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Classification of tree species based on their accumulation and exclusion of heavy metals, and the influence of heavy metals on species abundance on Copperbelt tailings dams in Zambia

Submitted by:

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DECLARATION

I hereby certify that this study is my own work and that where work of others has been used duly acknowledgement has been given. I also certify that no plagiarism was committed in writing this thesis.

Signed ________________

Nalukui Matakala

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ABSTRACT

Classification of tree species into excluders or accumulators is vital for ecological restoration of mine tailings dams via phytostabilization. The study dealt with classification of indigenous tree species found on tailings dams and also how heavy metal concentration influence the abundance and distribution of tree species on tailings Storage facilities in the Copperbelt Province, Zambia. Among the studied species a number of them were classified into excluders and accumulators of the 12 metals. For example, *Rhus longipes, Syzigium guineense, Senegalia polyacantha*, *Ficus craterostoma, Bauhinia thonningii, Albizia adianthifolia*, *Combretum molle, Peltophorum africanum* and *Ficus sycomorus, Albizia antunesiana, Albizia versicolor, Azanza garckeana, Bauhinia petersiana, Brysorcapus orientalis*, *Combretum molle, Combretum microphyllum, Dichrostachys cinerea, Dodonaea viscosa, Ficus capensis, Lannea discolor*, *Phyllanthus guineensis, Senna singueana, Terminalia mollis, Terminalia stenostachya, Combretum apiculatum* and *Vachelia sieberiana* were classified as Cu excluders while *Dombeya rotundifolia, Albizia amara* and *Parinari curatellifolia, Combretum zeyheri, Annona senegalensis* and *Ozoroa insignis* were classified as Cu accumulators. All the species were classified as Mn accumulators while *Albizia adianthifolia*, *Albizia antunesiana, Combretum apiculatum* and *Ficus capensis* were classified as Zn excluders and the other 28 species classified as Zn accumulators. The dominance of species in terms of importance values varied from (a species with the lowest IVI value) to (Species with the highest IVI value). Species with high IVI values dominated the tailings dams than those with low IVI values. *Rhus longipes*, *Syzygium guineense*, *Senegalia polyacantha*, *Ficus craterostoma*, *Bauhinia thonningii*, *Albizia adianthifolia* and *Combretum molle* were identified as the most dominant species on Copperbelt tailings dams. Low species richness (composition) and abundance was observed in high metal concentration. Most species showed high affinity to heavy metals in low concentration while a few species (*Rhus longipes, Albizia versicolor, Albizia amara Dombeya rotundifolia*, *Albizia antunesiana*, *Phyllanthus guineensis* and *Dichrostachys cinerea)* showed high affinity to metals in high concentration. Furthermore, the study identified Ni, Cu, S, Cr, Al, Co, Mn, B, Mo and Cd as metals influencing species richness and abundance on the Copperbelt tailings dams.

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ACRONYMS

TF – Translocation factor

BF – Bioconcentration factor

CCMERC - Canadian Council of Ministers of the Environment Remediation Criteria

MT – Million tons

M ha – Million hectares

TSF – Tailings Storage Facilities

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background

Mining of copper in Zambia is the major economic activity with the largest export percentage standing at 60% of the country's total exports (Zambia Central Statistics 2016). Mining accounts for 12% of Zambia's Gross Domestic Product (GDP) and constitutes 62% of direct foreign investment (World Bank 2016). It is also the second major employer after government, currently employing 62 236 people (Zambia Chamber of Mines 2016). The history of mining in Zambia dates back to the early 1900s when the first commercial mine was opened in Luanshya in 1928 (Simutanyi 2008). In 2011, Zambia was ranked as the world's third-largest copper producer (Zambia Development Agency 2011). Due to an increase in demand for copper products and other minerals on the international market, mining activities in Zambia continue to rise. According to Lad and Samant (2014), the increase in demand for copper products is because of increasing population, advances in technology, modern housing and infrastructure development.

Globally, mining is one of the major contributors to economic growth in many countries, and it contributes to socio-economic development through job creation, income generation and foreign exchange (Li 2006; Schueler et al. 2011; Lange et al. 2016). Since the early stone age, 1150 million tons of metal ores have been mined globally (Edraki et al. 2014). In Africa, small scale mining dates to the Iron Age period. However, commercial mining began during the colonial period with an increase observed from as early as the 21st Century (Miller 2002; Festin et al. 2018). Even though mining is a major contributor to economic growth for many countries, it generates vast amounts of waste that cause environmental, social and economic problems. Mining generates wastes that directly and indirectly effect the environment, the social and economic status of the communities living near mines (Bradshaw 1993). Direct effects include loss of agricultural land, forests, foraging land while indirect effects consist of air, land, water pollution and siltation of rivers (Bradshaw 1993). River siltation and clearance of forests lead to biodiversity loss and loss of economic wealth through loss of natural capital such as clean water, forests and aquatic biodiversity. The effects of mine wastelands on the environment result in land transformation and degradation, thereby affecting local livelihood (Schueler et al. 2011). The loss of economic wealth through the loss of natural capital (agriculture, foraging and forest land) reduces household income generation thus resulting in increased poverty levels (Lindahl 2014).

Mine tailings pose several health effects on the humans living near mines and mine wastelands. Heavy metals in tailings ingested through the food chain, drinking contaminated water and inhaling dust particles affect human health (Cheyns et al. 2014, Zhang et al. 2015, Xiao et al. 2017). A study by Xiao et al. (2017) evaluated soil heavy metal concentrations and health risks of artisanal gold mining in Tongguan, Shaanxi, China. The study reports that children in the study area were prone to chronic diseases due to high accumulation of Pb and Hg estimated in their daily intake (EDI) that was associated with high exposure to heavy metals from tailings. In the Katanga, Cheyns et al. (2014) also reported high Co in urine of children and adults as a result of high exposure to heavy metals. High consumption of Hg causes damage to the nervous and immune systems of humans (Zahir et al. 2015) while high Pb consumption destroys the circulatory, nervous, endocrine, skeletal and enzymic systems (Chen et al. 2015). High Cd intake has been reported to cause kidney dysfunction, pulmonary adenocarcinomas (lung cancer), hypertension and bone fractures (Chen et al. 2015, Banza et al. 2009). High Cd intake has also been associated with high calcium excretion and low mineral bone density (Staessen et al. 1999, Banza et al. 2009). Excess human consumption of Zn causes esophagitis, burning pain in the mouth and throat, abdominal cramps and lethargy (Walsh et al. 1994). Cu accumulation in humans has been associated with neurological defects, liver disease and death in severe cases (Uriu-Adams and Keen 2005). Hence, the impacts of heavy metals in tailings on human health necessitate the need to restore mine wastelands especially tailings dams.

Reports of effects of mine wastelands on the environment, health and social economic status of people living near mines have been reported in various regions of the world namely China (Li 2006), Ghana (Schueler et al. 2011), Zambia (Lindahl 2014), South Africa (Fairbanks et al. 2000), USA (Dudka and Adriano 1997) and many other regions (Lange et al. 2016, Pourret et al. 2016). Mining pollution is mainly from wastelands and smelting processes. In China, Li (2006) reported that mining produced massive amounts of wastelands that caused water pollution, soil erosion and other environmental problems. By 2002, the mining industry in China had produced "265.4 million tonnes (MT) of tailings, 130.4 MT gangue and 107.8 MT slag" (Li 2006). Land degradation by mining in China had reached 3.2 Million hectares by 2004 (Li, 2006). In Ghana, Schueler et al. (2011) reported that only 0.2% of land was used for surface mining representing 33 ha in 1986 in Wassa West district, the country's most important mining region. However, as of 2002, land used for surface mining in Wassa West district had increased to 41.9% representing about 6 864 ha (Schueler et al. 2011). Schueler et al. (2011)

also reported that, the greatest extensive land cover changes in Wassa West were conversions of forests and farmlands into mining pits with a total of "3 168 ha of forests and 4 935 ha of farmland" converted into mine pits. A study by Doso et al. (2014) shows that about one third of a region in Ghana is leased to large-scale mining companies with a total area of about 23 921km^2 which is equivalent to 10% of Ghana's total land area. Land transformation from arable or forest land to mine wasteland has been observed in many mining countries including Zambia. Cooke and Johnson (2002) state that, the area of land transformed by mining globally is quite small, however individual country estimates show substantial amount of mining generated wastelands. For the mining wasteland in the USA is estimated to be 3.7 M ha (Dudka and Adriano 1997) and the amount in South Africa is estimated to be 0.2 M ha (Fairbanks et al. 2000). Love-land et al. (2003) confirmed that conversion of forestland to mine wastelands was the major land use change in the past decades in Appalachian region of the USA. In Zambia, mining has created an ecological footprint through generation of mine wastelands (Figure 1.1). For example, Copperbelt Province alone has approximately "9125 ha of land that is estimated to contain 791 MT of tailings, 1899 Mt of overburden material cover an estimated area of 20 646 ha" (Environmental Council of Zambia 2004; Festin et al. 2018). 77 MT of waste rock covers an estimated 388 ha and an additional 279 ha of land contains about 40 MT of slag (Environmental Council of Zambia 2004; Sikaundi 2013; Festin et al. 2018). The history of copper production shows that the "average ore grade decreased from 4% in 1900 to 0.5% in 1975" (Mining Annual Review 1985). The decrease in ore grade resulted in an increase in tailings produced rising from 17 to 290MT a^{-1} globally over the same time (Cooke and Johnson 2002). The reduction in ore grade led to an increase in tailings production, leading to an increase in mine wastelands.

Figure 1.1: Mine wastelands at Konkola Copper mines, Copperbelt, Zambia

Mine wastelands are associated with environmental problems that affect the economic, health and social status of local communities living adjacent to the mines. This has necessitated the need for mine wasteland restoration. “Mining is a temporal land use as the mineral deposits are finite in nature” (Cooke and Johnson 2002). However, mine wastelands when left unattended remain wastelands for an extended period of time. Kambing’a and Syampungani (2012) reported that tailings in Zambia can remain un-vegetated for a prolonged period due to the difficulty of vegetation establishment as the Tailings Storage Facilities (TSF) tend to have elevated concentration of heavy metals.

Mine wasteland restoration has been an issue of global concern for the past two centuries. This is due to the environmental, social and economic problems associated with mine wastelands. Several restoration techniques ranging from chemical, physical and biological have been documented globally (Li 2006; Festin et al. 2018). These techniques have their pros and cons, however, the biological method known as phytoremediation has received more attention from scientists /researchers (Koelmel et al. 2015) and environmental managers. This is due to its cost-effectiveness, pollution control effect and environmental friendliness (Wong 2003; Li 2006; Chenregani et al. 2009).

“Phytoremediation is the use of plants and related microorganisms to reduce effects of toxic contaminants on the environment” (Mendez and Maier 2008). The use of phytoremediation techniques (phytostabilization & phytoextraction) to remove and stabilize metalliferous sites have been applied in many mining regions (Wong 2003) with both successes and failures recorded. “Phytoextraction is the use of plants for uptake and translocation of heavy metals from the roots to aboveground parts of the plant” (Mendez and Maier 2008). Phytostabilization on the other hand uses plants to confine (restrict) heavy metal immobility through adsorption and accumulation on roots (Wong 2003). Using phytoextraction to remove environmental contaminants may be appropriate in regions where such plants are processed for the purpose of extracting minerals. However, in places where such is not common, this may be problematic environmentally as such have potential of deposition of heavy metals back in the soil after the plants die. Others include plant tissue disposal cost, low biomass production by hyperaccumulators and metal toxicity which disturbs plant growth when heavy metals are in high concentrations. Phytostabilization, therefore, provides a potential alternative for mine wasteland stabilization and restoration in such areas. Unlike phytoextraction which employs the use of accumulators (especially the hyperaccumulators), phytostabilization employs heavy metal excluders to clean environmental contaminants. Hyperaccumulators translocate heavy metals to aboveground parts of the plant while excluders inhibit translocation and mobility of heavy metals. Translocation of heavy metals to aboveground parts makes it possible for metals to be put back into the soil when a plant dies making this technique unideal for environment clean up but more ideal for phytomining.

Mine wasteland restoration attempts using plants have been made in Zambia. However, more failures than success have been recorded (Kambinga and Syampungani 2012). This could be due to lack of understanding of the nutrient dynamics of the dumps and the functional traits of the species used in the re-vegetation of these wastelands. Plant establishment is difficult on the wastelands due to the prohibitive physical and chemical properties of tailing soil (Wong 2003). The tailings properties inhibit vegetation establishment and growth leaving the wastelands without vegetation for a long time (Wong 2003). The failure to achieve sustainability in restoration could be attributed to inadequate information on the suitability of species for such programs. As such, the mining sector in Zambia has faced numerous challenges in undertaking the restoration of mining generated wastelands. With the expected increase in mining activities in the country, the need for mine wasteland restoration techniques through plant establishment on wastelands is inevitable. The use of native tree species in the restoration programs has more advantages than the foreign species in that they are already adapted to the prevailing environment and that the possibility of becoming invasive are minimal (Festin et al. 2018). The presence of indigenous tree species on the Copperbelt Province tailings dams (see Figure 1.2) provides an opportunity for evaluating the potential of using such tree species in phytoremediation. Additionally, their presence poses an opportunity to evaluate their heavy metal accumulation abilities and the influence of heavy metals on their abundance. This will provide an understanding of the use of plant species on copper tailings for phytoremediation.

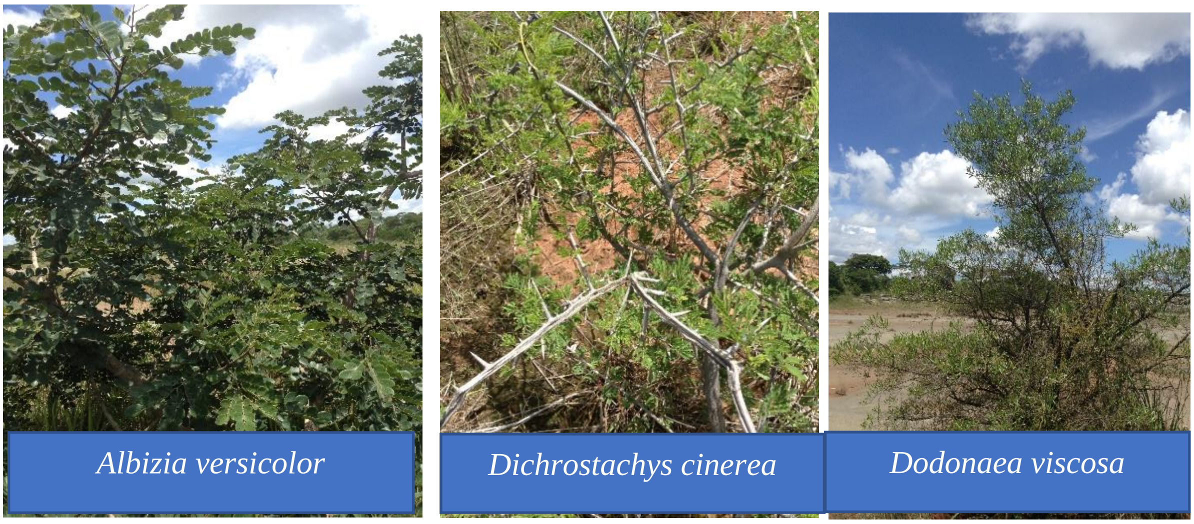


Figure 1.2: Indigenous tree species occurring on a mine tailing TD25 in Kitwe

1.2 Problem statement and justification of the study

Mining in Zambia is a major contributor to economic growth. However, mining produces wastes that when dumped on land render it derelict and are associated with environmental, social and economic problems. Increase in mining activities in the country entails an increase in waste generation and consequently an increase in mine wastelands which calls for developing an understanding of mine wasteland restoration. With the current global call for implementation and attainment of the sustainable development goals (SDGs) by 2030, mine wasteland restoration is key. If mining is to contribute effectively to sustainable development, there is need to develop and constantly apply “all-encompassing environmental management practices to reduce in and off-site environmental impacts” (Mulizane et al. 2005) such as ecological degradation. Mine wastelands hinder sustainable development due to the social, economic, health and environmental problems associated with wastelands that affect human well-being. Sustainable development goals number one, three, six, thirteen, fourteen and fifteen emphasize the need to end poverty, promoting human well-being and healthy lives, provision of clean water, combat climate change and biodiversity conservation. All these SDG’s are infringed by mining due to its unsustainable nature. Even though mining is an unsustainable activity, by putting certain measures in place for environmental protection and conservation, the activity could be sustainable. The protection of land, water and air from mine waste pollution and contamination will aid in protecting the environment for sustainable development and mine wasteland restoration is one of the measures that could transform traditional mining to sustainable mining.

Mine wasteland restoration has become an integral part of modern day mine development and closure. Mendez and Maier (2008) noted that, “unclaimed mine sites are a worldwide problem, with thousands of unvegetated exposed tailings presenting a source of contamination for nearby communities”. Mining is a temporal land use as the minerals are finite (Cook and Johnson 2002). However, the waste produced during mining remains waste for a long time if left unvegetated. Mine wastelands such as tailings in Zambia are left unvegetated for a long time (Kambin’ga and Syampungani 2012), hence increased mining activities will consequently increase waste production resulting in more mine wastelands. Mine wastelands contaminate water, land and air thereby affecting the local people socially and economically. They cause significant environmental problems in the surrounding environment if the area containing mine wastelands is left unrehabilitated (Ssenku et al. 2014). Managing mine wastelands especially tailing dams need methodical management for long-term storage stability and disposal facilities. This will help to prevent and reduce water and air pollution. Stabilization of mine wastelands can be achieved through wasteland revegetation. Plant cover influences soil protection and ecosystem processes enhancement (Bonet and Pausas 2004). This entails the role that plants play in restoring wastelands such as tailings which are loose and easily carried by water into aquatic systems. Plants have the capacity to stabilize the wastelands through the establishment of full functioning ecosystems such as forests that provide long-term cover on the wastelands (Wong 2003). Heavy metal immobilization by excluding plants reduces the concentration of toxic contaminants in the environment thereby reducing water, soil and air pollution.

Restoration or reclamation of mine wastelands through the establishment of vegetation cover (phytoremediation) aid in stabilizing the wastelands and reduce toxic heavy metal contamination of the environment. The use of phytoremediation to restore mine wastelands is preferable over the chemical and physical methods due to its cost effectiveness, environmental friendliness and additional benefits realised from plants used. Additional benefits may include biochar, phytoproducts, woody plants, ornamental plants, biodiesel, energy, aromatic essential oils and paper biomass, among others (Pandey et al. 2019). Phytoremediation reduces environmental, social and economic problems, including respectively, deforestation, migration, loss of cultural and spiritual sites, and lack of jobs and income due to loss of natural capital as discussed in chapter 2. Successful implementation of using phytoremediation to reclaim mine wastelands has been reported in China (Brombal et al. 2015), United States of

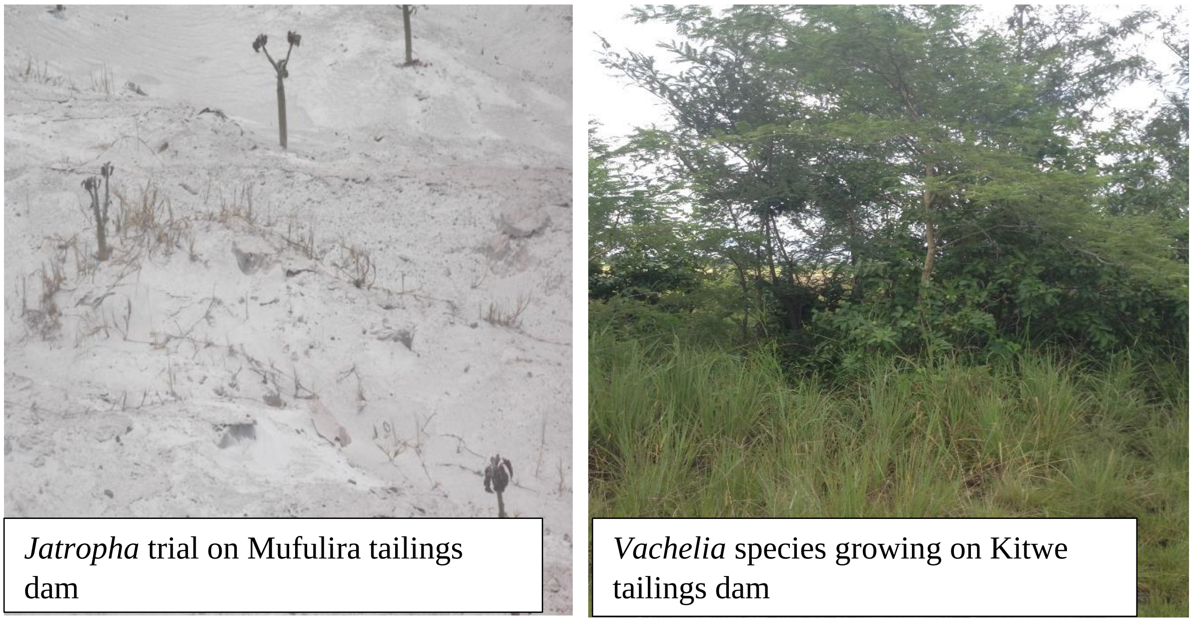
America (USA) (Nwaichi and Dhankher 2016), France (Ladislas et al. 2014) and Peru (Bech et al. 2012). In Africa, commercial application of phytoremediation to reclaim contaminated sites has not been fully exploited (Festin et al. 2018, Odor et al. 2019) due to lack of technical know-how, funding and research, unreliable policies and their poor implementation (Odor et al. 2019). However, successful restoration of plant cover on copper contaminated sites using phytostabilization has been reported in the DR Congo (Shutcha et al. 2015).

Using plants to remove toxic pollutants and stabilize mine wastelands has been recorded in Zambia, with more failures than success (Kambing'a and Syampungani 2012). This is evident from the continued pollution of nearby surroundings by tailings and the failure of plant establishment on the TSFs. However, a relic of plants including indigenous tree species exist on the walls of the TSFs indicating the ability of local plant species to adapt to contaminated sites. Lessons drawn from the phytoremediation trials in Zambia include the ability of plants to naturally colonize contaminated sites and the ability of soil amendments to enhance plant establishment and growth on the TSFs.

Restoration failures could be attributed to species selection, species heavy metal accumulation abilities and lack of research to determine species growth performance on the tailings dams over time. Classification of species according to their heavy metal accumulation abilities will aid in the selection of candidate species for restoring tailings dams. This is in line with Mganga's observation that classification of species according to their heavy metal accumulation allows for the identification of plants suitable for phytoremediation (Mganga et al. 2011).

Studies on mine wasteland restoration also focused on using grass to revegetate mine wastelands owing to grass's capacity to grow and establish in short periods of time. However, Ssenku et al. (2014) indicated that the use of indigenous and exotic tree species to reclaim copper tailings is a sustainable solution. This is due to the long-term cover tree species provide, the root system aids in stabilizing mine wasteland soils fast by reducing erosion and nutrient leaching (Sarrailh and Ayrault 2001). The use of tree species especially indigenous trees has other benefits such as carbon sequestration and timber and non-timber forest products harvesting. using indigenous tree species to rehabilitate tailings is important not only for the environment but for cultural and economic benefits of the local community where these tailings occur (Sarrailh and Ayrault 2001).

In Zambia, attempts were made to use both indigenous plant species (such as *Vachelia* species) and exotic species (*Jatropha* species) for the re-vegetation of tailings dams on the Copperbelt Province. *Vachelia* species showed good growth performance even though their performance in terms of how they deal with heavy metals have not been evaluated while *Jatropha* species showed poor growth performance (Figure 1.3). The poor growth performance could be attributed to harsh environmental conditions on the wastelands. Successful revegetation of mine wastelands (tailings dams) depends on the plants' adaptability to metalliferous soils, ability to immobilize heavy metals and good growth performance. It has been shown that screening plant species growing on metalliferous sites provides an opportunity to evaluate their phytostabilization potential (Li, 2006; Yang et al. 2014; Santos et al. 2017). What is known about species growing on Copperbelt tailings dams is the growth performance of some tree species and herbaceous plants based on Kambing'a and Syampungani (2012) and Mwangi (2017), respectively. There is little or no research on the accumulation strategies of the indigenous tree species, therefore, species are not categorised into either accumulators or excluders. This calls for screening of indigenous tree species growing on the TSFs for adaptability and heavy metal accumulation abilities. Classification of plant species with high metal tolerance abilities into either excluders or accumulators will aid in selecting species suitable for restoration of mining generated wastelands. In addition, determining the influence that heavy metal accumulation has on species abundance on the TSFs will enhance our understanding of how soils may be manipulated to support tree species growth.



Jatropha trial on Mufulira tailings dam

Vachelia species growing on Kitwe tailings dam

Figure 1. 3: Vegetation restoration trials on Mufulira tailings dams in the Copperbelt, Zambia

This dissertation seeks to evaluate the phytoremediation potential of indigenous tree species occurring on Copperbelt tailings dams and assess the influence of heavy metals on their abundance. The results of this study will generate valuable information that will be used in re-vegetating copper tailings in Zambia and other copper mining countries. The study will classify indigenous tree species into either excluders or hyperaccumulators and analyse the performance of these species on tailings dams. This information is necessary for establishing re-vegetation plans for mine wastelands such as tailings hence enabling restoration of ecosystems on contaminated soils. The study will inform decision makers and help with restoration planning based on the type of intended ecosystem to be restored.

1.3 Main objective

The study aimed at developing an understanding of the ability of tree species to either accumulate or exclude heavy metals on Copperbelt tailings dams, and the influence of heavy metals on the distribution and abundance of tree species on the Copperbelt tailings dams.

