 (0.145-0.073)	0.383 (0.476-0.279)	0.137 (0.174-0.112)
<i>A. schweinfurthii</i>	Tree (n=4)	0.002 (0.003-0.002)	0.047 (0.068-0.032)	0.146 (0.180-0.107)	0.330 (0.358-0.277)	0.149 (0.171-0.117)
Mean for shrubs and tress		0.00±0.00a	0.06±0.01a	0.15±0.06a	0.40±0.15a	0.18±0.07a
<i>P-Value</i>		0.072ns	0.153ns	0.263ns	0.180ns	0.372ns

¹The Mean concentration per plant group followed by the same letter in each column are not significantly different from each other based on 5% level of significance. ns: not significant

4.2.3.4 Composition, classification and heavy metal(loid)s concentration of plants in the vicinity (0.5 to 2km) of tailings dumpsite of the abandoned Monarch Gold Mine

There were 166 plant samples collected in the VTD (0.5 to 2km) of the abandoned Monarch Gold Mine (Table 4.2.6). A total of seven grass species were identified: *Cenchrus biflorus*, *Bolboschoenus nobilis* (Ridl.) Goetgh. & Simpson. *Oxycaryum cubense* (Poepp. & Kunth), *Dactyloctenium aegyptium* (L.) Willd., *Stipagrostis uniplumis* (Licht.), *Bothriochloa insculpta* (Hochst.), and *Panicum congoense* Franch. *Cenchrus biflorus* was the only grass that grew in both the TD and in the VTD (0.5-2km). Three different types of herbs species were found near the TD: *Portulaca oleracea*, *Gossypium herbaceum*, *Withania somnifera*. Four different shrubs (*Fadogia homblei* De Wild, *Lantana dinteri* Moldenke, *Maytenus senegalensis*, *Monechma leucoderme*) were also identified and a total of ten different trees (*Dichrostacys cineria*, *Schinus mole*, *Colospospermum mopane* (Kirk Ex Benth.), *Melia azedarach*, *Boscia albitrunca* (Burch.) Gilg & Gilg-Ben.), *Acacia mellifera* (M.Vahl) Benth.), *Acacia dudgeonii* (Craib ex Holl), *Acacia schweinfurthii* (Brenan & Exell), *Acacia leuderitzii* (Engl.var.) and *Grewia bicolor* A. Juss.) were found in the VTD. The most dominant shrub was *Lantana dinteri* with seven samples whereas the most dominant tree species was *Melia azedarach* with twelve samples.

Heavy metal contents in shoots of different plants growing in the VTD of the abandoned Monarch Gold Mine are also presented in Table 4.2.6. In general, grasses had a higher As content compared to herbs and trees. The highest concentration of As for the grasses was recorded in *Panicum congoense* Franch. (56.57 mg kg⁻¹) and for the trees it was *Fadogia homblei* De Wild. With respect to Mn, trees have a higher average concentration than

herbs and grasses. Among the shrubs and tree species, *Grewia bicolor* Juss. had the highest Mn concentration at 393.74 mg kg⁻¹; *Dactyloctenium aegyptium* (L.) Willd. at 190.37 mg kg⁻¹ for grasses, the *Gossypium herbaceum* (165.7 mg kg⁻¹). The shrubs and trees had higher average concentrations of Cu than herbs and grasses.

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

Table 4.2.6 Heavy metal(loid)s contents in shoots of different plants growing within 0.5 to 2km away from the tailings dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana¹

Plant Species	Classification	As	Mn	Cu	Cr	Zr
		(mg kg ⁻¹)				
<i>P. oleracea</i>	Herb/Succ (n=10)	22.49 (35.17-16.43)	136.5 (240.14-47.28)	20.11 (33.12-16.10)	47.73 (61.75-33.14)	16.2 (19.31-11.93)
<i>W. somnifera</i>	Herb (n=5)	4.16 (6.48-3.45)	122.4 (148.23-88.04)	105.4 (138.24-87.46)	53.31 (58.47-43.89)	42.13 (48.77-39.61)
<i>G. herbaceum</i>	Herb (n=8)	4.37 (5.23-2.47)	165.7 (187.23-68.94)	52.47 (87.46-38.46)	46.21 (60.12-33.49)	38.62 (46.78-24.36)
Mean for Herbs		10.34±10.52b	141.53±22.08a	59.33±43.06a	49.08±3.74a	32.32±14.07a
<i>C. biflorus</i>	Grass (n=12)	51.07 (112.03-28.47)	89.89 (168.25-64.14)	38.98 (142.06- 16.40)	87.38 (122.08-59.42)	55.91 (73.90-38.03)
<i>Bolboschoenus nobilis</i>	Grass (n=6)	35.37 (41.40-25.36)	71.85 (95.86-57.86)	27.14 (36.42-25.47)	98.47 (104.74-89.47)	3.91 (6.47-3.47)
<i>Oxycaryum cubense</i>	Grass (n=5)	43.68 (65.47-40.69)	155.22 (179.68-143.75)	117.03 (120.45-96.75)	130.1 (155.46-110.42)	5.58 (6.45-4.35)
<i>Dactyloctenium aegyptium</i>	Grass (n=6)	38.67 (42.25-37.52)	190.37 (228.42-162.45)	66.63 (83.12-55.24)	783.92 (105.24-82.47)	8.63 (13.22-6.42)
<i>Stipagrostis uniplumis</i>	Grass (n=9)	40.85 (46.13-32.18)	75.29 (87.24-68.47)	83.92 (135.42-41.43)	6.14 (10.33-3.47)	8.63 (13.65-6.42)
<i>Bothriochloa insculpta</i>	Grass (n=6)	44.99 (48.76-43.46)	114.05 (142.80-96.30)	112.47 (116.47-88.10)	3.13 (4.58-2.87)	16.48 (19.14-10.46)
<i>Panicum congoense</i>	Grass (n=6)	56.57 (62.47-55.24)	73.18 (90.14-55.46)	133.25 (163.47-101.38)	12.12 (22.19-11.42)	21.84 (31.49-18.75)
Mean for Grasses		44.46±7.31a	109.98±46.44a	82.77±40.58a	160.18±279.70a	17.28±18.16a
<i>Fadogia homblei</i>	Shrubs (n=6)	73.24 (103.68-58.42)	173.34 (185.42-158.71)	550.35 (768.45-486.72)	975.77 (1832.35-687.46)	4.46 (40.25-3.49)
<i>L. dinteri</i>	Shrubs (n=7)	6.47 (10.17-4.68)	113.87 (124.86-101.46)	121.3 (135.33-106.41)	33.46 (42.87-28.65)	33.16 (35.46-28.97)
<i>M. senegalensis</i>	Shrubs (n=3)	16.45 (22.37-14.75)	133.9 (146.89-127.66)	113.54 (133.29-98.71)	39.48 (44.23-31.86)	32.49 (43.86-28.77)
<i>M. leucoderme</i>	Shrubs (n=3)	8.46 (9.18-6.33)	167.33 (175.28-138.92)	158.66 (173.46-135.89)	33.48 (39.77-29.33)	27.46 (34.36-22.87)
<i>Grewia bicolor</i>	shrub (n=6)	29.36 (39.47-25.48)	393.74 (432.18-348.46)	195.97 (216-39-185.46)	76.42 (94.13-64.78)	13.45 (18.43-12.98)
<i>D. cineria</i>	Tree (n=8)	51.47	60.39	28.34	115.46	16.34

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

<i>S. mole</i>	Tree (n=5)	(59.14-39.48) 39.89	(88.68-38.42) 60.48	(39.88-17.18) 112.75	(145.61-85.30) 68.72	(34.16-12.47) 32.48
<i>C. mopane</i>	Tree (n=6)	(44.86-32.99) 43.26	(69.22-54.11) 144.95	(158.22-98.72) 134.72	(88.49-54.33) 96.78	(36.69-24.83) 13.38
<i>M. azedarach</i>	Tree (n=12)	(65.12-38.45) 27.15	(198.67-134.82) 114.41	(168.39-102.42) 21.27	(131.39-86.42) 102.25	(23.19-10.42) 53.42
<i>Boscia albitrunca</i>	Tree (n=6)	(36.28-22.43) 40.74	(297.11-48.76) 138.64	(36.46-14.33) 243.31	(46.25-24.58) 158.77	(67.25-42.12) 23.78
<i>Acacia mellifera</i>	Tree (n=6)	(65.42-34.78) 38.41	(165.74-112.41) 186.42	(265.44-222.13) 197.46	(168.47-144.73) 68.79	(35.44-21.17) 27.13
<i>Acacia dudgeonii</i>	Tree (n=5)	(46.23-32.71) 38.33	(211.37-176.48) 189.46	(238.16-185.47) 98.14	(84.93-52.13) 32.34	(33.42-22.49) 22.32
<i>Acacia schweinfurthii</i>	Tree (n=7)	(46.57-28.98) 18.48	(212.68-75.48) 213.82	(112.49-73.46) 178.47	(45.27-28.47) 34.98	(24.85-18.42) 18.79
<i>Acacia leuderitzii</i>	Tree (n=7)	(24.64-15.48) 9.79	(245.78-188.49) 47.01	(211.39-156.47) 57.35	(43.26-28.76) 35.9	(22.88-16.79) 31.26
Mean for trees and shrubs		(15.24-6.48) 31.54±18.77ab	(68.47-38.49) 152.70±86.27a	(69.11-42.75) 157.97±130.02a	(47.12-28.45) 133.76±245.37a	(38.14-28.13) 24.99±11.92a
<i>P-Value</i>		0.382ns	0.652ns	0.812ns	0.528ns	0.428ns

¹The Mean concentration per plant group followed by the same letter in each column are not significantly different from each other based on 5% level of significance.

ns: not significant

4.2.3.5 Heavy metal accumulation and the bioaccumulation factor in plants found in the vicinity (0.5 to 2km) of tailings dumpsite of the abandoned Monarch Gold Mine

The heavy metal(loid)s concentration in the shoots of plants growing in the VTD of Monarch Gold mine is presented in Table 4.2.7. Based on the plant type, the total As concentration in the *C. biflorus* grass species ranged from 0.008 to 0.076 mg kg⁻¹; Mn concentration ranged from 0.014 to 0.343 mg kg⁻¹; Cu ranged from 0.121 to 0.986 mg kg⁻¹; the Cr range was 0.232 to 0.937 and the Zr concentration ranged from 0.041 to 0.546 mg kg⁻¹. There were three different types of herbs found in the TD of the mine. Of the three different species of herbs, Cr had the highest concentration of heavy metal accumulation. Trees and shrubs were the most dominant species in the TD. *S. mole* and *C. mopane* (Kirk Ex Benth.) had high concentrations of Cr. In *S. mole*, Cr ranged from 0.970-0.510 mg kg⁻¹ whereas for *C. mopane* (Kirk Ex Benth.) the Cr concentration ranged from 0.808-0.608 mg kg⁻¹. For trees and shrubs, Cu had the second-highest heavy metal concentration.

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

Table 4.2.7 Bioaccumulation factor of metal(loid)s contents in shoots of different plants growing within 0.5 to 2km away from the tailing's dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana¹

Plant Species	Classification	As	Mn	Cu	Cr	Zr
Concentration in shoots/Concentration in soil						
<i>P. oleracea</i>	Herb/Succ (n=10)	0.128	0.168	0.084	0.300	0.107
		0.201-0.094	0.295-0.058	0.139-0.068	0.388-0.208	0.128-0.079
<i>W. somnifera</i>	Herb (n=5)	0.024	0.150	0.443	0.335	0.279
		0.037-0.020	0.182-0.108	0.0581-0.367	0.367-0.276	0.322-0.262
<i>G. herbaceum</i>	Herb (n=8)	0.025	0.204	0.220	0.290	0.255
		0.030-0.014	0.230-0.085	0.367-0.162	0.378-0.210	0.309-0.161
Mean for Herbs		0.06±0.06a	0.17±0.03a	0.25±0.18a	0.31±0.02a	0.21±0.09a
<i>C. biflorus</i>	Grass (n=12)	0.291	0.111	0.164	0.549	0.370
		0.639-0.162	0.207-0.079	0.597-0.069	0.767-0.373	0.489-0.251
<i>B. nobilis</i>	Grass (n=6)	0.202	0.088	0.114	0.619	0.026
		0.236-0.145	0.118-0.071	0.153-0.107	0.658-0.562	0.043-0.023
<i>O. cubense</i>	Grass (n=5)	0.249	0.191	0.492	0.817	0.037
		0.374-0.232	0.220-0.177	0.506-0.406	0.977-0.694	0.043-0.029
<i>D. aegyptium</i>	Grass (n=6)	0.221	0.234	0.280	4.926	0.057
		0.241-0.214	0.281-0.200	0.349-0.232	0.661-0.518	0.087-0.042
<i>S. uniplumis</i>	Grass (n=9)	0.233	0.093	0.353	0.039	0.057
		0.263-0.184	0.107-0.084	0.569-0.174	0.065-0.022	0.090-0.042
<i>B. insculpta</i>	Grass (n=6)	0.257	0.140	0.473	0.020	0.109
		0.278-0.248	0.176-0.118	0.489-0.370	0.029-0.018	0.127-0.069
<i>P. franch</i>	Grass (n=6)	0.323	0.090	0.560	0.076	0.144
		0.356-0.315	0.111-0.068	0.687-0.426	0.139-0.072	0.208-0.124
Mean for Grasses		0.25±0.04a	0.14±0.06a	0.35±0.17a	1.01±1.76a	0.11±0.12a
<i>F. de wild</i>	Shrubs (n=6)	0.418	0.213	2.312	6.131	0.029
		0.592-0.333	0.228-0.195	3.23-1.97	11.51-4.32	0.266-0.023
<i>L. dinteri</i>	Shrubs (n=7)	0.037	0.140	0.510	0.210	0.219
		0.058-0.027	0.153-0.125	0.569-0.447	0.269-0.180	0.234-0.192
<i>M. senegalensis</i>	Shrubs (n=3)	0.094	0.165	0.477	0.248	0.215
		0.128-0.054	0.181-0.157	0.560-0.4415	0.278-0.199	0.290-0.190
<i>M. leucoderme</i>	Shrubs (n=3)	0.048	0.206	0.667	0.210	0.182
		0.052-0.036	0.215-0.171	0.729-0.571	0.250-0.184	0.227-0.151
<i>D. cineria</i>	Tree (n=8)	0.294	0.074	0.119	0.725	0.108
		0.337-0.225	0.109-0.047	0.168-0.075	0.915-0.536	0.226-0.082
<i>S. mole</i>	Tree (n=5)	0.228	0.074	0.474	0.432	0.215
		0.256-0.188	0.085-0.067	0.665-0.415	0.556-0.341	0.243-0.164

Impact of Heavy Metal(loid)s Contamination on Soil and Plant Communities in Mining Areas

<i>C. mopane</i>	Tree (n=6)	0.247 0.372-0.219	0.178 0.244-0.166	0.566 0.707-0.430	0.608 0.826-0.543	0.088 0.153-0.069
<i>M. azedarach</i>	Tree (n=12)	0.155 0.207-0.128	0.141 0.365-0.060	0.089 0.153-0.060	0.642 0.291-0.154	0.353 0.445-0.278
<i>B. albitrunca</i>	Tree (n=6)	0.232 0.373-0.198	0.170 0.204-0.138	1.022 1.12-0.933	0.998 1.06-0.909	0.157 0.234-0.140
<i>A. mellifera</i>	Tree (n=6)	0.22 0.264-0.187	0.23 0.259-0.217	0.83 1.00-0.779	0.43 0.534-0.328	0.18 0.221-0.149
<i>A. dudgeonii</i>	Tree (n=5)	0.22 0.266-0.165	0.23 0.261-0.093	0.41 0.473-0.309	0.20 0.284-0.179	0.15 0.164-0.122
<i>A. schweinfurthii</i>	Tree (n=7)	0.11 0.141-0.088	0.26 0.302-0.232	0.75 0.888-0.657	0.22 0.272-0.181	0.12 0.151-0.111
<i>A. leuderitzii</i>	Tree (n=7)	0.06 0.087-0.037	0.06 0.084-0.047	0.24 0.290-0.180	0.23 0.296-0.179	0.21 0.252-0.186
<i>G. bicolor</i>	Tree (n=6)	0.17 0.225-0.145	0.48 0.531-0.428	0.82 0.909-0.779	0.48 0.591-0.407	0.09 0.122-0.086
Mean of trees and shrubs		0.18±0.11a	0.19±0.10a	0.66±0.55a	0.84±1.54a	0.17±0.08a
<i>P-Value</i>		0.584ns	0.721ns	0.821ns	0.661ns	0.754ns

¹The Mean concentration per plant group followed by the same letter in each column are not significantly different from each other based on 5% level of significance. ns: not significant

4.2.3.7 Metal Intake by Grazing Animals

The maximum and estimated daily heavy metal intake of grazing animals based on the concentration of heavy metal(loid)s from the different plant groups and the average daily intake of forage and soils are shown in Table 4.2.8. Assuming that cattle will consume 16.9 kg of food per day consisting of 75% grasses, 23% woody plants, and 2% forbs from the TD and the VTD, the cattle daily intake of heavy metal(loid)s would be 1363.45 mg day⁻¹ for As, 1130.09 mg day⁻¹ for Mn, 2321.58 mg day⁻¹ for Cu, 1223.52 mg day⁻¹ for Cr and 725.58 mg day⁻¹ for Zr. For goats, which have a daily feed consumption of 0.90 kg/day with a diet that consists largely of shrubs (78%) and grasses (20%), and herbs (2%), the daily intake of heavy metal(loid)s would be 191.93 mg day⁻¹ for As, 94.87 mg day⁻¹ for Mn, 180.46 mg day⁻¹ for Cu, 57.76 mg day⁻¹ for Cr and 55.89 mg day⁻¹ for Zr. With an average daily forage intake of 2.50 kg for sheep which consists of 74% grasses, 21% of shrubs and woody species, and 5% herbs, the daily intake of heavy metal(loid)s would be 281.20 mg day⁻¹ for As, 224.03 mg day⁻¹ for Mn, 294.51 mg day⁻¹ for Cu, 197.20 mg day⁻¹ for Cr and 112.35 mg day⁻¹ for Zr.

Table 4.2.8 Maximum and estimated daily intake of heavy metal(loid)s of cattle, goat and sheep grazing on plants inside and near tailings dumpsite of abandoned Monarch Gold Mine, Francistown, Botswana

Type of Ruminant	As	Mn	Cu	Cr	Zr
Daily Intake of Metals at TD (mg day ⁻¹)					
Cattle	1363.45	1130.09	2321.58	1223.52	725.58
Goat	191.93	94.87	180.46	57.76	55.89
Sheep	281.20	224.03	294.51	197.20	112.35
Daily Intake of Metals VTD (mg day ⁻¹)					
Cattle	771.94	2281.96	1660.35	2421.73	449.04
Goat	48.42	206.48	141.69	138.22	38.12
Sheep	111.29	368.68	210.64	322.57	74.01
Maximum Allowable Daily Intake (mg day ⁻¹)					
Cattle	32-640 ^b	2000 ^c	1000-2000 ^d	2029.10 ^a	No data
Goat	2-40 ^b	150 ^c	150-180 ^d	366.44 ^a	
Sheep	5-100 ^b	375-400 ^c	230-250 ^d	274.05 ^a	

^aBased on 700 mg Cr/kg of diet in cattle's and 1000 mg Cr /kg of diet in sheep and goat which result to reduced body weight gain (Headley et al., 2008). ^bBased on the 30 mg kg⁻¹ maximum acceptable limit for mineral mixture (Marçal et al., 2005). ^cBased on Nouri & Haddioui (2016). ^dBased on the 1-2g Cu/day to cause chronic poisoning in calves and daily ingestion of 3.5 mg Cu/kg BW of sheep and goat on Outridge and Scheuhammer (1993).

As for the heavy metal intake by cattle, goats, and sheep from plant species which are in the VTD, the daily intake of heavy metal(loid)s by cattle would be 771.94 mg day⁻¹ for As, 2281.96 mg day⁻¹ for Mn, 1660.35 mg day⁻¹ for Cu, 2421.73 mg day⁻¹ for Cr and 449.04 mg day⁻¹ for Zr. For goats, the daily intake of heavy metal(loid)s would be 48.42 mg day⁻¹ for As, 206.48 mg day⁻¹ for Mn, 141.69 mg day⁻¹ for Cu, 138.22 mg day⁻¹ for Cr and 38.12 mg day⁻¹ for Zr. With an average daily forage intake of 2.50 kg for sheep, the daily intake of heavy metal(loid)s would be 11.29 mg day⁻¹ for As, 368.68 mg day⁻¹ for Mn, 210.64 mg day⁻¹ for Cu, 322.57 mg day⁻¹ for Cr and 74.01 mg day⁻¹ for Zr.

4.2.4 Discussion

The 100-year operation of the Monarch Gold Mine in Francistown, which ended with its permanent closure in 1996 (Lewis, 2001) has altered the soil properties not only in the mine site but also for the surrounding areas and for the river sediments. The current data shows this alteration is due to the presence of elevated heavy heavy metal(loid)s, which has led to serious environmental pollution as a consequence of ineffective waste management and a lack of enforcement of environmental laws. In the TD, samples showed a relatively high pH of up to 8.7 while in the VTD, the pH ranged from 6.84 to 8.38 (Table 4.2.3). The alkaline pH of the tailings is attributed to the extraction process of gold which uses an alkaline solvent of sodium cyanide solution. During the extraction process, the ore is alkalinized with sodium hydroxide and calcium carbonate or calcium oxide before the addition of the cyanide solution to leach the gold (Wu et al., 2021). This alkaline condition was observed even on the surface sediments collected from the adjacent Ntshe river and these values are comparable to the pH recorded in the study of Vogel and Kasper (2002).

The electrical conductivity (EC) of the samples from the TD site (Table 4.2.2) exceeded the optimal range for EC for most crops with a range of between 800-1800 $\mu\text{S}/\text{cm}$, and an upper limit of 2500 $\mu\text{S}/\text{cm}$. In contrast, the soils from the VTD and from RS had EC which were relatively compared to those in the TD, and they were within the optimum EC range for plant growth. The high EC in the TD implies there is a high concentration of soluble salts in the tailings that may inhibit the growth of the plants. A high level of EC in the TD would also imply there is a high risk of transporting soluble substances into vulnerable environmental compartments e.g., ground and surface water. On the other hand, the low EC level coupled with a high pH in the VTD, and the sediments implies that the concentration of soluble heavy

metal(loid)s and metalloids are low compared to those at TD (Violante et al., 2010). High EC in the TD could be attributed to the high concentration of residual sodium since sodium is used during the gold extraction process. In addition, the low EC observed in the river sediment could be attributed to its light texture which has a low capacity to retain soluble substances; Hence soluble salts are being leached down to lower levels (Kanthle, 2016).

On average, As can be regarded as the main contaminant in the TD and VTD as its high quantities and available concentrations (Table 4.2.3) exceed the critical concentrations (0.5-50ppm) proposed by Albanese et al. (2007). It is followed by Mn, Cu, Zr, and lastly Cr. Most heavy metal(loid)s followed a decreasing trend based on the distance from the mine with the highest levels found at the TD; the next highest in the VTD and the lowest at the RS which is farthest from the mine. The TD serves as a point source of contamination of heavy metal(loid)s in the abandoned Monarch area. Elevated levels of heavy metal(loid)s found in the VTD are due to the dispersion of these elements both downstream and downslope by wind and water. The occurrence of heavy precipitation during the short rainy season (December to February) may have contributed to the dispersion of contaminants in the vicinity of the abandoned gold mine (Navarro et al, 2008). In addition, the strong wind velocity during the dry season could also disperse the heavy metal-laden particles from the TD to the VTD because of the low vegetation cover in the study area.

The high PLI in the tailings dump indicates that heavy metal concentrations exceed the background concentrations and show potential heavy metal contamination. The PLI in the TD is considered as highly contaminated with an average value of 2.86 (2.67-3.25), which implies that this area should be subjected to active decontamination and rehabilitation. Similarly, the PLI in the VTD and in RS is slightly lower at 2.11 and 1.89 respectively, but they

are still considered highly contaminated and should be subjected to the same remediation treatment as the TD. The high PLI values in the TD could be attributed to the high contribution of the As contamination factor (CF) followed by the contamination factor of Zr ($10.03 > \text{Cu } (7.61) > \text{Cr } (5.82) > \text{Mn } (1.93)$). Similar to the TD, the PLI value in the VTD was also increased mainly by As concentration but at a lower magnitude (CF of $\text{As}_{\text{TD}} = 178.5$ vs CF of $\text{As}_{\text{VTD}} = 17.5$). The CF for Cr (7.96), Zr (7.56) and Cu (5.95) remained relatively constant as compared to its CF in TD, contributing more to the observed PLI in the VTD. Based on the total concentration and the CF of the different metals under consideration, the concentration of As significantly decreased from the TD to the VTD by almost 10 times while other elements remained relatively unchanged. This would imply that As has emanated from the mine tailings after gold extraction, thus serving as the point source. The As originated from gold ore which is an arsenopyrite and is able to release As upon oxidation (Hutchison et al., 2018). Considering that there is a big difference between the As concentration in the samples from TD and VTD, it appears that the main mode of As dispersion is through the transport of particles that remain after gold extraction.

The other metals are inherently common between the tailings and the surrounding soil as evidenced by almost similar concentrations in the samples from the TD and the VTD. Environmental factors like erosion also contribute to the increase of heavy metal(loid)s , especially As in the VTD because the tailings are partially exposed to environmental factors (Singh 2018). Observation of the study site during sampling also showed the prevalence of gullies along the slope of the TD. Particle dispersion from the TD occurs during both the rainy season and the dry season especially because of low vegetation cover. Therefore, gully erosion disperses the tailings to the surrounding area, thereby increasing the concentration

of these heavy metal(loid)s in surface soils. This is also evident in terms of Cu, Mn, and Cr which have higher concentrations than the background concentrations (Table 4.2.2).

For the river sediments, the PLI was 1.89, which was due to high concentrations of Mn and Cu. This indicates that Mn and Cu concentrations may be associated with sand and silt particles that are dominant in the river sediment. The lower concentration of As may be due to its solubilization and loss from the matrix during the deposition process (Devesa et al., 2001). The significance of finding high amounts of Zr in the study area is this element has been found to significantly decrease plant growth and alter enzyme activity in plants (Liu and Huang 2002). Plants that are exposed to high amounts of Zr tend to develop various defense mechanisms to cope with Zr toxicity (Shahid et al., 2012). Some of these mechanisms include among others, Zr sequestration on plant roots and activation of numerous antioxidants (Shahid et al., 2012). Its presence may also have contributed to abiotic stress on plants (Fodor et al., 2005); hence only a few plants have been able to survive in the TD site.

The concentration of heavy metal(loid)s in the available fraction is considered a better index for assessing the potential risk to living organisms. The available fraction of As, Cu, and Cr are much higher in TD than VTD and RS while for Mn and Zr, the available fraction is much higher in TD than VTD. Comparing the availability of these metals, Mn is the most common element in all three sites (TD, VTD, RS) followed by Cu > Cr > As > Zr. The high availability of As at the TD was attributed to the presence of soluble salts. For concentrations, the available As is higher in the sediment than in soils in the VTD. This may have occurred because the soluble salts which are carried from the TD, cannot be retained in the surface soil due to its sandy texture, so they dry up in the sediment and are then retained in the sediment matrix.

(Sherene, 2010). The high availability of Mn may be due to its soluble nature at a pH of 7.0 and above (Hem, 1963).

The pH in all three sites was above 7. In addition, gold particles are associated with sulfide minerals such as pyrite, arsenopyrite, chalcopyrite, and bornite which has led to a high concentration of As in the tailings (Feng and van Deventer 2010; Kaksonen et al., 2014). Furthermore, the cyanidation process converts several As species into As(V). Paktunc et al. (2004) found that As and Fe-bearing minerals were composed of iron (III) oxyhydroxides, scorodite, ferric arsenates, arseniosiderite, Ca-Fe arsenates, pharmacosiderite, jarosite, arsenopyrite; and that As occurred exclusively as As (V) resulting from cyanidation which is more easily transported within a soil column and also preferentially fixed with iron oxides and sulphides. Mn was the highest available element recorded in the TD, VTD, and RS because Mn could be easily mobilized upon changes in the chemical properties of the host environment, and it can also be easily extracted from an oxidizable phase (Dehghani et al., 2017). Furthermore, Neves (2012) stated that Mn in poorly aerated soils and at a pH 6 will have high availability and can be toxic to plants.

After being abandoned for almost 20 years, the vegetation in the TD has reestablished itself with one grass species (*C. biflorus*), three herb species (*P. oleracea*, *G. herbaceum*, *W. somnifera*), eight tree species dominated by (*D. cineria*, *C. mopane*, *B. albitrunca*) and three shrub species (*M. senegalensis*, *L. dinteri*, *M. leucoderme*). All these species were able to accumulate As, Cu, Cr, Mn, Zr above the normal concentration in plants indicating their ability to withstand very high concentrations of these elements. In addition, these plants can tolerate high concentrations of these metals simultaneously. Based on the bioaccumulation factor (BAF) for the different elements, it appears that these plants could regulate the uptake of

these elements at very high concentrations, resulting in their ability to thrive in the TD. Trees like *C. Mopane*, *D. Cineria* and *B. albitrunca* are able to survive because they belong to the Fabaceae family which are known as hyperaccumulators (Germishuizen and Meyer ,2003). As for the herbs, *P. oleracea* and *G. herbaceum*, these plants are heavy metal tolerant and can bioaccumulate As, Cu, Cd, and Pb (Negi, 2018). These trees and herbs are common in other heavy metal-polluted areas such as the BCL mine in Selebi Phikwe, Botswana (Manyiwa et al., 2021).

With respect to the composition of the vegetation in the VTD, six additional grass species (*B. nobilis*, *O. cubense*, *D. aegyptium*, *S. uniplumis*, *B. insculpta* and *P. congoense* Franch), and two more shrub species (*G. bicolor* and *F. De Wild*) and one tree species (*A. leuderitzii*) were also found in the VTD. Grasses like *B. nobilis*, *O. cubense*, *B. insculpta* are hyperaccumulators that can accumulate high levels of Cu, Zn, and Pb. These heavy metal(loid)s are commonly found in metalliferous environments and in areas that are contaminated by heavy metal(loid)s due to mining activities (García-Salgado et al., 2012). Furthermore, the tolerance of heavy metal(loid)s like Pb displayed by *O. cubense* may be linked to the root anatomy of the grass which has an exodermis that acts as an apoplast barrier (Redjala et al., 2011). For plant species that possess an exodermis, ionic selectivity occurs very close to the periphery of the root, blocking the accumulation of potentially toxic substances in the cortex (Enstone et al., 2002). Tree species like *A. leuderitzii*, an acacia species, including those in the subgenera of *Aculeiferum* and *Acacia*, as well as those in the series Filicinae and other mimosoid legumes, contain hydrolyzable tannins that act as adsorbents of heavy metal(loid)s (Batiha et al., 2022).

There is also an increasing number of plants surviving in the VTD compared to the TD. Certain plant species found in the VTD but not in TD, are considered more sensitive and vulnerable to elevated concentrations of heavy metal(loid)s. For example, in the TD, only one grass species survived compared to eight grass species in the VTD. The *C. biflorus* which has survived in an As polluted environment, can accumulate up to 47.14 mg kg⁻¹ of As and has 0.26 BAF. Its ability to thrive with high As concentrations has been attributed to exclusion mechanisms which prevent the uptake of As (El-Shabasy, 2021). Similarly, except for *Acacia dudgeonii* and *Gossypium herbaceum* and some plants with high BAF, all plants in TD were able to survive with high levels of HM concentration, especially As. Therefore, these plants are non-hyper (Viehweger, 2014).

At the time of sampling, the abandoned Monarch Gold mine did not have a security fence to prevent the entry of ruminants. Livestock in the area frequent the abandoned mine and graze on the vegetation in the area, which puts them at risk for exposure to the contaminated vegetation. Among the heavy metal of interest in this study, only As is estimated to exceed the maximum allowable daily intake (MADI) from ruminants grazing on the plants from the TD and VTD while Mn, Cu, and Cr are below or comparable to the MADI. No reference value was established for Zr. The estimated daily intake of As in TD is much higher than in VTD for cattle, goat and sheep which may be attributed to having a much higher concentration of As in the soil/tailings and in the plants growing in the TD. Although ruminants with chronic exposure to As do not usually exhibit toxicity symptoms, there is a risk for human exposure to As through the consumption of meat or milk products from these ruminants (Datta, 2010). Therefore, there is a need for assessment and close monitoring of

the As levels of the ruminants in the study area. Such information is necessary for the prevention of As exposure in humans.

Overall, the study has shown that the TD, VTD, and the sediments from the Ntshe river near the abandoned mine tailings dam of the Monarch gold mine are severely contaminated based on the PLI ranging from 1.89 to 2.86. The high PLI was primarily due to very high As concentrations in tailings and also in part due to high concentrations of Cu, Cr, and Zr. The solubility of As, Cu, Cr, and Zr in the TD is much higher than the VTD and the RS which could indicate a higher risk for these metals to be absorbed by plants or transported to nearby environments and to the groundwater. Due to high concentrations of As in the tailings, as well as the alkaline pH and very high electrical conductivity, only a few species were thriving in the TD which was dominated by *C. biflorus*, three herbs, and eleven trees and shrubs. The grass and herbs in the TD have concentrations of As, Cu, and Cr which are above the critical levels while the trees have concentrations within or slightly above the critical levels of plants. The number of plants thriving in the VTD is much higher than in the TD. The BAF of these plants are less than one indicating that these plants can survive in these environments by using exclusion (Xu et al., 2022). Furthermore, estimates of the daily intake of these metals by cattle, goat, and sheep through grazing and browsing of these plants and intake of soil indicate potential As exposure risk and probable transfer of As to humans through the consumption of these ruminants. It is therefore recommended that proper management of the tailing dam and its vicinity should be implemented to prevent the spread of pollutants and the exposure of animals and humans to heavy metal(loid)s, especially As emanating from the TD.

4.2.5 Conclusions

The main contaminant in Monarch Gold Mine is As and some Cu, Cr, and Zr which were found to be slightly above the critical values in soil. TD recorded a high availability of these metals compared to VTD and the river sediments. The most dominant species in The TD was *C. biflorus*, three herbs, and eleven trees and shrubs and the concentration of As, Cu, and Cr was above the critical levels estimates of the daily intake of these metals by cattle, goat and sheep through grazing and browsing of these plants. The intake of soil indicates a potential risk of As exposure and probable transfer to humans through the consumption of these ruminants. It is recommended that proper management of the tailings dam and its vicinity should be implemented to prevent the spread of pollutants and exposure of animals and humans to heavy metal(loid)s especially As emanating from the tailings dam

4.2.6 General Conclusions of Chapter 4

The two publications presented an assessment of heavy metal(loid)s in two study areas Selibe Phikwe (BCL Cu-Ni mine) and Francistown (Monarch Gold mine). The assessment was conducted in soil and plants growing in the mine tailings and the vicinity. The data indicated that the pollution load index was above one, indicating that there is pollution in soils and plants growing in the two study areas since the heavy metal(loid)s (metalloids) were above the critical limits. Using the data from the study areas and applying a formula, an estimation of the potential risk of livestock feeding on the plants and soil was estimated. Analysed heavy metal(loid)s were above the critical levels estimates of the daily intake of these metals by cattle, goats, and sheep through grazing and browsing of these plants. The intake of soil could indicate a potential risk of toxic metal exposure and probable

transfer to humans through the consumption of these ruminants. The estimates of the daily intake of these metals due to the consumption of plants at the vicinity of the BCL and Monarch Gold mines warrant for evaluation of actual levels of heavy metal(loid)s in grazing animals near the study area and in other mining areas in Botswana.

4.2.7 References

- Aladesanmi, Omolara Titilayo, et al. (2019). "Bioaccumulation factor of selected heavy metals in Zea mays." *Journal of Health and Pollution* 9.24.
- Albanese S., De Vivo, B., Lima, A., & Cicchella, D. (2007). Geochemical background and baseline values of toxic elements in stream sediments of Campania region (Italy). *Journal of Geochemical Exploration*, 93(1), 21-34.
- Batiha, G. E. S., Akhtar, N., Alsayegh, A. A., Abusudah, W. F., Almohmadi, N. H., Shaheen, H. M. & De Waard, M. (2022). Bioactive Compounds, Pharmacological Actions, and Pharmacokinetics of Genus Acacia. *Molecules*, 27(21), 7340.
- Bingham, F. T., Sposito, G., & Strong, J. E. (1986). The Effect of Sulfate on the Availability of CADMIUM1. *Soil science*, 141(2), 172-177.
- Datta, B. K., Mishra, A., Singh, A., Sar, T. K., Sarkar, S., Bhattacharya, A., & Mandal, T. K. (2010). Chronic arsenicosis in cattle with special reference to its metabolism in arsenic endemic village of Nadia district West Bengal India. *Science of the total environment*, 409(2), 284-288.