1.3.1 Specific Objectives and related questions

a) Specific Objective 1: To determine the diversity and response of native tree species to heavy metals across various tailings dams on the Copperbelt Province

Questions

i) What plant species occur on tailings dams of the Zambian Copperbelt Province?
ii) What species dominate the Zambian Copperbelt tailings dams?
iii) What are the concentrations of heavy metals across the Tailings Storage Facilities of the Copperbelt Province, Zambia?

b) Specific Objective 2: To determine the translocation and bioconcentration factors of metals by indigenous tree species occurring on Copperbelt Tailings dams

Questions

i) What is the ratio of root: soil heavy metal concentration in these plant species?
ii) What is the ratio of root: shoot heavy metal concentrations in these plant species?

c) Specific objective 3: To determine the influence of heavy metals on species abundance on Copperbelt tailings dams

Questions

i) What environmental species groups exist on Copperbelt tailings dams?

ii) What heavy metals influence plant species abundance on mine tailings dams of the Copperbelt?

1.4 Hypotheses

The study hypothesized that metal exclusion or accumulation ability of tree species have an influence of their distribution across the Tailings Storage Facilities of the Copperbelt Province, Zambia.

1.5 Thesis structure

This dissertation is divided into five Chapters. The first chapter covers the general introduction and it deals with research background, study justification and research objectives. Chapter two deals with the literature review and gives an account of studies on the study topic while chapter three deals with the evaluation of tree species growing on Copperbelt tailings dam's phytoremediation potential. Chapter four deals with the influence of heavy metals on species abundance across the Tailings Storage Facilities or tailings dams of the Copperbelt while chapter five synthesizes all the findings of the study and provides an overall conclusion in terms of the applicability of the findings.

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CHAPTER TWO: RESTORATION OF MINE WASTELANDS: A REVIEW

Abstract

Abandoned mine wastelands are of great environmental concern in mining areas of the world. Mining generates mine wastelands that remain waste for a long time and pose health and environmental risks. Mine wastelands contain heavy metals in high concentrations which contaminate water, air and land in areas near mine wastelands. The effects of mine wastelands on land, water and air necessitated the need for mine wasteland restoration to alleviate the impacts of mine wastelands on the environment and human livelihood. Literature describes various techniques used to restore mine wastelands with phytoremediation taking the lead owing to its cost effectiveness and environmental friendliness. Studies on the use of phytoremediation to restore mine wastelands have been reported globally with both success and failures. This review discusses the ecological restoration of mine wastelands through re-vegetation and the application of phytoremediation in mining regions of the world. It emphasizes the use of phytostabilization in mine wasteland restoration and phytoextraction in phytomining. The questions that the review focused on include; (1) what species occur on mine wastelands? (2) What criteria are used to select species for phytoremediation? (3) How do heavy metals influence species richness and abundance on wastelands? Native and planted species have been used in mine wasteland restoration with literature supporting the use of native species owing to their high adaptability to local conditions. Evaluation of the species performance on the tailings coupled with the pattern of heavy metal accumulation aids in selecting suitable species for phytostabilization. This review discusses the responses of plant species to heavy metals, phytostabilization efficiency and accumulation of heavy metals of different plant species.

Keywords: Phytoremediation, Phytostabilization, Mine wasteland restoration, Indigenous tree species

2.1 Introduction

Mining is a major economic activity globally as it contributes towards economic development of many nations (Lam et al. 2017; Festin et al. 2018). Massive investments aimed at improving logistic and power infrastructure, expanding automotive and telecommunication industries continue to increase demand for mineral products at global level.

For the past decades, mining of Copper, Zinc, Lead and Coal has created an ecological foot print in many mining regions through the generation of vast amounts of waste in form of tailings, slag, overburden materials and waste rock (Figure 2.1). Waste rock is the waste generated during underground shaft mining (Sikaundi 2013). Waste rock dumps comprise of mixed, course-grained rocks which are stored on mine site (Broda et al. 2014). It contains minerals in low concentration that could be reprocessed with advanced technology which possess little or no harm to the environment. However, the metal sulphides present in the internal structure of the dump effect groundwater and the environment due to acid mine drainage from metal sulphides (Broda et al. 2014). Overburden waste is the near-surface soils and rocks from open pit mining (Sikaundi 2013; Vela-Almeida et al. 2016). Mostly, overburdens have low impacts on the environment due to their content (soil and rock). "Tailings are waste materials produced from the concentration of copper to copper concentrates" (Sikaundi 2013). They are fine ground particles (sandy like particles) that contain leftover chemicals and metals from copper concentrate. They are difficult to manage due to their size and quantity produced and cause severe environmental and health problems (Lin et al. 2005). For example, "to produce one ton of copper, 350 tons of waste is generated, of which 147 tons are tailings" (Kangwa 2008). Tailings remain unvegetated for long periods of time due to its contents. Slag is waste produced during metal concentrate smelting (Sikaundi 2013). It contains silicate, calcium, phosphorus and sulphur and disposed of in molten or granulated form (Sikaundi 2013).

The non-metallic waste has less impact on the environment due to its content. Slag is used in roads, railway and infrastructure development as a concrete aggregate. Its use in roads, railways and infrastructure development has a long history while its use as cement and uses such as covering landfills to avoid dust and odours are gaining ground globally.

Figure 2.1: Mine generated wastes in Zambia

Decades of mining resulted in environmental liabilities in mining towns (World Bank 2016; Lam et al. 2017; Festin et al. 2018). Environmental liabilities in the form of waste rocks, overburden materials, slag and tailings occupying huge areas of land, have changed the landscape and created environmental problems that have safety and health implications. Mining has been observed to result in the generation of tailings which contain high levels of metals (Wong 2003; Li 2006; Lam et al. 2017) which affects human health and the environment.

2.2 Ecological Restoration of Mine Wastelands

The past two decades have seen a growing increase in mine wasteland restoration research globally due to an increase in mine waste generation. It has become a global agenda as the world faces increased population that requires arable land for agriculture and settlement. Restoration of degraded landscapes such as mine wastelands is known as ecological restoration. “Ecological restoration is the process of assisting the recovery of a degraded, destroyed or damaged ecosystem” (Society for Ecological Restoration 2002; Li 2006). Usage of the word’s restoration, reclamation, remediation and rehabilitation interchangeably in literature is observed (Seabrook et al. 2011). Presently, no term exists in restoration ecology that encompasses reclamation, rehabilitation, restoration and replacement (Li 2006; Festin et al. 2018). Rehabilitation involves engaging strategies and processes that aim at returning the environment to its original state before the introduction of an industry (Li 2006). Rehabilitation of mining generated wastelands involves the use of precise approaches of re-vegetating and regenerating the natural ecosystem that existed on a land before mining was introduced. Bradshaw (2000) defined rehabilitation as “the development towards the restoration of the original ecosystem”. “Replacement is defined as the creation of another ecosystem to the original while restoration refers to the re-establishment of the pre-mining ecosystem in its structural and functional characteristics” (Bradshaw 2000; Li 2006). In the mining framework, reclamation denotes the overall procedure of returning mine wastelands to a valuable or usable state (Cooke and Johnson 2002). The words restoration, rehabilitation, reclamation and replacement are used interchangeably in practice as they can be “described as resetting the ecological clock” (Cairns 1993; Li 2006). In this study, the words restoration, rehabilitation, replacement and reclamation were used interchangeably as they refer to one general term restoration. The use of the words interchangeably is seen in old and current literature (Cooke and Johnson 2002; Li 2006; Lima et al. 2016). However, the use of restoration is more established in literature nowadays.

Mining produces huge quantities of waste such as waste rocks and tailings that are stored on arable land. Mine waste contents such as heavy metals, leftover chemicals and acids degrade the land (Wong 2003) resulting in massive wastelands. Restoration of vegetation on mine wastelands presents considerable challenges. These range from re-creating the natural ecosystem, amending the physicochemical properties of tailings soil (Wong 2003; Festin et al. 2018) to establishing physically and functionally complex forest ecosystems (Macdonald et al.

2015). These characteristics in natural systems develop slowly over a long time. Available evidence has shown that unaided natural colonization can deliver completely established and functional ecosystems after long period of time (Weiersbye et al. 2006). The natural colonization is very sluggish and takes very long time and rarely occurs on adverse conditions such as mine tailing dams and waste rock (Li 2006). A few plant species which are tolerant to high metals, toxicity and high acidity colonize mine wastelands and establish open vegetation (Cooke and Johnson 2002). For example, in Zambia, 21 species were observed on a tailings dam of more than 40 years in age Kambing'a and Syampungani 2012) which is lower than the 55 species reported in natural forests (Festin et al. 2018). Low species richness and cover on tailings is due to elevated heavy metal concentrations such as Cu, Ni, Pd, Cr, As, V, Ba, Co, As, and Cd that hinder the development of many other species (Wong 2003; Chileshe et al. 2019).

The choice to use natural colonization (passive restoration) or human assistance (active restoration) depends on the ecosystem resilience, the goals of the restoration, landscape context and the cost of restoration (Holl and Aide 2011). Active restoration aids in achieving restoration goals on time, hence the need for its adoption in mine wasteland restoration. Globally, the rate of restoration has been increasing steadily even though the progress is slow (Li 2006). Reclaimed mined land can be used for different developmental use. For example, in China reclaimed mine land has mostly been used for agriculture and forestry (Miao and Marrs 2000). In Zambia, active restoration of mining generated wastelands appears to have been undertaken in the past even though the actual period the restoration began is not documented. Natural colonization of species could have happened, and this is evident from the relic of indigenous tree species that occur on mine tailings across the Copperbelt Province. Over the years, several mine wasteland restoration techniques have been developed (Li 2006) as human assisted restoration provides an opportunity to attain restoration within a much shorter time frame hence offsetting the negative environmental, social, economic and health impacts of mine wastes.

2.2.1 Types of Restoration Methods

There are three types of restoration techniques namely chemical, physical and biological methods. The physical method focuses on recreation of the landform through grading, smoothing, ploughing and topsoil placement (Seenivasan et al. 2015; Festin et al. 2018). The method improves soil physical structure making mine wastelands more habitable ecologically. The method employs processes such as topsoil application (Wong 2003; Li 2006; Sheoran et al. 2010; Festin et al. 2018) use of techno sols (Asensio et al. 2013a), use of limestone (Shutcha et al. 2015), use of organic residues or compost (Asensio et al. 2013b; Carlson et al. 2015) and biochar application (Abassi and Anawar 2015). These processes improve soil physical and chemical properties hence enhancing plant establishment and survival on mine wastelands (Watkinson et al. 2017; Festin et al. 2018). However, the application of topsoil, techno sols and compost are costly making the method unideal for large areas. For example, Bradshaw (2000) reported that the cost of topsoil application alone ranges from US$50,000 to US$100,000 per hectare.

The chemical method employs the use of chemicals and other synthetic and natural based materials to improve soil quality. The method aims at correcting pH and removal of contaminants such as metalloids and heavy metals in soil (Tutu et al. 2008; Mensah 2015). Fertilizer is one of the chemicals applied to soils for enhanced soil properties. Fertilizer application modifies soil pH and increases nutrient availability (Mensah 2015). Chemical application such as limestone increases the pH value of soil and is reported to buffer AMD (Juwarkar et al. 2009; Seenivasan et al. 2015; Festin et al. 2018). Biological resources like organic wastes are also employed in pH modification (Seenivasan et al. 2015). The use of litter fall and litter decomposition could be a potential way to input into the food web of the soil and correct pH. “Organic matter is the major source of nutrients such as Nitrogen, available P and K in unfertilized soils” (Sheoran et al. 2010). In Zambia, fertilizer application to amend mine wasteland soils has not been commonly applied because of the cost implication. Current technology is the use of chelators to alter heavy metal solubility and bioavailability (Saifullah et al. 2009; Pereira et al. 2010) and application of nanoparticles (Liu and Lal 2012; Festin et al. 2018). This method has limitations ranging from the need for skilled workers, costly machines, and chemicals through to potential contamination of groundwater and soil when applied in excess (Wu et al. 2012).

The biological method (phytoremediation) employs plants and other microorganisms to remove and reduce contaminants in the environment (Mendez and Maier 2008; Festin et al. 2018). As opposed to the physical and chemical methods, phytoremediation is cost effective environmentally friendly, enhances TSFs stabilization, hence reducing contamination and pollution of air, water and land in nearby surroundings (Mendez and Maier 2008; Mahar et al. 2016). Phytoremediation provides other benefits such as biochar, phytoproducts, woody plants, ornamental plants, biodiesel, energy, aromatic essential oils and paper biomass, sustainable soil management and carbon sequestration (Mahar et al. 2016; Pandey et al. 2019). Unlike the physical and chemical methods, phytoremediation provides beneficial benefits from the extraction of metals from plants making it a win-win solution with both economic and environmental benefits (Mahar et al. 2016). With phytoremediation, sustainable soil management is guaranteed as plants increase soil fertility steadily (Mahar et al. 2016) thereby enhancing plant production and adding economic value (Vangronsveld et al. 2009; Mahar et al. 2016), making it the ideal method for tailings dams restoration in Zambia. The use of plants used in phytoremediation for biofuel production (Datsyar et al. 2019), paper biomass, biochar and other phytoproducts makes phytoremediation a suitable method for the remediation of copper tailings dams in Zambia. The role of the plant species used in phytoremediation and biochar produced from these plants in reducing atmospheric carbon is worth noting (Jansson et al. 2010). Carbon sequestration reduces the effects of climate change which is important for human lives, animals and plants as well as future generations. A reduction in extreme weather changes, fires, droughts, floods and cyclones reduces loss of property and enhances human livelihoods and ecosystem health. These reasons make phytoremediation more suitable for mine wasteland restoration

2.3 Phytoremediation

Phytoremediation is defined as "the use of plants and plant related organisms to clean up contaminants from the environment (Mclntyre 2003). It takes advantage of plants´ ability to take up, store, accumulate and degrade inorganic and organic substances (Mclntyre 2003). The use of phytoremediation to restore mine wastelands has been documented globally (Wong 2003; Li 2006; Mahar et al. 2016; Mendez and Maier 2008; Mganga et al. 2011; Zou et al. 2012; Festin et al. 2018, Odor et al. 2019). Soils on mine wastelands are of poor quality and they take longer to develop naturally hence, using plants to decontaminate and enhance soil properties results in successful restoration of mine wastelands such as tailings (Conesa et al. 2007a; Conesa et al.2007b). Phytoremediation has the capacity to stabilise mine wasteland soils and establish permanent plant communities (Li 2006). By removing pollutants from the soil or rendering them harmless, phytoremediation restores fully functioning ecosystems and prevents contamination of soil, air and water system (Salt et al. 1998).

Phytoremediation encompasses five methods namely, phytoevaporation, rhizofiltration, rhizodegradation, phytoextraction and phytostabilization (Mahar et al. 2016). Focus in this study is on phytostabilization and phytoextraction as the widely used soil reclamation methods.

Baker (1981) defined phytoextraction as "the uptake and translocation of heavy metals to aboveground parts of the plant". "Phytostabilization is the use of plants and soil amendments to confine heavy metals through absorption and accumulation by roots, adsorption onto roots, or precipitation within the rhizosphere" (Mendez and Maier 2008; Bolan et al. 2011). Phytoextraction uses hyperaccumulators while phytostabilization uses excluders (Zou et al. 2012). Phytoremediation methods, particularly phytoextraction, fail short of large-scale restoration programs due to some Constraints. These include "slow growth of naturally occurring hyperaccumulator species, their low aboveground biomass production and the long time it takes to remediate the contaminated soil" (Mendez and Maier 2008). Furthermore, the limited metal bioavailability, the potential for heavy metal recycling back in the soil ecosystem if improperly disposed and its limited application to sites with less metal concentration (Zou et al. 2012; Sarwar et al. 2017). These short comings make phytoextraction unideal for mine wasteland restoration, particularly tailings dams (Zou et al. 2012). Even though phytoextraction is not ideal for mine wasteland restoration, its application in phytomining is gaining ground. Phytomining employs the use of metal hyperaccumulators to harvest the elements in a process where metal hyperaccumulator plants are grown on contaminated sites for metal harvesting (Sheoran et al. 2009). Phytomining is ideal in areas where conventional mining presents a costly method. Phytostabilization however, concentrates on heavy metal sequestration in roots and rhizosphere (Zou et al. 2012), separates and stabilizes heavy metals making them harmless hence preventing contaminants from dispersing into the environment (Ojuederie et al. 2017). Heavy metal immobilization enhances plant establishment and growth on tailings dams hence creating a vegetation cap. This makes phytostabilization ideal for tailings dam restoration due to the stabilization of wastelands and permanent vegetation establishment (Mendez and Maier 2008; Bolan et al. 2011).

Phytostabilization is an in-situ remediation technology that uses plants to stabilize contaminated soils and mine tailings (Santos et al. 2017). It uses plants and soil amendments to immobilize and stabilize contaminated sites (Mendez and Maier 2008). Soil amendments used in phytostabilization include top soil application and application of compost and biochar. The use of soil amendment practices in phytostabilization is derived from the physical and chemical methods of restoration. This makes phytoremediation a comprehensive method that combines techniques derived from the chemical and physical methods. Using vegetative compost has been reported to increase microbial activity and offer protection against erosion

(Carlson et al. 2015). Of these soil amendments, biochar application has received more attention (Paz-Ferreiro et al. 2014) owing to its cost effectiveness, heavy metal immobilizing ability and soil quality enhancing properties (Beesley et al. 2011). Biochar is “a low-density carbon-rich material produced by pyrolysis of plant biomass at temperatures ranging between 300 and 1000 ºC “(Zhang et al. 2012). The carbon rich material contains essential plant nutrients such as such as nitrogen, phosphorus, potassium, calcium, magnesium, iron, and zinc (Haefele et al. 2011) which are important for plant establishment and growth. Application of biochar to mine wastelands raises pH and porosity thereby increasing the water holding capacity ((Paz-Ferreiro et al. 2014; Carlson et al. 2015). Studies suggest that biochar can affect heavy metal behaviour in soil by changing metal “solubility, availability, transport and spatial distribution” (Barrow 2012; Thomas and Gale 2015), thus immobilizing heavy metals (Park et al. 2011) which reduces transportation of metals to the shoot system of a plant.

After site amendment, planting a diverse range of tree species would enable the creation of a continuing vegetation ecosystem (Singh et al. 2014b). Tree species have deep roots that reduce soil compaction and soil density thereby enhancing the stability of mine wasteland soils such as tailings (wa Ilunga et al. 2015). Trees aid in creating a new layer of topsoil, increase organic matter and available plant nutrients (Singh et al. 2004a). Using native tree species enhances tree species adaptability resulting in reduced mortalities from pests (Bozzano et al. 2014). Native species are well adapted to the local conditions while exotic species may become invasive and threaten local biodiversity (Li 2006; Pourret et al. 2016). Literature shows that legumes and pioneer species have high survival rates on contaminated soils. Examples of such include species from the genera *Albizia*, *Vachelia*, *Senegalia* and *Leucaena* (Gathuru 2011; Mensah 2015).

The use of transgenic plants and nanoparticles has gained much attention recently (Seth 2012) due to their ability to decontaminate contaminated sites on time. Transgenic plants are plants that are” genetically engineered through transgenes insertion for increased bioaccumulation and heavy metal degradation”. For example, Farwell et al. (2007) assessed “the growth performance of transgenic canola (*Brassica napus*), expressing a gene for the enzyme 1-aminocyclopropane-1-carboxylate deaminase under different flooding conditions and elevated soil nickel concentration”. The study reported “increased Nickel accumulation and greater shoot biomass in transgenic than non-transgenic canola under low flood-stress conditions”. In

Zambia, the use of transgenic plants has not been applied and this can be attributed to inadequate technology

For phytostabilization to be successful, selection of suitable candidate species for precise ecological conditions is key (Santos et al. 2017). Suitable plant species should be heavy metal tolerant, heavy metal immobilizers (Mendez and Maier 2008), 0 have a wide root system and large amount of biomass on contaminated sites (Zou et al. 2012). The phytostabilization ability of a plant is enhanced by soil amendments. For example, addition of biochar and compost modifies organic matter content and pH thereby, increasing plant yield and heavy metal immobilization (Ojuederie et al. 2017). The availability of native plant species on tailings provides an opportunity for surveys to screen plant species for phytostabilization potential (Santos et al. 2017). Attempting to re-vegetate mine wastelands without knowledge of species interaction with wasteland soils and their suitability could be costly and unsustainable (Pourret et al. 2016).

2.3.1 Classification of plant species for phytoremediation potential

Plants depend on the physicochemical properties of the soil to survive, grow and reproduce in natural environments. However, in contaminated soils, plants are forced to adapt to the harsh conditions (Mganga et al. 2011). Plants adapt to metalliferous soils using two strategies namely accumulation and exclusion (Sainger et al. 2011). From the two strategies three types of plant species are observed namely; accumulators, excluders and indicators (Baker and Walker 1990, Sainger et al. 2011). “Heavy metal accumulators are plants that accumulate heavy metals in their above-ground parts to leaves more than those in their below-ground parts”(Baker and Walker 1990; Mganga et al. 2011). From this group, a subgroup called hyperaccumulators exists. “Hyperaccumulators take up heavy metals from the contaminated soils in huge amounts through the roots and accumulate them in above-ground parts of a plant at 100-1000 times higher in concentration than those found in non-hyperaccumulating plants” (Jabeen et al. 2009; Ojuederie et al. 2017). These plants grow on contaminated sites without signs of metal phytotoxicity (Rascio and Navari-Izzo 2011; Sainger et al. 2011; Bech et al. 2012; Ojuederie et al. 2017). Literature (Yoon et al. 2006; Bech et al. 2012; Ojuederie et al. 2017), reports that more than 500 plant taxa consisting of 101 families are hyperaccumulators. Families such as Brassicaceae, Fabacaeae, Asteraceae, Caryophyllaceae, Cumouniaceae, Cyperaceae, Flacourtiaceae, Poaceae, Lamiaceae, Euphobiaceae and Violaceae are part of the hyperaccumulator families (Yoon et al. 2006; Bech et al. 2012; Ojuederie et al. 207). Based on

Van der Ent's criteria, a hyperaccumulator has metal concentrations of "Co 300, Pb 1000, Cr 300, Cd 100, Ni 1000, Cu 300 and Zn 3000 μg/g" in dry foliage (Van der Ent et al. 2013). Hyperaccumulators as employed in phytoextraction are ideal for phytomining as opposed to metalliferous soil remediation. This is because hyperaccumulators put the heavy metals back in the soil when they die hence their utilization in mine wasteland restoration would only postpone the environmental problems associated with heavy metals hence the failure for complete remediation of metalliferous sites.

Excluder plants restrict the transportation of heavy metals to above-ground parts of the plant by maintaining low heavy metal concentrations in shoots than in soil (Walker 1990; Mganga et al. 2011; Ghanderian and Ravandi 2012). Excluders immobilize heavy metals through adsorption of heavy metals on rhizomes (Mendez and Maier 2008), making them ideal for contaminated soil remediation (Mganga et al. 2011; Ghaderian and Ravandi 2012). Metal excluders which are metal-tolerant have great phytostabilization potential and ultimate re-vegetation of metalliferous sites (Ghaderian and Ravandi 2012). Indicators are plant species that "accumulate metals in their above-ground parts and the levels of concentration of metals in above-ground parts is equal to heavy metals in soils" (Baker and Walker 1990; Ghosh and Singh 2005; Mganga et al. 2011). Indicators die off with continued heavy metal uptake however, their presence in an area is an indication of pollution hence providing ecological or biological functions (Kvesitadze et al. 2006; Mganga et al. 2011).

2.3.2 Screening species for phytoremediation potential

The process of screening and selecting plant species based on their phytoextraction and phytostabilization ability is the initial point for selecting candidate species for re-vegetating tailings dams (Dongmei and Changqun 2008; Parra et al. 2016). Classification of plant species based on their heavy metal accumulation abilities is based on the bioconcentration and translocation factors (Ali et al. 2013). Bioconcentration is the uptake of a substance by a living organism from the medium such as tailings dam soil through roots, to the stem, leaves and shoots (Baby et al. 2010). Bioconcentration factor (BF) is "the ratio of elemental concentration in shoot tissues/ element concentration in mine tailing's soils" (Mendez and Maier 2008; Mganga et al. 2011; Rezvani and Zaefarian 2011). BF evaluates a root's ability to accumulate heavy metals (Zou et al. 2012). Translocation factor (TF) on the other hand is "the ratio of element concentration in shoots/ element concentration in roots". TF >1 means heavy metals are translocated to a plant's shoot system from the root (Rezvani and Zaefarian 2011; Ojuederie et al. 2017) hence a low TF is an important characteristics for species with phytostabilization potential (Zou et al. 2012). The BF and TF values should be < 1 for a plant species to be characterized as an excluder. A ratio that exceeds 1 means the plant species under consideration is an accumulator (Mendez and Maier, 2008; Zou et al. 2012).