- Dehghani S, Moore F, Keshavarzi B, Beverley AH. (2017). Health risk implications of potentially toxic metals in street dust and surface soil of Tehran, Iran. *Ecotoxicology and environmental safety*, 136, 92–103.
- Devesa, V., Macho, M. L., Jalón, M., Urieta, I., Muñoz, O., Súnier, M. A., ... & Montoro, R. (2001). Arsenic in cooked seafood products: study on the effect of cooking on total and inorganic arsenic contents. *Journal of agricultural and food chemistry*, 49(8), 4132-4140.
- Dick, R. P., Thomas, D. R., & Halvorson, J. J. (1997). Standardized methods, sampling, and sample pretreatment. *Methods for assessing soil quality*, 49, 107-121.
- Ekosse, G. (2005). General health status of residents of the Selebi Phikwe Ni-Cu mine area, Botswana. *International journal of environmental health research*, 15(5), 373-381.
- Ekosse, G. I. E., Ngila, J. C., & Forchheh, N. (2005). Multivariate analyses of heavy metals in soils and *Colophospermum mopane* leaves around the Selebi Phikwe nickel-copper mine and smelter/concentrator plant area, Botswana. *Journal of Applied Sciences and Environmental Management*, 9(1), 177-185.
- El-Shabasy, A. (2021). Effect of Heavy metals in the cement dust pollution on morphological and Anatomical Characteristics of *Cenchrus ciliaris* L. *Saudi Journal of Biological Sciences*, 28(1), 1069-1079.
- Enstone, D. E., Peterson, C. A., & Ma, F. (2002). Root endodermis and exodermis: structure, function, and responses to the environment. *Journal of Plant Growth Regulation*, 21, 335-351.
- Feng, D., & Van Deventer, J. S. J. (2010). Thiosulphate leaching of gold in the presence of ethylenediaminetetraacetic acid (EDTA). *Minerals engineering*, 23(2), 143-150.

- Fodor, M., Hegedus, A., & Stefanovits-Banyai, E. (2005). Zirconium induced physiological alterations in wheat seedlings. *Biologia plantarum*, 49(4), 633-636.
- García-Salgado, S., García-Casillas, D., Quijano-Nieto, M. A., & Bonilla-Simón, M. M. (2012). Arsenic and heavy metal uptake and accumulation in native plant species from soils polluted by mining activities. *Water, Air, & Soil Pollution*, 223, 559-572.
- Germishuizen, G., Meyer, N.L (eds.) (2003). Plants of southern Africa: an annotated checklist. Strelitzia 14. National Botanical Institute, Pretoria, South Africa. pp 1231.
- Headley, S. A., dos Reis, A. C. F., Nakagawa, T. L. D. R., & Bracarense, A. P. F. R. L. (2008). Chronic copper poisoning in sheep from Northern Paraná, Brazil: Case report. *Semina: Ciências Agrárias*, 29(1), 179-184.
- Hem, J. D. (1963). Chemical equilibria affecting the behavior of manganese in natural water. *Hydrological Sciences Journal*, 8(3), 30-37.
- Hutchison, A. R., & Bortel, C. F. (2018). The Fate of arsenic in Noah's Flood. In *Proceedings of the International Conference on Creationism* (Vol. 8, No. 1, p. 26).
- Kaksonen, A. H., Morris, C., Rea, S., Li, J., Usher, K. M., McDonald, R. G., & du Plessis, C. A. (2014). Biohydrometallurgical iron oxidation and precipitation: Part II—Jarosite precipitate characterization and
- Kanthle, A. K., Lenka, N. K., Lenka, S., & Tedia, K. acid recovery by conversion to hematite. *Hydrometallurgy*, 147, 264-272. (2016). Biochar impact on nitrate leaching as influenced by native soil organic carbon in an Inceptisol of central India. *Soil and Tillage Research*, 157, 65-72.
- Liu, X., & Huang, B. (2002). Cytokinin effects on creeping bentgrass response to heat stress: II. Leaf senescence and antioxidant metabolism. *Crop Science*, 42(2), 466-472.

- Lewis, R. (2001). An outline history of Monarch Gold Mine Francistown. *Botswana Notes & Records*, 33(1), 41-46.
- Maleki, A., Amini, H., Nazmara, S., Zandi, S., & Mahvi, A. H. (2014). Spatial distribution of heavy metals in soil, water, and vegetables of farms in Sanandaj, Kurdistan, Iran. *Journal of environmental health science and engineering*, 12(1), 1-10.
- Manyiwa, T., Ultra, V. U., Rantong, G., Opaletswe, K. A., Gabankitse, G., Taupedi, S. B., & Gajaje, K. (2021). Heavy metals in soil, plants, and associated risk on grazing ruminants in the vicinity of Cu-Ni mine in Selebi-Phikwe, Botswana. *Environmental Geochemistry and Health*, 1-16.
- Marçal, W.S, Pardo P.E, Nascimento M.R.L, Veras, E.B, Moreno A.D (2003). Levels of lead in mineral salt commercial mixtures for beef cattle. *Journal of Veterinary Science*, 4, 235–238.
- Meliker, J. R., Wahl, R. L., Cameron, L. L., & Nriagu, J. O. (2007). Arsenic in drinking water and cerebrovascular disease, diabetes mellitus, and kidney disease in Michigan: a standardized mortality ratio analysis. *Environmental Health*, 6(1), 1-11.
- Motswaiso, F. S. (2019). Geochemical investigation of metals and trace elements around the abandoned Cu-Ni mine site in Selibe Phikwe, Botswana. *Journal of Geoscience and Environment Protection*, 7(05), 275.
- Navarro MC, Pérez-Sirvent C, Martínez-Sánchez MJ, Vidal J, Tovar PJ, Bech J. (2008). Abandoned mine sites as a source of contamination by Heavy metals : a case study in a semi-arid zone. *Journal of Geochemical exploration*, 96 (2-3), 183–193.
- Negi, S. (2018). Heavy metal accumulation in *Portulaca oleracea* Linn. *Journal of Pharmacognosy and Phytochemistry*, 7(3), 2978-2982.

- Neves MO, Figueiredo VR, Abreu MM. (2012). Transfer of U, Al, and Mn in the water–soil–plant (*Solanum tuberosum* L.) system near a former uranium mining area (Cunha Baixa, Portugal) and implications to human health. *Science of the total environment*, 416, 156–163.
- Nouri, M., & Haddioui, A. (2016). Human and animal health risk assessment of metal contamination in soil and plants from Ait Ammar abandoned iron mine, Morocco. *Environmental monitoring and assessment*, 188, 1-12.
- Outridge, P. M., & Scheuhammer, A. M. (1993). Bioaccumulation and toxicology of nickel: Implications for wild mammals and birds. *Environmental Reviews*, 1(2), 172–197.
- Paktunc, D., Foster, A., Heald, S., & Laflamme, G. (2004). Speciation and characterization of arsenicin gold ores and cyanidation tailings using X-ray absorption spectroscopy. *Geochimica et Cosmochimica Acta*, 68(5), 969-983.
- Redjala, T., Zelko, I., Sterckeman, T., Legué, V., & Lux, A. (2011). Relationship between root structure and root cadmium uptake in maize. *Environmental and Experimental Botany*, 71(2), 241-248.
- Shahid, M., Ferrand, E., Schreck, E., & Dumat, C. (2012). Behavior and impact of zirconium in the soil–plant system: plant uptake and phytotoxicity. *Reviews of Environmental Contamination and Toxicology Volume 221*, 107-127.
- Sherene, T. (2010). Mobility and transport of heavy metals in polluted soil environment. In *Biological forum—an international journal* (Vol. 2, No. 2, pp. 112-121).
- Singh, R., Ahirwar, N. K., Tiwari, J., & Pathak, J. (2018). Review on sources and effect of heavy metal in soil: Its bioremediation. *International Journal of Research in Applied, Natural and Social Sciences*, 2018, 1-22

- Soltani, R. D. C., Rezaee, A., Khataee, A. R., & Safari, M. (2014). Photocatalytic process by immobilized carbon black/ZnO nanocomposite for dye removal from aqueous medium: Optimization by response surface methodology. *Journal of Industrial and Engineering Chemistry*, 20(4), 1861-1868.
- Ultra, V. U., & Manyiwa, T. (2021). Influence of mycorrhiza and fly ash on the survival, growth and heavy metal accumulation in three Acacia species grown in Cu-Ni mine soil. *Environmental Geochemistry and Health*, 43(4), 1337-1353.
- Ultra Jr, V.U. (2020). Fly ash and compost amendments and mycorrhizal inoculation enhanced the survival and growth of Delonix regia in Cu-Ni mine tailings. *Philippine Journal of Science*, 149(3): 479-489
- Viehweger, K. (2014). How plants cope with Heavy metals. *Botanical Studies* 55.1 1-12.
- Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. G., & Pigna, M. (2010). Mobility and bioavailability of Heavy metals and metalloids in soil environments. *Journal of soil science and plant nutrition*, 10(3), 268-292
- Vogel, H., & Kasper, B. (2002). Mine soils on abandoned gold mine tailings in Francistown. *Report by the Bundesanstalt für Geowissenschaften und Rohstoffe and Department of Geological Survey (Environmental Geology Division). Lobatse, Botswana.*
- Wu, H., Feng, Y., Li, H., Li, H., & Ju, J. (2021). Effect of sodium carbonate on alkaline self-leaching of gold from flotation gold ore. *Separation and Purification Technology*, 256, 117499.
- Xu, Y., Yang, R., Zhang, J., Gao, L., & Ni, X. (2022). Distribution and dispersion of heavy metals in the rock-soil-moss system of the black shale areas in the southeast of

Guizhou Province, China. *Environmental Science and Pollution Research*, 29(1), 854-867.

CHAPTER 5

Influence of Different Soil Amendments on Heavy Metal (loid)s Uptake, Survival, and Phytoremediation Efficiency of Selected Tree Species

The purpose of this Chapter is to address objective two of the study which is to evaluate the influence of different soil amendments on selected plants with respect to heavy metal uptake; survival; and phytoremediation efficiency, by assessing the influence of these amendments (compost, fly ash, arbuscular mycorrhiza, cow manure, and sewage sludge) on soil chemical properties, heavy metal availability, and the overall phytoremediation potential of these plants. The heavy metal(loid)s under consideration includes As Cu, Mn, Ni Pb, Zn and Cr which are dominant in both BCL mine and Monarch gold mine tailings. Some of the metals like Pb and Cr were not included in the analysis under Publication 5.2 due to limitations on available resources and analytical equipment during the conduct of this experiment. These two elements, however, were included in the evaluation of the performances of *E. globulus* and *Vachellia* species as indicated in Publication 5.2 and 5.3. Therefore, Pb and Cr should be considered in future studies on the assessment of the performance of *C. mopane* in the phytoremediation of BCL mine tailings.

Findings from this study indicated that phytoremediation potential of the different tree species were influenced by the addition of fly ash and compost amendments together with mycorrhiza inoculation. These amendments enhanced the survival and growth and dry matter yield of all the tree species grown in heavy metal-contaminated soils. Compost and mycorrhiza reduced heavy metal availability in tailings but increased the biomass yield of

these plants, and subsequently the total uptake of heavy metal(loid)s by plants, thus enhancing the phytoremediation potential of *C. mopane* in Cu-Ni mine tailings. Likewise, the addition of fly ash alone or in combination with mycorrhiza enhanced the phytoremediation potential of *C. mopane* by increasing the overall heavy metal uptake through enhanced biomass production, hence the overall potential removal of reducing the heavy metal load in the soil.

The study on the assessment of the effect of amendments on the *Vachellia* (*acacia*) species indicated that fly ash improved the growth and lowered the heavy metal concentration of different *Vachellia* species by neutralizing the acidic pH of soil and reducing the availability of heavy metal(loid)s. The concentration of heavy metal(loid)s in plants was reduced due to the addition of FA. Mycorrhiza inoculation successfully colonized the roots of different *Vachellia* species and selectively enhanced the growth and altered the heavy metal accumulation in plants. Enhancement of growth by mycorrhiza was only observed in *V. luederitzii* in soils without fly ash, while growth retardation was observed in *V. albida* and *V. tortilis* in tailings without fly ash. Therefore, amendments improved the soil quality of mine tailings to a state suitable for plant growth.

E. globulus species could not survive in pure mine tailings from the BCL mine and Monarch Gold Mine. However, the addition of amendments increased the survival of this species in mine tailing and increased the uptake of heavy metal(loid)s by enhancing the biomass yield, thus its phytoremediation potential in BCL and Monarch Gold Mine tailings.

This Chapter is comprised of two publications and one manuscript by the author that has been reproduced as published.

Publication 5.1;

Published as: **Manyiwa, T.**, & Ultra Jr, V. U. (2022). Soil Amendments and Arbuscular Mycorrhiza Influenced the Growth and Heavy Metal Accumulation of *Colospospermum mopane* (Kirk Ex Benth.) In Heavy Metal Contaminated Soil. Soil and Sediment Contamination: An International Journal, 31(1), 81-96.
<https://doi.org/10.1080/15320383.2021.1910623>

Publication 5.2;

Ultra, V.U., **Manyiwa, T.** (2021). Influence of mycorrhiza and fly ash on the survival, growth and heavy metal accumulation in three *Acacia* species grown in Cu-Ni mine soil. *Environmental Geochemistry & Health* 43, 1337-1353 (2021).
<https://doi.org/10.1007/s10653-020-00627-x>

Manuscript 5.3;

Submitted to Soil Science & Environment Journal: Manyiwa, T., & Ultra Jr, V. U., Gajaje, K., Gaotlhobogwe, K. & Sefatlhi, K. (202x). Enhancing the phytoremediation potential of *Eucalyptus globulus* in three different mine tailings of Botswana through soil amendments. *Soil Science & Environment*.

Authors Contribution:

Trust Manyiwa is the main author and my supervisor, **Prof. Venecio Ultra** is the co-author for Publication 5.1 and submitted manuscript 5.3, while the candidate was the co-author for publication 5.2 with the supervisor as the first author. **Manyiwa T-** conceptualization and

planning, data gathering and analysis, validation of methodology, interpretation of results, writing and revision of manuscript. **Ultra VU-** project administration, conceptualization and planning; supervision of fieldwork and laboratory analyses; Interpretation of results; and review and editing of manuscript. For Manuscript 5.3, **Gajaje, K., Gaotlhobogwe, K. & Sefatlhi, K-** Fieldwork and sample collection; sample preparation and analysis, data interpretation and analysis, writing and review of different sections of the manuscript.

5.1 Soil Amendments and Arbuscular Mycorrhiza Influenced the Growth and Heavy Metal Accumulation of *Colospospermum mopane* (Kirk Ex Benth.) In Heavy Metal Contaminated Soil

Abstract

The potential of *Colospospermum mopane* for phytoremediation of heavy metal (HM) contaminated soil was evaluated. *C. mopane* seedlings were planted on soils from the vicinity of Cu-Ni mine as affected by different soil amendments (Compost and fly ash-FA) and mycorrhizal inoculation in terms of growth and HM accumulation through a 2-factorial pot experiment. The survival and dry matter yield of *C. mopane* seedlings was enhanced by FA and compost as well as mycorrhiza. Soils with compost had high survival and dry matter yield and were enhanced by mycorrhiza. FA also enhanced the survival and dry matter yield but at a lower magnitude. Except for Mn, FA and compost reduced the concentration of Cu, Ni, As, and Zn in the shoots and roots of the *C. mopane* seedlings. Mycorrhiza reduced the Cu, Ni, and Zn concentrations in shoots but not with As. FA and compost modified the soil pH, EC, and the total and available contents of heavy metal(loid)s in the soil thus the difference in growth and uptake of HMs. Overall, results showed that FA or compost together with mycorrhiza can enhance the survival and growth of *C. mopane* seedlings in HM-contaminated soils by reducing its availability and uptake by plants.

Keywords: Compost, fly ash, BCL mine tailings, heavy metal availability, translocation factor

5.1.1 Introduction

The economy of Botswana is largely driven by the mining sector. Aside from gold, copper, nickel, and coal, Botswana is the world's largest diamond producer. These mining activities generate several forms of waste that lead to serious and widespread environmental pollution by heavy metal(loid)s, and such environmental degradation was observed in the locality of Francistown (Vogel and Kasper, 2002). The continued economic dependence on the mining industry of Botswana in the near future calls for a comprehensive rehabilitation program against heavy metal pollution that threatens all life forms and ecological balance (Ayangbenro and Babalola, 2017; Likuku et al., 2013; Moreki et al., 2013).

In Botswana, the Environmental Impact Assessment Act of 2011, states that a company or a person awarded mining rights should rehabilitate the area affected by the adverse environmental impact of the implemented activity, and a company or a person that fails to rehabilitate the area, commits an offense and is liable to a fine, or imprisonment, or to both (Botswana Government Gazette, 2011). Unfortunately, this act is not fully enforced by the government that leads to negligence to rehabilitate the mined area by some mining companies.

Currently, the mining industry in Botswana is the major source of government's revenue and that the mineral resources exploration and processing is continuously expanding (Koitsiwe and Adachi, 2017). Such expansion of mining and smelting activity generates enormous waste that needs to be managed properly (Motswaiso, 2019). According to Letshwenyo (2016), in some instances, mine tailings and mine waste from active and abandoned mines are the main point source of heavy metal pollution causing serious health

hazards and possible threats to food chain contamination. Therefore, there is a need to develop site specific phytoremediation systems for the different mine waste in Botswana and these could be achieved by identifying suitable plants coupled with appropriate cultural and nutrient management to successfully established plants and achieve the phytoremediation goal.

To enhance the success of phytoremediation of heavy metal contaminated environment, some amendments are applied in soil especially during the plant establishment. These amendments include the application of lime in acidic sites, fly ash (FA), compost and other materials that would modify the physicochemical properties of soil (Han et al., 2012; Hussain et al., 2018; Ultra, 2020). Most of these amendments can significantly alter the growth and survival of plants and even the accumulation and uptake of heavy metal(loid)s in plants (Ultra and Manyiwa, 2021). The FA is a good amendment because of its texture and the fact that it contains almost all the essential plant nutrients except for N (Pathan, et al., 2003). Using FA as an amendment improves soil physicochemical properties and microbial activity (Singh and Pandey, 2013). However, studies by Lee et al. (2007) and Pandey and Singh (2010), have found out that FA contains heavy metal(loid)s such as Cu, Ni, Cr, Cd and others that may exhibit metal toxicity to plants.

On the other hand, compost as an amendment in the heavy metal contaminated environment also modifies the bio-physicochemical properties of soils and provides nutrients for plant growth and development (Huang et al., 2016). Studies by Saum, Jiménez, and Crowley (2018) and Huang et al. (2016), have shown that compost amendments could reduce heavy metal availability by providing humus as sorption sites of cationic metals, and soluble organic substances that could serve as chelating agents of heavy metal(loid)s in soils.

However, the application of compost can increase the concentrations of heavy metal(loid)s in plants because it may contain elevated heavy metal(loid)s, and some contain toxic substances (Petruzzelli, 1996). Therefore, caution should be taken in applying composts in phytoremediation systems. Depending on the source and quality, compost can also cause pollution, but the severity of pollution depends on the chemical form of heavy metal(loid)s present in compost and the source of the raw materials (Castaldi, et al., 2005). Other studies found out that the use of compost accelerates the removal and stabilization of organic pollutants present in the soil and plants by providing a favorable environment for microbial growth (Farrell and Jones, 2010).

In India, mycorrhizal fungi were utilized by several phytoremediation studies in heavy metal-contaminated soils but the inoculation effects on heavy metal concentration and alleviation of heavy metal toxicity in plants are contradictory and not clear. A study by Riaz et al. (2020) reports that mycorrhiza may not affect, decrease, or increase the uptake of toxic metals by plants. Dhalaria et al. (2020) and Riaz et al. (2020), discovered that mycorrhizal fungi protect plants from the toxic effect of highly concentrated heavy metal(loid)s by possibly binding the metals in their hyphae or by reducing the translocation of metals to the plant tops (Krishnamoorthy et al., 2019).

Therefore, there is a need to evaluate the effect of fly ash and compost amendments in combination with mycorrhizal inoculation on the potential of *C. mopane* for the phytoremediation of heavy metal contaminated soils near a Cu-Ni mine. Specifically, this study will assess the growth and the heavy metal uptake in *C. mopane* as influenced by compost, fly ash and mycorrhizal inoculation; and determine their influence on the chemical properties and availability of heavy metal(loid)s in soils.

5.1.2 Materials and Methods

This study used *C. mopane*, a leguminous tree belonging to the non-nitrogen fixing subfamily Caesalpinioideae and is widespread and locally dominant over significant areas in the drier parts of southern Africa, Botswana included (Timberlake, et al., 2010). It is estimated that the area under *C. mopane*-dominated vegetation covers 550 000 Km². It thrives in heavier-textured and nutrient-rich soils in the valleys of Southern Africa with an elevation of 200 to 1200 meters (Mapaure, 1994). Based on the survey of Ekosse (2005), *C. mopane* leaves from the vicinity of BCL mine in Selibe-Phikwe, Botswana had Cu contents in the range of 4–116 mg kg⁻¹, Zn between 17–79 mg kg⁻¹, Cr between 0.03–11 mg kg⁻¹, indicating that *C. mopane* can tolerate high levels of heavy metal(lloid)s.

5.1.2.1 Soil and fly ash sampling and pot preparation

The heavy metal contaminated soil used in this experiment was collected randomly from the surface soil (0–20 cm depth) at the vicinity (500 meters from the mine tailings pond) of the BCL mine in Selebi-Phikwe, Botswana. The collected soil was combined to make a composite sample used in the pot experiment. After air-drying, soils for cultivation and chemical analysis were passed through a 5 mm and 2 mm mesh sieve respectively. The properties and the heavy metal contents of the soils are presented in Table 5.1.1 The soil is highly acidic, sandy loam with very low organic matter and nutrient content and has high amounts of Cu, Ni, Mn, and As. Freshly dumped fly ash was collected from the dumpsite of the Morupule B Power Plant Station, Palapye, Botswana. The Morupule B Power Plant has 33 MW coal-fired units, with coal supplied from the adjacent Morupule Colliery and operated by the Botswana Power Corporation providing 90% of the country's domestic power generation

(Zhai et al., 2009). Morupule B generates 540,000 tons per annum of fly ash that are deposited in the dumpsite adjacent to the power plant. Collected fly ash was air-dried and sieved using a 2 mm diameter mesh before application. The compost used in the experiment was obtained commercially (Culterra Compost, Culterra, South Africa) from a local supplier. The chemical properties of fly ash and compost are also presented in Table 5.1.1. Plastic pots (25 cm diameter x 30 cm height) were filled with 8 kg soil or a mixture of soil + fly ash before treatment assignments and plant establishment.

Table 5.1.1 Chemical properties and heavy metal contents in soils and fly ash.

Properties	Soil	Fly Ash	Compost
pH (1:2, soil:water)	4.35	8.8	7.4
Electrical Conductivity ($\mu\text{S}/\text{m}$, 1:2)	1423	2370.0	794
% Organic Matter	0.265	0.012	43.15
% Total N	0.042	0.00	4.92
Particle Size Distribution			
%Sand	65.92	21.05	
%Silt	31.29	45.25	
%Clay	2.79	33.7	
Total Metal(loid)s (mg kg^{-1})			
As	124.31	10.08	0.00
Cu	768.13	63.20	36.50
Mn	745.18	396.71	425.12
Ni	634.26	78.25	5.26
Zn	79.35	25.31	438.26
Water Soluble Metal(loid)s (mg kg^{-1})			
As	1.81	1.23	0.00
Cu	9.25	0.07	6.47
Mn	8.74	0.04	42.18
Ni	16.64	0.01	0.00
Zn	0.12	0.04	56.47

5.1.2.2 Description of the treatments and plant culture

C. mopane seedlings were subjected to three types of soil amendments (pure soil, 10% fly ash (FA) by weight; and 10% compost (CP) by weight) and two levels of arbuscular mycorrhizal inoculation (–AM and +AM), comprising six-treatment combinations. Each treatment was designated as Control (Soil alone) -no mycorrhizal and no amendment, soil alone with mycorrhizal (soil +AM), soil with FA and no mycorrhizal (Soil +FA-AM), soil with

FA and mycorrhizal (Soil +FA +AM), soil with compost but no mycorrhizal (Soil +CP- AM), and soil with CP and mycorrhizal (Soil +CP +AM). An equivalent to 10% (by weight) of FA and CP was mixed with soil thoroughly before potting. The seeds of the *C. mopane* were collected from identified trees inside the university campus. The seeds were washed ten times with sterile distilled water and germinated on sterile Petri plates lined with moistened filter paper. Three pre-germinated seed was then planted at the center of the pots and was maintained at a moisture content of 40% of the field capacity under screen house conditions. For the AM treatments, five grams of sterile (121° C, 30 min for three consecutive days) or non-sterile AM inoculants (MYKOVAM®, National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Banos, Philippines) were mixed with the soil at the center of pot just below the roots of the seedlings of the – AM and +AM treatments, respectively (Ultra and Manyiwa, 2021). The MYKOVAM® is a soil-based biofertilizer contains vesicular arbuscular mycorrhiza. Four replicates were used for each treatment laid out in a complete randomized design. The pots were rearranged every week throughout the experimental period. Three weeks after transplanting the seedlings were thinned to 1 seedling per pot. The plants were irrigated regularly and monitored for survival and growth inside the shade house (80% exposure).

5.1.2.3 Plant sampling and chemical analysis

During the 40-weeks cultivation period, plants were periodically monitored for survival and growth. At harvest, the shoots were excised, and the roots were carefully separated from the shoots, and their fresh weights were determined. To assess the mycorrhizal infection, a portion of the fresh roots from each plant (0.2 g) was subjected to

trypan blue staining before the microscopic examination (Brundrett and Abbott, 1994). The colonization rate on the roots by AMF was quantified using the gridline intersection method (Giovannetti and Mosse, 1980). The shoots and the remaining roots were oven-dried at 70°C for 3 days, dry weights were recorded and ground for chemical analysis. To determine the heavy metal concentration in tissues, the tissues were digested using a nitric acid and hydrogen peroxide mixture in an open vessel at 120° C for a minimum of 4 hours (Ultra et al., 2016). The digests were analyzed for heavy metal(loid)s using Atomic Absorption Spectrometer (AAS, Analytik Jena ContrAA800, Denmark). The accuracy of the heavy metal contents in each sample was verified by subjecting a randomly drawn sample from the whole experimental samples for analysis at the Analytical Service Laboratory, University of Botswana. Based on the duplicate analysis from two laboratories, the variation of the values of the same sample was less than 6%. After calculating concentrations in plant tissues, the translocation factor for each metal was calculated as a ratio of the shoot divided by the root concentration.

5.1.2.4 Soil sampling and chemical analysis

For the assessment of the chemical properties after seedling growth, the soil from each pot was collected after the roots were removed from the pots. The collected samples were passed through a 2 mm sieve, air-dried and stored at 4°C until chemical analysis. The soil pH and electric conductivity (EC) were measured in water suspension (1:5) after shaking for 1 hour while the total heavy metal content was measured after wet acid digestion in an open vessel at 120 °C for 6 hours using HNO₃: HCl mixture (1:3) in a hot plate followed by quantification using AAS. The water-soluble (WS) fractions of heavy metal in soils were

determined to estimate the available fraction by extracting soil samples with distilled water (1:100) for 2 hours at room temperature. The WS fractions were analyzed in duplicates for the different heavy metal(loid)s using AAS.

5.1.2.5 Statistical Analysis

The data for each parameter were analyzed using a 2-factor ANOVA in CRD. Tukey's test was performed for treatment mean comparison when significant differences existed at a 5% level of significance.

5.1.3 Results

5.1.3.1 Survival, Mycorrhizal Colonization, and Dry matter yield

Soil amendments and mycorrhizal inoculation resulted in an interaction effect on the survival rate of seedlings, mycorrhizal roots, and the dry matter of *C. mopane* seedlings (Table 5.1.2). In soils contaminated with heavy metal(loid)s without amendments and mycorrhiza, *C. mopane* seedlings had 70% survival. Application of fly ash regardless of mycorrhiza resulted in 100% survival while those treatments with compost alone had 70% survival like the control. Combined application of compost and mycorrhiza enhanced the survival of *C. mopane* by up to 100%.

Plants grown in pure soils and soils with FA had zero mycorrhizal roots, while those inoculated with AM in pure soil had 32% root length colonized by mycorrhiza. The colonization was enhanced by the presence of FA and compost to about 54% and 68%, respectively. Soil with compost had mycorrhizal roots of 26%.

The dry matter yield was significantly influenced by amendments and mycorrhiza. Shoot dry weight was enhanced by FA and compost. The AM enhanced the shoot, roots and the total dry weights of *C. mopane* in soil alone and soil with compost and mycorrhiza but reduced the dry matter yield in soil with FA. The combination of compost and mycorrhiza is the best in enhancing dry matter yield compared to all other amendments.

Table 5.1.2 Survival, mycorrhizal root colonization and dry matter yield of Mopane in heavy metal contaminated soils as affected by soil amendments and mycorrhiza inoculation¹

Treatments	Survival rate (%)	Mycorrhizal Roots (Root length Colonized)	Dry Matter Yield (g/plant)		
			Shoots	Roots	Total
Soil Alone					
-AM	70 c	0 d	1.60d	0.50 d	2.10 e
+AM	90 b	32 c	2.40 c	1.40 c	3.80 d
Soil +10% FA					
-AM	100 a	0 d	4.10 b	1.00 c	5.10 c
+AM	100 a	54 b	2.90 c	0.90 c	3.80 d
Soil + 10% Compost					
-AM	70 c	26 d	4.90 b	3.10 b	7.90 b
+AM	100 a	68 a	8.70 a	4.70 a	13.40 a
P-values					
Amend	0.000***	Ns	0.000***	0.000***	0.000***
AM	0.048*	0.000***	0.000***	0.000***	0.000***
Amend x AM	0.004**	0.000***	0.000***	0.000***	0.000***

¹ Means followed by the same letter (s) are not significantly different from each other based on Tukey's Test at 5% level of significance. *=significant; **=highly significant; ***=very highly significant; ns=not significant

5.1.3.2 Heavy metal concentrations in tissues of *C. mopane*

The heavy metal concentrations in shoots and roots of *C. mopane* were significantly affected by the interaction of amendments and mycorrhizal (Table 5.1.3). The FA and compost generally reduced the As, Cu, Ni, and Zn concentrations in shoots and roots but not for Mn. Similarly, AM inoculation in soil alone reduced the concentration of these metals in the shoots and roots of *C. mopane*. The As concentration in shoots and roots was significantly reduced by AM in soil alone by about 30% and 60%, respectively, based on the control. Whereas, FA amendments without AM had shoots and root As lower than the control by 58% and 44%, respectively, while soil with compost but without AM had the lowest As concentration in shoots and roots by 65% and 82%, respectively, than the control. Mycorrhiza inoculation in soil with FA enhanced the As in shoots and roots but decreased in soil with compost.

Influence of Different Soil Amendments on Heavy Metal(loid)s Uptake, Survival and Phytoremediation Efficiency of Selected Tree Species

Table 5.1.3 Concentrations of heavy metal(loid)s in *C. mopane* as influenced by different amendments and mycorrhizal inoculation¹

Treatments	Concentrations (mg kg ⁻¹)									
	As		Cu		Mn		Ni		Zn	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Soil Alone										
-AM	21.20 a	66.18 a	63.92 a	705.88 a	37.42 c	29.41 b	34.30 a	112.75 a	54.56 a	98.04 a
+AM	15.00 b	25.55 c	40.62 b	250.11 b	48.95 c	25.01 c	20.83 b	66.10 b	28.12 b	39.30 c
Soil +10% FA										
-AM	8.94 c	36.82 b	22.36 c	85.16 d	48.94 c	30.06 b	3.02 d	22.54 d	19.94 c	30.06 d
+AM	11.69 b	39.79 b	18.92 d	109.57 c	66.20 b	54.79 a	2.58 d	28.84 d	16.34 c	60.55 b
Soil + 10% Compost										
-AM	7.38 c	11.63 d	22.54 c	147.47 c	86.05 a	36.87 b	12.29 c	49.98 c	21.51 bc	32.77 d
+AM	4.15 d	8.13 d	25.96 c	106.30 c	92.00 a	49.96 a	17.02 c	43.05 c	33.75 b	22.85 e
P-values										
Amend	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
AM	0.000***	0.002**	0.040*	0.032*	0.028*	0.000***	0.014*	0.038*	0.050*	0.022*
Amend x AM	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

¹ Means followed by the same letter (s) are not significantly different from each other based on Tukey's Test at 5% level of significance. *=significant; **=highly significant; ***=very highly significant

Based on the control, the Cu concentration in shoots and roots were reduced by 36% and 65%, respectively, due to AM inoculation. The Cu concentration in shoots of *C. mopane* from the soil with FA and compost was 65% lower than the control while, in the roots, the reduction due to FA was 88% and 80% due to compost. Mycorrhiza inoculation further decreased the Cu in shoots but increased in roots while in soils with compost, AM did not significantly influence the concentration in tissues.

The Ni concentration in shoots and roots was reduced significantly by FA and compost. The reduction due to FA is about 90% and 80% in shoots and roots, respectively and this magnitude is much greater than compost which reduced the Ni concentration to 64% and 56% for the shoots and roots, respectively. Mycorrhiza also reduced the Ni concentration in shoots and roots to about 39% and 41%, respectively in pure soils. Similarly, Zn concentration in shoots was reduced by FA amendments by 63% and 60% due to compost, respectively. In soil without amendment, AM reduced the shoot Zn concentration by 48%. In roots, Zn concentration was reduced by 60% due to AM inoculation in pure soil, 69% due to FA with AM, and 66% due to compost. Mycorrhiza in soil with FA had higher Zn concentration in roots compared to plants grown in soil with FA without mycorrhiza. Unlike the other metals, the Mn concentration in shoots was enhanced by AM and compost but not with FA amendments. The mycorrhiza inoculants, however, increased the Mn in shoots in the presence of FA. In roots, AM enhanced the Mn concentration regardless of the amendments.

5.1.3.3 Translocation of heavy metal(loid)s in the *C. mopane*

Significant interaction effects were observed on the translocation of heavy metal(loid)s from roots to shoots in *C. mopane* and different heavy metal(loid)s exhibited different translocation patterns (Table 5.1.4). The translocation factor (TF) of As and Mn was enhanced by mycorrhiza and compost amendments but not by FA. For Cu, the TF was reduced by AM in pure soil and soil with FA but increased in soil with compost. The TF of Ni and Zn were highest in soil with compost and AM. *C. mopane* grown in soils with FA amendments reduced the TF of Ni regardless of AM treatment. The TF of Zn in soil with FA was reduced with AM inoculation. The TF of Ni and Zn was not affected by AM in pure soils.

Table 5.1.4 Translocation factor of different heavy metal(loid)s in Mopane as affected by soil amendments and mycorrhizal inoculation¹

Treatments	TF (Shoot/Root Concentration Ratio)				
	As	Cu	Mn	Ni	Zn
Soil Alone					
-AM	0.32 b	0.09 c	1.27 b	0.30 b	0.56 b
+AM	0.59 a	0.16 b	1.96 a	0.32 b	0.72 b
Soil +10% FA					
-AM	0.24 b	0.26 a	1.63 ab	0.13 d	0.66 b
+AM	0.29 b	0.17 b	1.21 b	0.09 d	0.27 c
Soil + 10% Compost					
-AM	0.63 a	0.15 b	2.33 a	0.25 c	0.66 b
+AM	0.51 a	0.24 a	1.84 a	0.40 a	1.48 a
P-values					
Amend	0.022*	0.010*	0.024*	0.024*	0.020*
AM	0.046*	0.023*	0.020*	0.036*	0.038*
Amend x AM	0.050*	0.012*	0.016*	0.018*	0.026*

¹ Means followed by the same letter (s) are not significantly different from each other based on Tukey's Test at 5% level of significance. *=significant

5.1.3.4 Soil chemical properties and heavy metal availability

Table 5.1.5 indicates that fly ash makes the soil alkaline, and increases EC, whereas the introduction of mycorrhiza had little influence on pH and EC since there was no significant difference between treatments with +AM and -AM. The addition of compost slightly increased the pH and EC but when combined with mycorrhiza, only a small influence on pH and EC was observed. There was no significant difference due to AM in soils without other additional amendments.