2.3.3 Plant response to heavy metal concentration

Plant's response to element stress caused either by excess or deficiency cannot be exactly defined due to the evolution that plants have gone through (Kabata-Pendias 2010). Plant biochemical mechanisms lead to plant tolerance and adaptation to new and contaminated sites (Kabata-Pendias 2010). Due to the differences in plant species response to heavy metals, investigations for plant response to heavy metals should be particular to each plant-soil system (Kabata-Pendias 2001; Zou et al. 2012). During the ecological adaptation process, different populations are affected by environmental conditions of a site. Populations with high heavy metal tolerance are distributed in tailings and mine wastelands (Zou et al. 2012). These plants adapt to metalliferous sites as either accumulators, indicators or excluders. Classification of plant species into either accumulators, excluders or indicators requires the determination of the specie's BF and TF (Kabata-Pendias 2001; Zou et al. 2012; Ojuederie et al. 2017). The investigation, screening, evaluation and selection of plant species with phytostabilization all depend on the BF and TF. Studies conducted in China (Yang et al. 2014, Yang et al. 2017), Tanzania (Mganga et al. 2011), Mexico (Santos et al. 2017), Pakistan (Khan et al. 2013) and other parts of the world (Ojuederie et al. 2017) employed BF and TF in classifying species with phytostabilization potential. "The amount of metal in rhizosphere soil available for uptake determines how efficient metals are moved within the plant and the success of phytostabilization process" (Yang et al. 2017). Several studies on the accumulation and translocation of heavy metals such as Pb, As, Cu, Co, Zn, Mn, Cr, and Cd in certain plant species have been undertaken across the globe. Zou et al. (2012) determined the BF and TF of lead (Pb) for nine plants in three growing seasons on a lead-zinc mine in Yingjing in three growing seasons namely the" early growth stage (ES), vigorous growth stage (VS) and the Late growth stage (VS)". The BF of most of the species was < 1 in all growing seasons with a few having BF > 1 in one or two seasons of growth except *Athyrium wardii* and *Equisetum arvense L* that had a high BF in all growth seasons. The translocation factor of all the species in all three growing seasons was < 1 except for *Anemone vitifolia* which had TF > 1 in one growing season. The study identified *Athriu wardii* and *Equisetum arvense* L as phytostabilizers due to their low TF and BF (Zou et al. 2012). In a similar study, Yang et al. (2014) evaluated the "phytostabilization potential of 12 dominant plant species growing on manganese mine tailings" in four sites for Pb, Cd, Mn and Zn (Table 2.2). The results show differences in heavy metal accumulation by species signifying variations in different heavy metal accumulation strategies (Yang et al. 2014). BF of most species was < 1 except" *Phytolacca acinosa Roxb* (for Cd and Mn) and *Alopecurus japonicus Steud, Commelina. Communis Linn and Chrysanthemum indicum* (for Mn)". Lower BF indicates plant species ability to adapt to metalliferous sites through the exclusion strategy. The study reports most plants species having comparatively high TF than BF value for the same metal except "*Alopecurus japonicus Steud*, *Commelina. Communis*, *Chrysanthemum indicum* and *Phytolacca acinosa Roxb* for Cd, Mn, Pb, and Zn which were >1" (Yang et al. 2014). The translocation factor of a specie is key in determining phytostabilization potential. Low translocation factor entails the ability of a specie to immobilize heavy metals in the soil unlike a high TF that means hyperaccumulation of metals in the shoot system (Zou et al. 2012). From Yang's study, "eight plant species namely, *Alternanthera philoxeroides*, *Artemisia princeps*, *Bidens frondosa*, *Bidens pilosa*, *Cynodon dactylon*, *Digitaria sanguinalis*, *Erigeron canadensis* and *Setaria plicata* accumulated lower concentrations of Cd, Mn, Pb, and Zn in the shoots compared to the roots than the other species indicating their phytostabilization potential" (Yang et al. 2014, Parra et al. 2016). However, *Alopecurus japonicus Steud*, *Commelina. Communis*, *Chrysanthemum indicum* and *Phytolacca acinosa*" had TF > 1 for Cd, Mn, Pb, and Zn were >1 making them hyperaccumulators ideal for phytomining.

Table 2.1: TF and BF of "dominant plants from Four tailings ponds" (Yang et al. 2014)

		Cd		Mn		Pb		Zn	
Sites	Species	BF	TF	BF	TF	BF	TF	BF	TF
ZX[I]	*Alternanthera philoxeroides*	0,25	0,57	0,1	0,51	0,06	0,62	0,15	0,59
	Bidens frondosa	0,72	0,34	0,18	5,37	0,12	1,71	0,19	2,64
	Bidens pilosa	0,16	0,93	0,09	0,91	0,03	0,75	0,07	1,06
	Digitaria sanguinalis	0,54	1,1	0,16	0,72	0,07	0,79	0,21	0,44
	Erigeron canadensis	0,14	0,06	0,06	0,17	0,02	0,35	0,08	0,61
	Phytolacca acinosa	0,62	1,93	1,48	10,49	0,05	4,07	0,21	2,68
GK[I]	*Artenisia princeps*	0,7	0,79	0,33	0,9	0,15	0,57	0,17	0,59
	Cynodon dactylon	0,45	0,75	0,1	1,56	0,02	0,95	0,17	1,92

	Erigeron canadensis	0,87	0,49	0,04	0,14	0,03	0,34	0,21	1,02
	Phytolacca acinosa	1,33	1,06	1,11	11,59	0,03	2,02	0,2	2,43
	Setaria plicata	0,49	1,74	0,09	2,28	0,03	1,13	0,22	2,24
ZX[II]	*Alopecurus japonicus*	0,35	1,56	0,81	1,55	0,16	0,82	0,44	1,06
	Alternanthera philoxeroides	0,16	0,28	0,03	0,24	0,02	0,33	0,36	0,52
	Bidens pilosa	0,27	0,52	0,06	0,59	0,02	0,5	0,12	0,62
	Commelina communis	0,31	0,52	1,02	3,07	0,16	1,11	0,57	0,73
	Cynodon dactylon	0,25	0,58	0,07	0,42	0,05	0,55	0,09	0,5
	Chrysanthemum indicum	0,2	0,29	1,05	1,39	0,1	1,39	0,7	0,75
	Erigeron canadensis	0,28	0,19	0,04	0,13	0,2	1,15	0,31	1,16
	Phytolacca acinosa	1	1,62	1,41	9,72	0,02	2,02	0,92	5,04
XY[II	*Alopecurus japonicus*	0,61	1,75	1,14	1,2	0,09	1,3	0,29	0,78
	Bidens pilosa	0,31	0,31	0,1	0,61	0,02	0,33	0,11	0,65
	Cynodon dactylon	0,41	0,95	0,11	0,61	0,08	0,98	0,28	0,81
	Chrysanthemum indicum	0,17	0,26	0,44	1,15	0,03	2,08	0,2	3,07
	Digitaria sanguinalis	0,25	0,94	0,16	2,05	0,12	1,21	0,4	1,44
	Erigeron canadensis	0,38	0,13	0,02	0,17	0,33	1,29	0,41	1,89
	Phytolacca acinos	0,94	1,76	0,98	8,71	0,05	3,7	0,79	3,56
	Setaria plicata	0,38	2,01	0,08	5,64	0,05	1,23	0,43	1,5

Source: Yang et al. (2014)

In Africa, few studies determined the bioconcentration and translocation factors of species growing on contaminated or mine wastelands (Festin et al. 2018). A study by Mganga et al. (2011) around the North Mara Gold mine in Tanzania investigated the hyperaccumulation ability of 14 plant species growing around the mine using the plants bioconcentration and translocation factors. The study classified *Ludwigia stolonifera* as a Pb and Cd indicator, *Sphaeranthus gomphrenoides* as a Cd and Ni excluder, *Leersia hexandra* as a Ni and Pb hyperaccumulator and *Fuirena umbrellata* Rottb as a Cd and Cr hyperaccumulator. Furthermore, *Commelina benghalensis* was classified as a Cd hyperaccumulator, *Typha capensis* Pers was identified as a Cd hyperaccumulator and Ni excluder, *Agave sisialana* Perr was classified as a Cr hyperaccumulator and a Pb, Zn and Ni indicator. *Pluchea dioscoridis* (L) DC was identified as Cr hyperaccumulator, Cd and Ni excluder and Zn and Pb indicator, *Sphaeranthus kirkil* Oliv was identified as a Pb indicator and *Cyperus articulatus* L was classified as a Cd excluder and Zn indicator. The study also classified *Cyperus exaltatus* L as a Zn, Ni and Cd hyperaccumulator and Pb excluder, *Hygrophylla auriculate (*Schumach) Heine as a Cd, Ni and Cr hyperaccumulator and Pb excluder, *Crinum papilosum* L as a Cr and Cd hyperaccumulator and Ni indicator and *Hoslundia opposita* Vahl was identified as a Cd, Pb and Cr hyperaccumulator. Similarly, Schachtschneider et al. (2017) used the bioconcentration and translocation factor to investigate the phytostabilization and phytoextraction ability of species along the Upper Olifants River in South Africa. The study identified *Typha capensis, Schoenoplectus corymbosus, Phragmites autralis* and *Juncus effuses* as potential phytostabilizers of South African buffer areas. A recent study by Mwangi (2017) investigated the phytostabilization potential of seven indigenous perennial and annual herbs growing on Chambishi Copper Mine tailings dams. The study classified *Smilax anceps* and *Celosia digyna* as Cu excluders, *Smilax anceps*, and *Stetaria sphacelate* as Co excluders and *Smilax anceps* as an excluder for Fe. Furthermore, *Laurambergia engleri* and *Blumea alata* were identified as Zn excluders while *Laurambergia engleri* and *Cynodon dactylon* were classified as Pb excluders (Mwangi 2017). *Pteris vittata* was classified as a Pb, Cu, Fe and Co accumulator and its application in phytostabilization programs was cautioned.

2.4 Influence of heavy metals on species abundance and distribution

Heavy metals are said to belong to the most dangerous pollutant group (Wierzbicka et al. 2015; Morkunas et al. 2018), however, heavy metal toxicity depends on various factors. Factors like metal concentration, pH, type of metal, duration of exposure to heavy metals and oxidation state influence metal toxicity (Morkunas et al. 2018). Heavy metals are beneficial for plant's physiological functions (Oves et al. 2013) in low concentrations, however in high concentrations they cause various injuries to plants resulting in growth inhibition and death (Mohanpuria et al. 2007; Guo et al. 2008; Ugulu 2015). For example, Cu plays a vital role in the assimilation of CO_2, in photosynthesis and respiration systems (Demirevska-Kepove et al. 2004). However, excess concentrations of Cu cause stress and plant injuries resulting in chlorosis and plant growth retardation (Lewis et al. 2001). Cd influences the uptake and transportation of water and vital elements such as Mg, P, K and Ca (Das et al. 1997) but when in high concentrations (above 0.05ug/g), cadmium causes "chlorosis, root tip browning, growth inhibition and death" (Di Toppi et al. 1999). Cr, an extensively distributed element on earth in low concentrations has severe effects on plants in high concentration (above 1.5ppm) (Ugulu

2015). Cr is harmful to plant growth and development due to its effect on enzyme activities, photosynthesis and electron transport (Ugulu 2015). Furthermore, Ni causes chlorosis and necrosis (death of cells due to injury or disease) (Rahman et al. 2005) while Mn causes crinkle leaf (plant disease that causes leaf wrinkling and distortion), chlorosis and reduce photosynthetic rates (Kitao et al. 1997). Excess Zn in the soil causes inhibited growth of roots and shoots, chlorosis "(Yellowing of leaves due to lack of chlorophyll)" of juvenile leaves and old leaves if exposure is prolonged even though it is essential for enzyme, proteins and auxin biosynthesis in low concentrations (Ugulu 2015). Even with all the effects that heavy metals have on plant growth and establishment; resilient metal tolerant species naturally colonize metalliferous sites.

Plants have been used as a bioindicators in various studies (Ugulu 2015), because this approach is cost effective as plants are readily available and provide an opportunity for long-term monitoring of the environment (Gadzala et al. 2004; Ugulu et al. 2012; Ugulu 2015). Metalliferous sites like tailings dams have harsh conditions that inhibit plant establishment and growth. The interaction between heavy metals and plants depends on heavy metal solubility and reactivity with inorganic and organic molecules of such plant species (Singh and Sinha 2005). Bioavailable metals are transferred from the soil to above plant parts through water, soil and air (Ojuederie et al. 2017).

Heavy metal availability is influenced by several factors including their concentration in the soil, soil type, climatic conditions, plant maturity, root system and species response to heavy metals (Ugulu 2015). It is known that species establishment and abundance is influenced by physical and chemical properties of mine wastelands (Anawar et al. 2013; Ugulu 2015). Literature has shown variations in species richness and abundance across various tailings due to variations in element concentration. In Zambia, Mwangi (2017) observed seven indigenous species from seven families on the Chambishi copper tailings dams while Kambing'a and Syampungani (2012) reported 21 indigenous and exotic species on Kitwe's tailing dam (TD25) of the Copperbelt Province. In China, Sun et al. (2016) observed 22 species from11 families occurring on a mine wasteland. Similarly, Li et al. (2007) observed 36 species from 22 families colonizing a manganese mine wasteland in Guangxi, South China. Shen et al. (2004) also observed a few plant species such as *Cynodon dactylon, Pteridium aquilinum var. latiusculum, Sesbania rostrate, Paspalum notatum, Vetiveria zizaniodes etc* on Pb and Zn mine wasteland in China. Tailings have different compositions based on the mineral and the quality of the ore body hence the variations in species richness.

2.5 Conclusion

The studies reviewed suggest that phytostabilization has potential for reducing environmental contamination in areas with mine wastelands. The studies indicate that revegetation of mine wastelands using species with phytostabilization potential is a cost effective and environmentally friendly solution to environmental contamination by mine wastes. The studies highlight the importance of employing indigenous plant species that are well adapted to the environmental conditions of the sites than exotic species that could be invasive. Plants growing on contaminated sites provide an opportunity to screen or assess their heavy metal accumulation strategies for possible application in phytostabilization. Classification of species into excluders and hyperaccumulators of heavy metals aids in identifying species suitable for re-vegetating mine tailings dams. This classification is dependent on their BF and TF as species with BF and TF < 1 are classified as excluders and those with BF and TF > 1 are classified as accumulators. This review suggests that plant species accumulate heavy metals differently as such care must be taken when applying plant species in phytostabilization by considering the contaminants present at the site. In general, the review supports the use of excluders in phytostabilization and accumulators in phytoextraction.

2.6 References

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CHAPTER THREE: EVALUATION OF THE PHYTOSTABILIZATION POTENTIAL OF PLANT SPECIES OCCURRING ON THE COPPER TAILINGS STORAGE FACILITIES OF COPPERBELT PROVINCE, ZAMBIA

Abstracts

Mine tailings dams present major environmental problems globally. Restoration of these metalliferous sites via phytostabilization presents a cost effective and efficient way of restoring the sites. Screening native tree species occurring on copper (Cu) tailings dams of the Copperbelt province in Zambia is vital for their restoration. The current study evaluated the potential of 32 native tree species from 13 families occurring on the tailings dams for phytostabilization potential of Cu, Co, Zn, Al, S, B, Cd, Cr, Ni, Mn and Mo. The study employed the use of the bioconcentration factor (BF) and translocation factor (TF) to evaluate the heavy metal accumulation strategy of tree species growing on Copperbelt tailings dams. Six of the 32 species showed dominance in the following sequence, *Rhus longipes* (IVI) (15.985 ± 3.428) > *Syzygium guineense* (13.316 ± 5.093) > *Senegalia polyacantha* (8.642 ± 3.822) > *Ficus craterostoma* (8.201 ± 2.069) > *Albizia adianthifolia* (6.477 ± 0.925) > *Bauhinia thonningii* (6.451 ± 3.032) > *Combretum molle* (5.106 ± 2.334). Heavy metal concentrations as well as the BF and TF varied from species to species with most species having BF and TF < 1 for various heavy metals. Significant differences were observed in heavy metal accumulation by roots and shoots. Out of the 32 studied species, the results suggest that 25 species (*Albizia adianthifolia, Albizia antunesiana, Albizia versicolor, Azanza garckeana, Bauhinia petersiana, Bauhinia thonningii, Brysocarpus orientalis, Combretum apiculatum, Combretum molle, Combretum microphyllum, Dichrostachys cinerea, Diospyros mespiliformis, Dodonaea viscosa, Ficus capensis, Ficus craterostoma, Ficus sycomorus, Peltophorum africanum, Phyllanthus guineensis, Rhus longipes, Senegalia polyacantha, Senna singueana, Syzygium guineense, Terminalia mollis, Terminalia stenostachya* and *Vachelia sieberiana*) have potential for phytostabilizing Cu contaminated sites.

Key words: Phytostabilization, Excluder, Accumulator, Tailings dams, Heavy metals

3.1 Introduction

Mining is a major contributor to economic growth in many countries, especially the developing world (Lindahl 2014). However, it produces vast amounts of waste that degrade land. As such mining is considered "one of the greatest land cover change activities in" most mining regions as forests and other ecosystems are converted to mine wastelands (Cooke and Johnson 2002; Schueler et al. 2011).

Mine generated wastes contain leftover chemicals and metals in high concentrations that negatively impact the environment (Yang et al. 2017) and cause human health problems (Lin et al. 2005; Lam et al. 2017). Mine wastelands remain un-vegetated for an extended period of time (Cooke and Johnson 2002; Kambing'a and Syampungani 2012) due to unfavourable conditions associated with them for plant establishment and growth (Wong 2003). Adverse conditions such as low pH, low nutrient and water retention and elevated metal concentrations inhibit plant growth (Conesa et al. 2007). These conditions make mine wastelands such as tailings dams a source of environmental pollution as they are prone to all forms of erosion (Lam et al. 2017). Unrestored tailings dams are a source of heavy metal contamination that negatively impact the environment (Santos et al. 2017). Social, economic and health effects of mine wastelands have been observed in many mining regions of the world namely, USA (Dudka and Adriano 1997), South Africa (Fairbanks et al. 2000), China (Li 2006), Ghana (Schueler et al. 2011), Zambia (Lindahl 2014), Democratic Republic of Congo (Pourret et al. 2016) and Australia (Nirola et al. 2015).

To reduce environmental contamination by mine wastelands, several techniques such as topsoil application, fertilizer and lime application, biochar and nanoparticle application have been developed for the restoration of mine wastelands (Liu and Lal 2012; Abassi and Anawar 2015; Mensah 2015; Seenivasan et al. 2015; Festin et al. 2018). However, these techniques are environmentally unfriendly and expensive (Conesa et al. 2007). The application of topsoil, techno sols and compost (physical method) are costly as topsoil is moved from a different site to the wastelands (Bradshaw 2000). Additionally, the production of techno sols and compost are also costly. The chemical method is expensive and environmentally unfriendly because of the need for skilled workers, costly machines, and chemicals as well as the potential contamination of groundwater and soil when applied in excess (Wu et al. 2012). Vegetation establishment on tailings dams via phytoremediation provides a cost effective and environmentally friendly option for tailings dam restoration (Mendez and Maier 2008). Phytoremediation uses plants to stabilize and reduce contaminants over an area (Lam et al. 2017; Santos et al. 2017). It includes phytostabilization and phytoextraction. "Phytoextraction is the uptake and translocation of heavy metals to aboveground parts of the plants" (Baker 1981) while phytostabilization is the "use of plants and soil amendments to confine heavy metals through absorption and accumulation by roots, adsorption onto roots, or precipitation within the rhizosphere" (Mendez and Maier 2008; Bolan et al. 2011; Santos et al. 2017). Since the 1990's, the use of phytostabilization for mine wasteland restoration has gained momentum (Salt et al. 1995). It aims at stabilizing the erosion prone soils and reduce heavy metal bioavailability hence reducing contamination (Conesa et al. 2007; Santos et al. 2017).

Mine wasteland restoration is fundamental for attaining sustainable development as mining is a major economic activity in many countries yet with numerous impacts on the environment. Successful phytostabilization is dependent on the use of native species with heavy metal exclusion capacity with high biomass that adapt to metalliferous sites (Yang et al. 2016; Santos et al. 2017). Phytostabilization starts with the identification of plant species with phytostabilization potential (Yang et al. 2017). Studies on screening of various plant species for phytostabilization potential have been undertaken globally (Li et al. 2007; Galal and Shehata 2015; Santos et al. 2017). However, studies that investigate the phytostabilization potential of plants occurring on mine tailings dams in southern Africa are limited with the focus being on herbaceous and grass species (Mganga et al. 2011; Shutcha et al. 2015; Mwangi 2017).As such, less is known about the phytostabilization potential of indigenous tree species occurring on tailings dams. However, some studies (Kambing'a and Syampungani 2012) have documented that several indigenous tree species occur on mining generated wastelands on the Zambian Copperbelt. Such plant species are observed to successfully colonize the mining generated wastelands of the Copperbelt Province in Zambia. The abundance of both the mining generated wastelands and the associated plant species provides an opportunity to assess the phytostabilization potential of those metallophytes on wastelands. The primary aim of this study was to assess the phytostabilization potential of indigenous tree species occurring on Copperbelt tailings dam for the restoration of the copper (Cu) tailings dams in Zambia. The study addressed three research questions namely; (1) What are the species growing on the Copperbelt Tailings Storage Facilities (TSF)? (2) What is the ratio of root: soil heavy metal concentration in these plant species? (3) What is the ratio of root: shoot heavy metal concentrations in these plant species?

3.2 Materials and methods

3.2.1 Site description and sampling procedure

The study was conducted in selected mining towns of the Copperbelt Province in Zambia (Figure 3.1). Copperbelt lies between latitude 13° 00′ 00″ and longitude 28° 00′ 00″. The

Province is the main mining province in the Country with the mining history dating back to the 1920s (Sikamo et al. 2016). All the towns on the Copperbelt Province namely, Mufulira, Kitwe, Chingola, Ndola, Chililabombwe and Luanshya are mining towns as shown on the map below.

Copperbelt soils are extremely weathered and leached with low base exchange capacity (Křībek et al. 2014). According to FAO-UNESCO (1997), Copperbelt soils are categorised as ferralsol comprising of acric, orthic or rhodic ferralsols. These soils are highly acidic in nature with low humus and low soil organic carbon (Křībek et al. 2014) with high exchangeable manganese and aluminium. The Province is one of the wetter regions of the country as it falls under the third (III) agro-ecological zone, the high rainfall area with average annual rainfall and temperature ranging between 1000 to 1500 mm and 7.8°C to 23.7°C, respectively (Norway 2007). "The rainy season is from November to March except for rare showers in August, a cool dry season from April to August and a hot dry season is experienced from August to November" (Aregheore 2009; Sracek et al. 2010). The study focused on mine towns with abandoned or decommissioned tailings dams in Kitwe, Luanshya and Mufulira.

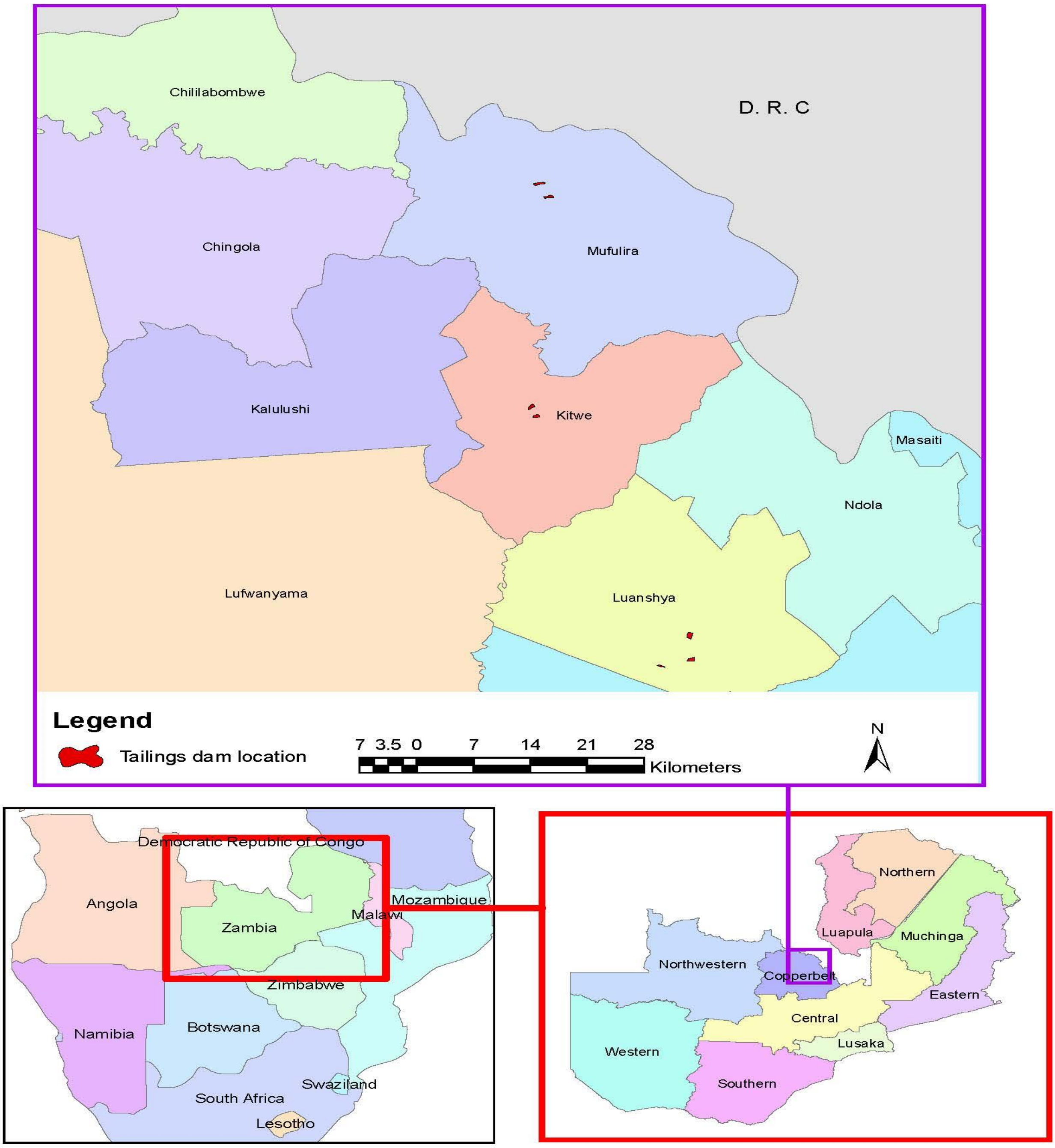

Figure 3.1: Location of study sites within Copperbelt Province in Zambia

3.2.2 Sampling procedure

Field surveys were carried out from December 2017 to May 2018 across seven decommissioned Tailings Storage Facilities (TSF) namely TD 25 & 26 (Kitwe), TD24, 25 & 26 (Luanshya) and TD 8 & 10 (Mufulira). The study adopted the transect method of sampling the vegetation on these TSFs due to the pattern of vegetation distribution and establishment on the TSFs. The transect method of sampling is a point-based sampling method used to determine plant cover, species occurrence and species abundance (Lancia et al. 2005). A transect or path is established along which a number of samplings points are established. The transect length and number of sampling points are dependent on the vegetation. Vegetation on tailings is in clusters running along certain sides of the TSF. Therefore, transects were established on areas with vegetation cover only as the vegetation was the focus of the study. Biasness was reduced or eliminated by randomly selecting the starting point of the first line transect and sampling points, thereafter, transects were established at every 200 m as recommended by Kambinga and Syampungani (2012) while sampling points were established at every 100m (see Mganga et al. 2011). The method is less costly, practical and efficient for studying most biological populations, plants inclusive (Varman and Sukumar 1995) making its application in this study feasible. A 20 m diameter circular plot was established at each sampling point using a distance tape for data observation. On each sampling point, the GPS coordinates were taken from the centre of the plot for location description. All tree species in a plot were identified and their respective diameter at breast height (DBH) measured and recorded.

3.2.3 Plant sampling

Plant samples (root and shoot) were collected from selected individuals of all individual species in a plot. Tree selection was based on DBH size and therefore individual species with large DBH were selected for inclusion in plant sampling. A kg of root and shoot samples each were collected from the selected individuals of species in each plot. After collection, root and shoot samples were packed in a plastic bag, clearly labelled with species name, site name, transect and plot number and were taken to the laboratory for analysis. Root and shoot samples were thoroughly washed with tap water and rinsed with deionised water to decontaminate the samples (Moraghan 1991; Richards 1993). The root and shoot samples were then dried in a microwave oven at 60˚ for 24 hours and 30 hours, respectively (Liao et al. 2016; Vymazal 2016).

3.2.4 Soil sampling

Soil sample collection was done at 0-30cm depth from three distinct locations within the sample plot using a stainless-steel auger. This is because soil biological factors which influence plant growth and active root zone are within the 0-30cm (Crepin and Johnson 1993). Dongmei and Changqun (2008) also suggest that the 0-30cm represents the most nutrient active zone of the soil. Previous studies elsewhere (Ssenku et al. 2014; Mganga et al. 2011; Chileshe et al. 2019) on tailing storage facilities have also sampled in the 0-30 cm zone. Collection of soil samples from the three distinct locations in the plot was done after Mganga et al. (2011) and Chileshe et al. (2019). Soil was collected from three randomly selected locations within a plot near the trees. The soil was then mixed to form a composite soil sample from which a kilogram was measured, labelled and packed in a plastic bag, and taken to the laboratory for analysis. The soil label had the site, transect number and plot number on it.

3.2.5 Plant and soil analysis

Total heavy metal concentrations in soil, shoots and roots of copper (Cu), zinc (Zn), manganese (Mn), aluminium (Al), barium (Ba), boron (B), nickel (Ni), cobalt (Co), sulphur (S), molybdenum (Mo) chromium (Cr) and cadmium (Cd) were determined using the Inductively coupled plasma atomic emission spectrochemistry ICP-OES after sample digestion using the standard EPA 3052 digestion method (Yang et al. 2017). In this method, 3g of soil, root and shoot samples were measured and placed in 50 ml inert microwave vessels and digested in 9 ml of Nitric Acid Suprapur for 10-20 minutes using a Multiwave 3000 manufactured by Perkin Elmer. The samples were left to cool for 20-25 minutes, then filtered, and each extract was diluted with deionised water to a 30 ml mark. Total heavy metal concentrations were then determined by Flame Atomic Absorption Spectrometry (FAAS). Precision and accuracy were achieved by measuring three blanks and results obtained in mg/l were converted to mg kg^{-1}

3.2.6 Ecological analysis

Tree species that occurred in the sampling plots were identified and their DBH measured. From the data, the relative density, relative frequency and relative dominance were used to calculate the species importance value index (IVI) and family IVI (Naidu & Kumar 2016). Abundance was used to determine species richness on the tailings (Santos et al. 2017) while importance values were used to determine the dominant species on the TSFs.

"Importance value index was calculated as a sum of relative frequency, relative density and relative dominance divided by three" (Naidu & Kumar 2016, Liu et al. 2017).

IVI = RF+ RD+ RD/3

Where (2) $RF = \frac{Frequency\ of\ a\ specie}{Total\ frequencies\ of\ all\ species}$

(3) $RD = \frac{Number\ of\ individuals\ of\ one\ species}{Total\ number\ of\ all\ individuals\ counted}$

(4) $RD = \frac{Basal\ area\ per\ species}{Total\ basal\ area}$

3.2.7 Phytostabilization potential assessment

To determine heavy metal mobility on the tailings and the phytostabilization potential of plant species occurring on Copperbelt tailings dams, the bioconcentration (BF) and translocation factors (TF) of Mn, Ni, Cr, Co, Cu, Cd, B, Ba, Mo, Al, Zn and S were determined. "Bioconcentration factor for each species was calculated by dividing the heavy metal concentration of the species in roots by heavy metal concentration in soil (tailings) while translocation factor was determined by dividing the heavy metal concentration in leaves by heavy metal concentration in roots" (Yang et al. 2014).

$$BF = \frac{\text{Heavy metal concentraion in roots}}{\text{Heavy metal concentration in soil (tailings)}}$$

$$TF = \frac{\text{Heavy metal concentration in leaves}}{\text{Heavy metal concentration in roots}}$$

BF < 1 indicates the inability of a plant to accumulate elements or metals from the soil to the roots as such it possesses excluding abilities while BF > 1 indicates heavy metal accumulation from the soil to the root (Rezvani & Zaefarian 2011). TF > 1 means that a plant accumulates heavy metals in aboveground parts while TF < 1 means a plant inhibits translocation of heavy metals to aboveground parts (Baker 1981; Rezvani & Zaefarian 2011; Lam et al. 2017). Plants with excluding abilities which are ideal for phytostabilization have TF and BF < 1 while accumulators have TF and BF > 1. Within the accumulator group exists another group called hyperaccumulators (Ojuederie et al. 2017). According to Baker and Brooks (1989), a hyperaccumulator is a plant that accumulates Cu, Co, Ni, and Cr concentrations > 1000 mg/kg, > 100 mg/kg for Cd and Zn and Mn concentrations > 10000 mg/kg (Liu et al. 2008).

3.2.8 Statistical analysis

Statistical analyses were done using SPSS version 25.0 and Microsoft excel. Significant differences of heavy metal concentrations in roots and shoots were determined using a two-way ANOVA at 0.05 level of significance. A paired T-test was then used to determine the differences between heavy metals in roots and shoots. Site specific heavy metal concentration and data with replicates were presented as mean ± standard error.

3.3 Results and discussion

3.3.1 Species composition

The study recorded 13 families, 22 genera and 32 native tree species (Table 3.1). From the 13 families, fabaceae and combretaceae dominated the tailings dams with 11 and 6 species, respectively. Anacardiaceae and moraceae had 3 species each while annonaceae, connaraceae, chrysobalanaceae, ebenaceae, phyllanthaceae, moraceae, myrtaceae, sapindaceae and sterculiaceae recorded 1 species each. Based on the family IVI (Table 3.1), mrytaceae had the highest IVI indicating dominance of the Copperbelt tailings dams followed by anacardiaceae, moraceae, fabaceae and combretaceae. At species level, *Rhus longipes* and *Syzigium guineense* had the highest IVI's with 15.99 % and 13.2%, respectively representing the most dominant species (see appendix 1). The IVI for other species ranged from 0.2 % to 8.43 % (Appendix 1). In terms of diversity, fabaceae had the highest number of species (11) followed by combretaceae (6), anacardiaceae (3) and moraceae (3) while the other 9 families recorded 1 species each. Based on density, fabaceae contributed 32.5% of stand density, followed by anacardiaceae (24%) and myrtaceae (17%). Combretaceae contributed 13% and Moraceae contributed 8.5% while the other families had densities between 0.2-1.1%.

Table 3.1: Dominant families on Copperbelt tailings dams based on IVI, number of species, genera, and relative density

Family	Number of genera	Number of species	Relative Density (%)	Relative IVI
Fabaceae	7	11	32.5	3.81
Combretaceae	2	6	13	2.15
Anacardiaceae	3	3	24	6.13
Moraceae	1	3	8.5	4.43
Annonaceae	1	1	0.9	0.71
Connaraceae	1	1	0.2	1.35
Chrysobalanaceae	1	1	0.2	0.31
Ebenaceae	1	1	0.2	0.79
Phyllanthaceae	1	1	0.9	0.87
Malvaceae	1	1	1.1	0.85
Myrtaceae	1	1	17	13.32
Sapindaceae	1	1	1.1	1.2
Sterculiaceae	1	1	0.4	0.40
Totals	22	32	100	36.32

The study has shown that native tree species are capable of colonizing mining generated wastelands despite the wastelands being contaminated (Appendix 1). The study's findings are in line with other studies elsewhere (Masvodza et al. 2013; Li et al. 2007; Hu et al. 2014; Sun et al. 2016) in which native tree species were reported to grow on contaminated sites. The tree species recorded on mining generated wastelands also occur in natural forests and local environments (Jew et al. 2016). Species that grow on contaminated sites with little or no signs of toxicity are considered for possible application in the remediation of contaminated sites for accelerated ecological restoration (Luo et al. 2015; Sun et al. 2016). Species richness and abundance on Copperbelt tailings is high (32 species) which indicates the adaptability of these species to Tailings dam environment (Conesa et al. 2007; Pérez-Esteban et al. 2013). Mulizane et al. (2005) reported similar results on gold mine tailings on the survival rate and growth performance of indigenous species. Within Zambia, Kambingá and Syampungani (2012) observed the good growth performance of most native species on a tailings dam in Kitwe. However, when compared to the species richness in the neighbouring local forests (55 species) (Festin et al. 2018), the richness on the Copperbelt tailings dams is low. Species richness on mine tailings has been reported in various regions, in China, 19 species (Liu et al. 2008), 51 species (Yang et al. 2014), Spain 31 species (Conesa et al. 2007), Mexico 42 species (Santos et al. 2017) and Tanzania 14 species (Mganga et al. 2011). Additionally, the high richness from previous studies is due to the inclusion of forbs and grasses which were not considered in the current study as it dealt with native tree species only which could be attributed to the low number of species when compared to other studies.

Of the 13 families occurring on the Copperbelt tailings dams, most species were from the fabaceae family (11 species from 7 genera). Previous studies have reported species from the fabaceae family to have colonized mine tailings elsewhere (Li et al. 2007; Ssenku et al. 2014; Yang et al. 2014). Evidently, fabaceae family species adapt to metalliferous sites, which makes their application in remediation viable. Hu et al. (2014) reported species from the moraceae, malvaceae and anacardiaceae family to have colonized the tailings, this study supports Hu's findings as species from moraceae, malvaceae and anacardiaceae among other families colonized the Cu tailings dams. Malvaceae species were also reported to have colonized abandoned Zn and Pb contaminated sites in China. Based on the family IVI (see table 3.1) fabaceae (3.81) was the fourth important family after mrytaceae (13.6) anacardiaceae (6.13) and Moraceae indicating their dominance of the area. These findings reflect those of Hu et al. (2014) who also found that the species *(Rhus chinensis*) from anacardiaceae occurred in 8 sampling plots out of the total 9 indicating its dominance of the area while species from the moraceae family occurred in 4 and 6 sampling sites. This suggests that species from these families adapt to contaminated or metalliferous sites therefore understanding their metal accumulation strategies would aid in determining suitable species for phytostabilization (Liu et al. 2017).

Individual species IVI however, had *Rhus longipes* (15.99) from anacardiaceae family with the highest IVI followed by Syzygium *guineense* (13.2*)* from the myrtaceae family. The high IVI's attest to the species dominance over an area, high density and good growth performance. Species suitable for phytostabilization should possess abilities such as good growth performance and production of large biomass besides immobilization of heavy metals (Sun et al. 2016). The presence of *Syzygium guineense* and *Rhus longipes* in all the study sites indicates their dominance, good growth performance on the tailings dams and their potential to grow and reproduce thereby satisfying the two requirements for phytostabilization species. Unlike the other species that showed good growth performance in areas where soil amendments had been applied, *Syzygium guineense* and *Rhus longipes* showed good growth performance even in areas where soil amendments were not applied. Species such as *Senegalia polyacantha, Ficus craterostoma, Bauhinia thonningii, Albizia adianthifolia, Combretum molle* also had high IVI's indicating their dominance and good growth performance. These species also showed exclusion properties for Cu, hence satisfying all the requirements of Cu excluders. They possess potential to phytostabilize Cu contaminated areas hence their possible application in phytostabilization of Copperbelt TSFs. Even though the other species IVI's were lower, some occurred in a few sampling sites indicating their adaptability and can therefore be considered in the stabilization of contaminated sites. Application of tree species in phytostabilization is ideal because the root system of trees has the ability to easily stabilize mine tailings (Sun et al. 2016) and produce large biomass that enhances soil properties for vegetation establishment and growth hence, their application in phytostabilization is viable.

3.3.2 Heavy metal concentration in tailing soils and Roots

Table 3.2 shows mean heavy metal concentrations of Copperbelt tailing soils. Site variations in heavy metal concentrations were observed between the sites. The One-Way Anova (Table 3.3) at P = 0.05 showed significant differences in heavy metal concentrations between the site

Table 3.2: Mean heavy metal concentrations of Copperbelt tailing soils in mg/kg

Sampling site	Cu	Mn	Zn	Ni	Cr	Co	Cd	B	Ba	Mo	Al	S
TD25 (Kitwe)	12638,44	9302,364	161,664	107,629	666,221	1868,593	-12,9286	503,95	1074,636	61,043	141409,6	3435,8
TD26 (Kitwe)	25789,84	17648,75	193,027	188,495	450,596	3242,508	-9,40714	406,096	2306,725	65,785	119130,8	4869,449
TD24 (Luanshya)	23140,79	8998,667	317,892	368,348	735,971	466,947	-13,6571	584,991	421,733	67,883	232487,5	2086,577
TD25 (Luanshya)	12523,25	10384,27	374,979	322,989	682,118	555,767	-12,5705	537,958	561,46	68,536	236100,8	2929,824
TD26 (Luanshya)	10668,05	9833,267	245,812	278,355	658,878	1262,733	-11,9305	500,631	1111,347	78,57	253414,2	6962,79
TD8 (Mufulira)	22494,25	11680,47	131,826	50,909	606,011	52,827	-3,89048	142,945	622,2	23,65	22797,5	1319,554
TD10 (Mufulira)	14724,52	11736,47	164,872	62,969	596,578	65,813	-4,43048	175,971	473,5	25,436	24240,83	2096,924

Table 3.3: One-Way ANOVA for mean heavy metal concentrations in tailing soils

		Sum of Squares	Df	Mean Square	F Value	P value
Concentration	Between Groups	1.351E + 11	11	1.228E +10	15.194	0.000
	Within Groups	5.891E + 10	72	808195232.8		
	Total	1.933E + 11	83			

Metal concentrations of the various species in roots are presented in table 3.4. The concentration varied from species to species per heavy metal. *Lannea discolor* recorded the highest concentration of Cu (220.38 mg/kg) while other species had concentrations ranging between 3.17 – 220.38 mg/kg. *Senegalia polyacantha* recorded the highest concentration of Mn (54.52 mg/kg) while other species ranged between 4.37 – 42.12 mg/kg). Similarly, Zn concentrations had *Albizia adianthifolia* having the highest concentration (24.29 mg/kg) and other species recorded the concentration within 1.02- 5.89 mg/kg range. Similar trend in metal concentration was observed across species, although most of the species are generally of lower concentrations as compared to Cu, Mn and Zn (table 3.4). Average concentrations of other elements were between 0.20 – 2.75 mg/kg for Ni, Cr (0.10 – 3.79 mg/kg), Co (0.01 – 4.35 mg/kg), Cd (-0.00 – 0.08 mg/kg), B (2.05 – 10.11 mg/kg), Ba (0.73 – 1560 mg/kg), Mo (0.43 – 9.79 mg/kg), Al (9.56 – 449.45 mg/kg) and S (156.67 – 2502.78 mg/kg), respectively. S recorded the highest concentrations in a number of species namely, *Azanza garckeana* and *Albizia antunesiana* (2502.78 mg/kg each), *Albizia adianthifolia* (2493.87 mg/kg) and *Bauhinia thonningii* (1240.81 mg/kg).

A Two-Way ANOVA (See table 3.5) at 0.05 level of significance revealed that there was a significant difference between the means of heavy metal concentrations in roots between species.

Table 3.4: Heavy metal concentrations in roots of species growing on Copperbelt tailings dams presented as mean ± SE

Species	Cu	Mn	Zn	Ni	Cr	Co	Cd	B	Ba	Mo	Al	S
Albizia adianthifolia	64.14 ± 64.14	12.07 ± 12.07	24.29 ± 24.29	2.75 ±2.75	2.25 ± 2.25	0.65 ± 0.65	(-0.01 ± 001)	5.41 ± 5.41	1.31 ± 1.31	9.79 ± 9.79	201.24 ± 201.24	2493.87 ± 2493.87
Albizia amara	66,37 ± 6.38	27,12 ± 6,78	5,45 ± 0,75	0,96 ± 0,57	2,17 ± 1,02	0.86 ± 0.54	0.06 ± 001	7.32 ± 2.79	33.59 ± 23.70	1.90 ± 0.50	243.70 ± 188.00	1193.45 ± 628.85
Albizia antunesiana	66.37 ± 6.38	22.49 ± 1.36	10.17 ± 3.73	0.58 ± 0.76	2.82 ± 0.51	2.39 ± 2.14	(0.01 ± 0.01)	7.34 ± 0.69	66.42 ± 17.41	3.37 ± 0.12	449.45 ± 341.78	2502.78 ± 1561.96
Albizia versicolor	102.99 ± 73.44	21.24 ± 11.89	5.89 ± 2.43	1.10 ± 0.81	2.74 ± 1.12	4.35 ± 3.37	0.05 ± 0.01	5.69 ± 1.13	13.67 ± 7.42	2.24 ± 1.10	259.14 ± 107.32	602.83 ± 61.46
Annona senegalensis	18.89 ± 7.93	16.94 ± 12.26	3.17 ± 1.57	0.79 ± 0.14	1.32 ± 0.39	0.24 ± 0.14	0.08 ± 0.06	6.13 ± 1.94	7.02 ± 4.81	5.23 ± 4.74	236.32 ± 114.91	501.03 ± 132.42
Azanza garckeana	49,80 ±49,80	6,67± 6,67	3,39 ± 3,39	0,20 ± 0,20	0,82 ± 0,82	0,06 ± 0,06	0,00 ±0,00	7,34 ± 0,69	66,42 ±17,41	3,37 ± 0,12	441,45 ± 341,78	2502,78 ±1561,96
Bauhinia petersiana	45,59 ±14,09	10,42 ± 3,03	5,19 ± 2,43	0,42 ± 0,60	1,16 ± 0,14	0,74 ±0,55	0,05 ± 0,03	5,38 ± 0,34	20,15 ±7,71	3,91 ± 1,27	93,68 ± 26,75	739,98 ± 2831,26
Bauhinia thonningii	45,71 ± 17,79	20,01 ± 3,34	8,04 ± 1,11	0,64 ±0,14	1,69 ± 0,10	1,96 ± 1,55	0,02 ±0,02	5,76 ± 0,66	13,86 ±4,16	3,08 ± 1,32	239,08 ± 108,47	1240,81 ± 236,46
Brysocarpus orientalis	39,78 ± 39,78	8,44 ± 8,44	1,39 ± 1,39	0,91 ± 0,91	0,68 ± 0,68	0,27 ± 0,27	0,01 ±0,01	2,05 ± 2,05	2,28 ± 2,28	1,20 ± 1,20	26,29 ± 76,29	208,52 ± 208,52
Combretum apiculatum	18.98 ± 18.98	9.27 ± 9.27	5.56 ± 5.56	0.63 ± 0.63	0.76 ± 0.76	0.38 ± 0.38	0.00 ± 0.00	3.69 ± 3.69	0.73 ± 0.73	1.56 ± 1.56	77.89 ± 77.89	640.19 ± 160.95
Combretum microphyllum	136,81 ± 63,48	30,21 ± 7,84	4,93 ± 1,39	0,88 ± 0,75	1,55 ± 0,39	0,22 ± 0,07	0,01 ± 0,00	6,87 ± 0,55	15,36 ± 1,30	1,67 ± 1,12	60,75 ± 32,96	710,50 ± 38,35
Combretum molle	83,12 ±43,21	27,25 ± 8,55	5,06 ± 1,18	2,19 ± 0,52	2,59 ± 0,70	0,67 ± 0,27	0,00 ± 0,01	7,84 ± 2,49	19,26 ± 7,77	2,80 ± 1,59	215,66 ± 106,45	1177,04 ± 555,10
Combretum zeyheri	48,10 ± 15,04	28,06 ± 1,03	4,14 ± 1,65	1,22 ± 0,01	0,77 ± 0,04	0,24 ±0,15	0,02 ± 0,01	4,60 ± 0,29	11,57 ± 0,85	1,59 ± 0,40	56, 93 ±23,24	682,88 ± 252,87
Dichrostachys cinerea	86,54 ± 41,60	29,96 ± 10,10	5,38 ± 1,72	2,40 ± 1,11	3,67 ± 1,53	0,87 ± 0,59	0,01 ± 0,02	6,30 ± 1,12	28,78 ± 24,33	1,76 ± 0,46	279,90 ± 167,02	382,51 ±42,51
Diospyros mespiliformis	20,34 ± 20,34	5,22 ± 5,22	1,48 ± 1,48	0,55 ± 0,55	0,54 ± 0,54	2,20 ± 2,20	0,00 ± 0,00	10,07 ± 1,07	1560± 1560	0,43 ± 0,43	40,74 ± 40,74	156,67 ± 156,67
Dodonaea viscosa	73,07 ± 60,86	15,25 ± 5,44	5,79 ± 0,80	0,89 ± 0,78	1,78 ± 0,64	2,85 ± 2,26	0,02 ± 0,02	5,93 ± 1,51	7,22 ± 0,94	6,28 ± 5,14	325,50 ± 202,88	576,88 ± 196,10
Dombeya rotundifolia	18,05 ± 18,05	9,13 ± 9,13	1,02 ± 1,02	0,88 ± 0,88	0,46 ± 0,46	0,01 ± 0,01	0,03 ± 0,03	2,84 ± 2,84	5,86 ± 5,86	0,68 ± 0,68	47,94 ± 47,94	198,67 ± 198,67
Ficus capensis	24,08 ± 24,08	8,33 ± 8,33	2,73 ± 2,733	0,35 ± 0,35	1,08 ± 1,08	0,15 ± 0,15	(-0,00 ± 0,01)	7,29 ± 7,29	52,15 ± 52,15	1,04 ± 1,04	46,59 ± 46,59	984,87 ± 984,87
Ficus craterostoma	48,91 ± 27,48	18,21 ± 17,14	4,19 ± 2,36	1,25 ± 1,02	1,78 ± 1,32	0,39 ± 0,30	0,04 ± 0,03	5,66 ± 2,30	20,74 ± 8,66	1,97 ± 1,98	98,48 ± 103,64	535,89 ± 257,81
Ficus sycomorus	79,45 ± 17,04	30,26 ± 6,24	5,77 ± 1,12	1,17 ± 0,30	2,53± 0,65	2,26 ± 1,96	0,04 ± 0,00	7,58 ± 1,98	62,90± 24,79	3,41 ± 1,70	72,55 ± 12,01	858,28 ± 157,34
Lannea discolor	220,38 ±167,35	32,23 ± 12,10	5,38 ± 2,01	1,26 ± 0,64	2,50 ± 0,90	1,91 ± 0,30	0,04 ± 0,01	10,11 ± 1,27	22,93 ± 6,67	6,64 ± 4,37	418,18 ± 145,45	1083,77 ± 675,67

Ozoroa insignis	6.36 ± 6.36	4.37 ± 4.37	1.69 ± 1.69	1.00 ± 1.00	0.82 ± 0.82	0.29 ± 0.29	(-0.00 ± 0.00)	7.29 ± 7.29	52.15 ± 52.15	1.04 ± 1.04	46.59 ± 46.59	984.87 ± 984.87
Parinari curatellifolia	3.17 ± 3.17	42.12 ± 42.12	1.30 ± 1.30	0.42 ± 0.42	1.33 ± 1.33	0.52 ± 0.52	0.07 ± 0.07	1.90 ± 1.90	4.19 ± 4.19	0.56 ± 0.56	44.59 ± 44.59	414.52 ± 414.52
Peltophorum africanum	141.51 ± 45.32	28.19 ± 6.47	6.15 ± 1.98	1.45 ± 0.60	3.48 ± 0.74	2.98 ± 0.96	0.02 ± 0.02	1.51 ± 1.51	2.36 ± 2.36	0.50 ± 0.50	132.69 ± 132.69	114.87 ± 115.87
Phyllanthus guineensis	103.36 ± 22.53	35.70 ± 19.77	4.57 ± 0.66	1.42 ± 0.04	0.10 ± 0.20	0.39 ± 0.27	0.03 ± 0.03	7.48 ± 1.01	29.95 ± 28.16	0.73 ± 0.06	73.62 ± 47.55	354.82 ± 133.68
Rhus longipes	95.56 ± 19.96	21.92 ± 5.02	4.63 ± 0.75	1.15 ± 0.26	1.94 ± 0.32	1.63 ± 0.80	0.04 ± 0.01	7.75 ± 1.46	26.82 ± 92	2.55 ± 0.56	108.15 ± 31.56	596.20 ± 78.51
Senegalia polyacantha	44.13 ± 12.08	54.52 ± 35.44	3.94 ± 0.62	0.40 ± 0.45	0.79 ± 0.10	0.68 ± 0.41	0.02 ± 0.01	9.63 ± 2.65	16.35 ± 3.34	3.98 ± 2.59	9.56 ± 46.31	916.77 ± 172.58
Senna singueana	48.52 ± 15.29	20.27 ± 7.42	5.56 ± 1.48	2.20 ± 0.85	1.62 ± 0.62	2.87 ± 2.22	0.02 ± 0.02	9.19 ± 3.18	17.10 ± 10.62	1.49 ± 0.66	152.14 ± 67.77	681.73 ± 189.18
Syzygium guineense	61.24 ± 11.33	27.10 ± 4.66	5.63 ± 0.84	2.2 ± 0.67	3.79 ± 1.20	2.05 ± 0.75	0.03 ± 0.02	8.62 ± 1.41	20.22 ± 4.19	3.58 ± 1.02	209.04 ± 54.46	871.53 ± 193.27
Terminalia mollis	93.62 ± 35.91	34.56 ± 6.61	4.27 ± 0.83	0.83 ± 0.25	1.79 ± 0.29	3.79 ± 3.33	0.07 ± 0.02	7.81 ± 1.63	15.73 ± 3.74	2.58 ± 0.41	218.41 ± 46.96	516.40 ± 146.55
Terminalia stenostachya	34.64 ± 12.67	20.47 ± 7.57	5.02 ± 0.94	1.38 ± 0.66	2.08 ± 0.23	1.81 ± 0.97	0.05 ± 0.03	8.58 ± 1.73	14.16 ± 6.49	2.53 ± 1.08	374.86 ± 262.76	640.19 ± 160.95
Vachellia sieberiana	8.55 ± 8.55	6.92 ± 6.92	1.60 ± 1.60	0.16 ± 0.16	0.77 ± 0.77	0.89 ± 0.89	0.03 ± 0.03	5.02 ± 5.02	5.36 ± 5.36	5.79 ± 5.79	74.94 ± 74.94	436.42 ± 436.42

Table 3.5: Two-way ANOVA for mean heavy metal concentrations in roots

Treatment		Sum of Squares	Df	Mean Square	F Value	P value
Elements	Between Groups	4454.917	323	13.792	6.834	0.000
	Within Groups	121.083	60	2.018		
	Total	4576.000	383			
Species	Between Groups	29069.383	323	89.998	1.473	.035
	Within Groups	3666.617	60	61.110		
	Total	32736.000	383			

Heavy metal concentrations in roots were generally lower than the concentration in their related soils except for average Cd which had a higher mean concentration in roots than in soils for some species (Table 3.4). These results are similar to Masvodza et al. (2013), Yang et al. (2014) and Hu et al. (2014)'s findings that reported, low metal concentrations in roots than that of their associated soils. Species with low concentrations of metals in roots than soil show the exclusion strategy of heavy metal accumulation while the ones with high concentration in roots show the accumulation strategy (Yang et al. 2014). Lam et l. (2017) indicated that plants with low root accumulation of heavy metals could be considered excluders. One interesting finding of this study is that all the tree species accumulated low amounts of heavy metals in the roots for all the elements except *Diospyros mespiliformis* for Ba and *Albizia adianthifolia, Combretum microphyllum*, *Bauhinia thonningii* and *Lannea discolor* for S indicating their accumulation ability of these elements. Species with high concentrations in roots may have an accumulation strategy (Yang et al. 2014). However, according to Baker (1981), a plant's leaf concentration determines its accumulation strategy (Nirola et al. 2015). Similarly, Schachtschneider et al. (2017) holds Fitamo and Leta (2010)'s view that suitable phytostabilizers are "plants with high metal concentrations in the root system". Other researchers suggest that high concentrations of metals in roots than soils indicate a species ability to accumulate metals, hence they could be categorised as bioaccumulators (Wei et al. 2008; Hu et al. 2014; Yang et al. 2014; Sun et al. 2016; Santos et al. 2017). Cu concentration on Copperbelt tailings (17425.59 mg/kg) was higher than what previous studies on Cu tailings reported in other mining region. For example, Ssenku et al. (2014) reported Cu concentration range of 28.16- 90.75mg/kg in 0-30cm depth and Zheng et al. (2006) reported a range of 2432-

7554 mg/kg. This may be attributed to differences in richness in copper between regions. With such high concentration of Cu, it is evident that tree species growing on Copperbelt tailings dams are highly tolerant to Cu and other heavy metals making their application in vegetation restoration programs on Cu tailings viable (Nirola et al. 2015). However, their application in phytostabilization is dependent on their ability to exclude heavy metals (Zou et al. 2012).