Table 5.1.5 The pH and electrical conductivity of soil after seedling growth as influenced by different amendments and mycorrhiza¹

Treatments	pH	EC (µs/cm)
Soil Alone		
-AM	4.35 c	255.38 c
+AM	4.67 c	193.80 c
Soil +10% FA		
-AM	8.34 a	696.10 a
+AM	8.27 a	673.22 a
Soil + 10% Compost		
-AM	6.04 b	328.40 b
+AM	4.67 c	193.80 c
P-values		
Amend	0.000***	0.000***
AM	Ns	Ns
Amend x AM	0.000***	0.000***

¹ Means followed by the same letter (s) are not significantly different from each other based on Tukey's test at 5% level of significance. ***=very highly significant; ns=not significant

The concentration of heavy metal(loid)s in water-soluble (WS) fractions after seedling cultivation were significantly affected by the interaction of amendments and mycorrhiza (Table 5.1.6). Water soluble heavy metal(loid)s refers to metals that can easily dissolve in water and can be absorbed easily by plants (Kinuthia et al., 2020). Specifically, WS-As and WS-Mn were reduced by FA and compost and in part by AM in unamended soil. However, there is no significant difference between WS-As due to AM inoculation in soils

with FA and compost. The reduction of WS-As due to FA is much higher than due to compost. Water-soluble Cu and Ni were also reduced significantly by FA regardless of the presence of mycorrhiza. Compost amendments also reduced the WS-Cu and WS-Ni but the reduction was lower in magnitude compared to FA. Mycorrhiza also reduced the WS-Cu and WS-Ni in pure soil but it increased slightly in soil with compost when compared to its counterpart. The WS-Zn were reduced by FA but not by compost and mycorrhiza.

Table 5.1.6 Concentrations of heavy metal(loid)s in *C. mopane* extracted using water as influenced by different amendments¹

Treatments	Water Soluble Concentration of heavy metal(loid)s (mg kg ⁻¹)				
	As	Cu	Mn	Ni	Zn
Soil Alone					
-AM	3.18 a	3.51 a	0.19 a	1.65 a	0.13 a
+AM	2.91 b	1.93 b	0.11 b	0.81 b	0.10 a
Soil +10% FA					
-AM	1.70 c	0.06 d	0.01 c	0.00 c	0.03 b
+AM	1.37 c	0.09 d	0.01 c	0.00 c	0.03 b
Soil + 10% Compost					
-AM	2.49 b	0.23 c	0.15 a	0.05 c	0.08 a
+AM	2.91 b	1.93 b	0.11 b	0.81 b	0.10 a
P-values					
Amend	0.000***	0.002**	0.002**	0.032*	0.000***
AM	0.042*	0.042*	0.028*	0.022*	ns
Amend x AM	0.050*	0.003**	0.000***	0.012*	ns

¹ Means followed by the same letter (s) are not significantly different from each other based on Tukey's Test at 5% level of significance. *=significant; **=highly significant; ***=very highly significant; ns=not significant

5.1.4 Discussion

Mycorrhizal inoculant is capable of colonizing *C. mopane* roots in acidic soils with high heavy metal contents as shown by 32% root length colonized plant inoculated with AM in pure soil. The colonization was enhanced by FA and compost. Compost also contained viable mycorrhiza that successfully colonized the roots of *C. mopane* seedlings.

The survival of *C. mopane* seedlings in soils contaminated with heavy metal(loid)s without amendments and mycorrhiza was about 70% survival and the application of mycorrhiza, fly ash regardless of mycorrhiza and the combined application of compost and mycorrhiza improved the survival of *C. mopane* seedlings (Table 5.1.2). This result indicates that either the mycorrhiza application or FA and compost, and its combination would be beneficial on the establishment of *C. mopane* seedlings in this acidic soil with low nutrients and high heavy metal contents. Similarly, the growth and development of *C. mopane* seedlings in soil with high metal contents were substantially enhanced by compost amendments and mycorrhiza inoculation (Table 5.1.2). Fly ash amendments also enhanced the growth when compared with plants from pure soil but the magnitude of enhancement is inferior to those of compost combined with mycorrhiza. Mycorrhiza inoculation alone in pure soil improved the growth and development of *C. mopane* seedlings as compared to those without mycorrhiza but its growth was inferior compared to plants grown in soils with FA or compost, with or without mycorrhiza.

These results indicate that in acidic soils with low nutrients and high heavy metal contents, a synergistic effect of compost and mycorrhiza on the growth of *C. mopane* will occur. The enhancement of survival, growth, and development of *C. mopane* seedlings can be attributed to the different roles these organisms and amendments influenced the *C. mopane* seedlings and the properties of soils. First, as shown from the studies of Aransiola et al. (2019) and Nie et al. (2017), mycorrhiza in roots could enhance the plant's ability to uptake water, and essential nutrients and tolerate unfavorable environments through several mechanisms that include the secretion of secondary plant metabolites such as siderophores, organic acids (a precursor in the biosynthesis of secondary metabolites); biosurfactants, etc.

that will affect numerous biogeochemical processes in the rhizosphere and improve plant growth especially in a stressful environment such as those with high heavy metal(loid)s contents. Through cell extension and transformation of nutrient elements when they are either applied to seeds or incorporated into the soil, mycorrhiza can enhance the supply and absorption of nutrients of the host plants (Aransiola et al., 2019; Ultra et al., 2007). Second, amendments such as compost and FA can modify the soil chemical, biological and physical characteristics, making the soil less stressful to seedlings (Balasubramaniam 2020; Sharma and Singh, 2019). Compost can enhance the soil buffering capacity and neutralized acidity aside from its ability to supply essential nutrients (Table 5.1.4). The high microbial load of compost will also provide the necessary microbes that will help in the transformation of nutrients and hasten nutrient availability and consequently absorption by plants (Akmal et al., 2019).

The fly ash from Morupule Power Plant was alkaline (classified as Class C, generally having $\geq 15\%$ CaO) and can neutralize the acidity of soil substantially as compared to compost (Table 5.1.5). It also contains almost all the essential nutrients for plants such as P, S, K, Ca, Mg, Cu, Mn, and Zn, except for nitrogen. The increase of pH due to FA will decrease the acidity stress on *C. mopane* seedlings and would enhance survival. Acidic soil is very detrimental to seedlings for none acid-tolerant plants because it can cause dehydration, oxidative stress to roots, and reduced nutrient availability (Dos Reis et al., 2018). It is apparent that the enhanced survival, growth, and development of *C. mopane* seedlings due to the combined effects of mycorrhizal inoculation, FA or compost amendments is due to these favourable effects on soil and plant's ability to acquire water, nutrients and tolerate the environmental stress associated with acidity and high heavy metal(loid)s in soils.

However, results also showed that AM inoculation negatively impacted the dry matter yield of *C. mopane* in soils with FA as compared to *C. mopane* seedlings grown in soils with compost (Table 5.1.2). In FA amended soils, the absence of nitrogen could be the limiting factor for dry matter production in the presence of mycorrhiza. Under limiting nutrient conditions, a decrease in biomass of mycorrhizal plants indicates that much of the carbon saved was used for fungal biomass that supplies nutrients to the plants rather than using the photosynthates on plant biomass production (Smith, 2000). Jakobsen, Smith, and Smith (2003) indicated that in mycorrhizal plants under limiting nutrient conditions, the production of hyphae is much more economical in terms of organic C than the production of an equivalent length of roots thus reducing the root biomass. The low nitrogen in FA amended soil did not favor the synergistic enhancement of AM and FA on biomass yield of *C. mopane* seedlings.

In soils without amendments, the concentrations of As, Cu, Ni and Zn in shoots and roots of *C. mopane* were reduced by mycorrhiza (Table 5.1.3). The reduction of heavy metal in shoots could be attributed to the capacity of mycorrhizal roots to retain heavy metal by localization at the external mycelium of the arbuscular mycorrhiza, selective exclusion of toxic and nontoxic metals by adsorption onto chitinous cell wall structure, or onto extracellular glycoprotein called glomalin, or intracellular crystallization which is a very important mechanism in reducing the plant's exposure to potentially toxic metals (Shi et al., 2019). Shabani and Sabzalain (2016) showed that for both mycorrhizal and non-mycorrhizal plants, Ni concentrations in shoots and roots increased according to the addition of the metal into the soil, but inoculation with *F. mosseae* led to significantly lower Ni translocation from roots to the aboveground parts compared to non-inoculated plants. They have attributed the decline of Ni translocation to a high accumulation of ABC transporter and metallothionein

transcripts *F. mosseae* colonized plants. Decreased Ni concentrations in mycorrhizal plants were also attributed to biomass dilution effect due to increased dry matter production thus improving plant tolerance to Ni stress (Amir et al., 2013; Vivas et al., 2006). Alternatively, the reduction of As, Cu, Ni, and Zn in shoots and roots could be due to plant dilution effect because the translocation factor for these metals did not decrease but rather increased due to mycorrhizal inoculation in pure soil (Table 5.1.4). Vogel-Mikuš, Drobne, and Regvar (2005) showed that mycorrhiza colonized plants showed significantly improved nutrient, higher biomass, and decreased Cd and Zn uptake, especially at higher soil metal contents as a strategy for metal tolerance. On the contrary, Mn concentration in shoots and roots was increased by mycorrhiza inoculation similar to the findings of Moon and Aggangan (2018) that mycorrhiza could enhance Mn uptake in eucalyptus up to 90% based on the non-mycorrhizal plants. They attributed the increase of Mn uptake to the larger volume of roots that would facilitate uptake. Mardukhi et al. (2015) attributed the enhanced Mn uptake of mycorrhizal plants to increase of exudates capable of chelating Mn and facilitate absorption by plants.

Fly ash and compost reduced the concentrations of As, Cu, Ni and Zn in shoots and roots of *C. mopane* seedlings grown in heavy metal(loid)s contaminated soils. This reduction in concentrations in plant tissues could be attributed to the capacity of these amendments to neutralize the soil acidity thus decreasing the heavy metal availability (Tables 5.1.5 and 5.1.6). FA neutralized soil acidity from the initial pH 4.35 to about pH 8.2 which resulted in various chemical changes as reflected in the reduction of the concentration of water-soluble metals (Table 5.1.6). The reduction of heavy metal availability due to pH increase is due to the precipitation of cationic heavy metal(loid)s or increased adsorption of heavy

metal(loid)s on soil matrix (Lwin et al., 2018). Fly ash amendments reduced the concentration of water-soluble As, Cu, Mn, Ni, and Zn (Table 5.1.6) similar to the findings of Sitarz-Palczak and Kalembkiewicz (2012). The high concentration of soluble elements in FA as reflected by high EC (Table 5.1.5) could have contributed to the anions that could precipitate heavy metal(loid)s (Leelarungroj et al., 2018). The reduction in availability resulted in a lower concentration of heavy metal(loid)s in plant tissues because the uptake of micronutrients including heavy metal contaminants is deduced to be a function of the concentration in soil within the rooting zone (Kutrowska et al., 2017). Likewise, compost could also neutralize the acidic soil, but the neutralizing power is lower compared to FA (Table 5.1.4). The low concentration of heavy metal(loid)s in plant tissue of *C. mopane* grown in compost-amended soils could be attributed mainly to the enhanced fixation of heavy metal(loid)s through complexation with oxygen-containing functional groups of humic acids and fulvic acids from compost amendments (Ding et al., 2019). This is evident in the low concentration of water-soluble heavy metal(loid)s in the soil after harvest of *C. mopane* seedlings (Table 5.1.6). The humic acids that are associated with compost amendments had high sorption capacity for heavy metal(loid)s that potentially reduce heavy metal availability and reduce the toxic impacts on plants and soil microorganisms. Humic substances can decrease the contents of free metal ions due to their complexation with dissolved organic substances and adsorption by humic substances, which enter the solid soil phase or adsorption into the mineral phase (Barsova et al., 2019).

The Interaction effect of soil amendments and mycorrhiza Inoculation on heavy metal concentration in shoots and roots of *C. mopane* are metal-specific and could be due to complex processes affecting availability, root absorption, and translocation mechanisms

(Bonanno et al., 2018). The translocation factor (TF) of As, Cu and Mn were enhanced by mycorrhiza and compost amendments but not by FA indicate that aside from availability, this is also supported by Ultra and Manyiwa (2021), the presence of mycorrhiza and other factors will influence the TF of different metals in plants. For instance, As translocation in fern (*P. vittata*) was enhanced by mycorrhiza colonization which could be due to more efficient As translocation to the fronds or on As exclusion mechanisms exerted by AM fungi on the fern's root. Arbuscular mycorrhiza in *H. lanatus*, whose resistance to As was enhanced by AM fungi through suppression of the high-affinity phosphate/arsenate uptake system (Gonzalez-Chavez et al., 2002).

The ericoid mycorrhizal fungus *Hymenoscyphus ericae*, which took up arsenate, reduced it to arsenite, and extruded this latter outside is an exclusion mechanism that would also be reflected as high TF because this will lower the concentration in the roots. In contrast, the TF of Cu was reduced by AM while those of Ni and Zn were increased in pure soil; and FA increased the TF of Cu but decreased the TF of Ni only. The observed differences on the effect of amendments and mycorrhiza on the TF of different metals could be attributed to the following: (i) metal uptake is performed by the younger parts of the root where the Casparian strips are not yet fully developed (Marschner, 2000), and the presence of mycorrhiza could enhance these mechanisms as root extensions; (ii) the translocation of metals to the shoot is performed in the xylem and this translocation is promoted by transpiration of water via leaves. It will follow that plants that have enhanced shoot development will create higher root pressure and enhance the translocation of xylem sap to shoots, thus increasing the TF. (iii) the translocation is also promoted for some metal ions by cation exchange at the negative charges of the xylem vessel walls (Wolterbeek, 1987) that

will lead to a separation of metal transport from water flow in an ion-exchange column (xylem) that has the potential to impede the movement of metals upwards (Momoshima and Bondietti, 1990); different metals have different affinity on charged surfaces thus resulting to differences in the TF; (iv) in the xylem vessels the metals are translocated in different forms and species, i.e., Zinc as chelated to organic acids, Cu in complex with amino acids, Cd as a divalent ion, and Ni as a Ni-peptide complex (JoJoshi et al., 2019).

Therefore, the different patterns of TF of different heavy metal(loid)s observed in *C. mopane* are the result of these different interacting factors affecting the translocation of the specific metals, the physiological characteristics of the plant as affected by mycorrhiza and the availability of nutrients in soils. Also, the TF of As, Cu, Ni, Mn, and Zn was highest in soils amended with compost and AM could be the results of improved uptake efficiency at low external concentrations of heavy metal(loid)s due to compost application. For example, Cd exhibited high TF because of low metal concentration per absorption area, giving low competition between ions at the uptake sites while the opposite occurs at high concentrations (Tang et al., 2019). It showed that the bigger the root size, the more effective is the uptake. Increased biomass production enhances the uptake of elements; however, the accumulation in the tissue will be diminished due to dilution caused by biomass increase and the uptake rate is lower than the rate of biomass production (Ekvall and Greger 2003). Therefore, the combined application of compost and mycorrhiza which resulted in high biomass yield and low external concentration of heavy metal(loid)s in soils promoted high TF of heavy metal(loid)s in *C. mopane* seedlings.

Overall, this study had demonstrated that compost amendment together with mycorrhiza inoculation can enhance the survival and growth of *C. mopane* seedlings in heavy

metal-contaminated soils by reducing heavy metal availability and uptake by plants, thus enhancing the phytoremediation potential of *C. mopane* in mine tailings of Cu-Ni mine. Fly ash alone or in combination with mycorrhiza could enhance the phytoremediation potential of *C. mopane* provided that additional nitrogen source should be supplied to plants to maximize growth. The application of FA and compost is effective in lowering the water-soluble heavy metal(loid)s which is beneficial in preventing leaching of pollutants to groundwater.

5.1.5 References

- Akmal, M., Maqbool, Z., Khan, K.S., Hussain, Q., Ijaz, S.S., Iqbal, M., Aziz, I., Hussain, A., Abbas, M.S. and Rafa, H.U. (2019). Integrated use of biochar and compost to improve soil microbial activity, nutrient availability, and plant growth in arid soil. *Arab Journal of Geoscience* 12(7):232.
- Amir, H., Lagrange, A., Hassaine, N. and Cavaloc, Y. (2013). Arbuscular mycorrhizal fungi from New Caledonian ultramafic soils improve tolerance to nickel of endemic plant species. *Mycorrhiza* 23(7):585-95.
- Aransiola, S.A., Ijah, U.J., Abioye, O.P. and Bala, J.D. (2019). Microbial-aided phytoremediation of heavy metals contaminated soil: a review. *European Journal of Biological Research* 9(2):104-25.
- Ayangbenro, A.S. and Babalola, O.O. (2017). A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *International Journal of Environmental Research and Public Health* 14(1):94.

- Balasubramaniam, P. (2020). Fly Ash as a Source of Silicon for Mitigating Biotic Stress and Improving Yield and Changes in Biochemical Constituents and Silicon in Rice Under Abiotic Stress. *Circular Economy and Fly Ash Management* 2020, pp 145-160.
- Barsova, N., Motuzova, G., Kolchanova, K., Stepanov, A., Karpukhin, M., Minkina, T. and Mandzhieva, S. (2019). The effect of humic substances on Cu migration in the soil profile. *Chemical Ecology* 35(1):86-101.
- Bonanno, G., VymazaL, J. and Cirelli, G.L. (2018). Translocation, accumulation and bioindication of trace elements in wetland plants. *Science of the Total Environment* 631:252-61.
- Botswana Government Gazette. 2011. Environmental assessment act, (2011). No.10 of 2011.
- Brundrett, M.C. and Abbott, L.K. 1994. Mycorrhizal fungus propagules in the jarrah forest: I. Seasonal study of inoculum levels. *New Phytologist* 127(3):539-46.
- Castaldi, P., Santona, L. and Melis, P. (2005). Heavy metal immobilization by chemical amendments in a polluted soil and influence on white lupin growth. *Chemosphere* 60(3):365-71.
- Dhalaria, R., D. Kumar, H. Kumar, E. Nepovimova, K. Kuča, M. Torequl Islam, and R. Verma. (2020). Arbuscular mycorrhizal fungi as potential agents in ameliorating heavy metal stress in plants. *Agronomy* 10 (6):815.
- Ding, H., Tang, L., Nie, Y. & Ji, H. (2019). Characteristics and interactions of heavy metals with humic acid in gold mining area soil at a upstream of a metropolitan drinking water source. *Journal of Geochemical Exploration* 200:266-75,
- Dos Reis, A.R., Lisboa, L.A., Reis, H.P, de Queiroz Barcelos, J.P., Santos, E.F., Santini, J.M., Meyer-Sand, B.R., Putti, F.F., Galindo, F.S., Kaneko, F.H. and Barbosa, J.Z. (2018).

- Depicting the physiological and ultrastructural responses of soybean plants to Al stress conditions. *Plant Physiology and Biochemistry* 130:377-90.
- Ekosse, G. (2005). Heavy metals concentrations in the biophysical environment around the Ni-Cu mine and the smelter/concentrator plant, Selebi Phikwe, Botswana. *Global Journal of Environmental Science* 4(2):97-110.
- Ekvall, L. and Greger, M. (2003). Effects of environmental biomass-producing factors on Cd uptake in two Swedish ecotypes of *Pinus sylvestris*. *Environmental Pollution* 121(3):401-11.
- Farrell, M. and Jones, D.L. (2010). Use of composts in the remediation of heavy metal contaminated soil. *Journal of Hazardous Materials* 175 (1-3):575-82.
- Giovannetti, M and, Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*. 1:489-500.
- Gonzalez-Chavez, C., Harris, P.J., Dodd, J. and Meharg, A.A. (2002). Arbuscular mycorrhizal fungi confer enhanced arsenate resistance on *Holcus lanatus*. *New Phytologist* 155 (1):163-71.
- Han, S.H., Kim, D.H., Ultra, V.U., Kim, P. and Aggangan, N.S. (2012). Physiological response of Cd-and Pb-treated *Populus alba* × *glandulosa* seedlings amended with SCB liquid fertilizer. *Asia Life Science* 21(2):481-98.
- Huang, M., Zhu, Y., Li, Z., Huang, B., Luo, N., Liu, C. and Zeng, G. 2016. Compost as a soil amendment to remediate heavy metal-contaminated agricultural soil: mechanisms, efficacy, problems, and strategies. *Water Air Soil Pollution* 227(10):359.
- Hussain, F., Hussain, I., Khan, A.H., Muhammad, Y.S., Iqbal, M., Soja, G., Reichenauer, T.G. and Yousaf, S. (2018). Combined application of biochar, compost, and bacterial consortia

- with Italian ryegrass enhanced phytoremediation of petroleum hydrocarbon contaminated soil. *Environmental Experimental Botany* 153:80-8.
- Jakobsen, I., Smith, S.E. and Smith, F.A. (2003). Function and diversity of arbuscular mycorrhizae in carbon and mineral nutrition. In *Mycorrhizal Ecology*. Springer, Berlin, Heidelberg. Pp 75-92.
- Joshi, R., Dkhar, J., Singla-Pareek, S.L. and Pareek, A. (2019). Molecular Mechanism and Signaling Response of Heavy Metal Stress Tolerance in Plants. In *Plant-Metal Interactions*, 29-47p.
- Koitsiwe, K., and T. Adachi. (2017). Linkages between mining and non-mining sectors in Botswana. *Mineral Economics*. 30 (2):95–105.
- Krishnamoorthy, R., Venkatramanan, V., Senthilkumar, M., Anandham, R., Kumutha, K., & Sa, T. (2019). Management of heavy metal polluted soils: perspective of arbuscular mycorrhizal fungi. *Sustainable green technologies for environmental management*, 67-85.
- Kutrowska, A., Małecka, A., Piechalak, A., Masiakowski, W., Hanć, A., Barańkiewicz, D., Andrzejewska, B., Zbierska, J. and Tomaszewska, B. (2017). Effects of binary metal combinations on zinc, copper, cadmium and lead uptake and distribution in *Brassica juncea*. *Journal of Trace Elements in Medicine and Biology* 44:32-9.
- Lee, C.H., Lee, Y.B., Lee, H. and Kim, P.J. (2007). Reducing phosphorus release from paddy soils by a fly ash-gypsum mixture. *Bioresource Technology* 98(10):1980-4.
- Leelarungroj, K., Likitlersuang, S., Chompoorat, T. and Janjaroen, D. (2018). Leaching mechanisms of Heavy metals from fly ash stabilized soils. *Waste Management Research* 36(7):616-23.

- Letshwenyo, M. (2016). Sulphur and heavy metals contents in soils and *Grewia bicolor* leaves around the Selibe Pikwe Cu-Ni mine (BCL), Botswana. *Journal of Environmental Chemistry and Ecotoxicology*, 8(8), 73-81.
- Likuku, A.S., Mmolawa, K.B. and Gaboutloeloe, G.K. (2013). Assessment of heavy metal enrichment and degree of contamination around the copper-nickel mine in the Selebi Phikwe Region, Eastern Botswana. *Environmental Ecology Research* 1(2):32-40.
- Lwin, C.S., Seo, B.H., Kim, H.U., Owens, G. and Kim, K.R. (2018). Application of soil amendments to contaminated soils for heavy metal immobilization and improved soil quality—a critical review. *Soil Science Plant Nutrition* 64(2):156-67.
- Mapaure, I. (1994). The distribution of *Colophospermum mopane* (Leguminosae-caesalpinioideae) in Africa. *Kirkia* 1:1-5.
- Mardukhi, B., Rejali, F., Daei, G., Ardakani, M.R., Malakouti, M.J. and Miransari, M. (2015). Mineral uptake of mycorrhizal wheat (*Triticum aestivum* L.) under salinity stress. *Communications in Soil Science and Plant Analysis* 46(3):343-57.
- Marschner, H.O. (2000). Mineral nutrient acquisition in nonmycorrhizal and mycorrhizal plants. *Phyton-Horn* 36:61-8.
- Momoshima, N. and Bondietti, E.A. (1990). Cation binding in wood: applications to understanding historical changes in divalent cation availability to red spruce. *Canadian Journal of Forest Research* 20(12):1840-9.
- Moon, H. K., & Aggangan, N. S. (2018). Growth and mineral uptake of *Eucalyptus pellita* in response to inoculation with different mycorrhizal inoculants. *Clonal Trees in the Bioeconomy Age: Opportunities and Challenges*. September, 10.

- Moreki, J.C., Woods, T.O & Nthoiwa, P.G. (2013). Estimation of the concentration of heavy metals in forages harvested around Dibete area, Botswana. *International Journal of Innovation and Research in Science Engineering, Technology* 2(8):3060-4071.
- Motswaiso, F. S., Nakamura, K., Watanabe, N., & Komai, T. (2019). Geochemical investigation of metals and trace elements around the abandoned Cu-Ni mine site in Selibe Phikwe, Botswana. *Journal of Geoscience and Environment Protection*, 7(5), 275-293.
- Nie, H., Nie, M., Xiao, T., Wang, Y. & Tian, X. (2017). Hexadecane degradation of *Pseudomonas aeruginosa* NY3 promoted by glutaric acid. *Science of Total Environment* 575:1423–1428.
- Pandey, V.C. and Singh, N. (2010). Impact of fly ash incorporation in soil systems. *Agriculture Ecology and Environment* 136(1-2):16-27.
- Pathan, S.M., Aylmore, L.A. & Colmer, T.D. (2003). Properties of several fly ash materials in relation to use as soil amendments. *Journal of Environmental Quality* 32(2):687-93.
- Petruzzelli, G. (1996). Heavy metals in compost and their effect on soil quality. In: de Bertoldi M., Lemmes B., Papi T. (eds,). *The science of composting*, 213–23. Dordrecht: Springer.
- Riaz, M., Kamran, M., Fang, Y., Wang, Q., Cao, H., Yang, G., ... & Wang, X. (2021). Arbuscular mycorrhizal fungi-induced mitigation of heavy metal phytotoxicity in metal contaminated soils: A critical review. *Journal of Hazardous Materials*, 402, 123919.
- Saum, L., Jiménez, M.B. & Crowley, D. (2018). Influence of biochar and compost on phytoremediation of oil-contaminated soil. *International Journal of Phytoremediation* 20(1):54-60.

- Shabani, L. & Sabzalian, M.R. (2016). Arbuscular mycorrhiza affects nickel translocation and expression of ABC transporter and metallothionein genes in *Festuca arundinacea*. *Mycorrhiza* 26(1):67-76.
- Sharma, B. & Singh, R.P. (2019). Physiological, Biochemical, Growth, and Yield Responses of Radish (*Raphanus sativus* L.) Plants Grown on Different Sewage Sludge–Fly Ash Mixture (SLASH) Ratios. In *Waste Valorisation and Recycling*. Springer, Singapore. pp. 539-552.
- Sharma, P. & Pandey, S. (2014). Status of phytoremediation in world scenario. *International Journal of Environmental Bioremediation and Biodegradation* 2(4):178-91.
- Shi, W., Zhang, Y., Chen, S., Polle, A., Rennenberg, H. & Luo, Z.B. (2019). Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants. *Plant Cell Environment* 42(4):1087-103.
- Singh, J.S. & Pandey, V.C. (2013). Fly ash application in nutrient poor agriculture soils: impact on methanotrophs population dynamics and paddy yields. *Ecotoxicology and Environmental Safety* 89:43-51.
- Sitarz-Palczak, E. & Kalembkiewicz, J. (2012). Study of remediation of soil contaminated with heavy metals by coal fly ash. *Journal of Environmental Protection* 3(10):1373.
- Smith, F.A. (2000). Measuring the influence of mycorrhizas. *The New Phytologist* 148(1):1-6.
- Tang, Y., Shi, L., Zhong, K., Shen, Z. and Chen, Y. (2019). Ectomycorrhizal fungi may not act as a barrier inhibiting host plant absorption of Heavy metals. *Chemosphere* 215:115-23.

- Timberlake, J., Chidumayo, E., & Sawadogo, L. (2010). Distribution and characteristics of African dry forests and woodlands. In *The dry forests and woodlands of Africa* (pp. 11-41). Routledge.
- Ultra, V. U. (2020). Fly ash and compost amendments and mycorrhizal inoculation enhanced the survival and growth of *Delonix regia* in Cu-Ni mine tailings. *Philippine Journal of Science*, 149 (3):479–89.
- Ultra Jr, V. U., Nunez, J. P., & Lee, S. C. (2016). Influence of charcoal-based soil amendments on growth and nutrient uptake of rice (*Oryza sativa*) in Cadmium contaminated soil. *Emirates Journal of Food and Agriculture*, 872-881.
- Ultra Jr, V. U., Tanaka, S., Sakurai, K., & Iwasaki, K. (2007). Arbuscular mycorrhizal fungus (*Glomus aggregatum*) influences biotransformation of arsenic in the rhizosphere of sunflower (*Helianthus annuus* L.). *Soil Science and Plant Nutrition*, 53(4), 499-508.
- Ultra, V. U., and T. Manyiwa. 2021. Influence of mycorrhiza and fly ash on the survival, growth and heavy metal accumulation in three acacia species grown in Cu–Ni mine soil. *Environmental Geochemistry and Health* 43:1337–53.
- Vivas, A., Biró, B., Németh, T., Barea, J. M., & Azcón, R. (2006). Nickel-tolerant *Brevibacillus brevis* and arbuscular mycorrhizal fungus can reduce metal acquisition and nickel toxicity effects in plant growing in nickel supplemented soil. *Soil Biology and Biochemistry*, 38(9), 2694-2704.
- Vogel, H., & Kasper, B. (2002). Mine soils on abandoned gold mine tailings in Francistown. Report by the Bundesanstalt für Geowissenschaften und Rohstoffe and Department of Geological Survey (Environmental Geology Division). Lobatse, Botswana.

- Vogel-Mikuš, K., Drobne, D. and Regvar, M. (2005). Zn, Cd and Pb accumulation and arbuscular mycorrhizal colonisation of pennycress *Thlaspi praecox* Wulf (Brassicaceae) from the vicinity of a lead mine and smelter in Slovenia. *Environmental Pollution* 133(2):233-42.
- Wolterbeek, H.T. (1987). Cation exchange in isolated xylem cell walls of tomato. I. Cd^{2+} and Rb^{+} exchange in adsorption experiments. *Plant Cell Environment* 10(1):39-44.
- Zhai, M., Totolo, O., Modisi, M.P., Finkelman, R.B., Kelesitse, S.M. and Menyatso, M. (2009). Heavy metal distribution in soils near Palapye, Botswana: an evaluation of the environmental impact of coal mining and combustion on soils in a semi-arid region. *Environmental Geochemistry and Health* 3

5.2 Influence of mycorrhiza and fly ash on the survival, growth and Heavy metal(loid)s accumulation in three Acacia species grown in Cu-Ni mine soil

Abstract

Acacia albida, *Acacia luederitzii* and *Acacia tortilis* are dominant acacia species in Botswana and have the potential to rehabilitate the heavy metal degraded environment. To establish this claim, experiments to assess the influence of mycorrhizal inoculation and fly ash amendments on the survival, growth and heavy metal accumulation of these species in mine tailings were conducted. A two-factor (AM inoculation x fly ash) in CRD was done on each of the three Acacia species consisting of four treatments: control, (no mycorrhizal, no fly ash added as -AM/-FA), with mycorrhizal but no fly ash (+AM/-FA), no mycorrhizal but with fly ash (-AM/+FA), and with mycorrhizal and with fly ash (+AM/+FA). After 24 weeks, results showed that the survival and dry matter yield of all Acacia species were enhanced by 10% fly ash amendments. However, mycorrhiza inoculation alone improved the survival of *A. albida* and *A. luederitzii* but reduced that of the *A. tortilis* in mine tailings. Fly ash amendments increased the pH of the mine tailings, reduced the availability of Cu, Ni, Pb, Mn and Zn, and consequently reduced the concentration of these metals in shoots. FA also increased the availability of As in the mine tailings. In addition, mycorrhizal inoculation reduced the concentration of these metals in shoots regardless of fly ash amendments. Overall, combined mycorrhizal inoculation and FA amendment enhanced the establishment of *A. luederitzii* in heavy metal-contaminated soils by reducing the heavy metal availability and metal uptake; thus, increasing the survival and dry matter yield of plants.

Keywords: *Acacia albida*, *Acacia luederitzii*, *Acacia tortilis*, heavy metal accumulation, mine tailings, heavy metals, bioremediation, contaminants, accumulation

5.2.1 Introduction

Resource-based economy and industrialization have resulted in various cases of pollution and contamination problems in the environment. The very common one is heavy metal contamination due to mining activities and water pollution from industries, which threaten human health and the ecosystem. Several technologies that are available entail high establishment and maintenance cost; hence, they are considered unsustainable to prevent further damage caused by pollution (Festin et al., 2019). On the other hand, phytoremediation, the use of plants to remediate a polluted environment is considered effective, environment-friendly and economically feasible procedure that will improve the overall soil health compared to other techniques (Hao et al., 2011; Burges et al., 2018). Existing phytoremediation technology used heavy metal hyperaccumulator species for the reclamation of metal-contaminated soils through phytoextraction, phytostabilization, phytovolatilization, and rhizofiltration. In developed countries, phytoremediation is already an integral component in the rehabilitation of mining areas (Koptsik, 2014), whereas in developing countries like Botswana, there is an urgent need to develop site-specific rehabilitation strategies for the abandoned and active mine areas where phytoremediation is the best option. Hence it is important to conduct research and development activities that will address the specific needs for the remediation and rehabilitation of several abandoned mine areas and provide an effective waste management strategy for existing operational mining facilities in Botswana.

Although the current practice of mining activities in Botswana are geared toward environmentally sound and sustainable operations, there are existing problems brought about by mining and smelting activities that should be addressed. Specifically, the rate of

mining activities in several mine sites in the country resulted to a corresponding increase in the tailings and other contaminants that are considered threats to the environment. Most strategies employed by mining companies are physical containment of mine waste, which are considered less stable and temporary, and need high operational cost (Liphadzi et al., 2005). In order to restore these mining-degraded areas into an ecologically stable ecosystem, long-term solution is needed; hence, phytoremediation.

The efficiency and effectiveness of phytoremediation, particularly phytoextraction is a function of the plant type and density on contaminated site; concentration, extent, and availability of pollutants; and soil and environmental condition which vary with location (Mahar et al., 2016). Variability of the physicochemical properties of contaminated sites will entail a unique approach for phytoremediation to become successful (Chileshe et al., 2019). Current technological advances use chelating agents to improve heavy metal availability and plant uptake (Ultra et al., 2005); genetically modified plants with high biomass and HM hyperaccumulating capabilities (Ibañez et al., 2016), soil amendments that reduce the metal availability and enhance the growth of plants, and recently, inoculation of beneficial soil microorganisms (Kong and Glick, 2017; Ultra et al., 2007).

The use of microorganisms associated with plants opens new prospects for phytoremediation technologies (Nie et al., 2011). The ability of microorganisms, particularly mycorrhiza to produce various metabolites (siderophores, organic acids, biosurfactants, etc.) that will affect numerous biogeochemical processes in the rhizosphere have been shown in some phytoremediation studies (Aransiola et al., 2019; Nie et al., 2011). Mycorrhiza can enhance the supply and absorption of nutrients through cell extension, detoxication of metals, and a decrease of abiotic stress in plants, production of

phytohormones and siderophores, and transformation of nutrient elements when they are either applied to seeds or incorporated into the soil. Mycorrhiza could accelerate the mobilization or immobilization of metals through the release of chelating agents, acidification, phosphate solubilization, induce metalloid transformation and redox changes (Motaharpoor et al., 2019, Hahl et al., 2018, Ultra et al., 2007, Gonzalez-Chavez et al., 2002). These functions are of great importance in the metal absorption by plants. Therefore, the plant-microbe interaction should be harnessed to improve the phytoremediation efficiency through modification of metal accumulation patterns (translocation) in plant tissues and stimulating their productivity, with emphasis on the limitations of a semi-arid environment.