Native plant's uptake of metals is mostly specific to metals and species. Other studies (Remon et al. 2013; Liu et al. 2017) have reported similar findings. The variations in metal accumulation exhibited by tree species entails the need for careful selection of plants in the remediation of contaminated sites as this indicated the variation in exclusion and accumulation strategies of plants (Yang et al. 2014).

3.3.3 Heavy metal foliar concentration

Average metal concentrations in leaves varied among species and between heavy metals (Table 3.6). *Dombeya rotundifolia* had the highest average Cu concentration in leaves with 104.09 mg/kg while the other species had average Cu concentrations ranging from 5.19 – 75.99 mg/kg with *Vachellia sieberiana* having the lowest concentration of 5.19 mg/kg. *Combretum zeyheri* contained the highest concentration of Mn (312.20 mg/kg) while other species had Mn concentration ranging from 0.7 – 212.15 mg/kg. Average Zn concentrations had *Peltophorum africanum* with the highest concentration (24.40 mg/kg) and other species ranged between 1.78 – 24.00mg/kg with *Ficus capensis* having the lowest concentration of 1.78 mg/kg. A similar trend as that of the root concentrations of heavy metals was observed with variations in concentrations between species and among metals. The other metals had concentrations lower than Cu, Zn and Mn except S, Ba and Al that had average concentrations ranging between 389.34 – 18535.37 mg/kg, 0.00- 235.25 mg/kg and 27.25 – 249.46 mg/kg, respectively. The other metals had concentrations ranging between 3.23 – 50.60 mg/kg for B, Mo (0.00 – 12.66 mg/kg), Ni (0.05 – 2.15 mg/kg), Cr (0.40 – 2.69 mg/kg), Co (0.12 – 6.01 mg/kg) and Cd (-0.02 – 0.07 mg/kg). The negative values obtained for Cd indicated low undetectable concentration of the metal.

A Two-Way ANOVA at 0.05 level of significance (Table 3.7) revealed significant differences in heavy metal concentrations in roots between species.

Table 3.6: Heavy metal concentrations in shoots (leaf foliar) of species growing on Copperbelt tailings dams presented as mean ± SE

Species	Cu	Mn	Zn	Ni	Cr	Co	Cd	B	Ba	Mo	Al	S
Albizia adianthifolia	10.33 ±10.33	212.15 ± 212.15	8.52 ± 8.52	0.47 ± 0.47	1.02 ± 1.02	0.36 ±0.36	(-0.12 ± 0.12)	4.70 ± 4.70	6.15 ± 6.15	0.26 ± 0.26	97.78 ± 97.78	895.89 ± 895.89
Albizia amara	75,99 ± 51,85	53,39 ± 20,10	10,81 ± 0,09	1,08 ± 0,57	1,62 ± 0,26	1,57 ± 0,64	0,01 ± 0,01	14,35 ± 4,70	10,88 ± 10,07	3,11 ± 2,32	167,66 ± 56,26	972,54 ± 51,10
Albizia antunesiana	16.00 ± 4.86	28.68 ± 0.52	5.21 ± 1.15	0.62 ± 0.41	0.79 ± 0.08	0.35 ± 0.21	0.00 ± 0.01	11.18 ± 3.54	44.28 ± 44.05	4.35 ± 3.09	64.31 ± 26.60	18535.37 ± 253.6
Albizia versicolor	27.18 ± 6.88	67.88 ± 14.97	5.22 ± 0.31	1.41 ± 0.37	2.69 ± 0.98	3.19 ± 1.52	(-0.02 ± 0.01)	11.59 ± 3.83	3.92 ± 2.18	12.66 ± 4.85	249.46 ± 104.00	1278.11 ± 36.94
Annona senegalensis	47.68 ± 32.68	31.09 ± 19.90	15.91 ± 5.36	2.15 ± 0.72	2.23 ± 1.44	0.61 ± 0.35	0.01 ± 0.02	14.68 ± 5.33	28.13 ± 19.84	1.28 ± 0.86	158.33 ± 150.51	2691.44 ± 1593.12
Azanza garckeana	15,6 ± 15,60	30,12±30,12	9,18 ± 9,18	1,06 ±1,06	0,63 ± 0,63	0,13 ± 0,13	0,03 ± 0,03	11,18 ± 3,54	44,28 ± 44,05	4,35 ± 3,09	64,31 ± 26,60	18535,37 ±253,6
Bauhinia petersiana	29,34 ± 12,37	59,95 ± 26,43	11,46 ±2,74	1,10 ± 0,28	0,62 ± 0,13	0,62 ±0,22	0,01 ± 0,01	18,93 ± 9,31	32,34 ±27,88	0,17 ± 0,02	33,04 ± 3,78	1314,164 ±322,63
Bauhinia thonningii	13,13 ± 2,64	49,89 ± 12,74	10,04 ± 1,96	1,35 ± 0,38	1,07 ± 0,08	0,60 ±0,29	0,02 ± 0,01	25,74 ±4,49	26,25 ± 6,47	0,91 ± 0,35	101,26 ± 8,92	1471,91 ± 156,92
Brysocarpus orientalis	6,59 ±28,28	76,25 ± 76,25	5,78 ± 5,78	0,76 ±0,76	1,07 ± 1,07	0,20 ± 0,20	0,00 ± 0,00	3,23 ± 3,23	16,60 ± 16,60	1,44 ± 1,44	94,88 ± 94,88	719,39 ± 719,39
Combretum apiculatum	7.56 ± 7.56	44.38 ± 44.38	5.29 ± 5.29	0.71 ± 0.71	0.61 ± 0.61	0.12 ± 0.12	0.01 ± 0.01	5.93 ± 5.93	3.1 ± 3.1	1.66 ± 1.66	49.68 ± 49.68	911.39 ± 911.39
Combretum microphyllum	49,28 ± 19,04	140,03 ± 22,17	11,85 ± 2,61	1,75 ± 0,77	1,78 ± 0,45	0,85 ± 0,32	0,04 ± 0,02	15,64 ± 3,32	69,78 ± 47,78	0,54 ± ,31	85,11 ± 32,60	2240,27 ± 412,31
Combretum molle	43,34 ± 28,28	91,64 ± 25,49	24,00 ± 7,13	1,64 ±0,36	1,76 ± 0,65	6,01 ± 5,11	0,05 ± 0,02	20,20 ± 5,15	26,06 ± 9,78	0,61 ± 0,20	111,59 ± 41,05	1100,10 ± 171,08
Combretum zeyheri	70,26 ± 66,21	321,20 ± 226,85	12,03 ± 3,48	1,16 ± 1,26	1,30 ± 0,72	1,80 ± 1,119	0,01 ± 0,02	14,25 ± 8,30	5,41 ± 3,10	2,03 ± 1,72	52,42 ± 0,79	1695,44 ± 151,67
Dichrostachys cinerea	19,00 ± 10,65	41,85 ± 6,15	8,88 ±1,73	1,20 ± 0,59	1,03 ± 0,34	0,26 ± 0,15	0,01 ± 0,01	17,83 ± 9,40	35,05 ± 19,46	1,79 ± 1,79	75,48 ± 24,97	1043,81 ± 314,41
Diospyros mespiliformis	6,66 ± 6,66	4,26 ± 4,26	3,05 ± 3,05	0,05 ± 0,05	0,40 ± 0,40	0,63 ± 0,63	0,00 ± 0,00	5,57 ± 5,57	235,25 ± 235,28	0,23 ± 0,23	27,25 ± 27,25	568,89 ± 568,89
Dodonaea viscosa	10,19 ± 1,93	41,24 ± 11,43	20,26 ± 2,02	0,72 ± 0,28	0,68 ± 0,06	2,57 ± 2,11	0,11 ± 0,04	22,04 ± 3,03	2,38 ± 0,69	2,97 ± 0,75	63,27 ± 12,69	1517,44 ± 288,43
Dombeya rotundifolia	104,09 ± 104,09	18,20 ± 18,20	3,83 ± 3,83	1,09 ± 1,09	0,78 ± 0,78	1,12 ± 1,12	0,00 ± 0,00	20,35 ± 20,35	4,7 ± 4,7	0,44 ± 0,44	36,05 ± 36,05	808,89 ± 808,89
Ficus capensis	16,80 ± 16,80	18,62 ± 18,62	1,78 ± 1,78	(-0,00 ± 0,00)	1,24 ± 1,24	0,09 ± 0,09	(-0,00 ± 0,01)	18,95 ± 18,95	26,19 ± 26,19	0,28 ± 0,28	31,54 ± 31,54	389,34 ± 389,34
Ficus craterostoma	16,70 ± 13,53	81,84 ± 90,15	12,99 ± 1,94	1,88 ± 0,5	1,09 ± 0,22	0,23 ± 0,06	(-0,00 ± 0,01)	60,63 ± 38,17	92,98 ± 65,41	2,08 ± 0,89	68,38 ± 41,48	1147,48 ± 418,90
Ficus sycomorus	51,28 ± 26,33	78,39 ± 10,13	12,88 ± 1,05	0,76 ± 0,43	1,93 ± 0,16	1,29 ± 0,67	0,07 ± 0,02	50,60 ± 17,37	110,22 ± 10,46	0,90 ± 0,15	90,21 ± 14,39	1490,92 ± 205,38
Lannea discolor	13,48 ± 1,50	45,48 ± 12,65	10,44 ± 1,72	0,85 ± 0,22	1,17 ± 0,34	1,05 ± 0,29	0,02 ± 0,01	20,83± 3,61	40,51 ± 12,09	1,53 ± 0,64	107,63 ± 21,73	3818,02 ± 499,30

Ozoroa insignis	8.26 ± 8.26	5.88 ± 5.88	4.55 ± 4.55	0.80 ± 0.80	1.18 ± 1.18	1.20 ± 1.20	(-0.02 ± 0.02)	18.95 ± 18.95	26.19 ± 26.19	0.28 ± 0.28	31.54 ± 31.54	389.34 ± 389.34
Parinari curatellifolia	10.08 ± 10.08	0.7 ± 0.7	4.04 ± 4.04	1.37 ± 1.37	1.26 ± 1.26	1.37 ± 1.37	0.02 ± 0.02	7.73 ± 7.73	0.00 ± 0.00	1.35 ± 1.35	139.83 ± 139.83	1937.39 ± 1937.39
Peltophorum africanum	17.09 ± 4.41	123.15 ± 37.37	24.40 ± 6.70	0.91 ± 0.05	1.30 ± 0.23	0.52 ± 0.12	0.02 ± 0.00	4.28 ± 4.28	23.87 ± 23.87	0.00 ± 0.00	63.33 ± 63.33	1381.39 ± 1381.39
Phyllanthus guineensis	28.51 ± 18.87	67.60 ± 4.84	13.55 ± 3.54	0.74 ± 0.47	0.74 ± 0.10	0.56 ± 0.31	0.02 ± 0.02	17.38 ± 9.01	37.45 ± 24.80	0.93 ± 0.45	53.24 ± 20.27	2697.52 ± 1226.25
Rhus longipes	29.01 ± 8.21	64.19 ± 11.36	14.52 ± 0.66	0.89 ± 0.18	1.14 ± 0.14	0.86 ± 0.19	0.03 ± 0.01	29.06 ± 4.20	50.72 ± 10.97	3.28 ± 1.06	85.40 ± 10.34	1347.18 ± 76.50
Senegalia polyacantha	34.19 ± 13.79	40.05 ± 8.24	8.84 ± 0.71	0.31 ± 0.14	1.26 ± 0.29	2.09 ± 1.07	(-0.01 ± 0.01)	25.76 ± 7.25	29.66 ± 3.70	11.07 ± 4.41	126.56 ± 31.35	1392.17 ± 170.28
Senna singueana	22.49 ± 11.66	75.39 ± 26.72	5.93 ± 0.89	0.56 ± 0.28	0.92 ± 0.15	1.26 ± 0.45	0.02 ± 0.01	50.51 ± 20.43	18.57 ± 8.50	1.63 ± 0.72	84.77 ± 20.45	1491.59 ± 198.33
Syzygium guineense	14.57 ± 5.53	45.10 ± 14.90	8.49 ± 0.67	1.16 ± 0.16	0.83 ± 0.08	0.32 ± 0.08	0.01 ± 0.01	21.98 ± 4.66	29.67 ± 10.72	1.35 ± 0.41	58.88 ± 7.25	1044.93 ± 106.94
Terminalia mollis	20.14 ± 7.11	97.88 ± 27.11	11.90 ± 1.05	1.03 ± 0.19	1.26 ± 0.44	1.23 ± 0.61	0.05 ± 0.01	14.86 ± 3.78	5.31 ± 2.99	0.76 ± 0.23	81.28 ± 38.62	1126.43 ± 347.31
Terminalia stenostachya	19.24 ± 3.99	75.14 ± 38.20	14.18 ± 3.32	1.60 ± 0.71	1.23 ± 0.68	1.78 ± 0.90	0.00 ± 0.01	18.87 ± 0.32	17.86 ± 12.47	0.28 ± 0.28	111.21 ± 59.80	1142.62 ± 108.43
Vachellia sieberiana	5.19 ± 5.19	17.75 ± 17.75	6.75 ± 6.75	0.53 ± 0.53	0.56 ± 0.56	0.4 ± 0.4	0.01 ± 0.01	17.90 ± 17.90	10.16 ± 10.16	1.27 ± 1.27	40.96 ± 40.96	516.86 ± 516.86

Table 3.7: Two-Way Anova for mean heavy metal concentrations in shoots

Treatment		Sum of Squares	Df	Mean Square	F Value	P value
Elements	Between Groups	4420.938	315	14.035	6.157	.000
	Within Groups	155.017	68	2.280		
	Total	4576.000	383			
Species	Between Groups	28568.233	315	90.693	1.480	.026
	Within Groups	4167.767	68	61.291		
	Total	32736.000	383			

The T-test on the mean heavy metals in roots and shoot concentrations showed significant differences between root and shoot concentrations as shown in Table 3.8.

Table 3.8: Paired T-test results for mean heavy metals in roots and shoots

	Mean (mg/kg)	N	Std. Deviation	Std. Error Mean	95% confidence interval of the difference Lower	Upper	t	df	Sig (P value)
Pair 1 Concentration Roots	98.766	384	300.663	15.343	--	--	--	--	--
Concentration shoots	222.800	384	1389.438	70.904	--	--	--	--	--
Pair1 Concentration Roots Concentration Shoots					-244.328	-3.741	-2.027	383	.043

A general trend of high metal concentration in shoots than roots was observed among the studied species in this study. Most species accumulated high concentrations in shoots compared to the roots for the various elements except for a few species for certain metals. Significant differences in heavy metal concentrations between root and shoot was observed among species. The results of this study reflect those of Sun et al. (2016) who also found variations in heavy metal concentration among plant species in shoots, leaves and roots. For example, in this study, *Albizia amara*, *Annona senegalensis*, *Combretum zeyheri*, *Dombeya rotundifolia*, *Ozoroa insignis* and *Parinari curatellifolia* accumulated more Cu in the shoots than the roots. These results reflect those of Nirola et al. (2015) who reported *Acacia pycnatha* and *Eucalyptus camaldulensis* to have accumulated more Zn and Cd in their shoots than roots. Tree species that accumulated higher concentration of metals in their shoots as compared to roots have the accumulation strategy of those metals (Yang et al 2014). The results suggest potential accumulation of Cu by *Albizia amara, Annona senegalensis*, *Combretum zeyheri*, *Dombeya rotundifolia, Ozoroa insignis* and *Parinari curatellifolia.* Species that translocate heavy metals to aboveground plant system's accumulate heavy metals and are not ideal for phytostabilization (Zou et al. 2012). Therefore, Albizia *amara*, *Annona senegalensis*, *Combretum zeyheri*, *Dombeya rotundifolia*, *Ozoroa insignis* and *Parinari curatellifolia* are not ideal for the phytostabilization of Cu contaminated as they translocate the metal to aboveground parts of the trees. These species could then be categorised as Cu accumulators. For Mn, the study found that all the 32 studied tree species accumulate the element in their leaves making their application in phytostabilization of Mn void. They could however be applied in the phytomining of the metal from mining generated wastelands (Sheoran et al. 2009).

3.3.4 Translocation and Bioconcentration factors of tree species

Table 3.9 shows species translocation factors (TF) and bioconcentration factors (BF). Species had different translocation factors for the various metals, for example, *Dombeya rotundifolia* had the highest TF for Cu (5.77) followed by *Parinari curatellifolia* (3.18), *Annona senegalensis* (2.52), *Combretum zeyheri* (1.46), *Albizia amara* (1.42) and *Ozoroa insignis* (1.3). All the other species had TF < 1 for Cu. Interestingly, none of species studied had TF < 1 for Mn with *Peltophorum africanum* recording the highest TF of 24.3 while only 4 species (*Albizia antunesiana* (0.46), *Ficus capensis* (0.65), *Albizia adianthifolia* (0.35) and *Combretum apiculatum* (0.95) had TF < 1 for Zn as the rest had TF > 1. A similar trend was observed with S and B that had four (*Albizia adianthifolia*, *Albizia antunesiana*, *Albizia amara* and *Ficus capensis*) and three species (*Albizia antunesiana*, *Albizia adianthifolia* and *Diospyros mespiliformis*) with TF < 1 while the rest had TF > 1. It is apparent from table 3.9 that, tree species had different BF and TF against each metal.

Conclusively, all the studied tree species had BF < 1 less for all the studied elements except *Diospyros mespiliformis* which had BF (1.16) for Ba and *Albizia adianthifolia* (5.99), *Bauhinia thonningii* (1.30), *Combretu m microphyllum* (1.51) and *Lannea discolor* (1.65) for S. The different BF and TF of species against the various elements is an indication of the tree species accumulation abilities per element. It shows that a species can accumulate more of an element than the other.

Table 3.9: Translocation (TF) and Bioconcentration (BF) factors of tree species occurring on Copperbelt tailings dams

	Cu		**Mn**		**Zn**		**Ni**		**Cr**		**Co**		**Cd**		**B**		**Ba**		**Mo**		**Al**		**S**	
Species	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**	**TF**	**BF**
Albizia adianthifolia	0,161	0,007	17,048	0,003	0,351	0,159	0,170	0,014	0,455	0,006	0,545	0,003	-20,321	0,001	0,869	0,018	4,695	0,007	0,026	0,260	0,486	0,002	0,359	5,989
Albizia amara	1,416	0,006	10,858	0,003	1,760	0,032	1,053	0,007	0,586	0,003	1,410	0,005	-0,545	-0,007	1,626	0,030	0,339	0,044	0,436	0,036	0,554	0,002	0,705	0,891
Albizia antunesiana	0,325	0,008	6,368	0,004	0,458	0,053	-1,186	0,016	0,309	0,005	0,556	0,005	-0,629	0,002	0,951	0,020	1,054	0,108	0,360	0,078	0,360	0,006	0,389	0,960
Albizia versicolor	0,883	0,007	5,033	0,002	1,450	0,027	1,710	0,008	1,595	0,005	3,009	0,003	-0,584	-0,004	3,142	0,012	0,539	0,018	11,488	0,037	1,602	0,002	2,117	0,174
Annona senegalensis	2,523	0,001	15,908	0,003	5,025	0,029	2,721	0,018	1,693	0,003	2,553	0,002	0,156	-0,015	2,395	0,039	4,010	0,022	0,245	0,058	0,670	0,006	5,372	0,299
Azanza garckeana	0,313	0,001	18,358	0,002	2,708	0,022	5,298	0,014	0,769	0,003	2,232	0,002	6,731	-0,012	4,282	0,031	24,157	0,017	0,212	0,092	2,238	0,005	1,095	0,288
Bauhinia petersiana	0,689	0,006	11,463	0,001	2,210	0,032	2,597	0,006	0,533	0,002	0,835	0,002	0,347	-0,002	3,521	0,051	1,605	0,017	0,045	0,042	0,353	0,001	1,776	0,303
Bauhinia thonningii	0,287	0,007	10,035	0,003	1,249	0,054	2,112	0,014	0,633	0,006	0,304	0,005	0,835	-0,010	4,473	0,037	1,894	0,067	0,297	0,102	0,424	0,003	1,186	0,971
Brysocarpus orientalis	0,166	0,006	11,558	0,004	4,159	0,069	0,836	0,002	1,588	0,004	0,752	0,002	-0,220	-0,001	1,577	0,016	7,295	0,034	1,201	0,050	1,244	0,002	3,450	1,300
Combretum apiculatum	0,399	0,003	10,578	0,002	0,951	0,022	1,129	0,008	0,810	0,006	0,315	0,001	1,731	-0,004	1,607	0,027	4,247	0,021	1,058	0,053	0,638	0,002	1,384	0,097
Combretum microphyllum	0,360	0,004	11,848	0,003	2,404	0,033	1,990	0,024	1,150	0,004	3,894	0,004	2,535	-0,004	2,278	0,034	4,543	0,055	0,323	0,058	1,401	0,002	3,153	0,827
Combretum molle	0,492	0,002	22,247	0,002	4,497	0,007	0,636	0,005	0,587	0,002	8,324	0,001	8,791	-0,002	3,106	0,007	1,403	0,011	0,254	0,035	0,510	0,001	1,111	0,094
Combretum zeyheri	1,461	0,006	12,025	0,003	2,907	0,034	0,948	0,002	1,688	0,002	7,602	0,003	0,258	-0,004	3,102	0,037	0,467	0,039	1,282	0,030	0,921	0,001	2,483	1,514