Coal fly ash is being utilized as soil amendments because of its favorable physicochemical properties including fine-sized particles, low bulk density, higher water-holding capacity, favorable pH (alkaline), and source of essential plant nutrients (Pandey and Singh, 2010). Fly ash is also used as a liming material and improves the soil buffering capacity but is dependent on the chemical characteristics on the material. For example, Morupule Power Plant fly ash is classified as Class C (generally having $\geq 15\%$ CaO) and could have high ability to raise pH. Aside from ameliorating the soil pH, fly ash also contains some useful nutrients, such as P, S, K, Ca, Mg, Cu, Mn, and Zn, which are beneficial for plant growth. Other benefits include modifying the soil texture, enhancing soil structure formation which ultimately reduces bulk density and improves aeration, percolation, and water retention. Some findings also showed the ability of fly ash to decrease the mobility and availability of metals in the soil due to the alkaline nature of fly ash (Shaheen et al., 2014). On the other hand, several harmful effects of fly ash had been reported also with low availability of associated nutrient because the elements present are occluded in the mineral matter, excess

salinity, low N contents and sometimes the presence of heavy metal(loid)s that may contaminate the soil. Several studies have been carried out to blend fly ash with varieties of organic and inorganic materials in ameliorating problem soils in agriculture applications. These include co-application of acid-forming organic material such as sewage sludge, poultry and animal manure (Belyaeva and Haynes, 2012) and other organic products. Co-application of fly ash with these materials resulted in greater advantages and higher efficiency in the overall improvement of soil health and increasing crop yield.

In this study, the potential use of fly ash from Morupule B power plant in Botswana is being evaluated as a soil amendment in combination with mycorrhizal inoculation on different *Acacia* (*Vachellia*) species as candidate plants for rehabilitation of mine soils and mine tailings of Bamangwato Concessions Limited (BCL) –Cu-Ni mine in Selebi-Phikwe Botswana. The mine is located geographically between longitudes 27° 47' East and 27° 53' East, and latitude 22° 55' South 22° 00' South. BCL mining operation started in 1963 for Cu and Ni; and produced the tailings dam of approximately 50 m high covering an area of about 1.2 km². The tailings dump contained sulfur-rich minerals producing acid mine drainage and of high risk for contaminating the immediate surrounding environment. This Cu – Ni mine is currently under liquidation stage and needs immediate solutions to treat mine waste generated during its operation.

The combined application of fly ash and mycorrhiza inoculant are deemed to influence heavy metal availability and uptake, and the translocation of metals from soil to plants (estimated as the concentration of metals in shoots divided by the concentration in roots). Therefore, this study evaluated the combined application of mycorrhiza and fly ash on the growth and survival of different *Acacia* species to contribute to finding solutions to

rehabilitate the negative impacts of mining operations to human and environmental health. Specific objectives were the following: to evaluate the effect of mycorrhiza inoculation and fly ash amendments on survival, growth and heavy metal uptake of seedlings of different plants species grown in the mine tailings from BCL mines; and to evaluate the effect of mycorrhiza inoculation and fly ash amendments on the chemical properties and heavy metal availability in the mine tailings from BCL mines.

5.2.2 Materials and methods

5.2.2.1 Soil and fly ash sampling and pot preparation

The mine tailings used in this experiment was collected from the tailings dump of the BCL mine in Selebi-Phikwe, Botswana. After air-drying, tailings for cultivation and chemical analysis were passed through 5 mm and 2 mm mesh sieves, respectively. The properties and the heavy metal contents of the tailings are presented in Table 5.2.1. The tailings are highly acidic, sandy loam with very low organic matter and nutrient content and have high amounts of Cu, Ni, Pb and As. Fly ash was collected from dumpsite of the Morupule B Power Plant Station, Palapye, Botswana. The Morupule B Power Plant has 33 MW coal-fired units, with coal supplied from the adjacent Morupule Colliery and operated by the Botswana Power Corporation providing 90% of the country's domestic power generation (Zhai et al., 2009). Morupule B generates 540 000 tons per annum of fly ash that are deposited in the dumpsite adjacent to the power plant. Collected fly ash was air dried and sieved using 2mm diameter mesh prior to application. The chemical properties of fly ash are also presented in Table 5.2.1. Plastic pots (25 cm diameter x 30 cm height) were filled with 8 kg tailings or a mixture of tailings + fly ash before treatment assignments and plant establishment.

Table 5.2.1 Chemical properties and heavy metal contents in mine tailings and fly ash.

Properties	Mine Tailings	Fly Ash	
pH (1:2, soil:water)	4.74	8.80	
Electrical Conductivity (1:2, soil:water, $\mu\text{S cm}^{-1}$)	1033.54	2290.00	
% Organic Matter (Walkley-Black, 1957)	0.655	0.015	
Total N (Kjeldahl distillation-acid titration)	36.42	0.78	
Particle Size Distribution (Laser diffraction)			
%Sand			
%Silt	65.92	21.05	
%Clay	31.29	45.25	
	2.79	33.70	
Metal(loid)s in Mine Tailings (mg kg^{-1})	Total	Water Soluble	1 N Ammonium Acetate Exch
As	154.31	1.81	2.95
Cu	778.23	4.05	10.71
Mn	799.08	5.73	8.60
Ni	656.06	15.24	25.40
Pb	452.81	16.69	9.11
Zn	17.27	0.18	0.25
Metal(loid)s in Fly Ash (mg kg^{-1})	Total	Water Soluble	Ammonium Acetate Exch
As	10.08	1.23	4.73
Cu	63.20	0.07	0.13
Mn	396.71	0.04	0.57
Ni	78.25	0.01	0.10
Pb	119.33	0.88	2.10
Zn	25.31	0.04	0.21

5.2.2.2 Description of the treatments and plant culture

Three acacia species (*A. albida*, *A. luederitzii*, and *A. tortilis*) were subjected to two levels of fly ash application (0% FA and 10% FA by weight) and two levels of arbuscular mycorrhiza inoculation (-AM and +AM), comprising four-treatment combinations for each plant species. Each treatment was designated as control-no mycorrhizal, no fly ash (-AM/-FA), with mycorrhizal but no fly ash (+AM/-FA), no mycorrhizal but with Fly Ash (-AM/+FA), and with mycorrhizal and with fly ash (+AM/+FA). For treatments with FA, an equivalent to 10% of the total weight of tailings was mixed thoroughly before potting. The seeds of the acacia species were collected from identified trees in the vicinity of the university. The seeds were scarified using concentrated sulfuric acid for 1 hour, then washed ten times with sterile

distilled water and germinated on sterile Petri plates lined with moistened filter paper. The post-germinated seeds were then planted at the center of the pots at 3 seedlings per pot and was maintained at a moisture content of 40% of the field capacity under shade house conditions. For the AM treatments, five grams of sterile (121°C, 30 min for three consecutive days) or non-sterile AM inoculants (MYKOVAM, National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Banos, Philippines) were mixed with the soil at the center of pot just below the roots of the seedlings of the –AM and +AM treatments, respectively. Five replicates were used for each treatment laid out in a complete randomized design per species. The pots in each group (species) were rearranged every week throughout the experimental period. The plants were irrigated regularly and monitored for survival and growth inside the shade house (80% exposure).

5.2.2.3 Plant sampling and chemical analysis

During the 24-week cultivation period, plants were periodically monitored for survival and growth. After cultivation, the shoots were excised and the roots were carefully separated from the growing media, and their fresh weights were determined. A portion of the fresh roots, 0.2g from each plant was subjected to trypan blue staining for microscopic examination of mycorrhizal infection (Brundrett and Abbott, 1994). The root length colonized by AMF was quantified using the gridline intersection method (Giovenetti and Mosse, 1980). The remaining roots and all the shoots were oven-dried at 70°C for 3 days, dry weights were recorded and ground for chemical analysis. The tissues were digested using a nitric acid and hydrogen peroxide mixture in an open vessel at 120°C for a minimum of 4 hours and analyzed for heavy metal(lloid)s using Atomic Absorption Spectrometer (AAS,

Analytik Jena ContraAA800, Denmark). To verify the accuracy of the heavy metal contents, a representative sample which was randomly drawn from the whole experimental samples were submitted for analysis at the Analytical Service Laboratory, University of Botswana. Variation in the values obtained between the two laboratories is less than 6%. Translocation factor for each metal was calculated as a ratio of the shoot divided by the root concentration.

5.2.2.4 Soil sampling and chemical analysis

After the plant roots were sampled, the soil (tailings or tailings +fly ash) from each pot were collected and passed through a 2 mm sieve. The soil was air dried and stored at 4°C until chemical analysis. Soil pH and electric conductivity (EC) were measured in water suspension (1:5) after shaking for 1 hour. Total heavy metal content was measured after wet acid digestion in an open vessel at 120 °C for 6 hours using HNO₃: HCl mixture (1:3) in a hot plate followed by quantification using AAS. The available fraction of heavy metal in soil was assessed by determining the water-soluble and ammonium acetate exchangeable. Soil samples were shaken with distilled water (1:100) for 2 h at room temperature to obtain a water-soluble (WS) fraction. The exchangeable fraction (Exch) was determined using 1N ammonium acetate (pH 7) at 1:100 ratio for 2 hours at room temperature. The WS and the Exch. fractions were analyzed for different heavy metal(lloid)s using AAS. Samples were analyzed in duplicates.

5.2.2.5 Statistical Analysis

The data were analyzed using a 2-factor ANOVA in CRD. When significant differences were observed, Tukey's test was performed for treatment mean comparison at 5% level of significance. Each parameter was analyzed separately per plant species.

5.2.3 Results

5.2.3.1 Survival and Dry Matter Yield

After 24 weeks, the survival of different Acacia species in mine tailings was significantly influenced by fly ash amendments and mycorrhiza inoculation (Table 5.2.2). Fly-ash amendments, regardless of mycorrhiza inoculation, improved the survival of the three Acacia species. On the other hand, mycorrhiza inoculation improved the survival of *A. albida* and *A. luederitzii* but reduced the survival of *A. tortilis* in mine tailings without Fly-ash.

Table 5.2.2 Percentage survival of different plant species in mine tailings from BCL mines as influenced by mycorrhiza inoculation and fly ash amendment¹

Treatments	<i>A. albida</i> (n=20)	<i>A. luederitzii</i> (n=20)	<i>A. tortilis</i> (n=20)
Mine Tailings No Fly Ash			
-AM	70b	70b	100a
+AM	90a	100a	30b
Mine Tailings with 10% Fly Ash			
-AM	100a	100a	100a
+AM	100a	100a	100a
p-Value			
FA	0.048*	0.062ns	0.083ns
AM	0.044*	0.052ns	0.058ns
FA x AM	0.042*	0.039*	0.048*

¹Means within the same column (plant species) followed by the same letter(s) are not significantly different from each other at 5% level of significance based on Tukey's test. * = significant; ns=nonsignificant.

The values of shoot dry weight of three *Acacia* species were significantly increased by fly ash amendments regardless of mycorrhizal inoculation. In contrast, mycorrhiza inoculation resulted in variable effects among the species (Table 5.2.3). In *A. albida*, mycorrhizal inoculation significantly reduced the shoot dry weight in pure mine tailings but not in the roots and the total dry weight of the same plant. There was no significant effect on the dry matter yield due to mycorrhizal inoculation in mine tailings with 10% fly ash. *A. tortilis* responded negatively to mycorrhiza inoculation in pure mine tailings but increased the shoots and the roots' dry matter yield to almost double (based on the control) under the fly ash amendments. In contrast, the shoots' dry weight was significantly reduced for mycorrhizal inoculation of *A. luederitzii*. However, the total dry matter yield of *A. luederitzii* was increased in mycorrhiza inoculation both for pure mine tailings and fly ash amendments. Among the three-acacia species, *A. luederitzii* produced the highest yield across all treatments.

Table 5.2.3. Shoots, roots and total dry weight of *Acacia* spp. grown in mine tailings as affected by mycorrhiza inoculation and fly ash amendment at 24 weeks after planting¹

Treatments	Dry Matter Yield (mg plant ⁻¹)								
	<i>A. albida</i> (n=20)			<i>A. luederitzii</i> (n=20)			<i>A. tortilis</i> (n=20)		
	Shoots	Roots	Total	Shoots	Roots	Total	Shoots	Roots	Total
Mine Tailings No Fly Ash									
-AM	8.12b	51.98b	60.09b	17.8d	58.6d	76.4d	9.49b	6.18b	15.68b
+AM	5.28c	62.28b	67.56b	27.4c	94.2c	121.6c	5.68c	4.83b	10.51c
Mine Tailings with 10% Fly Ash									
-AM	93.02a	185.26a	278.28a	286.8b	344.1b	630.9b	10.54 b	5.64b	16.18b
+AM	89.56a	173.51a	263.08a	326.3a	400.8a	727.0a	24.43 a	9.48 a	33.91a
<i>P</i> -values									
FA	0.042*	0.032*	0.038*	0.018*	0.022*	0.012*	0.036*	0.083ns	0.014ns
AM	0.048*	0.142ns	0.202ns	0.024*	0.036*	0.022*	0.028*	0.046*	0.026*
FA x AM	0.032*	0.132ns	0.186ns	0.012*	0.002**	0.024*	0.016*	0.048*	0.032*

¹For each plant species, means within the same column followed by the same letter (s) are not significantly different from each other at 5% level of significance based on Tukey's test. ** = highly significant; ns = non-significant

5.2.3.2 Mycorrhizal Infection

All plants from mine tailings and those grown in contaminated mine tailings with 10% fly ash amendments without mycorrhizal inoculation had zero roots colonized by mycorrhiza, while those inoculated with AM, a significantly higher colonization rate was observed on the different Acacia species grown in mine tailings with 10% fly ash (Table 5.2.4). Among the different species, the colonization rate was observed to be in the order: *A. tortilis* > *A. luederitzii* > *A. albida*. Ocular inspection of the roots also showed nodule formation in *A. luederitzii*, but not in *A. tortilis* and *A. albida*.

Table 5.2.4 Root colonization of different acacia species with arbuscular mycorrhiza grown in mine tailings as affected by mycorrhiza inoculation and fly ash amendment at 24 weeks after planting¹.

Treatments	Root Colonized (% root length colonized)		
	<i>A. albida</i> (n=20)	<i>A. luederitzii</i> (n=20)	<i>A. tortilis</i> (n=20)
Mine Tailings no fly ash			
-AM	ND	ND	ND
+AM	12b	18b	28b
Mine Tailings with 10% fly ash			
-AM	ND	ND	ND
+AM	32a	42a	52a
<i>P</i> -value			
FA	0.183ns	0.083ns	0.093ns
AM	0.000**	0.000**	0.000**
FA x AM	0.000**	0.000**	0.000**

¹Means within the same column (plant species) followed by the same letter(s) are not significantly different from each other at 5% level of significance based on Tukey's test. ND=not detected; ** = highly significant; ns = non-significant

5.2.3.3 Concentration of Heavy Metal(loid)s in Plants

Different species resulted in different patterns of heavy metal concentration in shoots and roots (Tables 5.2.5-5.2.7). Depending on the type of metal and plant species, mycorrhizal inoculation and fly ash amendments could either increase or decrease the metal concentration in the tissues. The concentration of heavy metal(loid)s in the shoots and roots

of *A. albida* (Table 5.2.5) grown in pure mine tailings without mycorrhiza inoculation in the was in this order: Cu > Pb > Ni > Zn > Mn > As.

The As concentrations in shoots were reduced significantly by fly ash amendments and such reduction was enhanced by mycorrhiza inoculation. Mycorrhizal inoculation alone also reduced the As uptake by 65% compared to the control. On the other hand, mycorrhizal inoculation reduced the shoot and root concentrations of Cu, Mn, and Ni in pure mine tailings, but fly ash amendments reduced the Cu, Mn, and Ni concentrations more than the reduction due to mycorrhizal inoculation. The reduction in Cu due to the combined application of fly ash and mycorrhiza was 96% in shoots and 80% in roots; 69% in shoots and 50% in roots for Mn; and 96% in shoots and 91% in roots for Ni. In contrast, the concentration of Pb was increased due to fly ash amendment by 2x in shoots and 4.6x in roots, while mycorrhizal inoculation reduced the Pb concentration by 61% in the shoot and 63% in roots as compared to its uninoculated counterpart. Fly ash amendments increased the Zn concentration in shoots and roots, but such an increase was countered by the presence of mycorrhiza. The % reduction of Zn in shoots due to mycorrhiza was about 88% in fly ash amended treatment and 46% in pure mine tailings. In *A. luederitzii*, the maximum shoot and root concentrations of heavy metal(loid)s were in the order: Cu > Pb > Ni > As > Zn > Mn in pure mine tailings without mycorrhizal inoculation (Table 5.2.6). As, Cu, Ni, and Zn concentrations in shoots and roots were reduced significantly by fly ash amendments, and such reduction was enhanced by mycorrhiza inoculation. The reduction in As due to the combined application of fly ash and mycorrhiza was 46% in shoots and 49% in roots; 97% in shoots and 95% in roots for Cu; 97% in shoots and 96% in roots for Ni; 10% in shoots and 69% in roots for Pb and 37% in shoots; and 82% in roots for Zn. The shoot concentration for Mn was enhanced

by fly ash amendments, but mycorrhiza inoculation reduced the Mn concentration both in the shoots and the roots.

Influence of Different Soil Amendments on Heavy Metal(loid)s Uptake, Survival and Phytoremediation Efficiency of Selected Tree Species

Table 5.2.5. Heavy metal(loid)s concentrations in shoot and roots of *A. albida* (n=20) grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 24 weeks after planting¹

Treatments	Heavy Metal(loid)s Concentration (mg kg ⁻¹)											
	As		Cu		Mn		Ni		Pb		Zn	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Mine Tailings No Fly Ash												
-AM	55.42a	53.28a	383.23a	336.87a	83.59a	73.48a	219.08a	194.66a	276.48b	245.26b	102.80b	90.9 b
+AM	42.11b	69.96b	138.08b	126.82b	61.71b	56.29b	78.55b	72.19b	168.44c	154.17c	55.31c	50.58c
Mine Tailings with 10% Fly Ash												
-AM	34.13c	67.20b	11.79c	51.53c	32.91c	48.99c	6.49c	17.52c	590.87a	1061.84a	268.94a	267.69a
+AM	16.54d	75.35b	16.55c	66.19c	26.22d	36.65d	9.37c	17.99c	566.47a	1121.29a	32.75d	80.39b
P-values												
FA	0.012*	0.062ns	0.038*	0.028*	0.032*	0.022*	0.032*	0.023*	0.014*	0.002**	0.022*	0.032*
AM	0.038*	0.042*	0.028*	0.024*	0.032*	0.042*	0.022*	0.046*	0.036*	0.006**	0.026*	0.018*
FA x AM	0.034*	0.032*	0.036*	0.022*	0.012*	0.020*	0.018*	0.048*	0.032*	0.002**	0.014*	0.018*

¹For each heavy metal, means within the same column followed by the same letter(s) are not significantly different from each other at 5% level of significance based on Tukey's test. ** = highly significant; ns = non-significant

Table 5.2.6. Heavy metal(loid)s concentrations in shoot and roots of *A. luederitzii* (n=20) grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 24 weeks after planting¹.

Treatments	Metal(loid)s Concentration (mg kg ⁻¹)											
	As		Cu		Mn		Ni		Pb		Zn	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Mine Tailings No Fly Ash												
-AM	39.44a	96.10a	445.90a	1212.2a	19.40c	52.37b	93.11a	265.1a	322.8a	918.6a	24.70a	70.31a
+AM	22.31c	65.19c	256.76b	759.25b	15.39c	46.64b	54.88b	173.3b	167.5b	562.15b	14.31b	46.42b
Mine Tailings with 10% Fly Ash												
-AM	32.25b	84.44b	157.27c	226.40c	130.54a	162.06a	30.54c	123.6c	240.0a	312.83c	29.43a	47.79b
+AM	21.27c	48.85d	15.52d	61.69d	47.74b	35.24b	2.82d	10.5d	290.0a	287.76c	15.52b	12.53c
P-values												
FA	0.042*	0.032*	0.038*	0.018*	0.022*	0.012*	0.036*	0.083ns	0.014ns	0.022*	0.012*	0.036*
AM	0.048*	0.142ns	0.202ns	0.024*	0.036*	0.022*	0.028*	0.046*	0.026*	0.036*	0.022*	0.028*
FA x AM	0.032*	0.132ns	0.186ns	0.012*	0.002**	0.024*	0.016*	0.048*	0.032*	0.002**	0.024*	0.016*

¹For each heavy metal, means within the same column followed by the same letter(s) are not significantly different from each other at 5% level of significance based on Tukey's test. ** = highly significant; ns = non-significant

The concentration of heavy metal(loid)s in the shoots and roots of *A. tortilis* (Table 5.2.7) grown in pure mine tailings without mycorrhizal inoculation was in the order of: Cu > Pb > Zn > Ni > Mn > As. The As concentrations in shoots were reduced significantly by mycorrhiza inoculation by around 50%. On the other hand, fly ash application reduced the shoot and roots

concentration of Cu, Mn, Ni, and Pb regardless of mycorrhiza inoculation. In pure mine tailings, mycorrhiza inoculation also significantly reduced the Cu, Mn, Ni and Pb concentrations in shoots and roots. On average, the reduction in Cu due to fly ash amendments was 83% in shoots and 54% in roots; 51% in shoots and 51% in roots for Mn; 90% in shoots and 52% in roots for Ni; 97% in shoots and 96% in roots; and 75% in shoots and 39% in roots for Zn. Mycorrhiza inoculation in pure mine tailings also reduced the heavy metal(loid)s contents to about 67% in shoots and 77% in roots for Cu; 29% in shoots and 10% in roots for Mn; 43% in shoots and 11% in root for Ni, and 55% in shoots and 40% in roots for Zn. There was no significant difference due to mycorrhiza inoculation in the shoot concentration of Pb; but in pure mine tailings, a significant increase in root Pb was observed.

Table 5.2.7. Heavy metal(loid)s concentrations in shoot and roots of *A. tortilis* (n=20) grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 24 weeks after planting¹

Treatments	Heavy Metal(loid)s Concentration (mg kg ⁻¹)											
	As		Cu		Mn		Ni		Pb		Zn	
	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Mine Tailings No Fly Ash												
-AM	44.42b	63.28b	473.33a	586.67a	103.49a	93.48a	119.08a	104.26a	176.48a	185.24b	132.85a	110.67a
+AM	22.11 c	49.96 c	158.14 b	136.72c	72.76b	84.23b	68.25b	92.15b	168.44a	224.27 a	59.38b	65.78b
Mine Tailings with 10% Fly Ash												
-AM	50.21a	77.01a	68.48c	243.23b	55.31c	77.02c	10.54c	40.54c	5.42b	8.26c	28.97c	44.59c
+AM	23.86c	72.57a	88.12c	293.74b	48.46c	71.92c	13.22c	60.27bc	6.61b	7.22 c	37.45c	89.63b
<i>P</i> -values												
FA	0.032*	0.012*	0.018*	0.026*	0.012*	0.042*	0.022*	0.018*	0.013*	0.004**	0.013*	0.012*
AM	0.036*	0.022*	0.034*	0.034*	0.046*	0.036*	0.048*	0.026*	0.036*	0.002**	0.018*	0.022*
FA x AM	0.038*	0.038*	0.022*	0.018*	0.028*	0.014*	0.037*	0.028*	0.032*	0.002**	0.016*	0.016*

¹For each heavy metal, means within the same column followed by the same letter(s) are not significantly different from each other at 5% level of significance based on Tukey's test. ** = highly significant; ns = non-significant

5.2.3.4 Translocation of heavy metal(loid)s in plants

The translocation factor (TF) of heavy metal(loid)s differed among the treatments (Table 5.2.8). In *A. albida*, the TF of As reduced in mycorrhizal inoculation and fly ash amendments. Whereas, the TF for Cu, Mn, Pb, and Ni were reduced in fly ash amendments only and not in mycorrhizal inoculation. For Zn, the TF was significantly reduced due to the combined effect of fly ash amendment and mycorrhizal inoculation. As to *A. luederitzii*, the TF of As reduced in plants grown in pure mine tailings with mycorrhizal inoculation. Fly ash amendments, on the other hand, reduced the translocation of Pb, Mn, and Zn from roots to shoots. In contrast, the TF of Cu increased only in fly ash amendments. In the case of Mn, both mycorrhiza and fly ash increased the translocation of Mn from roots to shoots. The TF of As in *A. tortilis* reduced by mycorrhizal inoculation and fly ash amendments. Whereas, the TF for Cu, Mn, Pb, and Ni reduced significantly in fly ash amendments. The reduction of TF due to mycorrhizal inoculation in pure fly ash was observed in As, Mn, Ni, Pb, and Zn, while a significant increase was observed in Cu.

Table 5.2.8. Translocation factor (shoot/root ratio) of heavy metal concentration of *A. albida*, *A. luederitzii* and *A. tortilis* grown in mine tailings as affected by fly ash amendment and mycorrhizal inoculation at 24 weeks after planting¹.

Treatments	Translocation Factor (Concentration in shoot/Concentration in Root)																	
	<i>A. albida</i> (n=20)						<i>A. luederitzii</i> (n=20)						<i>A. tortilis</i> (n=20)					
	As	Cu	Mn	Ni	Pb	Zn	As	Cu	Mn	Ni	Pb	Zn	As	Cu	Mn	Ni	Pb	Zn
Mine Tailings No Fly Ash																		
-AM	1.04a	1.14a	1.14a	1.13a	1.13a	1.13 a	0.41 a	0.37 b	0.37 c	0.35 a	0.35 c	0.35 c	0.71 a	0.80 b	1.11 a	1.14 a	0.95 a	1.20 a
+AM	0.60 b	1.09 a	1.10 a	1.09 a	1.09 a	1.09 a	0.34 b	0.34 b	0.33 c	0.32 a	0.30 c	0.31 c	0.44 c	1.16 a	0.86 b	0.74 b	0.75 b	0.91 b
Mine Tailings with 10% Fly Ash																		
-AM	0.51 b	0.23 b	0.67 b	0.37 b	0.56 b	1.00 a	0.38 a	0.69 a	0.81 b	0.25 b	0.77 b	0.62 b	0.65 b	0.28 c	0.72 b	0.26 c	0.66 b	0.65 c
+AM	0.22 c	0.25 b	0.72 b	0.52 b	0.51 b	0.41 b	0.43 a	0.25 b	1.35 a	0.27 b	1.01 a	1.24 a	0.33 d	0.30 c	0.67 b	0.22 c	0.91 c	0.42 d
P-values																		
FA	0.013*	0.028*	0.013*	0.004**	0.018*	0.0182ns	0.122ns	0.141ns	0.021*	0.016*	0.022*	0.032*	0.042*	0.016*	0.038*	0.036*	0.042*	0.022*
AM	0.036*	0.026*	0.236ns	0.092ns	0.318ns	0.024*	0.036*	0.046*	0.029*	0.334ns	0.028*	0.016*	0.036*	0.012*	0.044*	0.038*	0.026*	0.018*
FA x AM	0.028*	0.028*	0.132ns	0.122ns	0.062ns	0.046*	0.048*	0.038*	0.026*	0.128ns	0.024*	0.024*	0.038*	0.038*	0.042*	0.028*	0.038*	0.044*

¹For each heavy metal, means within the same column followed by the same letter(s) are not significantly different from each other at 5% level of significance based on Tukey's test. ** = highly significant; ns = non-significant

5.2.3.5 Soil chemical properties and heavy metal availability

The pH and electrical conductivity of mine tailings after seedling growth differed significantly due to fly ash amendments without mycorrhiza inoculation (Table 5.2.9). Fly ash increased the pH by more than 4 units and the EC by more than 9 times. Similarly, 10% fly ash amendments significantly reduced the water-soluble (WS)- AS, WS-Cu, WS-Mn, WS-Ni, WS-Pb, and WS-Zn. Mycorrhiza inoculation reduced the WS-Cu, WS-Mn, and WS-Ni, but not the WS-As, WS-Pb WS-Zn. On the other hand, the effects of fly ash amendments on the amount of exchangeable heavy metal(loid)s varied with specific metals. For example, exch-As and exch-Mn increased but exch-Cu, exch-

exch-Ni, exch-Pb, and exch-Zn decreased due to fly ash amendments. Mycorrhiza inoculation did not result in significant differences on the amounts of exchangeable metals in soil in the presence or absence of fly ash.

Table 5.2.9. Chemical properties of soils (n=60) after cultivation of plants¹

Treatments	pH	EC (dSm)	Water Soluble (mg kg ⁻¹)						NH ₄ OAc Extractable (mg kg ⁻¹)					
			As	Cu	Mn	Ni	Pb	Zn	As	Cu	Mn	Ni	Pb	Zn
Mine Tailings – Fly Ash														
-AM	4.18 b	343.5 b	3.86 a	3.58 a	0.17 a	1.79 a	6.49 a	0.12 a	4.62 b	2.88 a	0.21 b	2.04 a	4.14 a	0.29 a
+Am	4.66 b	273.4 b	3.70 a	1.35 b	0.11 b	0.65 b	6.45 a	0.10 a	4.72 b	2.66 a	0.25 b	2.03 a	4.30 a	0.24 a
Mine Tailings + 10% fly ash														
-AM	8.41 a	2700.0 a	2.94 b	0.06 c	0.01 c	0.01 c	3.99 b	0.04 b	5.79 a	0.61 b	1.26 a	0.56 b	3.35 b	0.14 b
+Am	8.36 a	2682.5 a	2.58 b	0.14 c	0.01 c	0.01 c	4.10 b	0.05 b	5.29 a	0.65 b	1.01 a	0.84 b	3.02 b	0.16 b
P-values														
FA	0.022*	0.001**	0.021*	0.016*	0.012*	0.002**	0.042*	0.012*	0.038*	0.036*	0.042*	0.022*	0.032*	0.042*
AM	0.136ns	0.246ns	0.089ns	0.334ns	0.128ns	0.116ns	0.126ns	0.222ns	0.124ns	0.134ns	0.226ns	0.118ns	0.216ns	0.236ns
FA x AM	0.148ns	0.238ns	0.126ns	0.048*	0.034*	0.036*	0.138ns	0.138ns	0.042*	0.098ns	0.019ns	0.144ns	0.124ns	0.138ns

¹ For each heavy metal, means within the same column followed by the same letter(s) are not significantly different from each other at 5% level of significance based on LSD test. * = significant; ** = highly significant; ns = non-significant

5.2.4 Discussions

Mycorrhizal inoculants successfully colonized the roots of *Acacia* spp. in mine tailings. The colonization rates were significantly higher in fly ash amended mine tailings indicating an improvement of mycorrhizal infection due to fly ash (Table 5.2.4). There were no mycorrhizal roots detected in –AM treatments indicating the absence of native mycorrhiza in the mine tailings used in the experiment.

Fly ash amendments and mycorrhizal inoculation improved the survival and growth of different *Acacia* species in soils collected from Cu – Ni mines in Selebi-Phikwe, Botswana (Tables 5.2.2 and 5.2.3). The increased survival and growth improvement of different *Acacia* species were contributed mainly by 10% fly ash amendments, which modified the chemical properties of soil affecting nutrient and heavy metal availability. Such survival and growth rate were also partly due to mycorrhizal inoculation. In addition, the nutrients contained in fly ash (Table 5.2.1) and the ability of mycorrhiza to mobilize and increase absorption of nutrients in soil improved the nutrition of different acacia species under this type of soil as evident in the heavier dry matter yield in plants (Table 5.2.3). Based on the study by He et al. (2017), FA increased the exchangeable phosphorus (P), magnesium (Mg), copper (Cu), zinc (Zn), molybdenum (Mo), and Se; and consequently, enhanced the dry matter yield, and the nutrient uptake of alfalfa grown in loessial soil. In the present study, it was apparent that the essential nutrient in FA helped the nutrition of different *Acacia* spp. in tailings contaminated with heavy metal(loid)s and enhanced their survival and growth. Similarly, mycorrhiza association improved plant growth in heavy metal-contaminated soils by improving nutrition (de Fátima Pedroso et al., 2018) and alleviation of toxic effects by enhancing the antioxidant defense system through up-regulating various antioxidant defense system enzymes such as SOD, CAT, POD, APX, and GR and regulate genes encoding proteins involved in ROS homeostasis

(Vilela et al., 2018). Colonized plants with mycorrhiza and other symbiotic microorganism also increased the concentrations of non-enzymatic antioxidants such as glutathione, flavonoids, ascorbic acid, phenolic compounds, alkaloids, tocopherol, and carotenoids. However, in this study, enhancement of survival and growth in heavy metal-contaminated mine tailings without FA due to mycorrhiza was only observed in *A. luederitzii* and not in *A. albida* and *A. tortilis*, suggesting that such beneficial effects of mycorrhiza can also be plant genotype-specific effect (Mora-Romero et al., 2015; Ravnskov and Larsen, 2016). Generally, mycorrhizal fungi are expected to improve the growth of the host plant, but in some cases, growth depression of the host plants could occur and could be attributed to several factors and mechanisms (Jin et al., 2017) not accounted in the present study.

In this particular case, plants without mycorrhiza are already exposed to heavy metal(loid)s stress such that the establishment and the maintenance of the mycorrhizal association could be costly to the host plant in terms of the demand of nutrients and photosynthates causing negative effects in host plants such as nutrient outflux (Mariotte et al., 2013) or growth depression (Graham, 2000), especially at seedling stage. Other authors have hypothesized that during root colonization process, the roots may perceive a need to defend against the potential colonization, e.g., by changing morphology for physical protection or producing secondary metabolic products (Jin et al., 2017) or maintenance of vesicle in roots which requires extra photosynthetic products and may result in growth depression if resources are limited. Werner and Kiers (2015) pointed out that AMF-host plant relations are context-dependent and can be mediated by abiotic factors, including soil nutrients, pH, water and sunlight. It can be deduced that the effectiveness of AMF ranges from mutualistic to parasitic depending mostly on soil

nutrient supply in natural or artificial ecosystems (Jin et al., 2017) and could be compounded by the presence of elevated heavy metal(loid)s in soils.

The alkaline pH of FA neutralized the acidity from the initial pH 4.74 of the soil to about pH 8.49 which resulted in various chemical changes (Table 5.2.9). This increase in soil pH decreased the solubility of heavy metal(loid)s in soil through different mechanisms, which include precipitation of cationic heavy metal(loid)s or increased adsorption of heavy metal(loid)s on soil matrix. This is very evident in the low concentration of water-soluble Cu, Mn, Ni, Pb and Zn, and exchangeable Cu, Ni, Pb, and Zn in fly ash amended soils (Table 5.2.9), thus effectively reducing the availability of these metal elements, which are similar to the findings of Sitarz-Palczak and Kalembkiewicz (2012). The reduction of available metals was attributed to increased sorption sites and metal precipitation in the form of hydroxides as the soil pH increases due to fly ash amendments (Lwin et al. 2018). On the other hand, fly ash amendments decreased the water-soluble As but increased the exchangeable As which could be attributed to changes in speciation and solubility of As in response to pH change (Signes-Pastor et al., 2007). Such a reduction could be due to uptake by plants. Consequently, Cu, Ni, Pb, Mn, and Zn uptake of different acacia species was reduced due to decreased amounts of available fraction of metals. The uptake of micronutrients including heavy metal contaminants is deduced to be a function of the concentration in soil within the rooting zone. However, this relationship is not linear because there existed extreme variability in trends between concentration in shoots and in soils indicating that there could be other factors that influence the uptake of heavy metal(loid)s. These could include the presence of competing ions, concentration ratio to other micro-and macro-nutrients, biochemical transformations in the rhizosphere induced by plant roots and microorganisms, and the presence or absence of transport facilities on plant membranes (Ultra et al., 2007).

Nonetheless, it is apparent that in these conditions, the reduction of available metals in the soil due to fly ash amendments reduced substantially the metal concentration in shoots.

The influence of mycorrhiza on metal uptake and on plant survival had been documented in several studies and most of these studies dealt with single to few metals in soils. In our study, mycorrhiza inoculation increased or decreased metal uptake depending on the properties of the soil, the plant species and the presence or absence of other factors affecting heavy metal availability. For example, results of this study showed that mycorrhizal inoculation reduced the As, Cu, Mn, Ni and Zn in shoots in all the three Acacia species but enhanced the Pb concentration in the shoots of *A. luederitzii* grown in soils with fly ash. These different responses to mycorrhizal inoculation among the Acacia species could be attributed to species variability with respect to their physiological attributes and on the consequential response to mycorrhizal colonization, and in part on the different uptake and transport mechanisms involved with different metals and nutrients (Becklin et al., 2016). First, the general observation of reduced concentration of different heavy metal(loid)s in *A. luederitzii*, *A. Albida* and *A. tortilis* due to mycorrhizal inoculation could be attributed to heavy metal localization at the external mycelium of the arbuscular mycorrhiza, selective exclusion of toxic and nontoxic metals by adsorption onto chitinous cell wall structure, or onto extracellular glycoprotein called glomalin, or intracellular crystallization which is a very important mechanism in reducing the plant's exposure to potentially toxic metals (Shi et al., 2018). Second, the outcome of metal accumulation could differ from one plant species to another or with different mycorrhiza species (Hahl et al., 2018). For example, localization of Cu accumulation in the extraradical mycelium (ERM) of different AM fungi differed in their capacity for sorption of Cu which was directly related to the cation exchange capacity of ERM of AM fungi

(Gonzalez-Chavez et al., 2002); thus altering the translocation of ions from roots to shoots. Results of the present study showed that the translocation from roots to shoots of As in all the three *Acacia* species was reduced by mycorrhiza so with the translocation of Mn, Ni, Pb, and Zn in *A. tortilis*, but not with the other two species (*A. luederitzii*, *A. albida*).

These differences could be attributed to the different degree mycorrhiza could affect the physiological and molecular controls of nutrients and heavy metal transport in the plants. Shi et al., (2018) indicated that the molecular processes for mycorrhizal-mediated heavy metal accumulation include upregulation of gene expression involved in uptake, translocation, detoxification, and sequestration in fungal cells, including P-ATPase, ABCs, met1, MFSs (major facilitator superfamily permease), ABC1, ZnT1, MTs, and GST which also vary among plant species; differential transcriptional regulation of the host genes involved in heavy metal accumulation in plants and induced transcriptional levels of genes related to the defense system of host plants and genes encoding antioxidative enzymes. Third, the presence of other metal ions may cause changes in the uptake and transport of other metals in plants. For example, both lead and copper are known to be transported from roots to shoots in terrestrial plants, though to different extent. Copper is thought to be widely translocated in an acropetal direction (Kutrowska et al., 2017). This indicates that the heavy metal(loid)s in the soil-plant system are dominated by competitive and inhibitory interactions with the major ions present, which control the concentrations of trace ions in soil solution and the cellular plasma membrane transport responsible for root uptake and translocations of these trace elements. Therefore, there is a high probability that such interaction will result in different patterns of heavy metal accumulation among the plants as observed in this study.

Overall, this study demonstrated the potential of fly ash and mycorrhiza in improving the survival rate and growth of different *Acacia* species in heavy metal-contaminated mine tailings from Cu-Ni mines. Generally, there was a significant interaction between fly ash and mycorrhizal in almost all parameter measured except for translocation factor of Mn, Ni and Pb in *A. albida*, and Ni in *A. luederitzii*; and the amount of ammonium acetate exchangeable metals in soils after seedling growth.

Fly ash improved the growth and lowered the heavy metal concentration of different *Acacia* species by neutralizing the acidic pH of soil and reducing the availability of heavy metal(loid)s. Consequently, the heavy metal concentration in plants was reduced due to FA amendments. Mycorrhiza successfully colonized the roots of different *acacia* species and selectively enhanced the growth and altered the heavy metal accumulation in plants. Enhancement of growth by mycorrhiza was only observed in *A. luederitzii* in soils without fly ash, while growth retardation was observed in *A. albida* and *A. tortilis* in tailings without fly ash. However, when mycorrhiza is combined with fly ash, dry matter yield was enhanced indicating that the benefits of mycorrhiza inoculation for *acacia* species is affected by the soil properties, availability of heavy metal(loid)s and the efficiency of plant-mycorrhiza association. In conditions with limited nutrients and high heavy metal(loid)s contamination, the combined application mycorrhiza inoculation and fly ash amendments synergistically enhanced the survival and growth of *A. Luederitzii*, primarily and to certain extent, the growth of *A. albida* and *A. tortilis*. Therefore, *A. Luederitzii* has more potential as phytoremediation agent in mine tailings provided fly ash and mycorrhiza will be utilized during its establishment.

5.2.5 References

- Aransiola, S.A., Ijah, U.J.J., Abioye, O.P. and Bala, J.D., (2019). Microbial-aided phytoremediation of Heavy metals contaminated soil: a review. *European Journal of Biological Research*, 9(2), 104-125.
- Becklin, K.M., Mullinix, G.W. and Ward, J.K. (2016). Host plant physiology and mycorrhizal functioning shift across a glacial through future [CO₂] gradient. *Plant Physiology*, 172(2), 789-801.
- Belyaeva, O.N. and Haynes, R.J. (2012). Comparison of the effects of conventional organic amendments and biochar on the chemical, physical and microbial properties of coal fly ash as a plant growth medium. *Environmental Earth Sciences*, 66(7), 1987-1997.
- Brundrett, M.C. and Abbott, L.K. (1994). Mycorrhizal fungus propagules in the jarrah forest. *New Phytologist*, 127(3), 539-546.
- Burges, A., Alkorta, I., Epelde, L. and Garbisu, C. (2018). From phytoremediation of soil contaminants to phytomanagement of ecosystem services in metal contaminated sites. *International journal of phytoremediation*, 20(4), 384-397.
- Chileshe, M.N., Syampungani, S., Festin, E.S., Tigabu, M., Daneshvar, A. and Odén, P.C. (2019). Physico-chemical characteristics and heavy metal concentrations of copper mine wastes in Zambia: implications for pollution risk and restoration. *Journal of Forestry Research*, pp.1-11.
- de Fátima Pedroso, D., Barbosa, M.V., dos Santos, J.V., Pinto, F.A., Siqueira, J.O. and Carneiro, M.A.C. (2018). Arbuscular Mycorrhizal Fungi Favor the Initial Growth of *Acacia mangium*, *Sorghum bicolor*, and *Urochloa brizantha* in Soil Contaminated with Zn, Cu, Pb, and Cd. *Bulletin of Environmental Contamination and Toxicology*, 101(3), 386-391.

- Festin, E.S., Tigabu, M., Chileshe, M.N., Syampungani, S. and Odén, P.C. (2019). Progresses in restoration of post-mining landscape in Africa. *Journal of Forestry Research*, 30(2), 81-396.
- Giovennetti, M. and Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, 84(3), 489-500.
- Gonzalez-Chavez, C., D'haen, J., Vangronsveld, J. and Dodd, J.C. (2002). Copper sorption and accumulation by the extraradical mycelium of different *Glomus* spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. *Plant and Soil*, 240(2), 287-297.
- Graham, J.H. (2000). Assessing costs of arbuscular mycorrhizal symbiosis in agroecosystems. *Current Advances in Mycorrhizae Research*, 127-140.
- Hahl, T., van Moorsel, S.J., Schmid, M.W., Zuppinger-Dingley, D., Schmid, B. and Wagg, C. (2018). Diversity loss selects for altered plant phenotypic responses to local arbuscular mycorrhizal communities. *bioRxiv*, 216986.
- Hao, X., Zhou, D., Wang, Y., Shi, F. and Jiang, P. (2011). Accumulation of Cu, Zn, Pb, and Cd in edible parts of four commonly grown crops in two contaminated soils. *International Journal of Phytoremediation*, 13(3), 289-301.
- He, H., Dong, Z., Pang, J., Wu, G.L., Zheng, J. and Zhang, X. (2018). Phytoextraction of rhenium by lucerne (*Medicago sativa*) and erect milkvetch (*Astragalus adsurgens*) from alkaline soils amended with coal fly ash. *Science of the Total Environment*, 630, 570-577.
- Ibañez, S.G., Paisio, C.E., Oller, A.L.W., Talano, M.A., González, P.S., Medina, M.I. and Agostini, E. (2015). Overview and new insights of genetically engineered plants for improving phytoremediation. *In Phytoremediation* (99-113). Springer, Cham.

- Jin, L., Wang, Q., Wang, Q., Wang, X. and Gange, A.C. (2017). Mycorrhizal-induced growth depression in plants. *Symbiosis*, 72(2), 81-88.
- Kinuthia, G. K., Ngure, V., Beti, D., Lugalia, R., Wangila, A., & Kamau, L. (2020). Levels of heavy metals in wastewater and soil samples from open drainage channels in Nairobi, Kenya: community health implication. *Scientific reports*, 10(1), 8434.
- Kong, Z. and Glick, B.R. (2017). The role of plant growth-promoting bacteria in metal phytoremediation. *In Advances in Microbial Physiology* (Vol. 71, 97-132). Academic Press.
- Koptsik, G.N. (2014). Problems and prospects concerning the phytoremediation of heavy metal polluted soils: a review. *Eurasian Soil Science*, 47(9), 923-939.
- Kutrowska, A., Małecka, A., Piechalak, A., Masiakowski, W., Hanć, A., Barańkiewicz, D., Andrzejewska, B., Zbierska, J. and Tomaszewska, B. (2017). Effects of binary metal combinations on zinc, copper, cadmium and lead uptake and distribution in *Brassica juncea*. *Journal of Trace Elements in Medicine and Biology*, 44, 32-39.
- Liphadzi, M.S., Kirkham, M.B. and Musil, C.F. (2005). Phytoremediation of soil contaminated with heavy metals: a technology for rehabilitation of the environment. *South African Journal of Botany*, 71(1), 24-37.
- Lwin, C.S., Seo, B.H., Kim, H.U., Owens, G. and Kim, K.R. (2018). Application of soil amendments to contaminated soils for heavy metal immobilization and improved soil quality—a critical review. *Soil Science and Plant Nutrition*, 64(2), 156-167.
- Mahar, A., Wang, P., Ali, A., Awasthi, M.K., Lahori, A.H., Wang, Q., Li, R. and Zhang, Z. (2016). Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: A review. *Ecotoxicology and Environmental Safety*, 126, 111-121.

- Mariotte, P., Meugnier, C., Johnson, D., Thébault, A., Spiegelberger, T. and Buttler, A. (2013). Arbuscular mycorrhizal fungi reduce the differences in competitiveness between dominant and subordinate plant species. *Mycorrhiza*, 23(4), 267-277.
- Mora-Romero, G.A., Cervantes-Gámez, R.G., Galindo-Flores, H., González-Ortíz, M.A., Félix- Gastélum, R., Maldonado-Mendoza, I.E., Pérez, R.S., León-Félix, J., Martínez-Valenzuela, M.C. and Lopez-Meyer, M. (2015). Mycorrhiza-induced protection against pathogens is both genotype-specific and graft-transmissible. *Symbiosis*, 66(2), 55-64.
- Motaharpour, Z., Taheri, H. and Nadian, H. (2019). *Rhizophagus irregularis* modulates cadmium uptake, metal transporter, and chelator gene expression in *Medicago sativa*. *Mycorrhiza*, 1-7.
- Nie, M., Wang, Y., Yu, J., Xiao, M., Jiang, L., Yang, J., Fang, C., Chen, J. and Li, B. (2011). Understanding plant-microbe interactions for phytoremediation of petroleum-polluted soil. *PloS one*, 6(3), e17961.
- Pandey, V.C. and Singh, N. (2010). Impact of fly ash incorporation in soil systems. *Agriculture, Ecosystems & Environment*, 136(1-2), 16-27.
- Ravnskov, S. and Larsen, J. (2016). Functional compatibility in cucumber mycorrhizas in terms of plant growth performance and foliar nutrient composition. *Plant Biology*, 18(5), 816-823.
- Shaheen, S.M., Hooda, P.S. and Tsadilas, C.D. (2014). Opportunities and challenges in the use of coal fly ash for soil improvements—a review. *Journal of Environmental Management*, 145, 249-267.
- Shi, W., Zhang, Y., Chen, S., Polle, A., Rennenberg, H. and Luo, Z.B. (2018). Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants. *Plant, Cell & Environment*. 42(4), 1087-1103.

- Signes-Pastor, A., Burló, F., Mitra, K. and Carbonell-Barrachina, A.A. (2007). Arsenic biogeochemistry as affected by phosphorus fertilizer addition, redox potential and pH in a west Bengal (India) soil. *Geoderma*, 137(3-4), 504-510.
- Sitarz-Palczak, E. and Kalembkiewicz, J. (2012). Study of remediation of soil contaminated with heavy metals by coal fly ash. *Journal of Environmental Protection*, 3(10), 1373.
- Ultra Jr, V.U., Tanaka, S., Sakurai, K. and Iwasaki, K. (2007). Arbuscular mycorrhizal fungus (*Glomus aggregatum*) influences biotransformation of arsenic in the rhizosphere of sunflower (*Helianthus annuus* L.). *Soil Science and Plant Nutrition*, 53(4), 499-508.
- Ultra Jr, V.U., Yano, A., Iwasaki, K., Tanaka, S., Kang, Y. and Sakurai, K. (2005). Influence of chelating agent addition on copper distribution and microbial activity in soil and copper uptake by brown mustard (*Brassica juncea*). *Soil Science & Plant Nutrition*, 51(2), 193-202.
- Vilela, L.A.F., Ramos, S.J., Carneiro, M.A.C., Faquin, V., Guilherme, L.R.G. and Siqueira, J.O. (2018). Cerium (Ce) and Lanthanum (La) promoted plant growth and mycorrhizal colonization of maize in tropical soil. *Australian Journal of Crop Science*, 12(5), 704.
- Werner, G.D. and Kiers, E.T. (2015). Partner selection in the mycorrhizal mutualism. *New Phytologist*, 205(4), 1437-1442.
- Zhai, M., Totolo, O., Modisi, M.P., Finkelman, R.B., Kelesitse, S.M. and Menyatso, M. (2009). Heavy metal distribution in soils near Palapye, Botswana: an evaluation of the environmental impact of coal mining and combustion on soils in a semi-arid region. *Environmental geochemistry and health*, 31(6), 759.

5.3 Enhancing the phytoremediation potential of *Eucalyptus globulus* in three different mine tailings of Botswana through soil amendments

Abstract

Three different soil amendments, fly ash (FA), cow manure (CM), and sewage sludge (SS) were used to assess the phytoremediation potential of the *Eucalyptus globulus* species on three different mine tailings: 1) Bamangwato Concessions Limited mine (BCL) tailings; 2) BCL smelter slag, and 3) Monarch Gold Mine tailings located in Selibe Phikwe and Francistown, respectively. All amendments resulted in a survival rate which went from 0% to 100%. The growth, dry matter yield and chlorophyll content of plants grown in these tailings were significantly high in the FA+SS treatment. The addition of 10% FA altered the soil pH of all mine tailing from acidic (4.15, 3.20, 4.5) to pH tolerable for plants (7.05-8.70). Additionally, the amendments increased the concentration of heavy metal(loid)s in the tissues and the biomass produced, thereby increasing the overall uptake of heavy metal(loid)s by *E. globulus*. A combination of sewage sludge and fly ash was found to be the most effective amendment, followed by 10% FA+CM. Overall, when amendments were used during plant establishment, the results indicated that *E. globulus* is a potential species for the phytoremediation of mine tailings in BCL-Selibe Phikwe and the Monarch Gold Mine in Francistown.

Keywords: Heavy metals, bioremediation, contaminants, accumulation.

5.3.1 Introduction

Phytoremediation is a type of bioremediation technology that uses plants to remove, transfer, stabilize, and destroy contaminants in the soil and water (Abdel-Shafy, 2018). It is a cost-effective and sustainable approach to removing heavy metal(loid)s and cleaning up contaminated soils and water. Heavy metal(loid)s are toxic to plants, animals, and humans, and can accumulate in the environment, posing a health risk to livestock and human beings (Ekosse, 2005; Manyiwa et al., 2023). As a way of managing risk, plants can be used to absorb and accumulate heavy metal(loid)s from the environment (Filipović-Trajković, 2012). Mining activities in Botswana are mainly concentrated in the coal, copper, nickel, gold, and diamond sectors (Jefferis, 2009). The most common heavy metal(loid)s found in the mining areas of Botswana are arsenic, lead, cadmium, chromium, and mercury. These metals are released into the environment and eventually pollute the air, soil and water. Contaminated soils, however, can be rehabilitated to reduce the concentrations of heavy metal(loid)s and hence the pollution impacts of these elements on the environment. According to Ye et al. (2001), a full understanding of the constraints and opportunities to successfully reclaim contaminated land can be achieved through the assessment of physical, chemical, and biological factors related to mine waste. Shrestha and Lal (2011) assert that effective reclamation will occur if these properties are conducive to plant growth. Soil properties that affect the growth of vegetation and metal uptake are therefore important factors to be considered in the rehabilitation of coal waste dumps or stockpiles. Plant metal uptake is influenced by soil factors including pH, electrical conductivity (EC), and the application of amendments such as fly ash (FA), cow manure, and sewage sludge that will modify or alter the physicochemical properties of the mine tailings (Han et al., 2012; Hussain et al., 2018; Manyiwa et al., 2021 Ultra, 2020). Ultra and Manyiwa (2021) stated that these

amendments could significantly influence the growth and survival of plants as well as the accumulation and uptake of heavy metal(loid)s in plants.

The use of sewage sludge (SS) as an amendment is good for plant growth because it contains essential elements and pathogens such as viruses and bacteria. It also contains valuable organic matter and nutrients such as nitrogen and phosphorus, therefore being very useful as a fertilizer or soil improver (Alvarenga et al., 2016). Alternatively, fly ash (FA) is a good amendment largely due to its texture and the fact that it contains all the essential nutrients required by plants except for nitrogen (Pathan et al., 2003).

The use of FA as an amendment improves soil's physicochemical properties and microbial activity (Singh and Pandey, 2013). The application of cow manure (CM) as a soil amendment is also beneficial since it can lower heavy metal availability by providing humus at sorption sites of cationic metals and soluble substances, that can act as chelating agents of heavy metal(loid)s in soils. Although cow manure is beneficial, its use has been found to elevate heavy metal(loid)s in plants (Petruzzelli 1996) while it also improves microbial activity (Farrell and Jones, 2010). Therefore, proper application of cow manure should be observed in phytoremediation studies.

One species that has been identified to be beneficial for the phytoremediation of heavy metal(loid)s is *E. globulus*. This tree species is effective in removing heavy metal(loid)s such as lead, cadmium, and zinc from contaminated soils (Arriagada et al., 2007). Some studies found that, on an annual basis, metalliferous *E. globulus* can produce more biomass and uptake more heavy metal(loid)s than nonmetalliferous plants (Luo, 2016). The tree is able to absorb these metals through its roots. Some studies stated that *E. globulus* has high adaptability and resistance to adverse climatic conditions and can grow in various harsh environments (Luo et al., 2015). Other studies indicated that the eucalyptus species occurs naturally in most African countries and is well known for its

shallow rooting system, quick growth, and high production of biomass (Bilal et al., 2014) in dry and poor soils (Almeida et al., 2007). It represents a low risk of entering food chains due to its reduced edibility. Furthermore, some studies have indicated that eucalyptus sp. has a high capability to bioaccumulate metals including Cd, Cu, Pb, Ni, Zn, and Cr, in its tissues, (Mok et al., 2013; Bilal et al., 2014; Luo et al., 2016; Nirola et al., 2016). This study evaluated different amendment applications on three different mine tailings to enhance the growth and survival of *E. globulus*. Specific objectives of this study were threefold. The first objective was to determine the effect of FA, CM, and SS on the growth and heavy metal accumulation of *E. globulus* grown in Bamangwato Concession Limited (BCL) copper and nickel mine tailings, BCL smelter slag and Monarch (MON) Gold Mine tailings. The second objective was to determine the effect of FA, CM and SS on the heavy metal availability in these mine tailings. The third objective was to determine the effects of these amendments on the physiological responses of plants, such as chlorophyll content. Studies indicated that that soil amendments improve soil quality which then leads to improved plant survival and growth, and increased chlorophyll content (Younis et al., 2015).

5.3.2 Materials and Methods

5.3.2.1 Experimental design and treatment application

The mine tailings used in this experiment were collected at the tailings dumpsite and from the copper slag tailings of the BCL mine in Selebi-Phikwe, Botswana. Other tailings were collected at the closed Monarch Gold Mine in Francistown. Mine tailings were air-dried before being transferred to pots for planting. Plastic pots (25 cm diameter x 30 cm height) were filled with 8 kg tailings and 10% of amendments namely FA, FA + CM, and FA + SS. Before cultivation, mine tailings and amendments were analyzed for

physicochemical properties. These are presented in Table 5.3.1. Generally, the tailings from all the mines have a silty sand textural class and are acidic, characterized by high electrical conductivity (EC). They also contain high levels of total heavy metal(loid)s. The amendments used have an alkaline pH (FA and CM).

Influence of Different Soil Amendments on Heavy Metal(loid)s Uptake, Survival and Phytoremediation Efficiency of Selected Tree Species

Table 5.3.1. Chemical properties of different mine tailings and amendments used in the study¹

Parameters	BCL Tailings	BCL Smelter slag	Monarch Tailings	FA	Cow Manure	Sewage Sludge	P-Value
pH	3.8±0.19d	2.6±0.13e	4.3±0.17c	10.8±0.57a	8.2±0.41a	6.70±0.37b	0.030
EC (µs cm ⁻¹)	3710±173.50c	7560±377.92b	1800±89.97e	2290±105.02d	12700±695.33a	650.00±33.33f	0.000
OM (%)	0.00±0.00c	0.00±0.00c	0.1±0.00b	0.00±0.00c	65.2±3.25a	65±3.32a	0.000
Texture	Silty sand	Silty sand	Silty sand	Clay	N/A	N/A	
Total N (mg kg ⁻¹)	0.00±0.00d	0.00±0.00d	0.001±0.00d	0.78±0.04c	12500.00±44987a	8.8±4.21b	0.000
Avail P (mg kg ⁻¹)	508.00±25.39c	0.00±0.00f	21.84±1.17e	165.0±8.25d	7100.00±365.79b	73100.00±3654.93a	0.000
Total K (mg kg ⁻¹)	10187.00±487.75b	5.36±0.28e	22.65±1.40d	545.8±271.73c	9500.00±436.90b	38690.00±2077.03a	0.000
Total metal(loid)s (mg kg ⁻¹)							
As	332.00±16.19a	98.00±4.80b	14.20±0.69c	4.05±0.20d	3.30±0.17e	3.50±0.19e	0.000
Cr	323.00±15.95b	1224.00±63.91a	56.20±2.74d	110.00±6.60c	23.80±1.72e	105.20±4.93c	0.000
Cu	1428.00±73.57b	4179.00±208.94a	336.00±16.73c	20.60±0.99d	14.55±0.71e	21.50±0.07d	0.000
Mn	1222.00±61.09b	1378.00±68.61a	413.00±20.09e	616.52±36.26c	68.00±3.40f	560.52±27.95d	0.000
Ni	1470.00±75.35b	1684.00±93.03a	39.00±256d	58.28±3.57c	24.85±1.58e	58.40±3.05c	0.000
Pb	89.00±4.34c	119.00±5.42b	27.40±1.55e	28.66±1.43e	160.11±8.73a	41.25±2.11d	0.000
Exchangeable metal(loid)s (mg kg ⁻¹)							
As	0.15±0.01d	0.05±0.00e	0.04±0.00e	2.10±0.10a	0.60±0.03c	1.30±0.07b	0.000
Cr	0.00±0.00e	0.02±0.00e	0.10±0.00d	0.55±0.03b	0.82±0.04a	0.20±0.01c	0.000
Cu	0.50±0.02c	0.25±0.01d	0.00±0.00f	0.65±0.03b	0.15±0.00e	10.05±0.55a	0.000
Mn	0.35±0.00e	0.38±0.02d	0.15±0.00f	2.45±0.12c	17.5±0.87b	102.55±5.13a	0.000
Ni	0.92±0.05c	0.60±0.03d	0.07±0.00f	0.30±0.01e	1.80±0.10b	3.11±0.16a	0.000
Pb	0.00±0.000d	0.00±0.00d	0.00±0.00d	0.08±0.00c	6.50±0.38a	0.31±0.02b	0.000

¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at 5% level of significance.

5.3.2.2 Plant sample processing, chemical analysis & dry matter yield

After 52 weeks of monitoring plants for survival and growth with regular watering, plants were sampled by emptying the pots so that their roots remained undisturbed. Fresh weights of the plant samples were determined. The plants were separated into roots, stems, and leaves. They were then air dried for 3 days and later taken to the oven for further drying at 70°C for 72 hours. Dry weights were recorded for dry matter yield. Plant samples were powdered for chemical analysis using a laboratory grinder. An Ethos Easy Maxi 44 Microwave digester was used to digest plant tissues using a mixture of nitric acid and hydrogen peroxide at a ratio of (4:1). Sample digestion was conducted for 60 minutes at 200°C and 1200w. After cooling, the contents in the vessels were transferred into a 25ml volumetric flask and diluted with deionized water. Diluted acid digests were filtered using the gravimetric method and Whatman filter paper No.42. The final filtering was conducted using 0.45µm nylon syringe filters. To determine the contamination, precision, and bias of the analysis, reagent blanks which represented 10% of the total digested sample population were also analyzed. The findings showed no signs of contamination.

5.3.2.3 Soil sample processing and total metal digestion

Air-dried soil samples were sieved using a 2mm sieve and 0.5g of the soil sample was digested in 12 mL of aqua regia (HNO₃: HCl, 1:3) at 200°C and 1200w for 60 minutes using the Ethos Maxi 44 Microwave digester. Digested soil samples were cooled, then filtered with 0.45µm filters, and final volumes were diluted to 25 mL using deionised water.

5.3.2.4 CaCl₂ Extractable Heavy Metal(loid)s

To extract soluble heavy metal(loid)s, 10g of air-dried soil and sieved soil (2mm) were weighed into a 200mL high-density polyethylene plastic bottle. 100mL of 0.01M CaCl₂ were then added to the samples. The bottles were shaken at 300rpm for 2 hours using a rotary shaker. The supernatant was filtered into a 50 mL centrifuge tube using a 0.45µm syringe filter. The filtrate was acidified with 4% HNO₃ at a ratio of 1:2 before metal analysis.

5.3.2.5 Analysis of Metals in Soil Digests and Extracts

Total heavy heavy metal(loid)s and CaCl₂ extractable metal(loid)s were analyzed using a Thermo Scientific ICAP 7400 Duo ICP-OES, serial number IC74DC141810. The following heavy metal(loid)s were analyzed: As, Cr, Cu, and Mn. Ni and Pb. A series of standard solutions from stock solutions were prepared to calibrate the ICP-OES according to Beer Lambert's Law.

5.3.2.6 Validation of the analytical method (ICP-MS)

The method was validated for linearity, limits of detection (LODs), limits of quantification (LOQs), accuracy, and precision. For each analyte, a calibration curve was created by plotting peak area versus concentration at eight different concentrations between 10 and 1000 pmol/mL under optimum extraction conditions. Table 5.3.2 indicates ICP-MS instrumental parameters employed for heavy metal determination. For all chemical analyses of soil and plant samples, precision and accuracy were guaranteed through batch inclusion of internal reference samples (soil and corn tissue) whose concentrations were cross-verified by three laboratories in Botswana and by repeat analysis of samples when the coefficients of variation of replicate analysis (n=2) were

*Influence of Different Soil Amendments on Heavy Metal(loid)s Uptake, Survival and
Phytoremediation Efficiency of Selected Tree Species*

more than 10%. The recovery of the internal standard sample was more than 95% for all elements measured (Table 5.3.2).

Table 5.3.2. ICP-MS instrumental parameters employed for heavy metal determination and % recovery of concentrations of internal reference sample

Element	ICP- instrumental parameters			Concentration of internal reference sample (mg kg ⁻¹)		Mean Recovery Concentrations (mg kg ⁻¹)		% Recovery	
	Wavelength (nm)	LOD (µg/L)	LOQ (µg/L)	Soil	Plant	Soil	Plant	Soil	Plant
As	189.042	0.074	0.28	62.25	10.15	60.23±2.92	9.73±0.47	96.34	95.86
Mn	257.610	0.033	0.11	720.50	42.05	715.25±34.72	40.32±1.96	99.27	95.89
Cu	224.700	0.12	0.39	260.11	35.50	250.08±11.95	34.18±1.67	96.14	96.28
Cr	283.563	0.19	0.65	80.30	10.22	78.38±3.91	9.88±0.48	97.61	96.67
Pb	217.00	1.85	0.62	96.07	5.69	92.46±6.02	5.45±0.36	96.24	95.78
Ni	231.60	0.10	0.30	43.11	8.45	41.61±2.31	8.22±0.43	96.52	97.28

5.3.2.7 Analysis of Survival Rate and Chlorophyll

The survival rate was obtained by counting the number of surviving plants in each treatment. The obtained number was divided by the number of plants originally planted and multiplied by 100 to express as a percentage of survival. To determine chlorophyll content, 0.5g of a leaf sample was weighed in a test tube and 6 mL of Dimethylsulfoxide (DMSO) was added. The samples were placed in a dark environment for 24 hours to allow for chlorophyll extraction. A Thermo Scientific Evolution 600 UV-VIS Spectrophotometer was used to obtain absorbance at 663 and 645 wavelengths and the following equations were used to calculate sample chlorophyll concentrations (Gu et al., 2016).

$$\text{Chlorophyll}_a \text{ (mg/mL)} = (12.7A_{663} - 2.69A_{645}) \quad [\text{Eq. 1}]$$

$$\text{Chlorophyll}_b \text{ (mg/mL)} = (22.9A_{645} - 4.68A_{663}) \quad [\text{Eq. 2}]$$

where:

A₆₄₅ = absorbance at a wavelength of 645 nm;

A₆₆₃ = absorbance at a wavelength of 663 nm.

$$\text{Total Chlorophyll (mg/mL)} = (\text{Chlorophyll}_a + \text{Chlorophyll}_b)$$

5.3.2.8 Statistical analysis

The data was analyzed using a two-factor ANOVA in CRD to determine the differences in heavy metal concentration, dry matter yield, and chlorophyll between tailings; amendments; and tailings and amendments combined. When significant differences were observed, Tukey's test was performed for tailings; amendments; and tailings and amendments combined comparison at a 5% level of significance.

5.3.3 Results and Discussion

The growth performance and the ability of *E. globulus* as a phytoremediation agent in different mine tailings was greatly improved through the modification of the rhizosphere during the plant establishment using different amendments. Amendments like fly ash, cow manure, and sewage sludge on different mine tailings modified the chemical characteristics of the tailings and favoured the survival, growth, and heavy metal uptake of *E. globulus* on these tailings. The following sections describe in detail the effect of different amendments on growth and survival; dry matter yield; chlorophyll; heavy metal accumulation of plants; the properties and the availability of heavy metal(loid)s in tailings; and the overall phytoremediation potential of *E. globulus*, on three different mine tailings.

5.3.3.1 Survival, growth, and physiological response of *E. globulus* in tailings with different amendments

The survival rate, dry matter yield, and chlorophyll content of *E. globulus* presented in Table 5.3.3 indicate that without amendments applied to the tailings, the survival rate of *E. globulus* species was 0%, i.e., plants could not survive in pure mine tailings from the BCL and Monarch Gold Mines. This was attributed in part to the acidic nature of tailings and high EC which could not support plant growth as previously reported by Manyiwa & Ultra (2022). Monarch Gold mine tailings contain high levels of arsenic due to arsenopyrite (Vogel and Kasper, 2002; Manyiwa et al., 2023). High levels of this element above 20 mg kg⁻¹ soil are very toxic to plants and therefore hamper the survival of *E. globulus*.

In all the three tailings used, (BCL tailings, BCL smelter slag, and Monarch tailings) the dry matter yield of leaves, stem and roots was high when a combination of FA and SS

were added, compared to other amendments like FA and FA+CM. The dry matter yield (DM) was 27.56 g.Pot⁻¹ (roots), 56.06 g.Pot⁻¹ (stem) and 45.11 g.Pot⁻¹ (leaves). The high DM was attributed to the addition of SS which is highly nutritious and contains high levels of nitrogen, organic matter, and other essential nutrients for plant growth (Cao et al., 2013). The addition of FA improved the soil pH from acidic to almost neutral, which is favorable for plant growth. FA amendments alone without organic amendments had the lowest DM in roots, stem, and leaves for *E. globulus* across the different mine tailings. This would indicate that FA can mitigate the acidity of the tailings but does not have all the necessary essential elements for plant growth. A combination of FA and cow manure had the second-best DM. This is mainly because cow manure contains high levels of nitrogen, organic matter, and other essential elements. Cow manure also improves soil microbial growth, increases soil bacterial diversity and improves the soil structure (Zhang et al., 2020). Balasubramaniam (2020); and Sharma and Singh (2019) stated that amendments such as cow manure and FA can modify the soil's chemical, biological, and physical characteristics, making it a less stressful environment for seedlings (Balasubramaniam 2020; Sharma and Singh, 2019). Cow manure can enhance the soil buffering capacity and neutralize acidity. It also has the ability to supply essential nutrients. The high microbial load of CM and SS also supplied the necessary microbes to help in the transformation of nutrients and improve nutrient availability, and consequently the absorption of nutrients by plants (Akmal et al., 2019). Although Monarch Gold Mine tailings had higher dry matter yield than BCL tailings, the discrepancy in growth and yield between the different tailings was largely attributed to the higher heavy metal content in BCL tailings compared to Monarch Gold Mine tailings (Table 5.3.3). It appears that the growth of *E. globulus* in these tailings could be improved by the

combined application of FA and organic amendments or other nutrient sources essential for plant growth.

Similar to dry matter yield, the highest total chlorophyll of *E. globulus* was recorded in treatments where a combination of SS and FA was applied. This is mainly due to the high nutritional content of SS and CM which also has high nitrogen content. This is supported by Burducea et al. (2019) and Najafi et al. (2013) who stated that the application of SS improves plant growth and photosynthesis leading to high chlorophyll content in plants. The addition of FA on its own without other amendments led to a decreased chlorophyll content. This is supported by Raja et al. (2014) who reported that the addition of FA reduces the photosynthetic rate and eventually decreases the chlorophyll content. The decrease in chlorophyll content is also attributed to the high levels of heavy metal content in mine tailings. Many studies have highlighted the negative effect of heavy metal(loid)s on the photosynthetic rate and efficiency of plants, eventually leading to low chlorophyll content (Sims and Gamon, 2002; Singh et al., 2015). A study by Doganlar et al. (2012) also reported that heavy metal(loid)s in the soil (e.g., Fe, Cd Pb Cu, Zn) lead to the reduction in total chlorophyll and carotenoid content.