Dichrostachys cinerea	0,220	0,002	8,882	0,003	1,650	0,021	0,500	0,021	0,281	0,001	0,295	0,002	0,897	-0,006	2,831	0,027	1,218	0,019	1,012	0,042	0,270	0,001	2,729	0,662
Diospyros mespiliformis	0,328	0,005	6,108	0,003	2,057	0,025	-0,089	0,013	0,735	0,005	0,284	0,004	-0,769	-0,001	0,553	0,023	0,151	0,061	0,536	0,037	0,669	0,003	3,631	0,679
Dodonaea viscosa	0,139	0,001	20,263	0,000	3,502	0,016	0,813	0,006	0,382	0,003	0,901	0,001	5,862	-0,001	3,714	0,056	0,329	1,156	0,473	0,011	0,194	0,001	2,630	0,049
Dombeya rotundifolia	5,767	0,005	7,668	0,002	3,760	0,025	1,234	0,007	1,718	0,004	18,361	0,002	0,066	-0,002	7,165	0,012	0,802	0,010	0,643	0,094	0,752	0,002	4,071	0,169
Ficus capensis	0,698	0,001	3,568	0,002	0,654	0,017	-0,011	0,048	1,145	0,001	0,563	0,002	2,179	-0,016	2,602	0,046	0,502	0,016	0,268	0,056	0,677	0,006	0,395	0,252
Ficus craterostoma	0,341	0,003	12,985	0,001	3,098	0,037	1,512	0,009	0,612	0,004	0,592	0,005	-0,135	0,003	10,711	0,077	4,481	0,162	1,055	0,080	0,694	0,003	2,141	0,636
Ficus sycomorus	0,645	0,004	12,883	0,003	2,233	0,022	0,648	0,012	0,763	0,004	0,571	0,003	2,074	-0,005	6,679	0,024	1,752	0,036	0,265	0,056	1,243	0,002	1,737	0,384
Lannea discolor	0,061	0,004	10,441	0,003	1,940	0,034	0,674	0,019	0,469	0,004	0,552	0,005	0,608	-0,007	2,060	0,039	1,767	0,098	0,230	0,069	0,257	0,002	3,523	0,612
Ozoroa insignis	1,298	0,018	9,098	0,004	2,692	0,028	0,801	0,020	1,450	0,004	4,178	0,002	18,077	-0,003	4,070	0,020	0,000	0,032	2,392	0,169	3,136	0,004	4,674	1,645
Parinari curatellifolia	3,180	0,001	8,088	0,001	3,112	0,016	3,290	0,007	0,948	0,002	2,620	0,000	0,296	0,000	2,836	0,008	10,134	0,006	0,000	0,014	0,477	0,000	11,922	0,078
Peltophorum africanum	0,121	0,001	24,399	0,011	3,965	0,014	0,628	0,010	0,373	0,003	0,176	0,001	1,107	-0,010	2,325	0,005	0,914	0,006	0,282	0,017	0,242	0,002	3,363	0,098
Phyllanthus guineensis	0,276	0,038	13,551	0,010	2,967	0,041	0,520	0,021	0,755	0,009	1,438	0,017	0,700	0,001	2,324	0,025	1,251	0,055	1,265	0,072	0,723	0,004	7,602	0,827
Rhus longipes	0,304	0,003	14,517	0,003	3,133	0,028	0,776	0,022	0,587	0,001	0,527	0,002	0,614	-0,002	3,748	0,037	1,891	0,070	1,285	0,019	0,790	0,001	2,260	0,261
Senegalia polyacantha	0,775	0,002	8,843	0,002	2,242	0,013	0,766	0,004	1,587	0,002	3,093	0,001	-0,692	-0,001	2,677	0,014	1,814	0,002	2,784	0,044	1,397	0,001	1,519	0,158
Senna singueana	0,464	0,005	5,926	0,002	1,065	0,035	0,257	0,015	0,569	0,004	0,439	0,003	0,838	-0,005	5,495	0,031	1,086	0,046	1,090	0,056	0,557	0,001	2,188	0,639
Syzygium guineense	0,238	0,003	8,494	0,002	1,508	0,026	0,529	0,013	0,219	0,003	0,155	0,002	0,338	-0,002	2,551	0,023	1,467	0,015	0,377	0,026	0,282	0,001	1,199	0,189

Terminalia mollis	0,215	0,004	11,896	0,002	2,787	0,022	1,206	0,011	0,705	0,004	0,310	0,003	0,687	-0,004	1,904	0,024	0,337	0,024	0,296	0,050	0,372	0,002	2,181	0,684
Terminalia stenostachya	0,556	0,004	14,179	0,003	2,826	0,022	1,160	0,006	0,593	0,005	0,986	0,003	0,087	-0,008	2,198	0,021	1,261	0,028	0,110	0,048	0,297	0,002	1,785	0,423
Vachellia sieberiana	0,607	0,003	13,498	0,004	4,206	0,020	3,330	0,011	0,738	0,001	0,450	0,001	0,299	-0,003	3,565	0,029	1,897	0,022	0,954	0,083	0,547	0,001	1,184	0,688

Selection of candidate species suitable for phytostabilization is a key aspect of phytostabilization (Wong 2003; Santos et al. 2017). Plant species with phytostabilization potential exclude heavy metals (Mendez and Maier 2008), have high ability to adapt to contaminated sites (Santos et al. 2017) and produce large biomass (Li et al. 2007; Anawar et al. 2013; Schachtschneider et al. 2017). Plant species ideal for phytostabilization are highly adaptable to contaminated sites. The selection of suitable indigenous tree species growing on Copperbelt tailings dams could be a suitable solution for revegetating the barren land (Mendez and Maier 2008). Based on the species performance and large biomass reflected by their high IVI's and low heavy metal accumulation, *Rhus longipes, Syzygium guineense, Senegalia polyacantha, Ficus craterostoma, Bauhinia thonningii, Albizia adianthifolia, Combretum molle, Peltophorum africanum* and *Ficus sycomorus* would be effective in the phytostabilization of Cu contaminated sites like Copperbelt tailings dams. In this study, 32 indigenous tree species were identified as accumulators and excluders of various metals, provides a basis for the selection of suitable tree species for the revegetation of Copperbelt tailings dams. The use of these species to stabilize mine wastelands provides other advantages such as provision of food, source of energy, timber and medicinal plants to the communities besides enhancing the soil development of the sites through increased organic matter and soil nutrients (Anawar et al. 2013). The study further identified accumulators of the 12 metals which could be considered for possible application in phytoextraction (Bhargava et al. 2012). The species application in phytoextraction however requires further research and evaluation.

There is little or no reports on the heavy metal accumulation of the 31studied tree species, except for *Senegalia polyacantha* which was reported as an accumulator of Cu (Masvodza et al. 2013) contrary to the findings of this study.

The study identified dominant species *Rhus longipes, Syzygium guineense, Senegalia polyacantha, Ficus craterostoma, Bauhinia thonningii, Albizia adianthifolia, Combretum molle, Peltophorum africanum* and *Ficus sycomorus* as potential phytostabilization candidates of Cu contaminated sites based on the species abundance, high IVI and low BF and TF (< 1). Other species (*Albizia antunesiana, Albizia versicolor, Azanza garckeana, Bauhinia petersiana, Brysorcapus orientalis, Combretum apiculatum, Combretum microphyllum, Dichrostachys cinerea, Diospyros mespiliformis, Dodonaea viscosa, Ficus capensis, Ficus sycomorus, Lannea discolor, Phyllanthus guineensis, Senna singueana, Terminalia mollis, Terminalia stenostachya* and *Vachellia sieberiana*) with phytostabilization potential for Cu but low biomass production could be considered potential phytostabilizers of Cu (Schachtschneider et al. 2017). The results of this study show that plant species respond to various metals differently for example, *Albizia versicolor* was identified as Cu, Cd and Ba excluder at the same time as a Zn, Ni, Co, Cr, Mn, B, Al, Mo and S accumulator. These results reflect those of Santos et al. (2017) who reported two species identified as phytostabilizers to accumulate Zn, Mo and Cu beyond acceptable animal levels. Therefore, Caution must be taken when considering these species for phytostabilizing contaminated sites.

3.4 Conclusion

The study set out to determine the heavy metal accumulation and exclusion strategies of indigenous tree species growing on Copperbelt tailings dams for possible application in phytostabilization. The study has characterized plant species based on their exclusion and accumulation strategies for each studied metal. A number of species have been observed to exclude Cu while other species have also been observed to exclude other elements. This suggests that phytostabilization of Cu TSF requires careful consideration in selecting plant species. The study has therefore provided information that would be important in implementing phytostabilization programs of Cu TSFs. The study has provided information that could provide for a well-targeted species combination to ensure that all the heavy metals on the TSFs are taken care of

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CHAPTER FOUR: INFLUENCE OF HEAVY METALS ON SPECIES RICHNESS AND ABUNDANCE ON COPPERBELT TAILINGS DAMS

Abstract

The current study assessed species richness and abundance and evaluated the influence of heavy metals on species richness and abundance on Copperbelt tailings dams. To determine heavy metal influence on species richness and abundance, ordination analysis methods "Detrended correspondence analysis (DCA) and Canonical correspondence analysis (CCA)" were employed to determine the relationship between heavy metals and tree species on Copperbelt tailings dams. The results show that Ni, Cu, S, Cr, Al, Co, Mn, B, Mo and Cd influence species richness and abundance on the Copperbelt tailings dams. Tree species generally correlated with heavy metals in low concentration with a few (*Rhus longipes, Albizia versicolor, Albizia amara Dombeya rotundifolia, Albizia antunesiana, Phyllanthus guineensis* and *Dichrostachys cinerea)* that showed correlation with some heavy metals in high concentration. The results of this study suggest low species richness and abundance in high heavy metal concentration. Species response to heavy metals was observed to vary from site to site with species showing correlations in low concentration in one site and showing correlation in high concentration in another site for the same metal. The results suggest significant variations in sites hence soil amendment programs and other re-vegetation programs should consider sites as different entities. The results of this study offer a basis for the development of soil amendment programs for the ecological restoration and management of copper mine tailings dams of the Copperbelt in Zambia.

Keywords: Heavy metals, Tailings dams, Species richness, Species abundance, Vegetation restoration.

4.1 Introduction

Species establishment and growth on tailings dams is a prerequisite to successful implementation of phytoremediation of tailings dams. Mine wasteland restoration has received considerable attention in both academic and mining sectors due to the landscape damage caused by the generation of mine wastelands (Lei et al. 2016). Mining itself is not sustainable in nature due to the non-renewable nature of the minerals, thus posing challenges on the mining industries. Restoration of mine wasteland aims at restoring and conserving degraded ecosystems and landscapes to promote sustainable development in mining areas (Lei et al. 2016). Available evidence (Lei et al. 2006; Anawar et al. 2013; Sun et al. 2016; Santos et al. 2017; Festin et al. 2018) suggests that mine wasteland restoration could contribute towards sustainable development.

Disposal of mine wastes such as tailings on land impacts on the soil, water quality, plant, animal and aquatic biodiversity (Hsu et al. 2006; Anawar et al. 2013). It is well documented that tailings have severe negative impacts on the environment, the economy and social aspect of human life (Bradshaw 2000; Wong 2003; Li 2006; Lin et al. 2005; Liu et al. 2008; Schueler et al. 2011, Lindahl et al. 2014; Sun et al. 2016; Santos et al. 2017; Festin et al. 2018) hence the need for restoration. Establishment of vegetation cover on TSFs limits water erosion and aeolian dispersion of toxic heavy metals and leftover chemicals into the environment (Vangronsveld et al. 1996; Festin et al. 2018) thereby reducing mine wastes impact on the environment. However, plant establishment on tailings dams has been reported to be difficult (Wong 2003; Yang et al. 2016; Skubala et al. 2016) due to the physicochemical properties of tailings (Tordoff et al. 2000; Anawar et al 2013; Skubala et al. 2016). Tailings contain elevated concentrations of metals and leftover chemicals and are characterized by poor nutrient and water retention capacity which makes plant establishment and growth on tailings dams difficult (Wong 2003; Yang et al. 2014). A study by Herrick and Friedland (1990) and Gawel et al. (1996) reported that “high metal concentrations in soil affected plant growth resulting in declined plant population over the contaminated area”. Even though plant establishment on tailings dams is difficult, reports of well adapted plants on tailings dams have been made in various regions globally, Japan (Takeuchi and Shimano 2009), China (Li et al. 2007; Liu et al. 2008; Zou et al. 2012; Yang et al. 2014), Tanzania (Mganga et al. 2011), Mexico (Santos et al. 2017), Uganda (Ssenku et al. 2014), Zambia (Kambin’ga and Syampungani 2012; Festin et al. 2018), Portugal (Anawar et al. 2013) and South Africa (Schachtschneider et al. 2017). Skubala et al. (2016) reported that metalliferous sites result in the emergency of new ecosystems with a specific composition of species. Physicochemical factors of tailings such as pH, organic matter, salinity, nutrient deficiency and high heavy metal concentration have been reported to inhibit plant establishment on tailings dams (Conesa et al. 2006; Ortiz-Calderon et al. 2008; Anawar et al. 2013; Parraga-Aguado et al. 2013; Ssenku et al. 2014). Studies elsewhere have reported heavy metal effects on the establishment and growth of plants on contaminated sites (Kandeler et al. 1996; Wong 2003; Anawar et al. 2013). Other studies such as Gawel et al.

(1996) observed the reduction in plant population over contaminated sites. Plant growth in soils contaminated with heavy metals is inhibited or disrupted due to cell membrane functional disturbance and destruction (Kandeler et al. 1996). Understanding the vegetation composition of a site is key in determining suitable management practices of various sites. Ecological data such as vegetation composition has been used "to determine the influence of environmental variables" on species richness and abundance (Vahdati et al. 2017) thereby informing precise decision making with regards to restoration of contaminated environments. Studies on the influence of environmental variables such as slope, elevation, organic matter, soil texture, pH, soil moisture content, grazing intensity, wildfires and surface roughness on flora and fauna of non-contaminated sites have been documented (Dorji et al. 2014; Serrat et al. 2015; Zhang et al. 2015; Vahdati et al. 2016) but little has been reported on the influence of heavy metals on species richness and abundance (Gawel et al. 1996; Takeuchi and Shimano 2009; Ficken and Byrne 2013; Šalamún et al. 2015). To date, few studies have investigated the influence of heavy metals on species richness and abundance on contaminated sites (Anawar et al. 2013) especially on copper mining generated wastelands. Previous studies reported the effect of physicochemical properties of the tailings on plant establishment with less reference to heavy metals (Ssenku et al. 2014, Santos et al. 2017, Chileshe et al. 2019). Understanding the influence of heavy metals on plant species abundance on tailings dams is cardinal for the successful implementation of phytoremediation strategy as it provides an understanding of the required soil amendments (Santos et al. 2017) and potential for manipulation of nutrients to enhance the survival of plants on degraded environments.

Mining activities on the Copperbelt have left an ecological footprint through the generation of mine wastelands like tailings dams that impact on the environment thereby affecting the social economic and health aspects of people. Copperbelt province is home to around 791 million tons of tailings occupying an estimated 9125 ha of land (Festin et al. 2018). The physicochemical properties and heavy metal concentrations of the Copperbelt tailings dams have been described (Festin et al. 2018; Chileshe et al. 2019). However, the influence of heavy metals on species richness and abundance on Copperbelt tailings dams has not been reported therefore, poorly understood. Previous studies highlighted the reduction of diversity and abundance in areas contaminated with heavy metals from mine wastelands (Mensah 2015; Festin et al. 2018), however, reports of how heavy metals influence species abundance are missing.

Indigenous tree species have been observed to colonize Copperbelt tailings dams in a patchy pattern. It is, therefore, important to assess the factors underlying the vegetation succession on Copperbelt tailings dams for restoration purposes. This study was set out to investigate the influence of heavy metals on species richness and abundance on Copperbelt tailings dams to provide for an understanding of the mechanism behind species succession or colonization of the metalliferous sites which is key for mine wasteland restoration programs. By determining the influence of heavy metal concentration on the species abundance at different sites, a sustainable ecological restoration of these wastelands could be probable.

4.2 Materials and methods

4.2.1 Site description and sampling procedure

The study was conducted in selected mining towns of the Copperbelt Province in Zambia (Figure 3.1). Copperbelt lies between latitude 13° 00′ 00″ and longitude 28° 00′ 00″. The Province is the main mining province in the Country with the mining history dating back to the 1920s (Sikamo et al. 2016). All the towns on the Copperbelt Province namely, Mufulira, Kitwe, Chingola, Ndola, Chililabombwe and Luanshya are mining towns.

Copperbelt soils are extremely weathered and leached with low base exchange capacity (Kříbek et al. 2014). According to FAO-UNESCO (1997), Copperbelt soils are categorised as ferralsol comprising of acric, orthic or rhodic ferralsols. These soils are highly acidic in nature with low humus and low soil organic carbon (Kříbek et al. 2014) with high exchangeable manganese and aluminium. The Province is one of the wetter regions of the country as it falls under the third (Ⅲ) agro-ecological zone, the high rainfall area with average annual rainfall and temperature ranging between 1000 to 1500 mm and 7.8°C to 23.7°C, respectively (Norway 2007). “The rainy season is from November to March except for rare showers in August, a cool dry season from April to August and a hot dry season is experienced from August to November” (Aregheore 2009; Sracek et al. 2010). The study focused on mine towns with abandoned or decommissioned tailings dams in Kitwe, Luanshya and Mufulira.

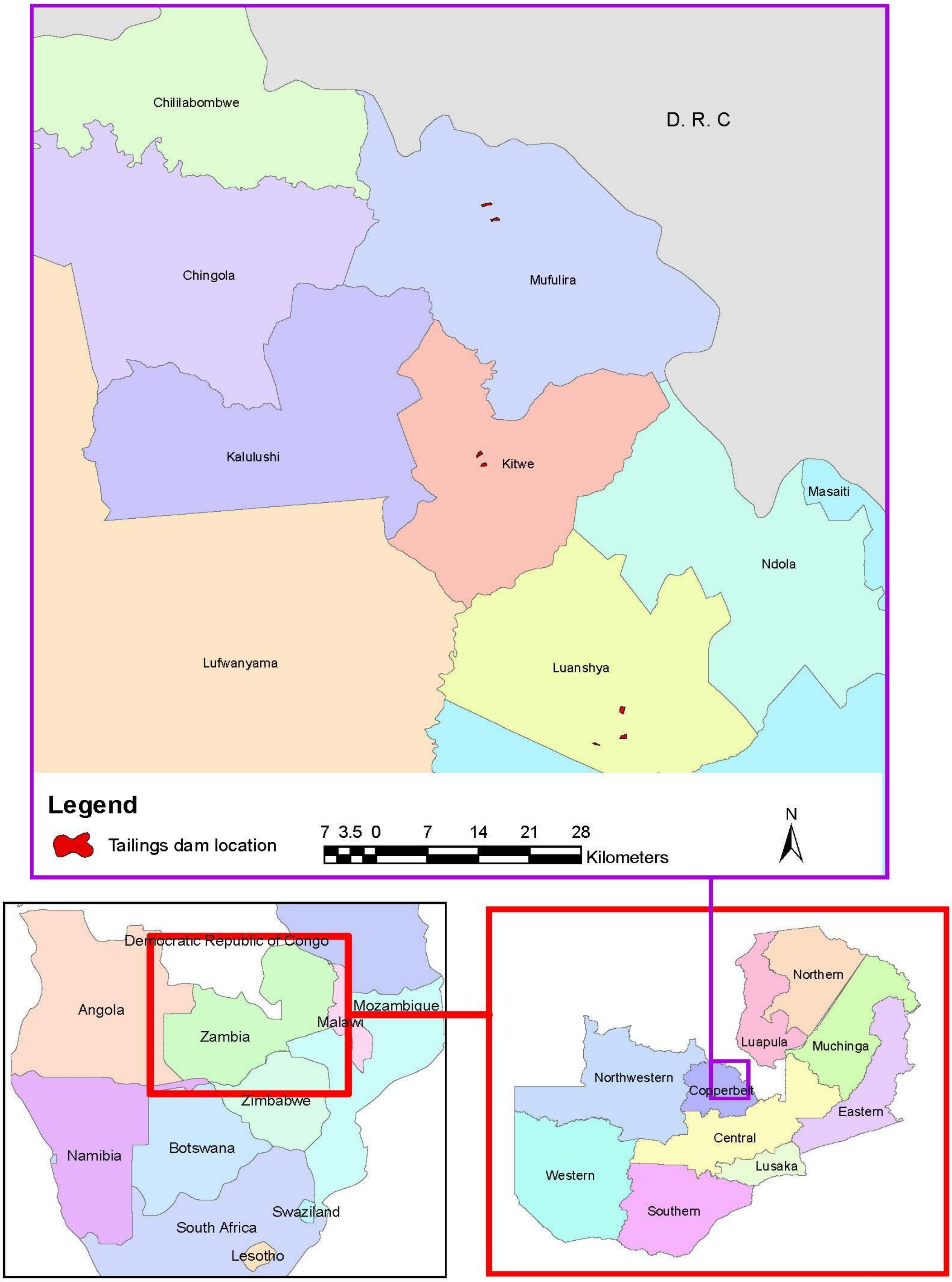

Figure 4.1: Location of study areas within Copperbelt Province

4.2.2 Sampling procedure

Field surveys were carried out from December 2017 to May 2018 across seven decommissioned Tailings Storage Facilities (TSF) namely TD 25 & 26 (Kitwe), TD24, 25 & 26 (Luanshya) and TD 8 & 10 (Mufulira). The study adopted the transect method of sampling the vegetation on these TSFs due to the pattern of vegetation distribution and establishment on the TSFs. The transect method of sampling is a point-based sampling method used to determine plant cover, species occurrence and species abundance (Lancia et al. 2005). A transect or path is established along which a number of samplings points are established. The transect length and number of sampling points are dependent on the vegetation. Vegetation on tailings is in clusters running along certain sides of the TSF. Therefore, transects were established on areas with vegetation cover only as the vegetation was the focus of the study. Biasness was reduced or eliminated by randomly selecting the starting point of the first line transect and sampling points, thereafter, transects were established at every 200 m as recommended by Kambinga and Syampungani (2012) while sampling points were established at every 100m (see Mganga et al. 2011). The method is less costly, practical and efficient for studying most biological populations, plants inclusive (Varman and Sukumar 1995) making its application in this study feasible. A 20 m diameter circular plot was established at each sampling point using a distance tape for data observation. On each sampling point, the GPS coordinates were taken from the centre of the plot for location description. All tree species in a plot were identified and their respective diameter at breast height (DBH) measured and recorded.

4.2.3 Plant sampling

Plant samples (root and shoot) were collected from selected individuals of all individual species in a plot. Tree selection was based on DBH size and therefore individual species with large DBH were selected for inclusion in plant sampling. A kg of root and shoot samples each were collected from the selected individuals of species in each plot. After collection, root and shoot samples were packed in a plastic bag, clearly labelled with species name, site name, transect and plot number and were taken to the laboratory for analysis. Root and shoot samples were thoroughly washed with tap water and rinsed with deionised water to decontaminate the samples (Moraghan 1991; Richards 1993). The root and shoot samples were then dried in a microwave oven at 60˚ for 24 hours and 30 hours, respectively (Liao et al. 2016; Vymazal 2016).

4.2.4 Soil sampling

Soil sample collection was done at 0-30cm depth from three distinct locations within the sample plot using a stainless-steel auger. This is because soil biological factors which influence plant growth and active root zone are within the 0-30cm (Crepin and Johnson 1993). Dongmei and Changqun (2008) also suggest that the 0-30cm represents the most nutrient active zone of the soil. Previous studies elsewhere (Ssenku et al. 2014; Mganga et al. 2011; Chileshe et al. 2019) on tailing storage facilities have also sampled in the 0-30 cm zone. Collection of soil samples from the three distinct locations in the plot was done after Mganga et al. (2011) and Chileshe et al. (2019). Soil was collected from three randomly selected locations within a plot near the trees. The soil was then mixed to form a composite soil sample from which a kilogram was measured, labelled and packed in a plastic bag, and taken to the laboratory for analysis. The soil label had the site, transect number and plot number on it.

4.2.4 Soil analysis

Total heavy metal concentrations in soil for copper (Cu), zinc (Zn), manganese (Mn), aluminium (Al), barium (Ba), boron (B), nickel (Ni), cobalt (Co), sulphur (S), molybdenum (Mo) chromium (Cr) and cadmium (Cd) were determined using the Inductively coupled plasma atomic emission spectrochemistry ICP-OES after sample digestion using the standard EPA 3052 digestion method (Yang et al. 2017). In this method, 3g of soil, root and shoot samples were measured and placed in 50ml inert microwave vessels and digested in 9ml of Nitric Acid Suprapur for 10-20 minutes using a Multiwave 3000 manufactured by Perkin Elmer. The samples were left to cool for 20-25 minutes, then filtered, and each extract was diluted with deionised water to a 30 ml mark. Total heavy metal concentrations were then determined by Flame Atomic Absorption Spectrometry (FAAS). Precision and accuracy were achieved by measuring three blanks and results obtained in mg/l were converted to mg/kg.

4.2.5 Ecological analysis

Species richness and abundance per site was determined. “Species richness was determined by counting the number of species” occurring on the site while abundance was “determined by counting the number of individuals” of a species per plot and consecutively by site using Microsoft Excel 2016. Tree species DBH was used to calculate the site species IVI for the determination of the overall species performance on the tailings and hence identifying the dominant (important) tree species on the tailings dams. The abundance was used to determine the species richness and cover on the tailings dams (Liu et al. 2008).

4.2.6 Statistical analysis

To determine the significant difference in species richness, abundance and heavy metal concentrations between the sites, a one-way Anova at p= 0.05 level of significance was conducted using SPSS version 25.0.

Ordination method, Detrended Cluster Analysis (DCA) was employed to investigate the relationship between environmental variables (heavy metals) and species abundance on the tailings dams. DCA analysis was used to determine the gradient length (Kent 2011) which is used to determine the suitable ordination method to apply to data (Zhang et al. 2015). Gradient lengths greater than 3 suggests the use of "unimodal ordination methods such as DCA and Canonical Correspondence Analysis (CCA) while gradient lengths less than 3 specify linear models" (ter Braak and Šmilauer 2002; Zhang et al.2015) such as Principal Component Analysis (PCA) and Redundancy Analysis (RDA) (Zhang et al. 2015; Vahdati et al. 2017). In the current study, the gradient length was 5.4, and therefore CCA was used to analyse the influence of Cu, Mn, Zn, Ni, Ba, B, Al, Co, Cd, S, Mo and Cr on species richness and abundance on Copperbelt tailings dams. DCA and CCA analysis were done using Canoco 5.1. to test the effect of environmental variables (heavy metals) on species abundance. To test the significance of heavy metal influence on species abundance, a Monte Carlo permutation test was employed.

4.3 Results

4.3.1 Species richness and abundance

In total, 32 species from 13 families and 22 genera were recorded on Copperbelt tailings dams (see appendix 1). Species richness varied at the seven sites with some sites having more richness than others (Table 4.1). The one - way Anova at p=0.05 showed significant differences in species richness between the studied sites. Species richness ranged from 20 species in TD24 (Luanshya) (site 3) which recorded the highest richness to 10 species in TD26 from Kitwe (site 2).

Species abundance also varied between the sites with TD24 (Luanshya) having the highest number of individuals (85) and TD26 (Kitwe) having the lowest individual trees (49). Even though there were variations in species abundance on the sites, the one-way Anova at p= 0.05 showed no significant differences in species abundance between the sites. What is striking about the data is that TD24 (Luanshya) had the highest species richness and abundance while TD26 (Kitwe) had the lowest richness and abundance.

Species abundance and DBH were used to calculate the IVI which measures a species dominance in an area (Liu et al. 2017). Species IVIs varied among and between species in the various sites with some species being dominant in one area and not on the others (Table 4.2). For example, dominant species on TD25 (Kitwe) were *Peltophorum africanum* (19.129), *Albizia versicolor* (13.049), *Bauhinia thonningii* (10.783), *Combretum molle* (9.551), *Lannea discolor* (8.984), *Syzygium guineense* (8.346) and *Albizia antunesiana* (7.895) while *Senegalia polyacantha* (30.421), *Rhus longipes* (23.602), *Syzygium guineense* (19.553) and *Ficus craterostoma* (7.092) dominated TD26 (Kitwe). TD24 (Luanshya) was dominated by *Rhus longipes* (22.445), *Bauhinia thonningii* (12.125), *Ficus craterostoma* (9.254) and *Senna singueana* (8.065) while TD25 (Luanshya) was dominated by *Bauhinia thonningii* (20.251), *Ficus craterostoma* (18.423), *Syzygium guineense* (14.142), *Rhus longipes* (10.064) and *Senna singueana* (7.899). TD26 (Luanshya) was dominated by *Syzygium guineense* (40.398), *Terminalia stenostachya* (14.157), *Rhus longipes* (8.942) and *Ficus craterostoma* (8.372). Additionally, *Ficus sycomorus* (23.516), *Rhus longipes* (21.949), *Bauhinia petersiana* (15.561), *Senegalia polyacantha* (8.583) and *Ficus craterostoma* (8.447) dominated TD10 (Mufulira) while *Rhus longipes* (23.593), *Combretum molle* (16.454), *Senegalia polyacantha* (9.190) and *Terminalia mollis* (7.607) dominated TD8 (Mufulira). Some species dominated more sites than others for example, *Rhus longipes* was dominant in six sites, *Ficus craterostoma* was dominant in five sites while *Syzygium guineense* dominated four sites. Based on the overall species performance on the tailings as characterized by species IVI (see appendix 1), *Rhus longipes*, *Syzygium guineense*, *Senegalia polyacantha*, *Ficus craterostoma*, *Albizia adianthifolia*, *Bauhinia thonningii*, *Combretum molle*, *Ficus sycomorus* and *Peltophorum africanum* were the most dominant species across tailings dams.

Table 4.1: Species richness and abundance on Copperbelt tailings dams

Town	Site	Species richness	Abundance
Kitwe	TD25(I)	15	62
	TD26(2)	10	49
Luanshya	TD24 (3)	20	85
	TD25(4)	16	70
	TD26 (5)	13	65
Mufulira	TD8 (6)	17	52
	TD10 (7)	13	81

Table 4.2: Species IVI per site

Species	IVI per Site						
	Kitwe		Luanshya			Mufulira	
	TD25	TD26	TD24	TD25	TD26	TD8	TD10
Albizia adianthifolia	0	0	6.477	0	0	0	0
Albizia amara	4.014	0	0	3.090	0	3.435	0
Albizia antunesiana	7.895	0	3.166	0	0	1.501	4.487
Albizia versicolor	13.049	0	2.357	2.909	3.111	0	0
Annona senegalensis	0	0	0	1.160	0	2.101	1.706
Azanza garckeana	0	0	0	0	0	0	5.924
Bauhinia petersiana	1.298	5.111	1.235	0	0	3.416	15.561
Bauhinia thonningii	10.783	1.999	12.125	20.251	0	0	0
Brysocarpus orientalis	0	0	1.353	0	0	0	0
Combretum apiculatum	0	0	0	1.398	0	0	0
Combretum microphyllum	0	0	1.233	0	0	1.914	1.742
Combretum molle	9.551	0	5.854	3.885	0	16.454	0
Combretum zeyheri	0	0	0	1.207	0	6.729	0
Dichrostachys cinerea	2.130	0	1.301	1.218	0	0	2.363
Diospyros mespiliformis	0	5.559	0	0	0	0	0
Dodonaea viscosa	4.562	0	0	2.392	1.430	0	0
Dombeya rotundifolia	0	0	0	1.281	0	1.501	0
Ficus capensis	0	0	0	0	0	0	1.990
Ficus craterostoma	0	7.092	9.254	18.423	8.372	5.817	8.447
Ficus sycomorus	0	2.411	1.301	0	0	6.437	23.516
Lannea discolor	8.984	0	1.779	0	3.582	0.993	0
Ozoroa insignis	0	0	0	0	1.574	0	0
Parinari curatellifolia	2.146	0	0	0	0	0	0
Peltophorum africanum	19.129	0	4.339	5.637	0	0	0
Phyllanthus guineensis	0	0	1.574	0	1.451	3.076	0
Rhus longipes	1.298	23.602	22.445	10.064	8.942	23.593	21.949
Senegalia polyacantha	5.329	30.421	3.309	0	3.662	9.190	8.583
Senna singueana	0	2.051	8.065	7.899	1.990	1.297	0
Syzigium guineense	8.346	19.553	5.829	14.142	40.398	2.951	1.990
Terminalia mollis	0	2.201	0	5.042	5.480	7.607	0
Terminalia stenostachya	1.484	0	0	0	14.157	0	1.742
Vachellia sieberiana	0	0	3.325	0	5.851	0	0

0 signifies absence of a species in a particular site.

4.3.2 Metal concentrations in Copperbelt tailings dams

Variations in heavy metal concentrations at the seven sites were observed with TD26 (Kitwe) having the highest concentration of Cu and Mn while TD24 (Luanshya) had the highest concentrations of Ni and Cr (Table 4.3). The one-way Anova revealed significant differences in heavy metal concentrations among the studied sites at p= 0.05.

Within the sites, variations in heavy metal concentrations along the transects were observed. The one-way Anova at p = 0.05 showed some metals being significantly different in some sites (see table 4.4). The one-way Anova showed significant differences in Cr, Cd and B concentrations between transects in TD25 (Kitwe) while the other metals (Cu, Mn, Zn, Ni, Ba, Al, S, Mo and Co) showed no significant differences. Contrary to the trend in TD25, significant differences in Cu, Mn, Ni, Cd, Co, B, Ba, Mo, and Al with an exception of S, Zn and Cr in TD26 (Kitwe) were indicated by the one-way Anova at p = 0.05. Comparably, significant differences in Zn, Co, Ba and Al concentrations between the transects were observed while the other elements showed no significant differences between transects at p= 0.05 in TD24 (Luanshya). In TD26 one-way Anova results indicate significant differences in Zn, Ni, Co, Cd, Ba and S concentrations between transects. Ba and Al showed significant differences in TD8 while Cu and Zn showed significant differences in concentration between transects in TD10 (Mufulira).

Table 4.3: Mean heavy metal Concentrations of Copperbelt tailings dams in mg/kg

Sampling site	Cu	Mn	Zn	Ni	Cr	Co	Cd	B	Ba	Mo	Al	S
TD25 (Kitwe)	12638,44	9302,364	161,664	107,629	666,221	1868,593	-12,9286	503,95	1074,636	61,043	141409,6	3435,8
TD26 (Kitwe)	25789,84	17648,75	193,027	188,495	450,596	3242,508	-9,40714	406,096	2306,725	65,785	119130,8	4869,449
TD24 (Luanshya)	23140,79	8998,667	317,892	368,348	735,971	466,947	-13,6571	584,991	421,733	67,883	232487,5	2086,577
TD25 (Luanshya)	12523,25	10384,27	374,979	322,989	682,118	555,767	-12,5705	537,958	561,46	68,536	236100,8	2929,824
TD26 (Luanshya)	10668,05	9833,267	245,812	278,355	658,878	1262,733	-11,9305	500,631	1111,347	78,57	253414,2	6962,79
TD8 (Mufulira)	22494,25	11680,47	131,826	50,909	606,011	52,827	-3,89048	142,945	622,2	23,65	22797,5	1319,554
TD10 (Mufulira)	14724,52	11736,47	164,872	62,969	596,578	65,813	-4,43048	175,971	473,5	25,436	24240,83	2096,924

Table 4.4: One-way Anova test results showing differences in heavy concentrations between transects

Variable	P value per site						
	Kitwe		Luanshya			Mufulira	
	TD25	TD26	TD24	TD25	TD26	TD8	TD10
Cu	0.407	0.002	0.075	0.961	0.164	0.009	0.047
Mn	0.257	0.035	0.535	0.811	0.315	0.474	0.812
Zn	0.428	0.124	0.018	0.462	0.000	0.785	0.007
Ni	0.318	0.001	0.331	0.736	0.000	0.895	0.313
Cr	0.043	0.249	0.131	0.363	0.115	0.609	0.296
Cd	0.006	0.016	0.105	0.766	0.013	0.123	0.310
Co	0.119	0.002	0.040	0.735	0.000	0.267	0.337
B*	0.046	0.019	0.060	0.783	0.010	0.308	0.143
Ba	0.154	0.013	0.002	0.768	0.000	0.005	0.635
Mo	0.797	0.015	0.013	0.712	0.094	0.252	0.153
Al*	0.955	0.013	0.023	0.898	0.083	0.021	0.132
S*	0.516	0.483	0.119	0.566	0.017	0.337	0.541

$P < 0.05$ = significant, $P > 0.05$ = not significant * denotes Non heavy metals

4.3.3 Heavy metal influence on species abundance

The CCA analysis revealed variations in heavy metals influence on species richness and abundance per site. The results for TD25 (Kitwe) show that, *Syzygium guineense* and *Dodonaea viscosa* were highly (positively) correlated to Cu in low concentrations while *Senegalia polyacantha, Terminalia stenostachya, Lannea discolor, Peltophorum africanum, Bauhinia thonningii* and *Albizia antunesiana* showed a negative correlation to Cu (Figure 4.2a). *Combretum molle, Albizia versicolor* and *Parinari curatellifolia* showed weak positive correlations with Cu in low concentrations while *Rhus longipes* showed strong positive correlation to Cu in higher concentrations than the others. A similar trend was observed with Mn (see appendix 2). *Albizia versicolor, Peltophorum africanum* and *Albizia antunesiana* showed strong positive correlations to Zn in low concentrations. *Rhus longipes, Parinari curatellifolia* and *Combretum molle* showed a correlation to Zn in even lower concentrations while the other species showed a negative correlation. *Lannea discolor* showed a positive correlation to Zn in high concentration with *Albizia amara* and *Dichrostachys cinerea* showing a weak correlation in high concentration too. *Peltophorum africanum, Dodonaea viscosa,* *Albizia versicolor* and *Rhus longipes* positively correlated to Ni in low concentration while *Dichrostachys cinerea* and *Albizia amara* showed a weak positive correlation in higher concentration to Ni with the other species showing negative correlation to Ni. *Parinari curatellifolia, Lannea discolor* and *Combretum molle* positively correlated with Cr in high concentration while *Peltophorum africanum, Terminalia stenostachya, Dodonaea viscosa, Albizia antunesiana* and *Bauhinia thonningii* positively correlated to Cr in low concentrations. A similar trend as that of Mn was observed for Co with *Rhus longipes* and *Combretum molle* adding to the positive correlation in low concentration and *Dichrostachys cinerea* and *Albizia amara* showing a weak correlation in high concentration. *Bauhinia thonningii, Albizia versicolor, Terminalia stenostachya, Syzygium guineense, Senegalia polyacantha* and *Rhus longipes* showed positive correlations to Cd in low concentrations while *Dichrostachys cinerea* and *Albizia amara* showed a weak positive correlation to Cd in higher concentrations than the other species. The rest of the species showed negative correlation to Cd. *Peltophorum africanum* showed strong positive correlation to B in low concentrations. Similarly, *Albizia antunesiana, Dodonaea viscosa, Combretum molle* and *Parinari curatellifolia* positively correlated with B in low concentrations while *Lannea discolor* showed positive correlation in high concentration. *Syzygium guineense, Albizia versicolor, Rhus longipes* and *Dodonaea viscosa* showed positive correlations with Ba in low concentrations while *Dichrostachys cinerea* and *Albizia amara* showed weak positive correlations in higher concentrations to Ba. The other species showed negative correlation to Ba. Only *Albizia antunesiana* and *Albizia versicolor* positively correlated with Mo in low concentration while *Combretum molle, Dodonaea viscosa, Rhus longipes* and *Parinari curatellifolia* showed a positive correlation to Mo in high concentration. *Albizia antunesiana, Dodonaea viscosa, Combretum molle, Rhus longipes, Parinari curatellifolia* and *Lannea discolor* showed positive correlations to Al in low concentrations with *Albizia antunesiana* showing strong correlation while *Dichrostachys cinerea* and *Albizia amara* showed a weak positive correlation in high concentration. Only *Albizia amara, Peltophorum africanum* and *Dichrostachys cinerea* showed positive correlation with S in low concentration while *Albizia versicolor* showed a weak correlation with S in high concentration. The other species showed negative correlation to S. The Monte Carlo permutation tests at ($p = 0.05$) showed no statistical significance on all axis ($F = 0.1$, $p = 1$) of the heavy metals indicating no influence of heavy metals on species richness and abundance.

For TD26 (Kitwe), *Diospyros mespiliformis* showed strong positive correlation with Cu in high concentration while *Rhus longipes* and *Ficus sycomorus* positively correlated with Cu in high concentration (Figure 4.2B). *Bauhinia thonningii, Bauhinia petersiana* and *Peltophorum africanum* showed positive correlation to Cu in low concentration. *Ficus craterostoma, Ficus sycomorus, Terminalia mollis* and *Rhus longipes* showed positive correlation to Mn in high concentration while *Diospyros mespiliformis, Bauhinia thonningii, Bauhinia petersiana* and *Peltophorum africanum* showed positive correlation in low concentration (appendix 3). The other species showed negative correlation to Mn. *Terminalia mollis* and *Ficus sycomorus* showed positive correlation to Zn in high concentration while *Syzygium guineense, Senegalia polyacantha, Bauhinia petersiana* and *Bauhinia thonningii* showed positive correlation with Zn in low concentration. *Terminalia mollis, Ficus sycomorus* and *Rhus longipes* showed positive correlation with Ni in high concentration while *Bauhinia petersiana, Bauhinia thonningii* and *Senegalia polyacantha* showed positive correlation with Ni in low concentration. *Syzygium guineense* and *Senna singueana* showed positive correlation with Cr in high concentration with *Terminalia mollis* showing a weak correlation in high concentration while *Senegalia polyacantha* positively correlated with Cr in lower concentration. The other species showed a negative correlation to Cr. *Senegalia polyacantha, Bauhinia thonningii* and *Bauhinia petersiana* positively correlated with Co in high concentrations while *Rhus longipes, Terminalia mollis* and *Ficus sycomorus* showed a positive correlation in lower concentrations. A weak correlation to Co was observed with *Bauhinia thonningii* in low concentrations and *Terminalia mollis* in even lower concentrations. A weak correlation was also observed between Cd and *Rhus longipes, Ficus craterostoma* and *Ficus sycomorus* in slightly high concentrations while *Diospyros mespiliformis, Peltophorum africanum* and *Bauhinia petersiana* showed a weak positive correlation in low concentration. A strong positive correlation was observed between *Syzygium guineense* and B in low concentration while *Senegalia polyacantha* and *Bauhinia thonningii* positively correlated with B in low concentration. *Senna singueana* and *Terminalia mollis* showed positive correlation to B in high concentration. *Senegalia polyacantha, Bauhinia petersiana* and *Bauhinia thonningii* showed positive correlation with Ba in high concentrations while *Terminalia mollis, Ficus sycomorus, Ficus craterostoma* and *Rhus longipes* showed a positive correlation to Ba in low concentration. *Diospyros mespiliformis, Rhus longipes, Ficus sycomorus* and *Terminalia mollis* positively correlated with Mo in high concentration while *Senegalia polyacantha, Peltophorum africanum* and *Bauhinia petersiana* showed a weak positive correlation in low concentrations. *Senegalia* *polyacantha, Syzygium guineense, Senna singueana* and *Bauhinia thonningii* positively correlated with Al in low concentration while *Terminalia mollis* showed a weak positive correlation with Al in high concentration. *Rhus longipes, Ficus craterostoma* and *Diospyros mespiliformis* showed a positive correlation with S in high concentration while *Bauhinia petersiana, Peltophorum africanum, Bauhinia thonningii* and *Senegalia polyacantha* showed a positive correlation in low concentration.

According to the Monte Carlo permutation tests results (F = 0.1, p = 1), no significant differences of the heavy metals were observed indicating no influence of heavy metals on species richness and abundance.

In TD24 (Luanshya), the CCA results show (figure 4.2C) that, *Combretum molle, Albizia antunesiana, Brysorcapus orientalis, Terminalia mollis, Albizia versicolor, Combretum zeyheri* and *Ficus craterostoma* showed a positive correlation to Cu in low concentration while other species showed a negative correlation with Cu with *Dichrostachys cinerea* showing a weak positive correlation in high concentration (see appendix 4). *Albizia antunesiana, Combretum molle, Combretum zeyheri, Lannea discolor* and *Albizia adianthifolia* showed a positive correlation with Mn in low concentration while the other species showed negative correlation to Mn. Equally, *Combretum zeyheri, Albizia adianthifolia, Bauhinia thonningii, Bauhinia petersiana* and *Combretum molle* positively correlated with Zn in low concentrations. *Ficus sycomorus, Albizia adianthifolia, Senegalia polyacantha, Bauhinia petersiana* and *Syzygium guineense* positively correlated with Ni in low concentration with *Dichrostachys cinerea* showing a similar trend as that of Cu. *Lannea discolor, Syzygium guineense, Albizia antunesiana, Bauhinia petersiana* and *Senegalia polyacantha* showed positive correlation with Cr in low concentrations while *Dichrostachys cinerea* showed a positive correlation with Cr in high concentration. *Bauhinia thonningii, Lannea discolor, Albizia adianthifolia* and *Syzygium guineense* and *Bauhinia petersiana* positively correlated with Co in low concentrations. *Dichrostachys cinerea* showed a similar trend of positive correlation in high concentration for Co. *Syzygium guineense, Combretum microphyllum* and *Bauhinia thonningii* showed a positive correlation to Cd in low concentrations with *Dichrostachys cinerea* showing a weak positive correlation in high concentration. *Senegalia polyacantha, Ficus sycomorus, Albizia versicolor, Bauhinia petersiana* and *Albizia antunesiana* positively correlated with B in low concentrations. Similarly, *Lannea discolor, Bauhinia thonningii, Bauhinia petersiana and Senegalia polyacantha* showed a positive correlation to Ba in low concentration while Lannea *discolor, Senna singueana, Albizia antunesiana, Brysorcapus orientalis* and *Peltophorum africanum* strongly correlated with Mo in low concentrations with *Dichrostachys cinerea* showing a weak positive correlation in high concentration. *Albizia antunesiana, Bauhinia petersiana, Lannea discolor* and *Albizia adianthifolia* positively correlated with Al in low concentrations while *Combretum molle, Bauhinia thonningii, Combretum zeyheri* and *Albizia adianthifolia* positively correlated with S in low concentration. Interestingly, *Dichrostachys cinerea* showed no positive correlation with any metal in low concentrations nor other species. The Monte Carlo tests showed no significant differences on all heavy metals on all axis ($F = 0.6$, $p = 0.198$).

The CCA results for TD25 (Luanshya) (Figure 4.2D) show that *Bauhinia thonningii, Dichrostachys cinerea, Senna singueana, Dodonaea viscosa, Ficus craterostoma* and *Terminalia mollis* positively correlated with Cu in low concentrations. A weak positive correlation between *Albizia amara* and Cu was observed in high concentrations (appendix 5). The other species showed negative correlation to Cu with *Combretum molle, Combretum apiculatum, Albizia versicolor* and *Dombeya rotundifolia* showing a strong negative correlation. A similar trend was observed for Mn with *Dodonaea viscosa* and *Syzygium guineense* as additional species showing a positive correlation with Mn in low concentration. Only *Ficus craterostoma, Bauhinia thonningii, Dichrostachys cinerea, Peltophorum africanum* and *Senna singueana* positively correlated with Zn in low concentrations, the other species showed negative correlations with *Dombeya rotundifolia, Combretum molle, Combretum apiculatum* and *Albizia versicolor* showing strong negative correlation. *Rhus longipes, Senna singueana, Peltophorum africanum, Dichrostachys cinerea, Annona senegalensis and Ficus craterostoma* are observed to positively correlate with Ni in low concentrations while *Combretum molle, Combretum apiculatum, Dombeya rotundifolia* and *Albizia versicolor* showed a weak positive correlation with Ni in high concentrations. Species that showed correlation with Cr in low concentration are *Rhus longipes*, *Peltophorum africanum, Lannea discolor, Senna singueana, Senegalia polyacantha, Annona senegalensis* and *Ficus craterostoma*. *Terminalia mollis, Senna singueana, Dichrostachys cinerea, Syzygium guineense* and *Bauhinia thonningii* positively correlated with Co in low concentrations while the rest of the species showed negative correlation with Co with *Combretum molle, Combretum apiculatum, Albizia versicolor* and *Dombeya rotundifolia* showing a strong negative correlation. *Rhus longipes, Dodonaea viscosa, Dichrostachys* *cinerea, Terminalia mollis* and *Syzygium guineense* positively correlated with Cd in low concentrations while *Albizia amara* and *Bauhinia thonningii* showed a positive correlation in high concentrations. *Rhus longipes, Dichrostachys cinerea, Senegalia polyacantha, Senna singueana, Ficus craterostoma, Lannea discolor* and *Annona senegalensis* showed a positive correlation with B in low concentrations. *Combretum molle, Combretum apiculatum, Dombeya rotundifolia* and *Albizia versicolor* showed a similar trend of weak positive correlation in high concentration with B, Mo and Al. The other species showed negative correlation with B with *Albizia amara* showing a strong negative correlation. *Senna singueana, Terminalia mollis, Ficus craterostoma, Annona senegalensis* and *Bauhinia thonningii* showed positive correlation with Ba in low concentration while the other species showed negative correlation. *Bauhinia thonningii, Dodonaea viscosa, Terminalia mollis, Dichrostachys cinerea* and *Senna singueana* showed a positive correlation with Mo in low concentrations with *Annona senegalensis* showing a weak correlation with Mo in low concentration. *Rhus longipes, Peltophorum africanum, Dichrostachys cinerea, Lannea discolor, Ficus craterostoma* and *Senegalia polyacantha* showed a positive correlation to Al in low concentrations while the other species showed negative correlation to Al. *Terminalia mollis, Senna singueana, Syzygium guineense* and *Bauhinia thonningii* positively correlated with S in low concentrations while *Albizia amara* showed a positive correlation to S in high concentrations. The other species showed negative correlation to S. *Dichrostachys cinerea* showed positive correlations in low concentrations unlike the other site (TD24 Luanshya and TD25 Kitwe). Interestingly, Albizia amara showed positive correlations in high concentration to Cu, Mn and S than in the other sites.

According to the Monte Carlo tests on all the heavy metals, there were no significant differences ($F = 0.9$, $p = 0.556$) however, individual metal analysis showed that Mn, Cr and Al significantly influenced species richness and abundance in TD25 ($F = 1.9$, $p = 0.018$; $F = 1.7$, $p = 0.048$; $F = 2.2$, $p = 0.008$).

TD26 (Luanshya) CCA results show that *Ozoroa insignis* had a strong positive correlation to Cu in low concentrations (Figure 4.3A). Equally, *Senegalia polyacantha, Lannea discolor, Peltophorum africanum* and *Ficus craterostoma* positively correlated with Cu in low concentrations with *Bauhinia petersiana* showing a weak positive correlation in low concentration (see appendix 6). *Rhus longipes, Bauhinia thonningii, Phyllanthus guineensis* and *Albizia antunesiana* showed a positive correlation to Cu in high concentrations. *Ozoroa insignis, Peltophorum africanum, Terminalia stenostachya* and *Albizia versicolor* showed a positive correlation with Mn in low concentration while *Bauhinia thonningii, Rhus longipes* and *Albizia antunesiana* showed a positive weak correlation to Mn in high concentration. *Ficus craterostoma, Vachelia sieberiana, Lannea discolor* and *Senegalia polyacantha* strongly (positively)correlated with Zn in low concentrations while *Phyllanthus guineensis* showed a positive correlation with Zn in high concentration. *Ozoroa insignis* was observed to strongly correlate with Ni in low concentration while *Vachelia sieberiana, Senegalia polyacantha, Lannea discolor* and *Ficus craterostoma* equally positively correlated with Ni in low concentration. *Phyllanthus guineensis* showed a similar trend as that of Zn while the other species showed a negative correlation to Zn. *Lannea discolor, Terminalia mollis, Syzygium guineense, Terminalia stenostachya, Albizia versicolor, Ficus craterostoma, Senegalia polyacantha,* and *Vachelia sieberiana* positively correlated to Cr in low concentrations with *Albizia versicolor* showing a strongest correlation. Only *Lannea discolor* and *Ozoroa insignis* showed a positive correlation to Cr in high concentration. *Ozoroa insignis, Terminalia mollis, Senna singueana, Lannea discolor, Senegalia polyacantha, Vachelia sieberiana, Terminalia stenostachya* and *Albizia versicolor* showed a strong positive correlation to Co in low concentration while the other species showed no correlation. *Terminalia mollis* strongly correlated to Cd in low concentration while *Senegalia polyacantha, Syzygium guineense, Albizia versicolor, Dodonaea viscosa, Bauhinia petersiana* and *Terminalia stenostachya* positively correlated with Cd in low concentration. *Albizia antunesiana, Rhus longipes* and *Bauhinia thonningii* showed a weak positive correlation to Cd in high concentrations. *Lannea discolor, Ozoroa insignis, Ficus craterostoma, Vachelia sieberiana* and *Senegalia polyacantha* positively correlated with B in low concentrations while *Phyllanthus guineensis* showed a positive correlation with B in high concentration. A similar trend of correlation for B was observed with Ba with *Terminalia mollis, Senna singueana* and *Syzygium guineense* adding to the species that positively correlated to Ba in low concentration. *Rhus longipes, Bauhinia thonningii* and *Albizia antunesiana* showed the consistent trend of a weak correlation in high concentrations to Ba just like the other metals. *Syzygium guineense, Terminalia mollis* and Lannea discolor strongly correlated with Mo in low concentrations while *Ozoroa insignis, Albizia versicolor, Senegalia polyacantha* and *Vachelia sieberiana* positively correlated with Mo in high concentrations. *Senegalia polyacantha, Terminalia mollis* and *Senna singueana* were observed to positively correlate to Al in low concentration while *Ozoroa insignis, Albizia versicolor, Lannea discolor, Vachelia sieberiana* and *Syzygium guineense* showed a positive correlation with Al in high concentrations. *Syzygium guineense, Vachelia sieberiana,* *Terminalia mollis*, *Lannea discolor*, *Ozoroa insignis* and *Bauhinia petersiana* positively correlated with S in low concentration while *Albizia versicolor*, *Rhus longipes*, *Albizia antunesiana*, *Bauhinia thonningii* and *Phyllanthus guineensis* showed a positive correlation with S in high concentration. Generally, *Rhus longipes*, *Bauhinia thonningii*, *Phyllanthus guineensis* and *Albizia antunesiana* showed positive correlations to metals in high concentration in this site.