Influence of Different Soil Amendments on Heavy Metal(loid)s Uptake, Survival and Phytoremediation Efficiency of Selected Tree Species

Table 5.3.3: Survival rate, dry matter (DM) yield in leaves, roots, stem, and chlorophyll content in *E. globulus*¹

Sample Code	Survival Rate (%)	DM-Roots (g.Pot ⁻¹)	DM-Stem (g.Pot ⁻¹)	DM-Leaves (g.Pot ⁻¹)	Chlorophyll (mg mL ⁻¹)	a Chlorophyll (mg.mL ⁻¹)	b Total Chlorophyll (mg.mL ⁻¹)
BCL Tailings Control	0.00c	-	-	-	-	-	-
BCL Tailings + FA	75.00b	8.08±0.40c	5.03±0.25e	4.66±0.23g	76.65±3.82d	20.83±1.14f	97.48±5.23b
BCL Tailings + FA + CM	100.00a	20.11±1.03b	37.18±2.15c	25.58±1.46c	129.41±5.90b	38.19±1.76c	167.60±8.38a
BCL Tailings + FA +SS	100.00a	27.56±1.28a	56.04±3.19a	45.11±2.46a	136.46±6.82a	40.20±1.84b	176.66±9.05a
BCL Smelter Slag Control	0.00c	-	-	-	-	-	-
BCL Smelter slag + FA	50.00b	5.03±0.26c	8.57±0.46e	12.05±0.64f	44.54±2.23f	11.85±0.54g	56.39±2.88b
BCL Smelter slag + FA + CM	100.00a	23.22±1.16b	16.21±0.79d	15.08±0.75d	93.64±5.21c	40.64±2.07b	134.28±7.37a
BCL Smelter slag + FA + SS	100.00a	26.02±1.26a	37.02±1.85c	28.33±1.57b	53.00±26.43	67.01±3.28a	120.01±5.98a
MON Tailings Control	0.00c	-	-	-	-	-	-
MON Tailings + FA	75.00b	14.34±0.84c	4.44±0.25e	14.02±0.70e	49.28±2.26e	7.23±0.35h	56.50±3.01b
MON Tailings + FA + CM	100.00a	24.07±1.35b	47.09±2.21b	24.45±1.22c	96.13±5.01c	25.70±1.31e	121.83±7.13a
MON Tailings + FA + SS	100.00a	28.88±1.47a	56.11±3.42a	28.09±1.40b	99.38±4.83c	28.01±1.50d	127.39±6.35a
P-Value							
Tailings	Ns	ns	Ns	Ns	0.002	0.004	ns
Amendments	0.000	0.000	0.000	0.003	0.005	0.006	0.004
Tailings x Amendments	Ns	ns	0.002	0.008	0.003	0.002	ns

¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at 5% level of significance. 5

5.3.3.2 Influence of amendments on the chemical properties of mine tailings

Table 5.3.4 shows the pH, EC, organic matter (OM), and total heavy metal(loid)s concentrations of different mine tailings as influenced by different amendments after the cultivation of *E. globulus*. The results indicate that without the addition of amendments, mine tailings had intolerable levels of pH and EC, and very low organic matter. This was observed in BCL tailings, BCL smelter slag, and Monarch tailings. As an example, BCL tailings had a pH of 4.1.5, 0.00% of OM, and BCL smelter slag had a pH of 3.20 and 0.00% of OM. This led to unfavorable conditions that could not support the growth of *E. globulus*. According to Azeez and Van Averbek (2012), extreme soil pH indicates toxicity, harms plants and causes nutrient deficiency in the soil. The addition of amendments greatly improved the pH, EC, and OM of the soil. FA is alkaline, with a pH of 10.8 (Table 5.3.1) leading to the neutralization of the tailings. FA is a good amendment due to its texture and because it contains almost all essential nutrients required by plants apart from Nitrogen (Pathan et al., 2003). FA also improves soil microbial activities leading to the availability of essential nutrients for plants (Singh and Pandey, 2013). Although FA improves the soil, it also introduces heavy metal(loid)s including Ni, Cu, and Cr at higher amounts which could be toxic to plants (Pandey and Singh, 2010). The addition of amendments such as cow manure and SS led to a reduction in EC levels and increased OM. This was also observed in all treatments across all mine tailings. Cow manure and sewage sludge generally have high OM content as indicated in Table 5.3.1, and as reported by Zbytniewski and Buszewski (2005).

Several researchers have proposed that organic residues (such as animal manures) might be used as an alternative liming material and that their application can increase plant growth in acidic soils by ameliorating aluminum (Al) toxicity. Recent findings have also indicated that animal manures could serve as substitutes in the liming

of acidic soils (Pocknee and Sumner, 1997; Wong et al., 1998; Mokolobate and Haynes, 2002).

All heavy metal(loid)s analysed (Table 5.3.4) were above the critical limits except for As in BCL tailings treatments and Cr in Monarch tailings (MON). After the addition of FA+CM and FA+SS in BCL tailings, the concentration of Cr was reduced to be below the critical limits (64.50 and 45.50 mg kg⁻¹) respectively. As concentration decreased from 12.59 mg kg⁻¹ to 5.25 mg kg⁻¹ in BCL tailings, Cr from 110.00 to 45.50 mg kg⁻¹, Mn from 1134.50 to 479.00 mg kg⁻¹, and Pb from 79.05 to 16.20 mg kg⁻¹. In the BCL smelter slag, a significant increase of heavy metal(loid)s was observed in Mn and Ni (1134.50 to 479.00 mg kg⁻¹ and 1121.50 to 345.16 mg kg⁻¹ respectively). In MON tailings, an inverse effect was observed as the addition of FA+SS increased the concentration of As, Mn, and Ni compared to the concentration of tailings without amendments. In BCL smelter slag tailings, the heavy metal(loid)s with high concentrations were ranked in this order: Ni>Cu>Mn>Cr>Pb>As. In BCL tailings, the levels of heavy metal(loid)s were Mn>Ni>Cu>Cr>Pb>As and in Monarch tailings, the concentrations of total heavy metal(loid)s were Mn>Cu>Cr>As>Ni>Pb. The results indicate that the addition of amendments led to the reduction of heavy metal(loid)s in the tailings as observed with almost all the heavy metal(loid)s analysed. This is an indication that amendments dilute heavy metal concentrations.

The addition of FA which has a binding effect, led to some heavy metal(loid)s being bonded to FA and reducing their mobility and availability. This is supported by Alinnor (2007) who reported that FA can act as a stabilizer or an adsorbent that can remove heavy metal(loid)s in the soil. The addition of FA improved the soil pH so that micro-organisms present in SS and CM can thrive and break complex compounds of heavy metal(loid)s, making them soluble and available to be absorbed by plants (Bhogal et al.,

2003). Smith (1996) states that different soil properties can influence the uptake of heavy metal(loid)s by plants. These include, among others, pH; organic matter; and soil texture. Soil with favourable pH and sandy texture tends to have a high uptake of heavy metal(loid)s by plants (Bhogal et al., 2003). Therefore, the cultivation of *E. globulus* in these soils also contributed to a reduction of heavy metal(loid)s after the addition of amendments, as some heavy metal(loid)s were taken up by plants.

Influence of Different Soil Amendments on Heavy Metal(loid)s Uptake, Survival and Phytoremediation Efficiency of Selected Tree Species

Table 5.3.4. pH, EC OM & Total heavy metal(loid)s concentration in different mine tailings with amendments after the cultivation of *E. globulus*¹

Sample Code	pH	EC (µs cm ⁻¹)	O.M (%)	As	Cr	Cu	Mn	Ni	Pb
				Concentration (mg kg ⁻¹)					
BCL Tailings Control	4.15±0.24c	3620.00±184.62c	0.00±0.00i	12.59±0.78e	110.00±5.34c	1998.50±99.64d	1134.50±56.55a	1121.50±56.06b	79.05±3.94b
BCL Tailings + FA	7.55±0.47ab	2010.00±126.63g	0.05±0.00h	7.15±0.33f	101.00±4.95c	1102.00±53.98e	906.50±44.41b	945.00±46.29c	63.80±3.13c
BCL Tailings + FA + CM	7.05±0.39b	2210.00±119.34f	2.01±0.09d	5.75±0.32g	64.50±3.22d	1223.00±60.98f	572.50±28.51d	447.00±22.28e	5.10±0.25i
BCL Tailings + FA +SS	7.32±0.45b	1580.00±94.80g	4.05±0.20a	5.25±0.28g	45.50±2.24e	1177.00±58.70f	479.00±25.08e	345.16±17.21f	16.20±0.79h
BCL Smelter Slag Control	3.20±0.16c	7325.00±307.65a	0.00±0.00i	76.70±3.93a	1203.50±58.72a	3499.00±167.57a	1141.50±55.92a	1374.40±68.50a	67.00±3.45c
BCL Smelter slag + FA	7.05±0.28b	5230.00±203.97b	0.01±0.00i	61.00±3.00c	1016.25±50.38a	2416.50±123.80c	883.50±44.06b	1149.50±56.08b	82.00±4.16b
BCL Smelter slag+FA+ CM	7.30±0.39b	3010.00±149.60d	2.35±0.12c	51.00±2.44d	711.20±35.32b	2044.50±100.95d	525.50±23.25d	895.50±44.65c	75.50±4.14b
BCL Smelter slag+FA+SS	7.65±0.44ab	2560.00±124.93e	3.98±0.20a	66.50±3.39b	589.00±29.37b	3149.50±156.78b	367.00±18.55f	696.50±34.67d	63.00±3.06c
MON Tailings Control	4.50±0.22c	1520.00±69.31h	0.50±0.03g	73.70±3.76a	52.02±2.60d	260.50±12.48e	523.00±23.08d	35.31±1.81i	33.08±1.73d
MON Tailings + FA	7.15±0.54b	1010.00±46.26i	0.65±0.03f	49.85±2.43d	46.00±2.24de	246.50±11.49f	620.00±30.36c	54.00±2.66f	29.50±1.50e
MON Tailings + FA + CM	8.70±0.51a	950.00±55.10j	1.82±0.10e	66.55±3.26b	41.03±2.04e	228.00±10.95g	575.50±28.78d	47.00±2.31g	26.30±1.31f
MON Tailings + FA + SS	8.20±0.52a	840.00±45.36k	3.56±0.17b	78.05±3.89c	48.20±2.40d	258.50±12.88f	586.50±28.05cd	66.65±3.86e	31.20±1.52d
<i>P-Value</i>									
<i>Tailings</i>	0.003	0.004	0.005	0.003	0.006	0.002	0.003	0.016	0.041
<i>Amendments</i>	0.002	0.003	0.04	0.002	0.004	0.005	0.006	0.023	0.033
<i>Tailings x Amendments</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Critical values				20.00***	100.00*	36.00*	200.00**	35.00*	85.00*

¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at 5% level of significance.

*Ogundele et al. (2015), **Shah et al. (2013), ***FAO (2006).

5.3.3.4 Effects of amendments on exchangeable heavy metal(loid)s in different mine tailings

The available heavy metal(loid)s in soils are quantified using mild extracting solutions which can dissolve metals from the soil matrix comparable to the strength of the soil solution (Chojnacka et al., 2005). These solutions can quantify the fractions of heavy metal(loid)s that can be mobilized in response to plant uptake and percolating water in the profile. Therefore, quantifying the available fractions of heavy metal contaminants in soil could be more useful in risk assessment and identifying remediation strategies. Exchangeable heavy metal(loid)s in different treatments indicated that the addition of amendments improved the uptake of heavy metal(loid)s by *E. globulus*. As concentration increased from 0.05 (mg kg⁻¹) to 1.31 (mg kg⁻¹) in BCL tailings. In BCL smelter slag, As concentration increased from 0.70 (mg kg⁻¹) to 0.85 (mg kg⁻¹) whereas in MON tailings, As concentration increased from 3.23 (mg kg⁻¹) to 3.85 (mg kg⁻¹). The concentration of Cr, Cu, and Mn was reduced by the addition of amendments across all the tailings. The concentration of Pb was reduced by other amendments (FA, FA+CM) and only increased when FA+SS were added. This was observed across all treatments. The addition of FA, and FA+CM led to a reduction in Cr, Cu, and Mn because FA has fine particles and clay-like properties, causing it to act as a binding agent and adsorbent. These heavy metal(loid)s were bonded to FA, therefore, reducing their mobility and availability. This is supported by Alinnor (2007) who reported that FA can act as a stabilizer, a binding agent or an adsorbent which can remove heavy metal(loid)s in the soil. FA+SS greatly increased the uptake of heavy metal(loid)s in all treatments compared to other amendments (Table 5.3.5). On average, Cr was the highest heavy metal that was available to *E. globulus*. This is largely because Cr is soluble in water (Kožuh et al., 1994). The addition of FA and FA+CM decreased the solubility of heavy metal(loid)s compared

to the control (Table 5.3.5). The alkaline pH of FA neutralized the acidity of mine tailings leading to various chemical changes. This increase in soil pH decreased the solubility of heavy metal(loid)s in the soil through different mechanisms, which include precipitation of cationic heavy metal(loid)s or increased adsorption of heavy metal(loid)s on the soil matrix. This is similar to the findings of Sitarz-Palczak and Kalembkiewicz (2012). The reduction of available cationic metals was also attributed to increased sorption sites and metal precipitation in the form of hydroxides as the soil pH increases due to fly ash amendments (Lwin et al., 2018).

Influence of Different Soil Amendments on Heavy Metal(loid)s Uptake, Survival and Phytoremediation Efficiency of Selected Tree Species

Table 5.3.5. 0.01M CaCl₂ exchangeable heavy metal(loid)s in different mine tailings with amendments after the cultivation of *E. globulus*¹

Sample Code	As	Cr	Cu	Mn	Ni	Pb
	Concentration (mg kg ⁻¹)					
BCL Tailings Control	0.05±0.00d	5.00±0.28a	5.80±0.27b	2.20±0.11a	2.10±0.10c	1.08±0.05ab
BCL Tailings + FA	0.04±0.00d	3.80±0.18bc	4.22±0.21c	1.06±0.05b	2.05±0.10c	0.85±0.42b
BCL Tailings + FA + CM	1.31±0.07c	3.10±0.16c	3.02±0.15d	1.11±0.06b	0.10±0.00d	0.45±0.02a
BCL Tailings + FA +SS	0.58±0.03d	2.30±0.11d	6.12±0.31b	2.45±0.12a	2.14±0.10c	1.75±0.08a
BCL Smelter Slag Control	0.70±0.04c	4.10±0.20b	7.00±0.35a	0.13±0.06d	3.39±0.16b	0.25±0.12d
BCL Smelter slag + FA	0.03±0.00d	3.92±0.20b	5.08±0.20b	0.80±0.04b	2.33±0.11c	0.23±0.11d
BCL Smelter slag+FA+ CM	0.13±0.06c	2.84±0.14c	4.01±0.37c	0.19±0.01d	2.45±0.12c	0.15±0.01d
BCL Smelter slag+FA+SS	0.85±0.02e	4.01±0.20b	7.52±0.38a	0.37±0.02c	4.02±0.20a	0.27±0.13d
MON Tailings Control	3.23±0.01a	5.92±0.29a	3.00±0.15d	0.11±0.01d	0.08±0.00d	1.00±0.05a
MON Tailings + FA	2.03±0.00b	4.05±0.20b	1.01±0.05e	0.06±0.003e	0.06±0.00d	0.85±0.42b
MON Tailings + FA + CM	1.08±0.00c	3.08±0.15c	0.88±0.04e	0.16±0.01d	0.05±0.00d	0.45±0.02c
MON Tailings + FA + SS	3.58±0.01a	5.98±0.29a	3.20±0.16b	0.04±0.00e	0.18±0.00d	1.50±0.07b
P-Value						
Tailings	0.051	ns	0.021	0.034	0.028	ns
Amendments	0.025	ns	ns	0.044	0.039	ns
Tailings x Amendments	0.033	ns	ns	0.042	0.020	ns

ns: not significant

¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at 5% level of significance.

5.3.2.2 Influence of amendments on heavy metal(loid)s accumulation of *E. globulus* grown in different mine tailings

Tables 5.3.6-5.3.8 show total heavy metal concentrations in roots, stems, and leaves of *E. globulus* in roots, the concentration of As was increased by the addition of FA+SS, from 1.19 to 5.65 (mg kg⁻¹). The increase in the uptake of heavy metal(loid)s after the addition of FA+SS was observed in all BCL tailings treatments as Cr, Cu, Ni, Pb, and Mn increased compared to other amendments. In BCL tailings generally, the results exhibit a similar trend. As increased with the addition of amendments (4.35 to 4.90 mg kg⁻¹). The greatest increases were observed in Cu (66.24 to 291.50 mg kg⁻¹), Mn (152.23 to 397.02 mg kg⁻¹), Ni (162.21 to 363.92 mg kg⁻¹), and Pb (62.90 to 297.99 mg kg⁻¹). In Monarch (MON) tailings, a similar trend was observed where the addition of FA+SS led to an increase of heavy metal accumulation in roots. The results indicated that the addition of amendments led to an increase in the uptake of heavy metal(loid)s by *E. globulus* plants. The stem tissues and leaves of *E. globulus* recorded a low uptake of heavy metal(loid)s across all treatments compared to the roots. The addition of FA+SS in all treatments increased heavy metal uptake in plant tissues compared to other amendments like the single addition of FA to treatments and FA+CM. This is attributed to the fact that the sewage sludge amendment improved soil conditions and led to high dry matter compared to other treatments. Therefore, the results indicate that dry matter yield is directly proportional to the uptake of metal(loid)s by *E. globulus*. The capability of the *E. globulus* to uptake heavy metal(loid)s from the soil indicates its great phytoremediation potential. Studies have indicated that *E. globulus* is a hyperaccumulator (Luo et al., 2016). *E. globulus* is one of the species that can survive in harsh conditions with high heavy metal content and still display low symptoms of toxicity (El Rasafi et al., 2021). It is a good candidate for phytoremediation with a huge potential

to stabilize pollutants and accumulate contaminants in its tissues (Luo et al., 2016). The results on heavy metal accumulation in plant tissues indicated that *E. globulus* has a high tolerance for heavy metal(loid)s. Studies by Mok et al. (2013); Hazrat et al. (2014); Luo et al. (2016); Nirola et al. (2016) and Motesharezadeh et al. (2017) revealed that *E. globulus* species had a high bioaccumulation capacity of metals in its tissues.

These metals include among others Cr, Cu, Zn, Ni, Cd, and Pb. The accumulation of heavy metal(loid)s in plant tissues was directly correlated to the total concentrations of heavy metal(loid)s in mine tailings which are taken up passively with the flow of water. (Kabata-Pendias, 2011). This was observed in the roots, stems, and leaves of the *E. globulus* plant species. Overall amendments increased the uptake of heavy metal(loid)s by the roots, stems, and leaves of *E. globulus* due to the addition of amendments. The increase was in this order FA>FA+CM>FA+SS. Although an increase in heavy metal(loid)s was observed, the concentrations were low. The low concentration of metal(loid)s in the plant tissue of *E. globulus* grown in cow manure-amended soils could be attributed mainly to the enhanced fixation of metal(loid)s through complexation with oxygen-containing functional groups of humic acids and fulvic acids from compost amendments (Ding et al., 2019). Padmavathiamma et al. (2007) and Prasad and Freitas (2002) reported that *E. globulus* is good for phytoremediation because it can survive in metalliferous sites by developing the capacity to accumulate huge quantities of indigenous metals without exhibiting symptoms of phytotoxicity. The high quantities of heavy metal(loid)s in roots compared to the stems and leaves indicated that rhizosphere engineering was greatly influenced by amendments (Hakim et al., 2021). Generally, results also indicate that the *E. globulus* accumulated more heavy metal(loid)s in its roots compared to the stem and leaves. This shows that heavy metal(loid)s reach the above tree parts in low quantities

therefore strengthening the phytoremediation potential of *E. globulus*. This finding is also supported by (Luo et al. 2016).

El Rasafi et al. (2021) studied the potential of *E. globulus* on the phytoremediation of mine soils. The results from their study indicated that the *E. globulus* species had a high bioaccumulation of metals in its tissues, such as Cd, Cu, Pb, Ni, Zn, and Cr without exhibiting signs of toxicity (Mok et al., 2013; Hazrat et al., 2014; Luo et al., 2016; Nirola et al., 2016; Motesharezadeh et al., 2017).

Table 5.3.6. Total heavy metal(loid)s concentration in the roots of *E. globulus* grown in different mine tailings with amendments¹

Sample Code	As	Cr	Cu	Mn	Ni	Pb
	Concentration (mg kg ⁻¹)					
BCL Tailings Control	-	-	-	-	-	-
BCL Tailings + FA	1.90±0.10g	54.75±2.98c	87.55±4.35e	144.00±7.20d	71.88±3.98	70.38±3.51e
BCL Tailings + FA + CM	2.30±0.11f	49.09±2.72d	51.45±2.57g	218.87±11.72b	89.04±4.45e	97.20±4.75d
BCL Tailings + FA + SS	5.65±0.29a	68.55±3.43b	156.35±7.66b	224.55±10.99b	95.24±4.75d	119.34±6.50b
BCL Smelter Slag Control	-	-	-	-	-	-
BCL Smelter slag + FA	4.35±0.23c	56.51±2.82c	66.25±3.6f	152.23±8.23c	164.21±8.56c	62.90±3.223
BCL Smelter slag+FA+ CM	4.83±0.26b	78.40±4.62a	116.00±5.80c	158.16±8.62c	192.00±9.39b	82.72±4.48c
BCL Smelter slag+FA+SS	4.90±0.25b	83.40±4.51a	291.50±14.16a	397.02±19.34a	363.92±18.60a	297.99±15.26a
MON Tailings Control	-	-	-	-	-	-
MON Tailings + FA	3.05±0.15e	9.70±0.48f	16.65±0.76h	82.11±4.31f	0.21±0.01h	0.00±0.00h
MON Tailings + FA + CM	3.90±0.20d	30.69±1.60e	104.00±5.33d	116.48±5.97e	1.28±0.06g	0.03±0.00g
MON Tailings + FA + SS	3.95±0.21d	55.39±3.12c	149.00±7.44b	142.45±7.30d	10.11±0.50f	9.03±0.50f
P-Value						
Tailings	0.037	0.052	0.062	0.029	0.027	0.044
Amendments	0.042	0.048	0.035	0.069	0.035	0.025
Tailings x Amendments	0.029	0.038	0.044	0.043	0.018	0.036
Critical Values	10-20*	75-100*	100.00**	200.00**	50-150**	20.00**

-: no survival

¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at 5% level of significance.

*Hajar et al. (2014) **Manyiwa et al. (2021)

Influence of Different Soil Amendments on Heavy Metal(loid)s Uptake, Survival and Phytoremediation Efficiency of Selected Tree Species

Table 5.3.7. Total heavy metal(loid)s concentration in the stem of *E. globulus*¹

Sample Code	As	Cr	Cu	Mn	Ni	Pb
	Concentration (mg kg ⁻¹)					
BCL Tailings Control	-	-	-	-	-	-
BCL Tailings + FA	2.50±0.12d	16.08±0.86b	2.80±0.14h	100.57±5.25d	4.67±0.24e	0.00±0.00f
BCL Tailings + FA + CM	4.20±0.21b	16.48±0.81b	13.35±0.74c	213.35±10.91a	22.05±1.08d	0.05±0.00f
BCL Tailings + FA +SS	5.22±0.26a	19.47±0.95a	17.00±0.87b	222.54±12.04a	36.41±1.86b	4.78±0.25c
BCL Smelter Slag Control	-	-	-	-	-	-
BCL Smelter slag + FA	1.20±0.06g	5.74±0.29e	11.10±0.54d	79.93±4.00e	2.71±0.14g	0.00±0.00f
BCL Smelter slag+FA+ CM	1.35±0.07f	6.08±0.32d	9.00±0.46e	142.00±7.32c	26.78±1.43c	8.32±0.43b
BCL Smelter slag+FA+SS	3.10±0.15c	19.07±0.97a	18.70±0.10a	164.18±8.39b	67.38±3.30a	10.06±0.54a
MON Tailings Control	-	-	-	-	-	-
MON Tailings + FA	0.00±0.00h	1.49±0.08g	0.00±0.00i	44.30±2.40f	2.82±0.14g	0.33±0.02e
MON Tailings + FA + CM	1.75±0.09e	4.71±0.25f	6.60±0.33g	79.67±3.97e	3.44±0.18f	3.33±0.16d
MON Tailings + FA + SS	3.25±0.17c	6.11±0.31d	7.50±0.37f	141.71±7.08c	4.21±0.24e	3.57±0.19d
P-Value						
Tailings	0.032	0.027	0.087	0.055	0.087	0.037
Amendments	0.042	0.068	0.054	0.045	0.095	0.091
Tailings x Amendments	0.033	0.034	0.021	0.039	0.050	0.042
Critical Values	10-20*	75-100*	100.00**	200.00**	50-150**	20.00**

-: no survival

¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at 5% level of significance.

*Hajar et al. (2014) **Manyiwa et al. (2021)

Influence of Different Soil Amendments on Heavy Metal(loid)s Uptake, Survival and Phytoremediation Efficiency of Selected Tree Species

Table 5.3.8. Total heavy metal(loid)s concentration in the leaves of *E. globulus*¹

Sample Code	As	Cr	Cu	Mn	Ni	Pb
	Concentration (mg kg ⁻¹)					
BCL Tailings Control	-	-	-	-	-	-
BCL Tailings + FA	0.00±0.00i	1.61±0.08g	0.00±0.00g	65.97±3.29h	4.09±0.20f	1.05±0.05h
BCL Tailings + FA + CM	1.80±0.09h	8.11±0.41c	9.15±0.51f	84.40±4.10g	9.76±0.50e	1.37±0.07g
BCL Tailings + FA +SS	2.80±0.14e	4.80±0.25d	71.00±3.68d	153.21±7.63e	11.52±0.61d	9.60±0.49a
BCL Smelter Slag Control	-	-	-	-	-	-
BCL Smelter slag + FA	2.35±0.13g	2.53±0.13f	166.95±8.70b	133.88±6.67f	16.24±0.83cb	3.44±0.18d
BCL Smelter slag+FA+ CM	2.56 ±0.14f	2.68±0.14f	177.12±9.08b	163.32±7.96d	17.09±0.91b	5.11±0.26c
BCL Smelter slag+FA+SS	3.95±0.18b	3.59±0.15e	216.60±9.47a	262.81±12.58c	19.38±0.99a	7.89±0.40b
MON Tailings Control	-	-	-	-	-	-
MON Tailings + FA	3.25±0.17d	4.49±0.24d	0.00±0.00g	153.75±7.50e	7.78±0.42g	2.30±0.11f
MON Tailings + FA + CM	3.45±0.18c	11.91±0.58b	18.05±0.92e	417.48±20.45b	14.75±0.74c	2.67±0.14e
MON Tailings + FA + SS	5.35±0.26a	14.09±0.74a	76.00±4.07c	847.56±42.27a	18.08±0.98b	9.25±0.40a
P-Value						
Tailings	0.038	0.041	0.043	0.044	0.038	0.041
Amendments	0.022	0.036	0.038	0.047	0.022	0.048
Tailings x Amendments	0.011	0.049	0.022	0.031	0.011	0.018
Critical Values	10-20*	75-100*	100.00**	200.00**	50-150**	20.00**

-:no survival

¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at 5% level of significance.

*Hajar et al. (2014) **Manyiwa et al. (2021)

5.3.4 Conclusions

E. globulus presented certain beneficial qualities that could be used for the phytoremediation of metals in mine tailings in Botswana. Throughout the experiment, *E. globulus* was able to recover from an initial state of oxidative stress, and while its growth, leaf development, and pigment content were somewhat impacted in mine soil when compared to the other soils, photosynthetic efficiency and chlorophyll content were not significantly improved by amendments. Furthermore, *E. globulus* bioaccumulated larger concentrations of metals in the roots, including Cu, Mn, and Ni, which reduced the likelihood of their transfer to the above ground tissues and decreased the risk of contamination spreading. Because of this, *eucalyptus* trees' inherent resistance to severe weather conditions (such as drought and high temperatures) common in semi-arid to arid locations, and their adaptability to survive in contaminated environments, make them good candidates for phytoremediation. However, without the addition of amendments, *E. globulus* species could not survive in pure mine tailings from the BCL mine and Monarch Gold Mine. The addition of amendments had a positive effect on tree growth, dry matter yield, and heavy metal uptake since they increased all these parameters, indicating the effectiveness of amendments. The addition of amendments increased the uptake of heavy metal(loid)s by *E. globulus* thus enhancing its phytoremediation potential in BCL and Monarch Gold Mine tailings by improving the survival, growth, and dry matter yield. Amendments also increased the uptake of heavy metal(loid)s while some decreased the uptake of heavy metal(loid)s by *E. globulus*. Therefore, *E. globulus* has a great potential as a phytoremediation species in mine tailings provided that fly ash, cow manure, and sewage sludge mycorrhiza are used during its establishment.

5.3.5 General Conclusions of Chapter 5

These studies revealed that adding amendments to mine tailings positively affected the growth of trees, and dry matter yield. The amendment can either reduce or increase heavy metal availability depending on the chemical characteristics of each metal under consideration. Overall results had shown increase in uptake by trees which is attributed mainly to improvement of growth and enhanced dry matter yield, thus enhancing the phytoremediation potential of these tree species. The study also established that a combination of amendments like compost and fly ash, compost and mycorrhiza, and fly ash and mycorrhiza greatly influenced growth, dry matter yield, and heavy metal uptake. Although the combination of amendments had a different influence according to tree species it appeared that the combination of these amendments had synergistic effect on the ability of these different tree species for phytoremediation. Further studies should be conducted to evaluate other readily available materials in Botswana like biochar and lignite on these mine tailings and how they could enhance the phytoremediation of these five tree species.

5.3.6 References

- Abdel-Shafy, H. I., & Mansour, M. S. (2018). Phytoremediation for the elimination of metals, pesticides, PAHs, and other pollutants from wastewater and soil. *Phytobiont and ecosystem restitution*, 101-136.
- Akmal, M., Gondal, T. A., Khan, K. S., Hussain, Q., Ahmad, M., Abbas, M. S., ... & Khosa, S. A. (2019). Impact of biochar prepared from leaves of *Populus euphratica* on soil

- microbial activity and mung bean (*Vigna radiata*) growth. *Arabian Journal of Geosciences*, 12, 1-8.
- Alinnor, I. J. (2007). Adsorption of heavy metal ions from aqueous solution by fly ash. *Fuel*, 86(5-6), 853-857.
- Almeida, A., Valle, R., Milke & M., Gomes, F. (2007) Tolerance and prospection of phytoremediator woody species of Cd, Pb, Cu and Cr Brazilian. *Journal of Plant Physiology* 9:83–98. <https://doi.org/10.1590/S1677-04202007000200001>
- Alvarenga, P., Farto, M., Mourinha, C., & Palma, P. (2016). Beneficial use of dewatered and composted sewage sludge as soil amendments: behaviour of metals in soils and their uptake by plants. *Waste and biomass valorization*, 7, 1189-1201.
- Arriagada, C. A., Herrera, M. A., & Ocampo, J. A. (2007). Beneficial effect of saprobe and arbuscular mycorrhizal fungi on growth of *Eucalyptus globulus* co-cultured with *Glycine max* in soil contaminated with heavy metals. *Journal of Environmental Management*, 84(1), 93-99.
- Azeez, J. O., & Van Averbek, W. (2012). Dynamics of soil pH and electrical conductivity with the application of three animal manures. *Communications in Soil Science and Plant Analysis*, 43(6), 865-874.
- Balasubramaniam, P. (2020). Fly ash as a source of silicon for mitigating biotic stress and improving yield and changes in biochemical constituents and silicon in rice under abiotic stress. In: Ghosh S., Kumar V. (eds.), *Circular economy and fly ash management 2020*, 145–60. Singapore: Springer.

- Bhogal, A., Nicholson, F. A., Chambers, B. J., & Shepherd, M. A. (2003). Effects of past sewage sludge additions on heavy metal availability in light textured soils: implications for crop yields and metal uptakes. *Environmental pollution*, 121(3), 413-423.
- Bilal, H., Ali, S. S., & Kim, K. M. (2014). Potential of Eucalyptus in the remediation of environmental problems: a review. *International Journal of Innovative Science and Research Technology*, 4(2), 136-144.
- Burducea, M., Lobiuc, A., Asandulesa, M., Zaltariov, M. F., Burducea, I., Popescu, S. M., & Zheljazkov, V. D. (2019). Effects of sewage sludge amendments on the growth and physiology of sweet basil. *Agronomy*, 9(9), 548.
- Cao, J. P., Li, L. Y., Morishita, K., Xiao, X. B., Zhao, X. Y., Wei, X. Y., & Takarada, T. (2013). Nitrogen transformations during fast pyrolysis of sewage sludge. *Fuel*, 104, 1-6.
- Chojnacka, K., Chojnacki, A., Gorecka, H., & Go'recki, H. (2005). Bioavailability of Heavy metals from polluted soils to plants. *Science of the Total Environment*, 337(1-3), 75-182.
- Ding, H., Tang, L., Nie, Y., & Ji, H. (2019). Characteristics and interactions of heavy metals with humic acid in gold mining area soil at a upstream of a metropolitan drinking water source. *Journal of geochemical exploration*, 200, 266-275.
- Banu Doganlar, Z., Doganlar, O., Erdogan, S., & Onal, Y. (2012). Heavy metal pollution and physiological changes in the leaves of some shrub, palm and tree species in urban areas of Adana, Turkey. *Chemical Speciation & Bioavailability*, 24(2), 65-78.
- Ekosse, G. (2005). Heavy metals concentrations in the biophysical environment around the Ni-Cu mine and the smelter/concentrator plant, Selebi Phikwe, Botswana. *Global Journal of Environmental Sciences*, 4(2), 97-110.

- El Rasafi, T., Pereira, R., Pinto, G., Gonçalves, F. J., Haddioui, A., Ksibi, M., ... & Marques, C. R. (2021). Potential of Eucalyptus globulus for the phytoremediation of metals in a Moroccan iron mine soil—A case study. *Environmental Science and Pollution Research*, 28, 15782-15793.
- Farrell, M. & D. L. Jones. (2010). Use of composts in the remediation of heavy metal contaminated soil. *Journal of hazardous materials*, 175 (1–3):575–82.
- Filipović-Trajković, R., Ilić, Z. S., Šunić, L., & Andjelković, S. (2012). The potential of different plant species for metal(loid)s accumulation and distribution. *Journal of Agriculture, Food and Environment*, 10(1), 959-964.
- Gu, D. D., Wang, W. Z., Hu, J. D., Zhang, X. M., Wang, J. B., & Wang, B. S. (2016). Nondestructive determination of total chlorophyll content in maize using three-wavelength diffuse reflectance. *Journal of Applied Spectroscopy*, 83, 541-547.
- Hajar, E. W. I., Sulaiman, A. Z. B., & Sakinah, A. M. (2014). Assessment of heavy metals tolerance in leaves, stems and flowers of Stevia rebaudiana plant. *Procedia Environmental Sciences*, 20, 386-393.
- Hakim, S., Naqqash, T., Nawaz, M. S., Laraib, I., Siddique, M. J., Zia, R., ... & Imran, A. (2021). Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability. *Frontiers in Sustainable Food Systems*, 5, 617157.
- Han, S. H., D. H. Kim, V. U. Ultra, P. Kim, & N. S. Aggangan. (2012). Physiological response of Cd-and Pb-treated Populus alba glandulosa seedlings amended with SCB liquid fertilizer. *Asia Life Sciences*, 21 (2):481–98.

- Hazrat, B., Ali S.S., & Kim K.M. (2014). Potential of Eucalyptus in the remediation of environmental problems: a review. *International Journal of Science and Innovative Research* 4: 136–144.
- Heikens, A. (2006). Arsenic contamination of irrigation water, soil and crops in Bangladesh: Risk implications for sustainable agriculture and food safety in Asia. *Rap Publication* (FAO).
- Hussain, F., I. Hussain, A. H. Khan, Y. Muhammad, M. Iqbal, G. Soja, T. G. Reichenauer, & S. Yousaf. (2018). Combined application of biochar compost and bacterial consortia with Italian ryegrass enhanced phytoremediation of petroleum hydrocarbon contaminated soil. *Environmental and Experimental Botany*, 153:80–88.
- Irshad, M., Malik, A. H., Shaukat, S., Mushtaq, S., & Ashraf, M. (2013). Characterization of Metal(loid)s in Livestock Manures. *Polish Journal of Environmental Studies*, 22(4).
- Jefferis, K. (2009). The role of TNCs in the extractive industry of Botswana. *Transnational corporations*, 18(1), 61-92.
- Kabata-Pendias, A. (2011). *Trace elements in soils and plants*. (4th ed.). CRC Press.
- King, D.J, Doronila A.I, Feenstra, C, Baker, A.J.M, Woodrow, I.E. (2008). Phytostabilisation of arsenical gold mine tailings using four Eucalyptus species: Growth, arsenic uptake and availability after five years. *Science of the Total Environment* 406:35;42.
- Kožuh, N., Štupar, J., Milačić, R., & Gorenc, B. (1994). Optimization of extraction procedure for determination of total water-soluble chromium and chromium (VI) in various soils. *International journal of environmental analytical chemistry*, 56(3), 207-217.

- Lwin, C. S., Seo, B. H., Kim, H. U., Owens, G., & Kim, K. R. (2018). Application of soil amendments to contaminated soils for heavy metal immobilization and improved soil quality—A critical review. *Soil Science and Plant Nutrition*, 64(2), 156–167.
- Luo, J., Qi, S., Peng, L., & Wang, J. (2016). Phytoremediation efficiency of Cd by *Eucalyptus globulus* transplanted from polluted and unpolluted sites. *International Journal of Phytoremediation* 18:308–314. <https://doi.org/10.1080/15226514.2015>.
- Luo, J., Qi, S., Peng, L., & Xie, X. (2015). Phytoremediation potential of cadmium-contaminated soil by *Eucalyptus globulus* under different coppice systems. *Bulletin of environmental contamination and toxicology*, 94, 321-325.
- Manyiwa, T., Ultra Jr, V. U., Hengari, M., & Rantong, G. (2023). Spatial variability of heavy metals in soils and vegetation and associated risk to grazing animals in the abandoned gold mine in Francistown, Botswana. *Environmental Pollutants and Bioavailability*, 35(1), 2254493.
- Manyiwa, T., Ultra, V.U., Rantong, G., Opaletswe, K.A., Gabankitse, G., Taupedi, S.B., & Gajaje, K. (2021). Heavy metals in soil, plants, and associated risk on grazing ruminants in the vicinity of Cu–Ni mine in Selebi-Phikwe, Botswana. *Environmental Geochemistry and Health*. 44 (2022):1633-1648. <https://doi.org/10.1007/s10653-021-00918-x>
- Manyiwa, T., & Ultra Jr, V. U. (2022). Soil Amendments and Arbuscular Mycorrhiza Influenced the Growth and Heavy Metal Accumulation of *Colospospermum Mopane* (Kirk Ex Benth.) In Heavy Metal Contaminated Soil. *Soil and Sediment Contamination: An International Journal*, 31(1), 81-96.
- Mok, H.F, Majumder, R., Laidlaw, W.S., Gregory, D., Baker A.J. & Arndt, S.K. (2013). Native Australian species are effective in extracting multiple heavy metals from biosolids.

Mokolobate, M. S., & R. J. Haynes. (2002). Comparative liming effect of four organic residues applied to an acid soil. *Biology and Fertility of Soils* 35:79–85.

Motesharezadeh, B., Kamal-poor, S., Alikhani, H. A., Zariee, M., & Azimi, S. (2017). Investigating the effects of plant growth promoting bacteria and *Glomus Mosseae* on cadmium phytoremediation by *Eucalyptus camaldulensis* L. *Pollution*, 3(4), 575-588.

Najafi, N., Mahmoudi, S. H., & Reyhanitabar, A. (2015). Effects of soil moisture and sewage sludge compost on leaf chlorophyll index and some growth traits of alfalfa in greenhouse conditions. *Journal of Soil and Plant Interactions-Isfahan University of Technology*, 5(4), 205-218.

Nirola, R., Megharaj, M., Aryal, R. & Naidu R. (2016). Screening of metal uptake by plant colonizers growing on abandoned copper mine in Kapunda, South Australia. *International Journal of Phytoremediation* 18:399–405.
<https://doi.org/10.1080/15226514.2015.1109599>.

Ogundele, D.T., Adio, A.A. & Oludele, O.E. (2015). Heavy Metal Concentrations in Plants and Soil along Heavy Traffic Roads in North Central Nigeria. *Journal of Environmental Analytical Toxicology* 5: 334. doi:10.4172/2161-0525.1000334.

Padmavathiamma, P. K., & Li, L. Y. (2007). Phytoremediation technology: hyper-accumulation metals in plants. *Water, Air, and Soil Pollution*, 184, 105-126.

Pandey, V. C., & Singh, N. (2010). Impact of fly ash incorporation in soil systems. *Agriculture, ecosystems & environment*, 136(1-2), 16-27.

- Pathan, S. M., Aylmore, L. A. G., & Colmer, T. D. (2003). Properties of several fly ash materials in relation to use as soil amendments. *Journal of environmental quality*, 32(2), 687-693.
- Petruzzelli, G. (1996). Heavy metals in compost and their effect on soil quality. In: de Bertoldi M., Lemmes B., Papi T. (eds,). *The science of composting*, 213–23. Dordrecht: Springer.
- Pocknee, S., & M. E. Sumner. (1997). Cation and nitrogen contents of organic matter determine its liming potential. *Soil Science Society of America Journal* 61:86–92.
- Prasad, M. N.V, & de Oliveira Freitas, H. M. (2003). Metal hyperaccumulation in plants: biodiversity prospecting for phytoremediation technology. *Electronic journal of biotechnology*, 6(3), 285-321.
- Pratt, P. F. (1984). Salinity, sodium, and potassium in an irrigated soil treated with bovine manure. *Soil Science Society of America Journal* 48:823–828
- Raja, R., Nayak, A. K., Rao, K. S., Puree, C., Shahid, M., Panda, B. B., ... & Gautam, P. (2014). Effect of fly ash deposition on photosynthesis, growth and yield of rice. *Bulletin of environmental contamination and toxicology*, 93, 106-112.
- Shah, A., Niaz, A., Ullah, N., Rehman, A., Akhlaq, M., Zakir, M., & Suleman Khan, M. (2013). Comparative study of Heavy metals in soil and selected medicinal plants. *Journal of Chemistry*, 2013. <https://doi.org/10.1155/2013/621265>.
- Sharma, B., & R. P., Singh. (2019). Physiological, biochemical, growth, and yield responses of radish (*Raphanus sativus* L.) plants grown on different sewage sludge–fly ash mixture (SLASH) ratios. In: Ghosh S. (eds,). *Waste valorisation and recycling*, 539–52. Singapore: Springer.

- Shrestha, R. K., & Lal, R. (2011). Changes in physical and chemical properties of soil after surface mining and reclamation. *Geoderma*, 161(3-4), 168-176.
- Sims, D. A., & Gamon, J. A. (2002). Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote sensing of environment*, 81(2-3), 337-354.
- Singh, J. S., & Pandey, V.C. (2013). Fly ash application in nutrient poor agriculture soils: Impact on methanotrophs population dynamics and paddy yields. *Ecotoxicology and Environmental Safety*, 89:43–51.
- Singh, S., Parihar, P., Singh, R., Singh, V. P., & Prasad, S. M. (2016). Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. *Frontiers in plant science*, 6, 1143.
- Sitarz-Palczak, E., & Kalembkiewicz, J. (2012). Study of remediation of soil contaminated with metal(loid)s by coal fly ash. *Journal of Environmental Protection*, 3(10), 1373.
- Smith, S.R., (1996). *Agricultural Recycling of Sewage Sludge and the Environment*. Cab International, Wallingford, UK.
- Ultra, V. U. (2020). Fly ash and compost amendments and mycorrhizal inoculation enhanced the survival and growth of *Delonix regia* in Cu-Ni mine tailings. *Philippine Journal of Science* 149 (3):479–89.
- Ultra, V. U., & T. Manyiwa. (2021). Influence of mycorrhiza and fly ash on the survival, growth and heavy metal accumulation in three acacia species grown in Cu–Ni mine soil. *Environmental Geochemistry and Health*, 43:1337–53.
- Wong, M. T. F., S. Nortcliff, & R. S. Swift. (1998). Method for determining the acid ameliorating capacity of plant residue compost, urban waste compost, farmyard manure, and peat

applied to tropical soils. *Communications in Soil Science and Plant Analysis* 29:2927–2937

- Younis, U., Qayyum, M. F., Shah, M. H. R., Danish, S., Shahzad, A. N., Malik, S. A., & Mahmood, S. (2015). Growth, survival, and heavy metal (Cd and Ni) uptake of spinach (*Spinacia oleracea*) and fenugreek (*Trigonella corniculata*) in a biochar-amended sewage-irrigated contaminated soil. *Journal of Plant Nutrition and Soil Science*, 178(2), 209-217.
- Zbytniewski, R., & Buszewski, B. (2005). Characterization of natural organic matter (NOM) derived from sewage sludge compost. Part 1: chemical and spectroscopic properties. *Bioresource technology*, 96(4), 471-478.
- Zhang, S., Sun, L., Wang, Y., Fan, K., Xu, Q., Li, Y. & Ding, Z. (2020). Cow manure application effectively regulates the soil bacterial community in tea plantation. *BMC microbiology*, 20(1), 1-11.
- Zhao, H., Yang, C., Zhang, X., Zhang, Y., & Qiu, G. (2021). *Biohydrometallurgy of Chalcopyrite*. Elsevier.
- Zhou, H., Liu, G., Zhang, L., Zhou, C., Mian, M. M., & Cheema, A. I. (2021). Strategies for arsenic pollution control from copper pyrometallurgy based on the study of arsenic sources, emission pathways and speciation characterization in copper flash smelting systems. *Environmental Pollution*, 270, 116203.

CHAPTER 6

Potential of Different Tree Species {*Eucalyptus Globulus*, *Colophospermum Mopane* and Three *Vachelia (acacia)* Spp.} For Phytoremediation of Mine Tailings

Chapter 6 addresses the last objective of the study which is to evaluate the potential of different tree species {*Eucalyptus globulus* (blokomo), *Colophospermum mopane*, *Vachellia tortilis* (Mosu), *Vachellia luederitzii* (Mokala) and *Fairherbia albida* (Mokosho)} for phytoremediation of mine tailings by comparative evaluation of their growth, and development, as well as examining heavy metal bioaccumulation. Using the data presented in Chapter 5, the comparative evaluation of five different tree species for their phytoremediation potential was conducted based on the survival rate, relative dry matter yield/week, and heavy metal accumulation and uptake capacity in plant tissues (shoots and roots). The evaluation was only based on the tree species grown in BCL mine tailings and those applied with the same amendments, namely, arbuscular mycorrhiza, Fly ash, and compost. The results indicated that of the five species, *V. luederitzii* had the highest survival rates and relative dry matter yield followed by *F. Albida* & *V. tortilis* in pure mine tailings. These tree species accumulated heavy metal(loid)s in roots more than the shoots and the order of accumulation as follows: *V. luederitzii* > *F. albida* > *V. tortilis* > *C. mopane* > *E. globulus*. Among these tree species, *V. luederitzii* and *F. albida* can accumulate heavy metal(loid)s or tolerate high concentrations of heavy metal(loid)s in the tailings without interference of their growth and development. This elucidated the mechanisms of how the different tree species performed under mine tailings and how the amendments influenced their

phytoremediation potential. This chapter is comprised of one manuscript by the author that has been reproduced as submitted for review and possible publication.

Manuscript 6.1;

Submitted to the Journal Soil Science and Environment as **Manyiwa, T.**, Ultra V.U., Gajaje, K., Gaotlhobogwe, K., Sefatlhi, K. (20xx). Variability of the phytoremediation potential of different tree species in BCL Cu-Ni mine tailings. *Soil Science and Environment*.

Authors Contribution:

Trust Manyiwa is the main author and my supervisor, **Prof. Venecio Ultra** is the co-author.

Manyiwa T- conceptualization and planning, data gathering and analysis, validation of methodology, interpretation of results, writing and revision of manuscript. **Ultra VU**- project administration, conceptualization and planning; supervision of fieldwork and laboratory analyses; Interpretation of results; and review and editing of manuscript. **Gajaje, K., Gaotlhobogwe, K. & Sefatlhi, K**- Fieldwork and sample collection; sample preparation and analysis, data interpretation and analysis, writing and review of different sections of the manuscript.

6.1 Variability of the phytoremediation potential of different tree species in BCL Cu-Ni mine tailings

Abstract

Various studies have used only one tree species to evaluate its phytoremediation potential. In this study, five different species were evaluated for their phytoremediation potential under the same mine tailings from the BCL Cu-Ni mine and amendments. The survival rate, dry matter yield/week, and uptake/removal capacity of heavy metal(loid)s by these species were used to evaluate which tree had the greatest phytoremediation potential. The tree species selected for the study were *Eucalyptus globulus*, *Colophospermum mopane*, *Vachellia tortilis*, *Vachellia luederitzii*, and *Fairherbia albida*. The amendments that were applied to enhance the phytoremediation potential of these trees were fly ash, compost, and arbuscular mycorrhiza. The best amendment that improved the survival rate and dry matter yield per week was the addition of fly ash (FA) combined with arbuscular mycorrhiza (FA+AM) followed by compost combined with arbuscular mycorrhiza (CM+AM). These amendments improved the survival rate of all species by 100%. The addition of compost alone was found to be the least effective amendment as tree species like *E. globulus*, *V. luederitzii*, *F. albida*, and *E. globulus* recorded a survival rate of 50-70%. *V. luederitzii* recorded the highest overall dry matter yield/week. The study indicated that rhizobacteria and mycorrhiza altered nutrient and heavy metal bioavailability in mine tailing deposits through the release of chelating substances, acidification of the microenvironment, and by influencing changes in redox potential, whereas fly ash altered the pH of the mine tailings. Compost modified the bio-physicochemical properties of soils and provided nutrients for plant growth and development. Overall, the results indicated that the addition of the correct

amendments to all treatments allows tree species to survive in BCL mine tailings. Heavy metal uptake capacity or accumulation was affected by the amendments, and each amendment affected each heavy metal differently, allowing each tree species to have phytoremediation potential depending on the heavy metal uptake.

Keywords: dry matter yield, heavy metal(loid)s, meta-analysis

6.1.1 Introduction

Mining activities tend to affect the environment through land degradation, and the presence of heavy metal(loid)s. Generally, mining activities cause disturbances to many soil properties, including pH, electrical conductivity, and cation exchange capacity (Saleem et al., 2020). Subsequently, metal(loid)s such as Arsenic (As), Copper (Cu), Lead (Pb), Zinc (Zn), Nickel (Ni), Cadmium (Cd), Chromium (Cr), Iron (Fe), Manganese (Mn) and other organic pollutants such as hydrocarbons, have been found at elevated concentrations in the soil and sediments in mining areas (Gutiérrez-Ginés et al., 2014; Liu et al., 2017b; Song et al., 2017). Therefore, tree species could offer a solution to remediate these heavy metal(loid)s from the environment through a process known as phytoremediation (Gomez et al., 2019). Phytoremediation is a method that utilizes plants to remediate contaminated environments. Phytoremediation is an inexpensive and environmentally favorable technique as it utilizes photosynthesizing plants to sequester or cleanse contaminants from contaminated soil and water (Ashraf et al., 2019). Different plants use one or more mechanisms to remediate different contaminants at different rates. Using a variety of methods, including genetic engineering, natural and chemical modifications, and natural microbial stimulation, the limitations of phytoremediation can be assessed (Kafle et al., 2022). Different plants utilize various strategies or combinations of strategies to remediate soil and water. The strategies should be contingent upon the pollutant types, structures, and media. Polluted groundwater can be remediated through phytodegradation, phytovolatilization, rhizo-filtration, rhizodegradation, and phytodegradation. Surface and wastewater pollutants can be treated by rhizofiltration, phytodegradation, or rhizodegradation. Soil, dregs, or slime defilements are

remediated through phytoextraction, phytodegradations, phytostabilization, rhizodegradation, or phytovolatilization (Kafle et al., 2022).

Rapid growth, a high biomass, and a high tolerance to heavy metal(loid)s are desirable qualities for the plants utilized in this process of phytoremediation (Marques, 2009). Immobilizing or limiting the mobility of heavy metal(loid)s is the goal of phytostabilization. Metals can precipitate in the rhizosphere, accumulate by the roots, or be absorbed on their surface (Podar and Maathuis, 2022). Plants with a strong root system and a high level of heavy metal tolerance are suitable candidates for phytostabilization. Additionally, these plants must have low metal translocation from roots to shoots and the ability to tolerate high metal buildup in the roots (Karczewska et al., 2013, Korzeniowska et al., 2011, Stanisławska-Głubiak et al., 2012). Therefore, the advantage of using multi-species outweighs the use of a single species for phytoremediation. Tree species are potential plants for phytoremediation due to their rapid growth and extensive root system (Shi et al., 2016). This study will evaluate the potential of five different tree species for the phytoremediation of BCL tailings. These are *C. mopane*, *V. tortilis*, *V. luederitzii*, *F. albida* and, *E. globulus*.

6.1.2 Materials and Methods

The data on survival rate, dry matter yield, and heavy metal accumulation by five different tree species from Chapter 5 was computed and analyzed through meta-analysis. Relative dry matter yield was calculated by dividing the dry matter yield of each species as affected by different amendments with the growing period (weeks) (Eq 1.).

$$\text{Relative Dry matter yield} = \frac{\text{Dry matter yield}}{\text{Number of weeks since planting trees}} \quad [\text{Eq.1}]$$

Total heavy metal(loid)s accumulated by different tree species as affected by amendments were estimated by multiplying the relative dry matter yield by the total HM concentration in the tissues to obtain heavy metal uptake capacity and removal rate through biomass extraction (Eq. 2).

$$\text{HM uptake capacity} = \text{Total HM} \times \text{Relative Dry matter yield} \quad [\text{Eq.2}]$$

The transformed data were used to assess the heavy metal uptake and removal rate by these five different species as influenced by different amendments like fly ash, cow manure, and sewage sludge. 10% of these amendments were applied singularly or in combination. The tree species that were used for meta-analysis were grown in a pot experiment in the same tailings from BCL and the same amendments were applied. *C. mopane* was grown for 40 weeks, *V. tortilis*, *F. albida*, and *V. luederitzii* had a growth period of 24 weeks, and *E. globulus* was grown for 52 weeks.

6.1.3 Statistical analysis

The transformed data was analyzed using a single-factor ANOVA in CRD to determine the differences in heavy metal concentration accumulation between tree species. When significant differences were observed, Tukey's test was performed for treatment mean (tree species) comparison at a 5% level of significance. Each parameter was analyzed separately per tree species.

6.1.4 Results and Discussion

6.1.4.1 Effects of amendments on the Survival Rate & Dry matter yield per Week of tree species

Table 6.3.1 indicates that all tree species grown in mine tailings without amendments (soil alone) had a survival rate below 100%. Specifically, *Eucalyptus globulus* had a 0% survival rate. *V. tortilis* had a 30% survival rate whereas *C. mopane*, *V. luederitzii*, and *F. albida* each had a survival rate of 70%. Similarly, those tree species that have survived had low relative dry matter yield especially *C mopane*. This survival rate and relative dry matter yield were attributed to the acidic nature of mine tailings and the high concentration of heavy metal(loid)s in the tailings which hinders plant growth due to the deficiency of essential nutrients and toxicity of some metals. With the addition of mycorrhiza, the survival rate of all tree species improved with *V. tortilis* and *V. luederitzii* recording a 100% survival rate and *E. globulus* improving from 0% to 70%. In addition, *V. luederitzii* recorded a highly significant faster growth rate and significantly higher relative dry matter yield (3.18g/plant/week) compared to the corresponding plants in pure mine tailings (control) due to mycorrhiza inoculation.

The addition of 10% FA on mine tailings significantly improved the survival rate and relative dry matter yield of all tree species as compared to those in the control. The relative dry matter yield of *V. luederitzii*, and *F. albida* is significantly higher than the increase of relative dry matter yield in the presence of Mycorrhiza alone. The combination of FA+AM resulted in 100% survival rate and significantly higher relative dry matter yield of all tree species as compared to control and FA alone, and in tailings amended with AM alone. The difference between FA alone and FA+AM on relative dry matter yield of *F. albida* and *V.*

luederitzii, is not significantly different as denoted by the same letters in the Table 6.3.1. The addition of FA+AM significantly improved the dry matter yield of *E. globulus* compared to soil alone, AM alone and FA alone.

The addition of 10% compost significantly improved the survival rate of tree species especially *E. globulus* and *V. tortilis* but not *F. albida*, *V. luederitzii*, and *C. mopane*. In terms of the relative dry matter yield, a significant increase was also observed due to compost application as compared to the control, but such increase was insignificant compared to treatments involving mycorrhiza (+AM). Among the different tree species that were added with compost, the highest relative dry matter yield was observed in *V. luederitzii*, as compared to *E. globulus* and *C. mopane*. The combination of compost and AM resulted to a much higher survival rate (100% for all species). The relative dry matter also indicated a significant increase due to combined application of compost and AM as compared to the control for each species. The highest increase due to the addition of compost and AM was observed in *F. albida*, and *V. luederitzii*.

Overall performance of these different tree species indicated that there is a significant increase in survival rate with the application FA, compost, AM and its combination leading to 100% survival rate. In terms of the relative dry matter yield, *F. albida*, and *V. luederitzii* produced significantly high dry matter compared to other tree species especially in the presence of FA+AM and FA alone. These results indicate that in acidic soils with low nutrients and high heavy metal contents, a synergistic effect of compost and mycorrhiza or FA +AM on the growth of tree species will occur. The enhancement of survival growth, development, and dry matter yield/week of tree species can be attributed to the different roles these organisms and amendments played in the tree species and the properties of soils (Thoms

and Gleixner, 2013). Studies by Aransiola et al. (2019) and Nie et al. (2017) indicated that mycorrhiza in roots can enhance the plant's ability to uptake water and essential nutrients and allow it to tolerate unfavorable environments through several mechanisms. These include the secretion of secondary plant metabolites such as siderophores, organic acids, biosurfactants, etc. that will affect numerous biogeochemical processes in the rhizosphere and improve plant growth, especially in a stressful environment such as those with high heavy metal contents. Through cell extension and the transformation of nutrient elements that occurs when they are either applied to seeds or incorporated into the soil, mycorrhiza can enhance the supply and absorption of nutrients of the host plants (Aransiola et al., 2019; Ultra et al., 2007). Second, amendments such as compost and FA can modify the soil's chemical, biological, and physical characteristics, making the soil less stressful to seedlings (Balasubramaniam, 2020; Sharma and Singh, 2019).

Compost can enhance the soil buffering capacity and neutralize acidity aside from its ability to supply essential nutrients (Sánchez et al., 2017). The high microbial load of compost will also provide the necessary microbes that will help in the transformation of nutrients and hasten nutrient availability, and consequently absorption by plants (Akmal et al., 2019). The third amendment, fly ash from Morupule Power Plant was alkaline enabling it to neutralize the acidity of soil substantially as compared to compost. With the exception of nitrogen, it also contains all essential nutrients for plants, including P, S, K, Ca, Mg, Cu, Mn, and Zn (Yunusa et al., 2006). The increase in pH due to FA will decrease the acidity stress on tree species and thus enhance survival. Acidic soil is very detrimental to seedlings for non-acid-tolerant plants because it can cause dehydration, oxidative stress to roots, and reduced nutrient availability (Dos Reis et al., 2018). The enhanced survival, growth, and development

of tree species seedlings due to the combined effects of mycorrhizal inoculation, FA, or compost amendments is due to these favorable effects on soil and the plant's ability to acquire water, nutrients and tolerate the environmental stress associated with acidity and high heavy metal(loid)s in soils (Ultra, 2020). However, results also showed that the addition of compost alone negatively impacted the survival rate, and dry matter yield/week for tree species like *E. Globulus*, *F. albida* *V. luederitzii*. This is largely because, without the addition of FA, compost alone could not alter the pH of the soil, leaving the soil acidic and toxic to plants (Latifah et al., 2018).

Potential of Different Tree Species for Phytoremediation of Mine Tailings

Table 6.3.1: Survival rate, dry matter yield and relative dry matter yield/week of five different species

Treatments	Species name	Survival Rate (%)	Dry matter yield (g/plant)	Number of weeks planted	Relative Dry matter yield/week (g/plant/week)
Soil Alone	<i>C. mopane</i>	70d	2.100	40	0.05i
	<i>V. tortilis</i>	30f	15.68j	24	0.65g
	<i>E. globulus</i>	0g	0.00m	52	0.00i
	<i>F. albida</i>	70d	60.09h	24	2.50e
	<i>V. luederitzii</i>	70d	76.40g	24	3.18e
Soil + AM	<i>C. mopane</i>	90b	3.80l	40	0.10i
	<i>V. tortilis</i>	100a	10.51k	24	0.44g
	<i>E. globulus</i>	75c	17.77j	52	0.34
	<i>F. albida</i>	90b	67.56h	24	2.82e
	<i>V. luederitzii</i>	100a	121.60e	24	5.07c
Soil+10% FA	<i>C. mopane</i>	100a	5.10l	40	0.13i
	<i>V. tortilis</i>	100a	16.18j	24	0.67g
	<i>E. globulus</i>	100a	82.87g	52	1.59f
	<i>F. albida</i>	100a	278.28c	24	11.60b
	<i>V. luederitzii</i>	100a	630.90b	24	26.29a
Soil+10% FA + AM	<i>C. mopane</i>	100a	3.80l	40	0.10i
	<i>V. tortilis</i>	100a	33.91i	24	1.41f
	<i>E. globulus</i>	100a	128.71e	52	2.48e
	<i>F. albida</i>	100a	263.08c	24	10.96b
	<i>V. luederitzii</i>	100a	727.00a	24	30.29a
Soil + 10% Compost	<i>C. mopane</i>	70d	7.90k	40	0.20h
	<i>V. tortilis</i>	100a	7.90k	24	0.33h
	<i>E. globulus</i>	50e	25.65i	52	0.49g
	<i>F. albida</i>	70d	110.25ef	24	4.59d
	<i>V. luederitzii</i>	70d	145.02e	24	6.04c
Soil + 10% Compost+AM	<i>C. mopane</i>	100a	13.40j	40	0.34h
	<i>V. tortilis</i>	100a	13.40j	24	0.56g
	<i>E. globulus</i>	100a	54.51h	52	1.05f
	<i>F. albida</i>	100a	161.33d	24	6.72c
	<i>V. luederitzii</i>	100a	188.54d	24	7.86c
P-value		0.000***	0.000***		0.000***

¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at a 5% level of significance;

*** very highly significant

6.1.4.2 Effects of amendments on heavy metal accumulation capacity of different tree species

Table 6.3.2 show the heavy metal accumulation of different tree species as influenced by the addition of amendments (AM, FA, FA+CM, and CM). Within the control (soil alone), there is a significant variation in the concentration of metals and metalloid(s) accumulated in different tissues by different tree species. The addition of AM reduces the uptake of As in shoots and roots of all tree species. AM also significantly reduces the uptake of Cu, Mn, Ni, Pb and Zn in shoots and roots in all tree species. Compared to heavy metal(loid)s accumulation in the control, the addition of FA also reduces the accumulation of all the analysed metals and metalloid(s). The addition of FA+AM significantly decreased the concentration of these metals and metalloid(s) in shoots and in roots of all tree species. The application of 10% compost and 10% + AM significantly decreased the presence of these metals in shoots and roots when compared to the control. The addition of 10% compost proved to be the amendment that had the highest significant reduction of metals and metalloids especially As, Cu, Mn in most species. The lowest concentration of As and Cu was observed in *E. globulus* (shoots and roots) when grown in tailings with 10% FA and AM respectively. As for Mn, the lowest concentration was observed in the shoots of *V. luederitzii* and in the roots of *F. albida* under the +AM treatment which are significantly lower compared to other treatments. Within the control, significantly higher uptake of As was found in *V. tortilis* and *F. albida*; while more Cu was significantly up taken by *V. luederitzii* and *V. tortilis* in shoots and roots. The uptake of Pb was significantly lower in the shoots of *E. globulus*'s grown in tailing with compost alone and the combination of compost and AM. Similarly, the shoots of *C. mopane* also had low concentration of Pb when grown in tailings with +FA+AM. The lowest Ni concentration was recorded in the shoots of *C. mopane* when grown in tailings with +FA+AM.

Potential of Different Tree Species for Phytoremediation of Mine Tailings

Table 6.3.2 Heavy metal accumulation by five tree species as influenced by amendments.

Treatments	Species	Heavy Metal(loid)s Concentration (mg kg ⁻¹)											
		As		Cu		Mn		Ni		Pb		Zn	
		Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Soil Alone	<i>C. mopane</i>	21.20c	66.18b	63.92f	705.88b	37.42h	29.41h	34.3f	112.75d	54.03j	68.15j	54.56d	98.04c
	<i>V. tortilis</i>	44.42b	63.28b	473.33a	586.67c	103.49c	93.48c	119.08b	104.26d	176.48e	185.24f	132.85b	110.67b
	<i>E. globulus</i>	-	-	-	-	-	-	-	-	-	-	-	-
	<i>F. albida</i>	55.42a	53.28c	383.23b	336.87d	83.59d	73.48d	219.08a	194.66b	276.48d	245.26e	102.8c	90.97c
	<i>V. luederitzii</i>	39.44b	96.1a	445.9a	1212.2a	19.4i	52.37f	93.11c	265.1a	322.8c	918.6b	24.7h	70.31e
Soil +AM	<i>C. mopane</i>	15.35d	25.55e	40.62g	250.11e	48.95g	25.01i	20.83g	66.1f	56.09j	66.32j	28.12g	39.3h
	<i>V. tortilis</i>	22.11c	49.96c	158.14d	136.72f	72.76e	84.23c	68.25d	92.15e	168.44e	224.27e	59.38d	65.78e
	<i>E. globulus</i>	1.08e	2.11h	4.21k	5.01j	45.28g	66.33e	25.03g	22.02k	32.05l	38.02k	45.88e	66.02e
	<i>F. albida</i>	42.11b	69.96b	138.08e	126.82f	61.71f	56.29f	78.55d	72.19f	168.44e	154.17f	55.31d	50.58g
	<i>V. luederitzii</i>	22.31c	65.19b	256.76c	759.25b	15.39j	46.64g	54.88e	173.3c	167.5e	562.15c	14.31j	46.42g
Soil+10% FA	<i>C. mopane</i>	8.94e	36.82d	22.36h	85.16g	48.94g	30.06h	3.02j	22.54k	25.33	41.08k	19.94i	30.06h
	<i>V. tortilis</i>	38.21b	77.01a	68.48f	243.23e	55.31f	77.02d	10.54i	40.54i	5.42n	8.26l	28.97g	44.59g
	<i>E. globulus</i>	2.50e	1.90h	2.81k	87.55g	100.57c	144b	4.67j	71f	8.20m	70.38i	41.08e	54g
	<i>F. albida</i>	34.13b	67.2b	11.79i	51.53h	32.91h	48.99f	6.49j	17.52l	590.87a	1061.84a	268.94a	267.69a
	<i>V. luederitzii</i>	32.25b	84.44a	157.27d	226.4e	130.54b	162.06b	30.54f	123.6d	240d	312.83ld	29.43g	47.79g
Soil+10% FA + AM	<i>C. mopane</i>	11.69d	39.79d	18.92i	109.57f	66.2f	54.79f	2.58	28.84j	3.07n	7.55	16.34j	60.55f
	<i>V. tortilis</i>	23.86c	72.57a	88.12e	293.74e	48.46g	71.92d	13.22h	60.27g	6.61m	7.22l	37.45e	89.63c
	<i>E. globulus</i>	5.02e	2.11h	5.45j	99.21fg	105.33c	154.07b	5.07j	23.22k	405.33b	305.21d	44.31e	65.02f
	<i>F. albida</i>	16.54d	75.35a	16.55i	66.19g	26.22i	36.65h	9.37i	17.99l	566.47a	1121.29a	32.75f	80.39d
	<i>V. luederitzii</i>	21.27c	48.85c	15.52i	61.69gh	47.74g	35.24h	2.82j	10.5m	290.00c	287.76e	15.52j	12.53j
Soil + 10% Compost	<i>C. mopane</i>	7.38e	11.63f	22.54h	147.47f	86.05d	36.87h	12.29h	49.98h	35.25l	28.02l	21.51h	32.77h
	<i>V. tortilis</i>	6.21e	36.13d	10.03j	42.08h	20.75i	28.21i	10.03i	11.54m	56.88j	52.08j	28.36g	15.53j
	<i>E. globulus</i>	4.20e	2.30h	16.48i	66.25g	13.35j	213.35a	22.05g	89.04e	0.05n	97.20h	10.08k	49.09g
	<i>F. albida</i>	10.52d	45.08cd	9.33j	40.01h	24.21i	30.85h	10.78i	10.25m	78.03h	66.21j	10.22k	25.02i
	<i>V. luederitzii</i>	15.22d	56.45c	15.09i	55.33h	31.12h	45.22g	15.31h	18.36l	118.02f	115.08g	18.21i	38.52h
Soil + 10% Compost +AM	<i>C. mopane</i>	4.15e	8.13f	25.96h	106.3f	92.03c	49.96f	17.02h	43.05i	45.21k	97.2h	33.75f	22.85i
	<i>V. tortilis</i>	10.55d	44.25cd	15.07i	52.24h	25.03i	35.44h	14.06i	13.74m	60.01i	58.22j	30.07f	18.25i
	<i>E. globulus</i>	5.22e	5.65g	17.25i	156.35f	222.54a	224.55a	22.06g	95.24e	4.67n	119.34g	19.47i	68.55e
	<i>F. albida</i>	5.32e	28.01e	5.21j	25.21i	21.08i	21.22i	9.25i	10.08m	65.02i	71.05i	11.08k	23.09i
	<i>V. luederitzii</i>	10.22d	44.22cd	23.07h	39.18h	20.09i	35.14h	19.22h	19.25k	98.22g	111.58g	17.21j	36.15h
P-value		0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

:- no-survival; ¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at a 5% level of significance; *** very highly significant

The lowest uptake of Zn was observed in the shoots of *F. albida* in treatments involving compost+AM, and in the roots of *V. luederitzii* in +FA+AM treatments.

This data showed that the different tree species have different capacities to uptake these metals and the different amendments had generally reduced the amount accumulated in the plant tissues of the different tree species. It appeared that FA, and compost would reduce the availability of these heavy metal(loid)s in soils, thus reducing its uptake and accumulation in plant tissues. The reduction in the concentration in plant tissues due to the colonization of mycorrhiza on these tree species could be attributed to the ability of mycorrhiza to retain these metals on the fungal tissues in roots. The combination of FA or compost with mycorrhiza synergistically enhanced the growth and the dry matter yield which also resulted to reduction in the concentration of these metals in tissue by dilution effect. The high capacity of *V. luederitzii* to accumulate these metals is consistent with other studies (Bech, 2021; Scalbert, 1992).

Table 6.3.3 shows the heavy metal uptake capacity and removal rate through biomass extraction for different tree species from the BCL mine tailings. The uptake capacity was based on the dry matter yield of plants and the concentration of metals in the tissues. Among the five species growing in pure BCL mine tailings, *V. luederitzii* and *F. albida* have the highest capacity to remove metals from the soil, as these metals were accumulated mostly in their shoots. The application of AM led to a significant reduction of these metals and metalloids in the tissues all tree species compared to the control, but the mycorrhizal fungus enhances the biomass yield resulting to higher potential removal of these metals from the tailings. Similarly, the application of 10% FA on tailings led to a significant increase of the uptake

Potential of Different Tree Species for Phytoremediation of Mine Tailings

Table 6.3.3 Meta-analysis showing heavy metal(loid)s uptake capacity and removal rate through biomass extraction for different tree species¹

Treatments	Species	Heavy Metal(loid)s Concentration (mg kg ⁻¹)											
		As		Cu		Mn		Ni		Pb		Zn	
		Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Soil Alone	<i>C. mopane</i>	1.06l	3.31m	3.20m	35.29j	37.42j	1.47m	1.72l	5.64l	2.70l	3.41l	2.73k	4.90l
	<i>V. tortilis</i>	28.87j	41.13j	307.66e	381.34f	67.27h	60.76j	77.40g	67.77i	114.71h	120.41g	86.35f	71.94i
	<i>E. globulus</i>	-	-	-	-	-	-	-	-	-	-	-	-
	<i>F. albida</i>	138.55e	133.20h	958.08c	842.18d	208.98e	183.70h	547.70b	486.65c	691.20e	613.15e	257.00d	227.43f
	<i>V. luederitzii</i>	125.42e	305.60e	1417.96b	3854.80b	61.69h	166.54h	296.09c	843.02b	1026.50c	2921.15c	78.55f	223.59f
Soil +AM	<i>C. mopane</i>	1.54l	2.56m	4.06m	25.01j	4.90l	2.50m	2.08l	6.61l	5.61l	6.63l	2.81k	3.93l
	<i>V. tortilis</i>	9.73k	21.98k	69.58i	60.16i	32.01j	37.06k	30.03j	40.55j	74.11i	98.68h	26.13i	28.94j
	<i>E. globulus</i>	0.37l	0.72m	1.43m	1.70k	15.40	22.55k	8.51l	7.49l	10.90l	12.93k	15.60j	22.45j
	<i>F. albida</i>	118.75f	197.29g	389.39e	357.63f	174.02f	158.74h	221.51d	203.58e	475.00f	434.76f	155.97e	142.64g
	<i>V. luederitzii</i>	113.11f	330.51e	1301.77b	3849.40b	78.03h	236.46g	278.24c	878.63b	849.23d	2850.10c	72.55f	235.35f
Soil+10% FA	<i>C. mopane</i>	1.16l	4.79m	2.91m	11.07k	6.36l	3.91m	0.39l	2.93l	3.29l	5.34l	2.59k	3.91l
	<i>V. tortilis</i>	33.64i	51.60j	45.88j	162.96h	37.06j	51.60j	7.06l	27.16k	3.63l	5.53l	19.41i	29.88j
	<i>E. globulus</i>	3.98l	3.02m	4.47m	139.20h	159.91f	228.96g	7.43l	112.89g	13.04k	111.90g	65.32g	85.86h
	<i>F. albida</i>	395.91c	779.52d	136.76g	597.75e	381.76c	568.28c	75.28g	203.23e	6854.09b	12317.34a	3119.70a	3105.20a
	<i>V. luederitzii</i>	847.85a	2219.93a	4134.63a	5952.06a	3431.90a	4260.56a	802.90a	3249.44a	6309.60b	8224.30b	773.71b	1256.40b
Soil+10% FA + AM	<i>C. mopane</i>	1.17l	3.98m	1.89m	10.96k	6.62l	5.48m	0.26l	2.88l	0.31l	0.76l	1.63k	6.06l
	<i>V. tortilis</i>	33.64i	102.32i	124.25g	414.17e	68.33i	101.41i	18.64k	84.98h	9.32l	10.18k	52.80g	126.38g
	<i>E. globulus</i>	12.45k	5.23m	13.52l	246.04g	261.22d	382.09e	12.57k	57.59i	1005.22c	756.92d	109.89e	161.25g
	<i>F. albida</i>	181.28d	825.84c	181.39f	725.44d	287.37d	401.68d	102.70f	197.17e	6208.51b	12289.34a	358.94c	881.07c
	<i>V. luederitzii</i>	644.27b	1479.67b	470.10d	1868.59c	1446.04b	1067.42b	85.42g	318.05d	8784.10a	8716.25b	470.10c	379.53d

:- no-survival; ¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at a 5% level of significance.; *** very highly significant

Potential of Different Tree Species for Phytoremediation of Mine Tailings

Table 6.3.3 (Cont). Meta-analysis showing heavy metal(loid)s uptake capacity and removal rate through biomass extraction for different tree species¹

Treatments	Species	Heavy Metal(loid)s Concentration (mg kg ⁻¹)											
		As		Cu		Mn		Ni		Pb		Zn	
		Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Soil + 10% Compost	<i>C. mopane</i>	1.48l	2.33m	4.51m	29.49j	17.21k	7.37m	2.46l	10.00l	7.05l	5.60l	4.30k	6.55l
	<i>V. tortilis</i>	2.05l	11.92l	3.31m	13.89k	6.85l	9.31m	3.31l	3.81l	18.77k	17.19k	9.36j	5.12l
	<i>E. globulus</i>	2.06l	1.13m	8.08m	32.46j	6.54l	104.54i	10.80k	43.63j	0.02l	47.63i	4.94k	24.05j
	<i>F. albida</i>	48.29h	206.92f	42.82j	183.65	111.12g	141.60	49.48i	47.05j	358.16g	303.90f	46.91h	114.84g
	<i>V. luederitzii</i>	91.93g	340.96e	91.14h	334.19f	187.96f	273.13f	92.47g	110.89g	712.84d	695.08e	109.99e	232.66f
Soil + 10% Compost +AM	<i>C. mopane</i>	1.41l	2.76m	8.83m	36.14j	31.29j	16.99l	5.79l	14.64l	15.37k	33.05j	11.48j	7.77l
	<i>V. tortilis</i>	24.78j	8.44l	29.25k	14.02k	19.85k	7.87m	7.69l	33.61k	32.60j	16.84k	10.22j	18.25k
	<i>E. globulus</i>	5.48k	5.93m	18.11l	164.17h	233.67de	235.78g	23.16j	100.00g	4.90l	125.31g	20.44i	71.98i
	<i>F. albida</i>	35.75i	188.23g	35.01k	169.41h	141.66f	142.60h	62.16h	67.74i	436.93f	477.46f	74.46f	155.16g
	<i>V. luederitzii</i>	80.33g	347.57e	181.33f	307.95f	157.91f	276.20f	151.07e	151.31f	772.01d	877.02d	135.27e	284.14e
P- value		0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

:- no-survival; ¹Means followed by the same letter (s) in each column are not significantly different from each other based on Tukey's test at a 5% level of significance.; *** very highly significant

of this metal(loid)s as compared to the control in most of the tree species. However, FA application did not significantly enhance the uptake of Cu, Ni and Mn by *C. mopane* and *V. tortilis* and *E. globulus* as compared to the control. Across the different tree species, all the metals except Mn, recorded a significantly high uptake in the roots compared to shoots. The addition of amendments did not significantly alter the pattern of accumulation of metals by roots compared to shoots. The only exception on this accumulation pattern were observed for As by *F. albida* (soil alone), *V. tortilis* and *E. globulus* (Compost+AM) wherein a higher concentration in shoots than in roots was recorded. The other exception on this accumulation pattern was observed in Cu accumulated in *V. tortilis* after the application of Compost+AM.

The addition of a combination of FA+AM and AM alone lead to a highly significant increase of heavy metal uptake capacity by *F. albida* and *V. luederitzi*. This increase was observed in all the metals under study. *E. globulus* species had a significantly low metal uptake capacity compared to all the species under study. Overall, *V. luederitzi* accumulated the highest concentration of heavy metal(loid)s in roots and shoots, followed by *F. albida*, *V. tortilis*, *C. mopane* and *E. globulus*.

Generally, the reduction of heavy metal in shoots compared to roots could be attributed to the capacity of mycorrhizal roots to retain heavy metal by localization at the external mycelium of the arbuscular mycorrhiza (Thoms and Gleixner, 2013); or selective exclusion of toxic and nontoxic metals by adsorption onto the chitinous cell wall structure; or onto extracellular glycoprotein called glomalin; or intracellular crystallization which is a very important mechanism in reducing the plant's exposure to potentially toxic metals (Shi et al., 2019). Shabani and Sabzalian (2016) showed that for both mycorrhizal and non-

mycorrhizal plants, Ni concentrations in shoots and roots increased according to the addition of the metal into the soil, but inoculation with *F. mosseae* led to significantly lower Ni translocation from roots to the aboveground parts compared to non-inoculated plants. This is attributed to the improved root system due to arbuscular mycorrhiza (Yang et al., 2015). Additionally, the reduction of As, Cu, Ni, and Zn in shoots of most tree species could be due to the plant dilution effect because the translocation factor for these metals did not decrease but rather increased due to mycorrhizal inoculation in pure soil (Baycu, 2006). Vogel-Mikuš et al. (2005) showed that mycorrhiza colonized plants showed significantly improved nutrients, higher biomass, and a decreased Cd and Zn uptake, especially at higher soil metal contents, as a strategy for metal tolerance. On the contrary, Mn concentration in shoots and roots was increased by mycorrhiza inoculation. This was also supported by the findings of Moon and Aggangan (2018) which showed that mycorrhiza could enhance Mn uptake in the tree species. This was attributed the increase of Mn uptake to the larger volume of roots that would facilitate uptake. Mardukhi et al. (2015) attributed the enhanced Mn uptake of mycorrhizal plants to the increase of exudates capable of chelating Mn and facilitating absorption by plants.

Fly ash and compost reduced the concentrations of As, Cu, Ni, and Zn in shoots of all five tree species. This reduction in concentrations in plant tissues could be attributed to the capacity of these amendments to neutralize the soil acidity thus decreasing the heavy metal availability (Yunusa et al., 2006). FA neutralized the acidity of soil from the initial pH 4.35 to about pH 8.2 which resulted in various chemical changes as reflected in the reduction of the concentration of water-soluble metals. The reduction of heavy metal availability due to pH increase is due precipitation of cationic heavy metal(loid) or increased adsorption of

metal(loid)s on the soil matrix (Lwin et al., 2018). Compost also neutralized the acidic soil (Khater, 2015), but the neutralizing power is lower compared to FA. The low concentration of heavy metal(loid)s in shoots of most plants compared to roots could be due to humic substances, which can decrease the content of free metal ions due to their complexation with dissolved organic substances and adsorption by humic substances, which enter the solid soil phase, or through adsorption in the mineral phase (Barsova et al., 2019).

The interaction effect of soil amendments and mycorrhiza inoculation on heavy metal concentration in shoots and roots of these tree species is metal-specific and could be due to complex processes affecting availability, root absorption, and translocation mechanisms (Bonanno et al., 2018).

6.1.4.3 Effects of amendments on tree species

Figure 6.1.1 summarizes the effect of amendments on the heavy metal uptake and other parameters that were analyzed. Overall, without the addition of amendments, tree species recorded survival rate below 100% and poor growth due to high levels of heavy metal(loid), which led to the reduced uptake of HM by plants, limiting their phytoremediation potential. A study by Sharma and Reynnells (2018) indicated that amendments are important to plant growth because they provide plants with soil nutrients and improve soil fertility which is very limited in heavy metal-contaminated soil. The addition of amendments like fly ash, compost, and arbuscular mycorrhiza increases the survival rate and growth of all tree species and lowered the availability of heavy metal(loid)s, and, in return, increases the uptake and stabilization of heavy metal(loid)s by all tree species, and increases their phytoremediation potential. This was also corroborated by Ultra (2020)

in the study of lemon grass species as affected by amendments. The results indicated that these plants could accumulate high concentrations of several heavy metal(loid)s while others can either accumulate one particular metal or maintain a low concentration of other metals to survive in an environment with multi-metal contamination. Furthermore, this was supported in a study by Rascio and Navari-Izzo (2011) who indicated that plants could accumulate high amounts of heavy metal(loid)s in their shoots while some plants are excluders. Therefore, mine tailings in the BCL mine and the vicinity can be phytoremediated with these identified tree species and the phytoremediation potential could be improved by the addition of amendments.

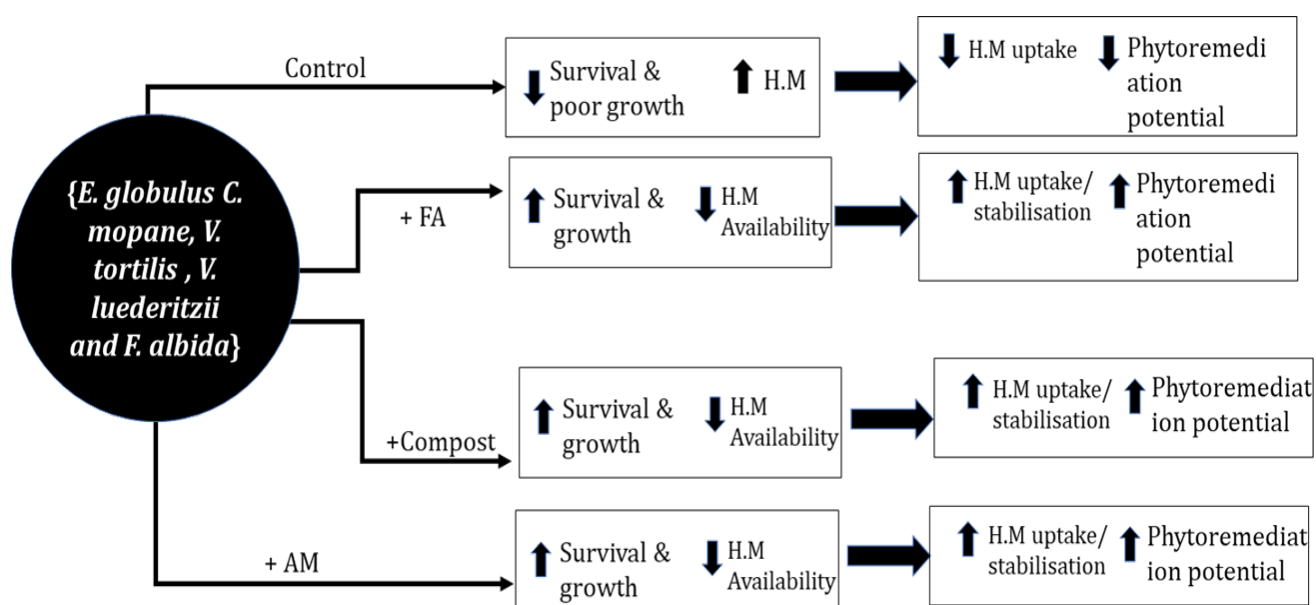


Figure 6.1.1 Summary of the effects of amendments on the phytoremediation potential of different tree species

6.1.5 Conclusions

In conclusion, of the five tree species, *V. luederitzii* had a high survival rate and dry matter yield/week followed by *F. Albida* & *V. tortilis*. Tree species accumulated concentrations of heavy metal(loid)s in roots and shoots in this order *V. luederitzii* > *F. albida* > *V. tortilis* > *C. mopane* > *E. globulus*. This indicates that these trees can accumulate heavy metal(loid)s or tolerate high concentrations of heavy metal(loid)s without interference with their growth and development. *V. luederitzii* species also accumulated high concentrations of metal(loid)s compared to other species. Other tree species were ranked according to the decreasing preference based on survival rate, dry matter yield and heavy metal accumulation. The rank was as follows: *V. luederitzii* > *F. albida* > *V. tortilis* > *C. mopane* > *E. globulus*. Therefore, these trees should be used in combination to successfully remediate BCL mine tailings through the addition of amendments. Future studies should explore the use of other indigenous tree species on their potential for phytoremediation through the application of readily available amendments in Botswana.

6.1.6 References

- Akmal, M., Maqbool, Z., Khan, K.S., Hussain, Q., Ijaz, S.S., Iqbal, M., Aziz, I., Hussain, A., Abbas, M.S. & Rafa, H.U. (2019). Integrated use of biochar and compost to improve soil microbial activity, nutrient availability, and plant growth in arid soil. *Arab Journal of Geoscience* 12(7):232.
- Aransiola, S.A., Ijah, U.J., Abioye, O.P. & Bala, J.D. (2019). Microbial-aided phytoremediation of heavy metals contaminated soil: a review. *European Journal of Biological Research* 9(2):104-25.

- Ashraf, S., Ali, Q., Zahir, Z. A., Ashraf, S., & Asghar, H. N. (2019). Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils. *Ecotoxicology and environmental safety*, 174, 714-727.
- Balasubramaniam, P. (2020). Fly Ash as a Source of Silicon for Mitigating Biotic Stress and Improving Yield and Changes in Biochemical Constituents and Silicon in Rice Under Abiotic Stress. In *Circular Economy and Fly Ash Management 2020* Springer, Singapore. pp 145-160.
- Barsova, N., Motuzova, G., Kolchanova, K., Stepanov, A., Karpukhin, M., Minkina, T. & Mandzhieva, S. (2019). The effect of humic substances on Cu migration in the soil profile. *Chemical Ecology* 35(1):86-101.
- Baycu, G., Tolunay, D., Özden, H., & Günebakan, S. (2006). Ecophysiological and seasonal variations in Cd, Pb, Zn, and Ni concentrations in the leaves of urban deciduous trees in Istanbul. *Environmental pollution*, 143(3), 545-554.
- Bech, J. (2021). Special Issue on “Metallophytes for soil remediation”- Preface. *Environmental Geochemistry and Health*, 43, 1319-1325.
- Bonanno, G., VymazaL, J. and Cirelli, G.L. (2018). Translocation, accumulation, and bioindication of trace elements in wetland plants. *Science of the Total Environment* 631:252-61.
- Dos Reis, A.R., Lisboa, L.A., Reis, H.P, de Queiroz Barcelos, J.P., Santos, E.F., Santini, J.M., Meyer-Sand, B.R., Putti, F.F., Galindo, F.S., Kaneko, F.H. & Barbosa, J.Z. (2018). Depicting the physiological and ultrastructural responses of soybean plants to Al stress conditions. *Plant Physiology and Biochemistry* 130:377-90.
- Gołda, S., & Korzeniowska, J. (2016). Comparison of phytoremediation potential of three grass species in soil contaminated with cadmium. *Environmental Protection and Natural Resources*, 27(1), 8-14.

- Gómez, L., Contreras, A., Bolonio, D., Quintana, J., Oñate-Sánchez, L., & Merino, I. (2019). Phytoremediation with trees. In *Advances in Botanical Research* (Vol. 89, pp. 281-321). Academic Press.
- Gutiérrez-Ginés, M. J., Hernández, A. J., Pérez-Leblic, M. I., Pastor, J., & Vangronsveld, J. (2014). Phytoremediation of soils co-contaminated by organic compounds and heavy metals: bioassays with *Lupinus luteus* L. and associated endophytic bacteria. *Journal of environmental management*, 143, 197-207.
- Kafle, A., Timilsina, A., Gautam, A., Adhikari, K., Bhattarai, A., & Aryal, N. (2022). Phytoremediation: Mechanisms, plant selection and enhancement by natural and synthetic agents. *Environmental Advances*, 8, 100203.
- Karczewska, A., Lewinska, K., Gałka, B. (2013). Arsenic extractability and uptake by velvet grass *Holcus lanatus* and ryegrass *Lolium perenne* in variously treated soils polluted by tailing spills. *Journal of Hazardous Materials* 262: 1014– 1021.
- Khater, E. S. G. (2015). Some physical and chemical properties of compost. *International Journal of Waste Resources*, 5(1), 72-79.
- Korzeniowska, J., Stanislawska-glubiak, E., Igras, J. (2011). Applicability of energy crops for metal phytostabilization of soils moderately contaminated with copper, nickel and zinc. *Journal of Food Agriculture and Environment* 9, 3/4: 693-697
- Latifah, O., Ahmed, O. H., & Majid, N. M. A. (2018). Soil pH buffering capacity and nitrogen availability following compost application in a tropical acid soil. *Compost Science & Utilization*, 26(1), 1-15.
- Liu, Y., Zeng, G., Zhong, H., Wang, Z., Liu, Z., Cheng, M., & Liu, S. (2017). Effect of rhamnolipid solubilization on hexadecane bioavailability: enhancement or reduction?. *Journal of hazardous materials*, 322, 394-401.

- Lwin, C.S., Seo, B.H., Kim, H.U., Owens, G. & Kim, K.R. (2018). Application of soil amendments to contaminated soils for heavy metal immobilization and improved soil quality—a critical review. *Soil Science Plant Nutrition* 64(2):156-67.
- Marques, A.P.G.C., Rangel, A.O.S.S. & Castro P.M.L. (2009). Remediation of Heavy Metal Contaminated Soils: Phytoremediation as a Potentially Promising Clean-Up Technology. *Environmental Science and Technology* 39: 622–654.
- Nie, H., Nie, M., Xiao, T., Wang, Y. and Tian, X. (2017). Hexadecane degradation of *Pseudomonas aeruginosa* NY3 promoted by glutaric acid. *Science of Total Environment* 575:1423–1428.
- Mardukhi, B., Rejali, F., Daei, G., Ardakani, M.R., Malakouti, M.J. and Miransari, M. (2015). Mineral uptake of mycorrhizal wheat (*Triticum aestivum* L.) under salinity stress. *Communications in Soil Science and Plant Analysis* 46(3):343-57.
- Moon, H.K. & Aggangan, N.S. (2018). Growth and mineral uptake of *Eucalyptus pellita* in response to inoculation with different mycorrhizal inoculants. *Clonal Trees in the Bioeconomy Age: Opportunities and Challenges*. 2018 Sep 10.
- Podar, D., & Maathuis, F. J. (2022). The role of roots and rhizosphere in providing tolerance to toxic metals and metalloids. *Plant, cell & environment*, 45(3), 719-736.
- Rascio, N., & Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting?. *Plant science*, 180(2), 169-181.
- Sánchez, Ó. J., Ospina, D. A., & Montoya, S. (2017). Compost supplementation with nutrients and microorganisms in composting process. *Waste management*, 69, 136-153.

- Scalbert, A. (1992). Tannins in woods and their contribution to microbial decay prevention. In *Plant polyphenols: synthesis, properties, significance* (pp. 935-952). Boston, MA: Springer US.
- Shabani, L., & Sabzalain, M.R. (2016). Arbuscular mycorrhiza affects nickel translocation and expression of ABC transporter and metallothionein genes in *Festuca arundinacea*. *Mycorrhiza* 26(1):67-76.
- Sharma, M., & Reynnells, R. (2018). Importance of soil amendments: survival of bacterial pathogens in manure and compost used as organic fertilizers. *Preharvest food safety*, 159-175.
- Shi, X., Chen, Y. T., Wang, S. F., Pan, H. W., Sun, H. J., Liu, C. X. & Jiang, Z. P. (2016). Phytoremediation potential of transplanted bare-root seedlings of trees for lead/zinc and copper mine tailings. *International Journal of Phytoremediation*, 18(11), 1155-1163.
- Shi, W., Zhang, Y., Chen, S., Polle, A., Rennenberg, H. & Luo, Z.B. (2019). Physiological and molecular mechanisms of heavy metal accumulation in nonmycorrhizal versus mycorrhizal plants. *Plant Cell Environment* 42(4):1087-103.
- Stanislawska-Glubiak, E., Korzeniowska, J. & Kocon, A. (2012). Effect of the reclamation of heavy metal contaminated soil on growth of energy willow. *Polish Journal of Environmental Studies* 21, 1:187–192.
- Song, W. Y., Ju Sohn, E., Martinoia, E., Jik Lee, Y., Yang, Y. Y., Jasinski, M., & Lee, Y. (2003). Engineering tolerance and accumulation of lead and cadmium in transgenic plants. *Nature biotechnology*, 21(8), 914-919.
- Thoms, C., & Gleixner, G. (2013). Seasonal differences in tree species' influence on soil microbial communities. *Soil Biology and Biochemistry*, 66, 239-248.

- Ultra Jr, V.U. (2020). Fly ash and compost amendments and mycorrhizal inoculation enhanced the survival and growth of *Delonix regia* in Cu-Ni mine tailings. *Philippine Journal of Science*, 149(3): 479-489
- Ultra Jr, V. U., Tanaka, S., Sakurai, K., & Iwasaki, K. (2007). Arbuscular mycorrhizal fungus (*Glomus aggregatum*) influences biotransformation of arsenic in the rhizosphere of sunflower (*Helianthus annuus* L.). *Soil Science and Plant Nutrition*, 53(4), 499-508.
- Ultra, V. U. (2020). Growth and yield of lemongrass (*Cymbopogon citratus*) in fly ash with nutrient amendments and Mycorrhiza for three-ratoon period. *International Journal of Phytoremediation*, 22(14), 1551-1561.
- Yang, H., Zhang, Q., Dai, Y., Liu, Q., Tang, J., Bian, X., & Chen, X. (2015). Effects of arbuscular mycorrhizal fungi on plant growth depend on root system: a meta-analysis. *Plant and Soil*, 389, 361-374.
- Yunusa, I. A. M., Eamus, D., DeSilva, D. L., Murray, B. R., Burchett, M. D., Skilbeck, G. C., & Heidrich, C. (2006). Fly-ash: an exploitable resource for management of Australian agricultural soils. *Fuel*, 85(16), 2337-2344.
- Zhao, X. Q., Chen, R. F., & Shen, R. F. (2014). Coadaptation of plants to multiple stresses in acidic soils. *Soil Science*, 179(10-11), 503-513.

CHAPTER 7

General Discussion, Conclusions, and Recommendations

7.1 General Discussion and Conclusions

Overall, the assessment of the impact of mining activities of BCL Cu-Ni mine and Monarch Gold Mine on the adjacent environment was presented through the quantification of heavy metal(loid)s on the soil and plants communities in the vicinity of these mining areas. Results showed that the soil and plants growing in these areas have pollution load index above one, indicating progressive pollution in these two study areas and estimates showed the possibility of bioaccumulation of the metal(loid)s on the grazing animals that have access on these abandoned mines (Publication 4.1 and 4.2). The data on the heavy metal accumulation of the different plants was used to identify potential plants or combinations of plants that could be utilized for the rehabilitation of the study area through phytoremediation. The data provided preliminary estimates on the possible risk associated with heavy metal contamination among grazing animals in the Selibe-Phikwe BCL mine and Monarch Gold mine. The estimates of the daily intake of the minerals due to the consumption of plants in the vicinity of the mine warrant evaluation of actual levels of heavy metal(loid)s in grazing animals near the study area and in other mining areas in Botswana. This will need to be ascertained by sampling blood and organs like the liver and kidneys from livestock in the study areas. In the study conducted at the Francistown Monarch Gold mine, the main contaminant was As but some Cu, Cr, and Zr were also found to be slightly above the critical values in the soil. The tailings dumpsite (TD) recorded a high availability of these metals compared to the vicinity and the river sediments. The most dominant species in the TD was *Cenchrus biflorus*. The most dominant species in the VTD were *Cenchrus biflorus* (n=12) and *Melia*

azedarach (n=12). The concentrations of As, Cu, Mn, and Cr were above the critical levels with respect to estimates of the daily intake of these metals by cattle, goat, and sheep through grazing and browsing. The intake of soil indicates a potential risk of As exposure and probable transfer to humans through the consumption of these ruminants. Therefore, as recommended in the BCL paper, the estimates of the daily intake of the minerals due to the consumption of plants in the vicinity of the mine warrant evaluation of actual levels of heavy metal(loid)s in grazing animals. Proper management of the tailings dam and its vicinity should be implemented to prevent the spread of pollutants and exposure of animals and humans to heavy metal(loid)s, especially As, emanating from the tailings dam.

The influence of different soil amendments on the survival, growth and biomass production, heavy metal uptake, and the overall phytoremediation efficiency of different tree species were presented in publications 5.1 and 5.2 and manuscript 5.3. Major findings showed that the addition of fly ash and compost amendments together with mycorrhiza inoculation significantly enhanced the survival and growth of *C. mopane* seedlings in heavy metal-contaminated soils. Compost and mycorrhiza significantly reduced heavy metal availability and uptake by plants, thus enhancing the phytoremediation potential of *C. mopane* in Cu-Ni mine tailings (Publications 5.1). The addition of fly ash, FA+AM, compost and a combination of compost and AM did not significantly increase the uptake of As in *C. mopane*.

The study on the effect of amendments on the *Vachellia (acacia)* species grown in BCL mine tailings indicated that fly ash improved the survival, growth and biomass yield, and lowered the heavy metal concentration of different *Vachellia* species by neutralizing the acidic pH of soil and reducing the availability of heavy metal(loid)s (Publications 5.2). The concentration of heavy metal(loid)s in plants was reduced due to the addition of FA.

Mycorrhiza inoculation successfully colonized the roots of different *Vachellia* species and selectively enhanced the growth and altered the heavy metal accumulation in plants. Enhancement of growth by mycorrhiza was only observed in *V. luederitzii* in soils without fly ash, while growth retardation was observed in *F. albida* and *V. tortilis* in tailings without fly ash. Therefore, amendments improved the soil quality of mine tailings to a state suitable for plant growth. In contrast, *E. globulus* did not survive in pure mine tailings from the BCL mine and Monarch Gold Mine. The addition of amendments (fly ash, compost, cow manure and sewage sludge) improved the survival, growth, biomass production, and heavy metal uptake of *E. globulus*, indicating the effectiveness of these amendments to enhance its phytoremediation potential (Manuscript 5.3). The addition of FA+SS significantly increased these parameters compared to the addition of FA alone and FA+CM.

The overall phytoremediation potential of different species (*E. globulus*, *C. mopane*, and three *acacia* spp.) on mine tailings as affected by different amendments were assessed based on the survival, relative dry matter, and heavy metal uptake capacity using the data from Chapter 5. Comparative assessment of the five species, *V. luederitzii* had high survival rates and dry matter yield followed by *F. Albida* & *V. tortilis*. Tree spp. accumulated concentrations of heavy metal(loid)s in roots and shoots in this order *V. luederitzii* > *F. albida* > *V. tortilis* > *C. mopane* > *E. globulus* (Manuscript 6.1). These plants can accumulate heavy metal(loid)s or tolerate high concentrations of heavy metal(loid)s without interference with their growth and development. Some of these plants have the ability to accumulate high concentrations of several heavy metal(loid)s while others can only accumulate one particular metal and maintain a low concentration of other metals in order to survive in an environment with multi-metal contamination. Therefore, amendments improved the soil condition and increased survival growth and plant dry

matter yield, resulting in high biomass production and increased the removal rate of heavy metal(loid)s from the soil due to increased uptake.

7.2 Recommendations

To curb or reduce pollution emanating from mining activities, this study recommends the enforcement of the EIA Act of 2011 and Environmental Management Policies and imposing high penalties for companies not complying with set limits by regulatory bodies. The EIA Act clearly states that mining companies should rehabilitate the environment after mining activities. This study further recommends the incorporation of phytoremediation as a component of waste management strategy in all mining activities in Botswana and the continuous and comprehensive monitoring of heavy metal contamination in the vicinity of all mining areas, including active and abandoned mining sites. This study further recommends fencing the perimeter of both active and abandoned mining sites to a radius of 5 Km to deny access to grazing animals, in order to prevent their ingestion of heavy metal-contaminated plants and soil. The study further recommends actual quantification of heavy metal(loid) bioaccumulation in blood and tissues of grazing animals in the study area. This information is necessary for the mitigation and strategic management of the impacts of mining on the cattle industry of Botswana.

To fully utilize the output of this study, it is recommended that parallel assessments be done in all active and abandoned mining areas in Botswana in order to establish a functional database on the extent of heavy metal contamination and environmental impact, especially on adjacent agricultural areas and the wildlife ecosystem. Additionally, field trials should be conducted to assess the potential of different tree species in different mining sites including BCL Mine in Selibe Phikwe and

Monarch Gold Mine in Francistown since this study was conducted in a screenhouse.

To further improve the research output of this study, the following recommendation should be considered. (i) Resources permitting, planting of all tree species should be conducted at the same time and for the same period, to allow for uniformity of comparisons; (ii) A speciation exercise to investigate heavy metal(loid)s that are organic bound, carbonate bound, iron and manganese oxide bound should be considered; (iii) Investigate the role of plant physiological characteristics like the root surface area, transpiration rates, and others; and microorganisms in the soil in relation to heavy metal(loid)s uptake and phytoremediation potential should be studied; (iv) The use of indigenous trees and beneficial trees that can produce by products like timber, pulp etc. should be used through the use of readily available amendments in Botswana. (v) Improvement of the phytoremediation potential of indigenous species using “omics” tools should be explored. Lastly, the analysis and dynamics of Pb and Cr on BCL mine tailings and plant species should be considered in future studies.

Appendices

7.3 List of Publications

Manyiwa, T., Ultra Jr, V. U., Hengari, M., & Rantong, G. (2023). Spatial variability of Heavy metals in soils and vegetation and associated risk to grazing animals in the abandoned gold mine in Francistown, Botswana. *Environmental Pollutants and Bioavailability*, 35(1), 2254493.

Manyiwa, T., & Ultra Jr, V. U. (2022). Soil Amendments and Arbuscular Mycorrhiza Influenced the Growth and Heavy Metal Accumulation of *Colospospermum Mopane* (Kirk Ex Benth.) In Heavy Metal Contaminated Soil. *Soil and Sediment Contamination: An International Journal*, 31(1), 81-96.

Manyiwa, T., Ultra, V. U., Rantong, G., Opaletswe, K. A., Gabankitse, G., Taupedi, S. B., & Gajaje, K. (2021). Heavy metals in soil, plants, and associated risk on grazing ruminants in the vicinity of Cu–Ni mine in Selebi-Phikwe, Botswana. *Environmental Geochemistry and Health*, 1-16.

Ultra, V. U., & **Manyiwa, T.** (2021). Influence of mycorrhiza and fly ash on the survival, growth and heavy metal accumulation in three *Acacia* species grown in Cu–Ni mine soil. *Environmental Geochemistry and Health*, 43, 1337-1353.

Other Publications

Veciana, A., Steiner, S., Tang, Q., Pustovalov, V., Llacer-Wintle, J., Wu, J., Chen, X.-Z., **Manyiwa, T.,** Ultra, V.U., Jr., Garcia-Cirera, B., Puigmartí-Luis, J., Franco, C., Janssen, D.J., Nyström, L., Boulos, S. and Pané, S. (2024), Breaking the Perfluorooctane Sulfonate Chain: Piezocatalytic Decomposition of PFOS Using

BaTiO₃ Nanoparticles. *Small Science*.
2400337. <https://doi.org/10.1002/smsc.202400337>

Gajaje, K., Monnakgotla, O., Ultra Jr, V. U., & **Manyiwa, T.** (2024). Pollution index of soils along and against the wind direction within a 40 km distance of Morupule Fly Ash Dumpsite, Botswana. *Environmental Quality Management*, 33(3), 283-294.

7.4 Conference Presentations & Posters

Presentation: Manyiwa, T. & Ultra, V.U. Jr. *Influence of chelating agents on heavy metal accumulation in plants (Eucalyptus globulus, and Schinus mole) in mine tailings*. Japan-Africa Hybrid Workshop on Promotion of Science, Engineering, and Technology in Innovative Solutions to Environmental Problems in Metal Mining Area. 14-16 December 2022, Kyoto University, Katsura Campus, **Japan**.

Presentation: Manyiwa, T. & Ultra, V.U. Jr. 'Growth and Heavy Metal Accumulation of *Colophospermum mopane* as Influenced by Compost, Fly Ash and Mycorrhizal Inoculation'. 5th World Congress on Risk: Development and Resilience – SETAC Africa 9th Biennial Conference. Cape Town International Convention Center, **Cape Town, South Africa**, 2019 May 6-8. Pp.26.

Presentation: Manyiwa, T. & Ultra, V.U. Jr. 'Heavy Metal Accumulation of Plants from the Vicinity of Copper-Nickel Mines in Selebi-Phikwe, Botswana'. 5th World Congress on Risk: Development and Resilience – SETAC Africa 9th Biennial Conference. Cape Town International Convention Center, **Cape Town, South Africa**, 2019 May 6-8. Pp.15.

Poster: Manyiwa, T. & Ultra, V.U. Jr. 'Temporal Dynamics of heavy metals from Different Mine Tailings and Contaminated Soils as Influenced by Soil Amendments Based on Lysimeter Studies'. Research & Innovation Symposium 2019 (RDAIS 2019) Botswana International University of Science and Technology, Palapye, Botswana. 2019 June 4 - 7. Pp. 229.

Appendices

Poster: Gaotlhobogwe, K., Ultra, V. U. Jr, & **Manyiwa, T.** (2023). Heavy metal contamination in food crops grown in farmlands near Cu-Ni mine in Central Botswana. Environmental data-driven for innovation & sustainable development. SETAC Africa 11th Biennial Conference, 8-11th October, 2023, Accra, **Ghana**.

Poster: Phiri, L.V.A, **Manyiwa, T.** & Ultra, V.U. Jr. *Enhancement of phytoremediation potential of Eucalyptus in Fly Ash using Biodegradable Chelating Agents*. BIUST Teaching, Research, & Innovation Symposium 2023 (TRDAIS 2023). September 18-19. Pp.27.

Poster: Molefi, K.T., **Manyiwa, T.** & Ultra, V.U. Jr. *Assessment of the Heavy Metal Contaminations in Soils and Plant Shoots in the Vicinity of the Kgwakgwe Manganese Mine in Kanye, Botswana*. BIUST Teaching, Research, & Innovation Symposium 2023 (TRDAIS 2023). September 18-19. Pp.27.

Poster: Lefadola, O., **Manyiwa, T.** & Ultra, V.U. Jr. *'Survival, Growth and Heavy Metal Accumulation of Vetiver in Different Mine- Affected Soils as Influenced by Fly Ash and Compost Amendments'*. Research & Innovation Symposium 2019 (RDAIS 2019) Botswana International University of Science and Technology, Palapye, Botswana. 2019 June 4 - 7. Pp.223.

Poster: Opaletswe, K.A., Hengari, M., **Manyiwa, T.** & Ultra V.U. Jr. *'Heavy Metal Concentration and Availability in Soil at the Vicinity of Abandoned Gold Mine, Francistown, Botswana'*. 5th World Congress on Risk: Development and Resilience – SETAC Africa 9th Biennial Conference. Cape Town International Convention Center, **Cape Town, South Africa**, 2019 May 6-8. Pp.13.

Poster: July, K.P., Opaletswe, K.A., **Manyiwa, T.** & Ultra, V U.Jr. *'Heavy Metal Concentration and Availability in Soils at the Vicinity of Cu- Ni Mine in Selebi-Phikwe, Botswana'*. 5th World Congress on Risk: Development and Resilience – SETAC Africa 9th Biennial Conference.

Cape Town International Convention Center, **Cape Town, South Africa**, 2019 May 6-8.
Pp.32.

Poster: Setuwe, B., **Manyiwa, T.** Rantong, G. & Ultra, V.U. Jr, 2019. *Heavy metal accumulation of plants growing in abandoned Monarch Gold Mine, Francistown, Botswana.* 8th Young Environmental Scientists Meeting. Ghent University, Belgium. 5–10 February 2019.

Poster: Ultra, V.U. & **Manyiwa, T.** 2018. *Influence of fly ash application and mycorrhiza on inoculation on Heavy metals availability and survival of different Acacia species in heavy metal contaminated soils from Selibe Phikwe, Botswana*, World Congress of Soil Science, Brazil. August 12-17, 2018.