The monte Carlo test results for all heavy metals shows no significant influence ($F = 1.2$, $p = 0.288$) of heavy metals on species richness and abundance, however, the individual element analysis shows that Cr ($F =2.0$, $p = 0.018$) significantly influence species richness and abundance on TD26.

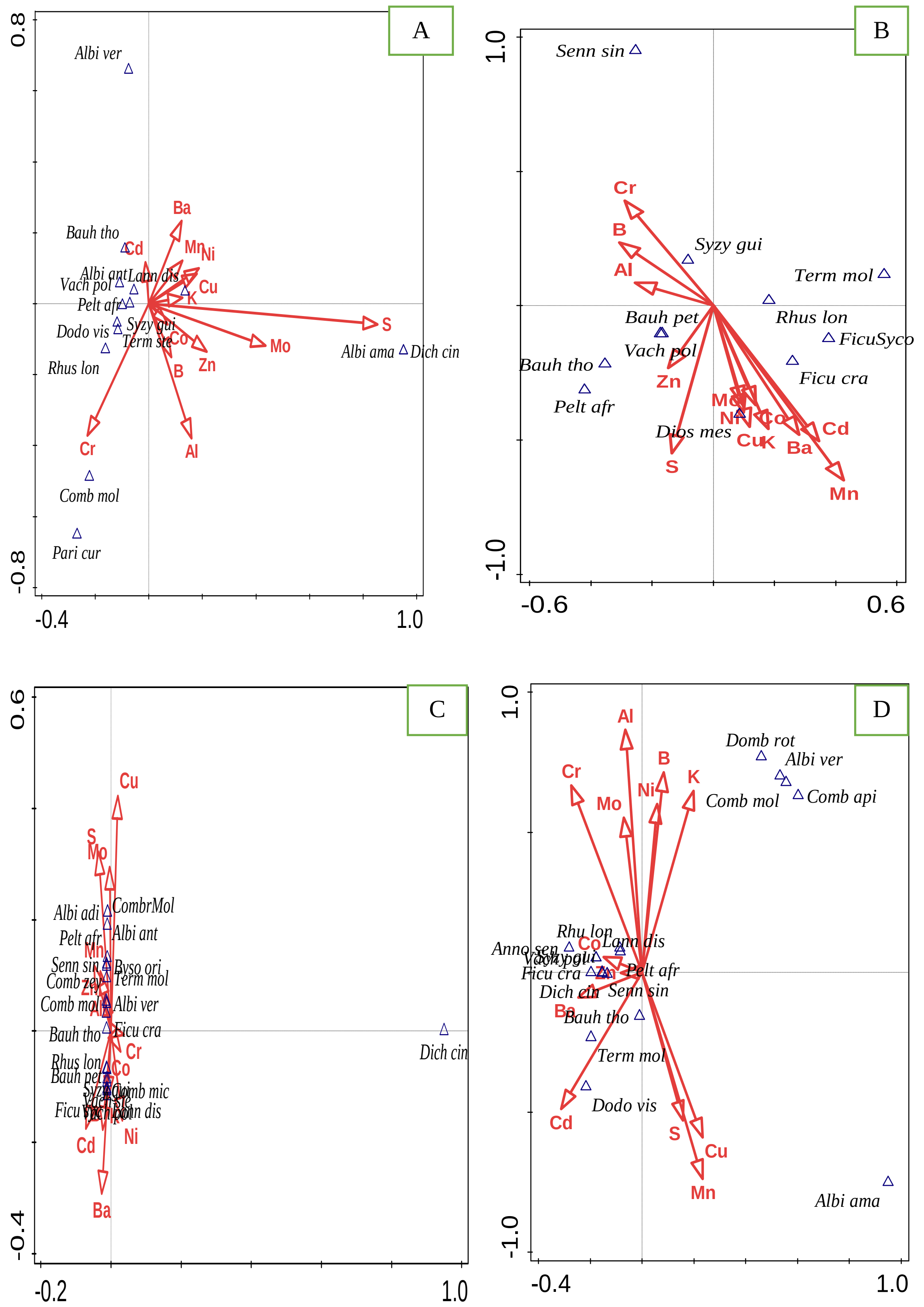

Figure 4.2: Ordination diagram showing CCA analysis results of heavy metals and species on TD25 (Kitwe) A, TD26 (Kitwe) B, TD24 (Luanshya) C, TD25 (Luanshya) D

In TD8 (Mufulira), *Dichrostachys cinerea*, *Senegalia polyacantha*, *Ficus capensis* and *Albizia antunesiana* showed a positive correlation with Cu in low concentration while and *Rhus longipes, Annona senegalensis and Terminalia stenostachya* positively correlated with Cu in high concentration (Figure 4.3B). The other species showed a negative correlation to Cu. (see appendix 7). *Rhus longipes*, *Ficus craterostoma* and *Combretum microphyllum* showed a positive correlation with Mn in low concentration while *Bauhinia petersiana* and *Senegalia polyacantha* showed a weak correlation in high concentrations. Only *Ficus sycomorus* showed a strong correlation to Zn in low concentration. *Ficus craterostoma* and *Combretum microphyllum* showed a weak correlation in low concentration. On the other hand, *Ficus capensis* and *Albizia antunesiana* showed a correlation with Zn in high concentration. *Annona senegalensis*, *Combretum microphyllum*, *Ficus craterostoma* and *Rhus longipes* showed correlation with Ni in low concentration while *Ficus sycomorus* and *Bauhinia petersiana* showed a weak correlation to Ni in high concentrations. The other species showed a negative correlation with Ni. *Ficus sycomorus* showed a strong correlation to Cr in low concentration with *Annona senegalensis*, *Terminalia mollis*, *Combretum microphyllum*, *Combretum molle*, *Combretum zeyheri* and *Ficus craterostoma* showing a positive correlation with Cr in low concentration. *Dichrostachys cinerea*, *Bauhinia petersiana* and *Senegalia polyacantha* showed a positive correlation with Cr in high concentration while the other species showed negative correlation to Cr. *Ficus capensis*, *Albizia antunesiana* and *Senegalia polyacantha* showed a positive correlation to Co in high concentrations while *Ficus craterostoma*, *Syzygium guineense*, *Combretum microphyllum* and *Terminalia stenostachya* showed a positive correlation with Co in low concentrations. *Combretum zeyheri*, *Rhus longipes*, *Terminalia stenostachya*, *Terminalia mollis* and *Combretum molle* showed a positive correlation with Cd in low concentrations while *Azanza garckeana* and *Albizia antunesiana* showed a weak positive correlation in high concentration. *Ficus craterostoma*, *Ficus sycomorus*, *Rhus longipes*, *Annona senegalensis*, *Syzygium guineense* and *Combretum microphyllum* showed a positive correlation to B in low concentrations while *Ficus capensis*, *Albizia antunesiana*, *Bauhinia petersiana*, *Dichrostachys cinerea* and *Senegalia polyacantha* showed a weak positive correlation with B in high concentrations. *Annona senegalensis* positively correlated with Ba in low concentration while *Albizia antunesiana* positively correlated with Ba in even lower concentration. *Terminalia mollis*, *Combretum zeyheri*, *Combretum molle*, *Rhus longipes* and *Terminalia stenostachya* showed a positive correlation with Ba in high concentration. *Ficus capensis*, *Ficus sycomorus*, *Bauhinia petersiana*, *Albizia antunesiana* and *Annona* *senegalensis* showed a positive strong correlation to Mo in low concentration while *Terminalia stenostachya, Senegalia polyacantha, Dichrostachys cinerea* and *Syzygium guineense* showed a positive correlation with Mo in high concentration. *Ficus sycomorus* showed a strong correlation to Al in low concentration while *Annona senegalensis, Ficus craterostoma* and *Combretum microphyllum* positively correlated with Al in low concentration. Similarly, *Ficus capensis, Albizia antunesiana, Bauhinia petersiana, Senegalia polyacantha* and *Dichrostachys cinerea* showed positive correlation to Al in high concentration. *Combretum zeyheri, Combretum molle, Terminalia mollis, Terminalia stenostachya, Azanza garckeana* and *Rhus longipes* positively correlated with S in low concentration with *Azanza garckeana* showing a weak correlation. *Albizia antunesiana* and *Ficus capensis* however showed a weak positive correlation with S in high concentration.

The Monte Carlo test results ($p = 0.05$), show no statistical significance in heavy metal influence of species richness and abundance ($F = 1.3$, $p = 0.066$). However, the individual heavy metal analysis show that Mo was significantly different ($F = 1.8$, $p = 0.004$) indicating its influence on species richness and abundance in TD8 (Mufulira).

The CCA results for TD10 (Mufulira) indicate that *Rhus longipes, Phyllanthus guineensis, Ficus craterostoma* and *Syzygium guineense* positively correlated with Cu in high concentration while *Combretum molle, Albizia amara, Bauhinia petersiana,* and *Combretum microphyllum* positively correlated with Cu in low concentration (Figure 4.3C). *Ficus sycomorus, Rhus longipes, Combretum microphyllum, Bauhinia petersiana, combretum molle* and *Albizia amara* positively correlated with Mn in low concentration while *Albizia antunesiana, Combretum molle, Dombeya rotundifolia* and *Senegalia polyacantha* positively correlated with Mn in high concentrations (Appendix 8). *Rhus longipes, Phyllanthus guineensis, Combretum molle* and *Albizia amara* positively correlated with Zn in low concentrations while *Combretum microphyllum, Bauhinia petersiana* and *Senegalia polyacantha* positively correlated with Zn in high concentrations. The other species showed no correlation to Zn. *Ficus sycomorus, Combretum molle, Bauhinia petersiana, Rhus longipes, Combretum microphyllum* and *Albizia amara* positively correlated with Ni in low concentrations while *Senegalia polyacantha* and *Albizia antunesiana* positively correlated with Ni in high concentrations. The other species showed negative correlation. Cr was observed to be positively correlated to *Combretum microphyllum, Rhus longipes, Combretum molle, Bauhinia petersiana* and *Albizia amara* in low concentrations while *Senegalia polyacantha,* *Lannea discolor* and *Dombeya rotundifolia* showed a positive correlation in high concentration. *Rhus longipes, Phyllanthus guineensis, Bauhinia thonningii* and *Combretum molle* were observed to positively correlate with Co in low concentration while *Annona senegalensis, Lannea discolor, Dombeya rotundifolia* and *Terminalia mollis* showed a positive correlation in high concentrations. *Ficus sycomorus* showed a strong correlation with Cd in low concentration with *Senna singueana, Combretum microphyllum, Syzygium guineense* and *Ficus craterostoma* showing a positive correlation in low concentration too. *Senegalia polyacantha, Annona senegalensis, Combretum molle, Combretum zeyheri* and *Albizia antunesiana* showed a positive correlation with Cd in high concentration. *Bauhinia petersiana* showed a strong positive correlation to B in low concentration with *Phyllanthus guineensis, Combretum microphyllum, Combretum molle* and *Albizia amara* also positively correlating with B in low concentration. *Senegalia polyacantha, Dombeya rotundifolia* and *Lannea discolor* showed a positive correlation to B in high concentration. *Rhus longipes, Syzygium guineense, Bauhinia petersiana* and *Combretum molle* positively correlated with Ba in low concentration while *Senegalia polyacantha, Ficus sycomorus* and *Albizia antunesiana* positively correlated with Ba in high concentration. *Phyllanthus guineensis, Bauhinia petersiana, Rhus longipes, Combretum microphyllum, Combretum molle* and *Albizia amara* positively correlated with Mo in low concentration while *Dombeya rotundifolia* and *Lannea discolor* showed a positive correlation with Mo in high concentration. Al showed positive correlation with *Phyllanthus guineensis, Combretum microphyllum, Combretum molle* and *Albizia amara* in low concentration while Ficus *sycomorus* showed positive correlation to Al in high concentration. *Phyllanthus guineensis, Syzygium guineense, Senna singueana, Ficus craterostoma* and *Annona senegalensis* were observed to positively correlate with S in low concentration while *Bauhinia petersiana, Combretum molle, Rhus longipes* and *Combretum microphyllum* were observed to positively correlate with S in high concentrations.

The Monte Carlo tests results ($F = 1.3$, $p = 0.214$) for all the metals shows no significant influence of heavy metals on species richness and abundance, however individual metal analysis results (see appendix 9) show that Cu ($F = 2.2$, $p = 0.002$) and S ($F = 1.7$, $p = 0.02$) significantly influence species richness and abundance on TD10.

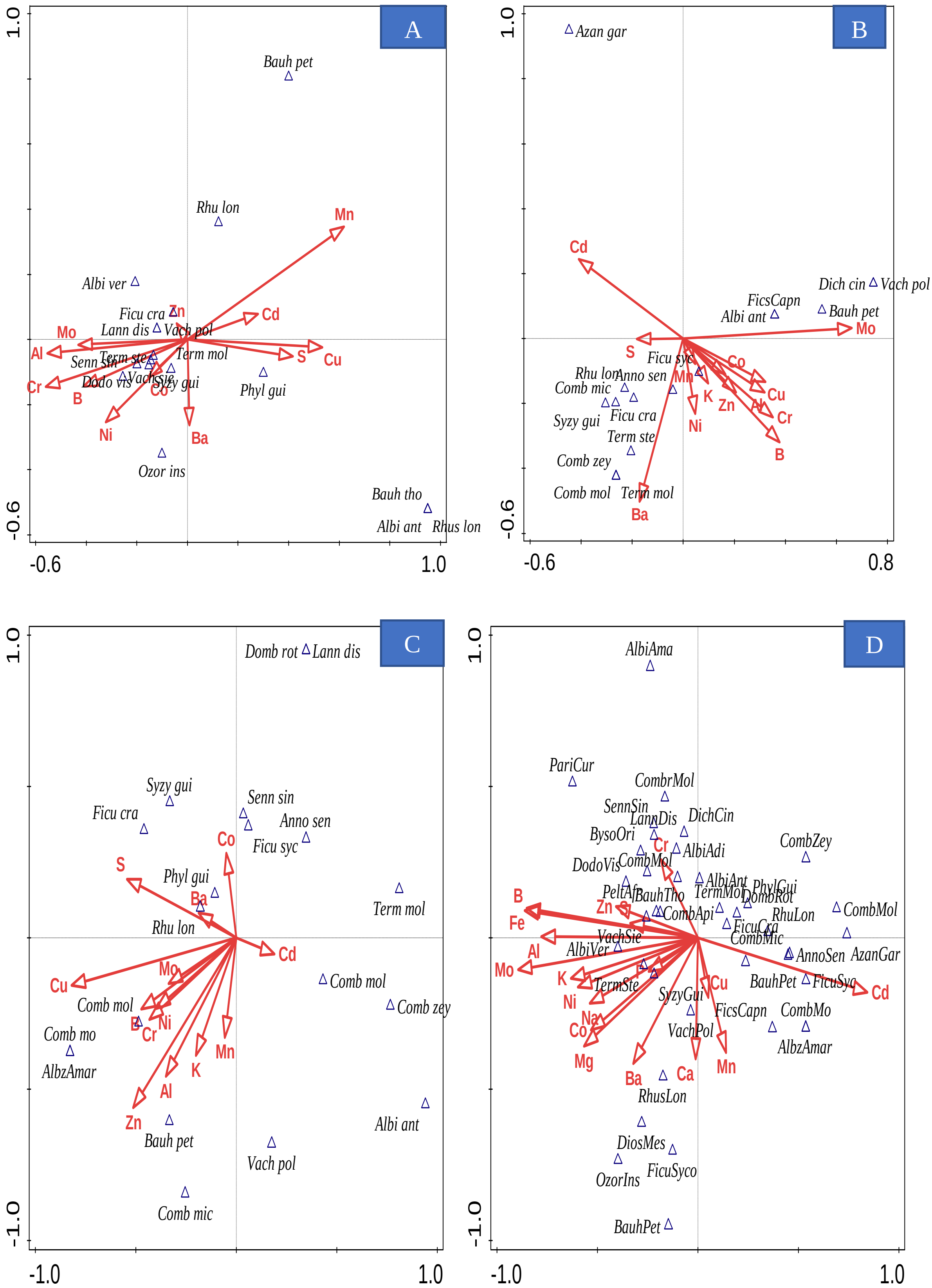

Figure 4.3: Ordination diagram showing CCA analysis results of heavy metals and species on TD26 (Luanshya) A, TD8(Mufulira) B, TD10 (Mufulira) C, CCA overall analysis results of heavy metals and tree species (D).

The pooled CCA results of all the Copperbelt sites (Figure 4.3 D) show that tree species correlate with different heavy metals differently. Generally, *Senegalia polyacantha, Ficus craterostoma, Combretum molle, Bauhinia petersiana, Parinari curatellifolia, Senna singueana* and *Albizia versicolor* positively correlated with Cu in low concentrations while *Rhus longipes, Albizia antunesiana, Dichrostachys cinerea* and *Phyllanthus guineensis* positively correlated with Cu in high concentrations. *Bauhinia petersiana, Albizia versicolor, Albizia adianthifolia, Dodonaea viscosa, Combretum microphyllum, Lannea discolor* and *Albizia antunesiana* were observed to positively correlate with Mn in low concentrations while *Rhus longipes, Albizia antunesiana, Senegalia polyacantha* and *Ficus sycomorus* positively correlated with Mn in high concentration. Zn had a positive correlation with *Bauhinia thonningii, Bauhinia petersiana, Peltophorum africanum, Albizia versicolor, Syzygium guineensis, Senegalia polyacantha, Lannea discolor, Rhus longipes* and *Vachelia sieberiana* in low concentrations while *Dombeya rotundifolia, Combretum apiculatum* and *Albizia versicolor* positively correlated with Zn in high concentration. *Rhus longipes, Ficus craterostoma, Ficus sycomorus* and *Combretum molle* showed positive correlations with Ni in low concentrations while *Dichrostachys cinerea, Combretum apiculatum, Dombeya rotundifolia, Phyllanthus guineensis, Senegalia polyacantha* and *Albizia antunesiana* showed positive correlations to Ni in high concentrations. Additionally, *Combretum molle, Albizia antunesiana, Parinari curatellifolia, Terminalia stenostachya, Syzygium guineense, Senegalia polyacantha* and *Ficus craterostoma* showed positive correlation to Cr in low concentration while *Dombeya rotundifolia* and *Dichrostachys cinerea* showed a positive correlation in high concentration. Similarly, Co positively correlated with *Lannea discolor, Rhus longipes, Bauhinia petersiana* and *Bauhinia thonningii* in low concentrations while *Ficus capensis, Albizia antunesiana, Senegalia polyacantha, Terminalia mollis, Annona senegalensis, Dombeya rotundifolia* and *Lannea discolor* showed positive correlation with Co in high concentration. *Combretum zeyheri, Combretum thonningii, Syzygium guineense, Terminalia mollis, Rhus longipes, Senna singueana, Albizia versicolor, Diospyros mespiliformis* and *Terminalia stenostachya* positively correlated with Cd in low concentration while *Albizia antunesiana, Senegalia polyacantha, Rhus longipes, Albizia amara* and *Azanza garckeana* showed positive correlation with Cd in high concentration. Correspondingly, *Peltophorum africanum, Combretum molle, Ficus craterostoma, Rhus longipes* and *Bauhinia thonningii* showed positive correlation with B in low concentrations while *Senegalia polyacantha, Phyllanthus guineensis, Dombeya rotundifolia* and *Lannea discolor positively* correlated with

B in high concentration. Additionally, *Senna singueana*, *Bauhinia thonningii*, *Terminalia mollis* and *Annona Senegalensis* showed positive correlations with Ba in low concentrations while *Rhus longipes* and *Senegalia polyacantha* showed positive correlations to Ba in high concentration. Mo showed positive correlations with *Annona senegalensis, Senna singueana, Bauhinia petersiana, Albizia antunesiana, Dichrostachys cinerea* and *Senegalia polyacantha* in low concentrations while *Terminalia stenostachya, Albizia versicolor* and *Combretum apiculatum* showed positive correlation with Mo in high concentrations. Furthermore, *Syzygium guineense*, *Senna singueana*, *Ficus sycomorus*, *Albizia antunesiana*, *Dodonaea viscosa* and *Senegalia polyacantha* positively correlated with Al in low concentration while *Rhus longipes, Dombeya rotundifolia, Combretum apiculatum and Albizia versicolor* positively correlated with Al in high concentration. Finally, *Ozoroa insignis*, *Syzygium guineense*, *Terminalia mollis, Senna singueana* and *Bauhinia thonningii* positively correlated with S in low concentration while *Rhus longipes*, *Albizia amara* and Dichrostachys cinerea showed positive correlation with S in high concentration. One interesting observation is that *Azanza garckeana* only showed correlation with Cd.

The overall Monte Carlo test at $p = 0.05$ show significant influence of heavy metals on species richness and abundance ($F = 1.4$, $p = 0.002$). The overall CCA analysis showed that Ni, Co, Cd, B, Mo, Al and S (Table 4.4) significantly influence species richness and abundance on Copperbelt tailings dams. Furthermore, the site individual heavy metal analysis show that S ($F = 1.7$, $p = 0.02$), Cu ($F = 2.2$, $p = 0.002$), Cr ($F = 2.0$, $p = 0.018$), Mn ($F = 1.9$, $p = 0.018$), Cr ($F = 1.7$, $p = 0.048$) and Al ($F = 2.2$, $p = 0.008$) (see appendix 9) significantly influenced species richness and abundance. Therefore, Ni, Co, Cd, B, Mo, Al, Cr, Mn and Cu influence species richness and abundance on Copperbelt tailings dams.

Table 4.5: Monte Carlo permutation test results for heavy metals

Heavy metal	F	P
Cu	1.8	0.006
Mn	1.3	0.134
Zn	1.7	0.064
Ni	2.7	0.002
Cr	1.3	0.172
Co	1.9	0.01
Cd	3.4	0.002
B	3.4	0.002
Ba	1.5	0.07
Mo	3.7	0.002
Al	3.1	0.002
S	1.6	0.03

4.4 Discussion

4.4.1 Species abundance

Tree species richness and abundance on Copperbelt tailings dams (seven sites) varied with TD24 (Luanshya) having the highest richness and abundance whilst TD26 (Kitwe) had the lowest richness and abundance. A probable explanation for this could be the variation in physical and chemical properties of the tailings (Ssenku et al. 2014, Yang et al. 2016a), such as the nutrient contents of tailings (Santos et al. 2017) and heavy metal concentrations (Wong 2003; Li 2006) across sites. Overall, this study observed species richness on the dams to be low as compared to species richness in local forests (Festin et al. 2018). Festin et al. (2018) reported 55 species occurring in local forests on the Copperbelt which is higher when compared to our observation. A number of studies have reported lower species richness in contaminated sites than in uncontaminated sites. For example, Dowo et al. (2013) reported eight (8) species in slimes and 21 species in a natural woodland in Zimbabwe. Similarly, Sun et al. (2016) reported 22 plant species on contaminated sites compared to 40 species in uncontaminated areas in China. The lower species richness in contaminated sites compared to uncontaminated sites gives an indication of the restrictive nature of contamination on plant development and survival. Even though species richness was significantly different on the tailings dams, a few individual species colonized most of the dams. *Rhus longipes* and *Syzygium guineense* occurred across sites while *Senegalia polyacantha* and *Ficus craterostoma* occurred in six and five sites, respectively thus indicating their adaptability to tailings dams environment. Such plant species may have the capacity to reduce metal toxicity thereby reducing environmental contamination (Wang et al. (2014).

The one-way Anova results indicated no significant differences in species abundance between sites even though variations in abundance were observed between sites. These results reflect Santos et al. (2017)'s findings which showed variations in species abundance between studied patches. In this study, the site with the highest species richness had the highest abundance and vice versa. This suggest a possible relationship between species richness and abundance and the environmental variables of a site such as heavy metals. Additionally, the age of the tailings dams could have a bearing on the species richness and abundance. A study by Arshi (2017) reported high species richness on old overburden dumps than the young dumps. This study confirms the results that species richness and abundance on tailings dams is associated with the age of the dam as the site with the highest species richness and abundance is one of the oldest dams on the Copperbelt.

Simon (1978) suggests that vegetation growth on metal contaminated sites depends on metal soil mobility, availability of metals to plants and the species ability to develop mechanisms for metal tolerance. These factors determine the vegetation structure and growth of plants on contaminated sites like tailings dams (Dumba 2013). Soil amendments applied to mine tailings enhance "the quality of the soil for plant establishment and growth" (Zanuzzi et al. 2009). Soil amendments increase the pH of a soil (Yang et al. 2016a), bioimmobilize heavy metals (Yang et al. 2016b) and reduce heavy metal bioavailability (Zhang et al. 2013) thereby increasing vegetation establishment, species richness and abundance on the sites. According to Yang et al. (2017), amended Cu tailings had vegetation cover established on them within 6 months with an enhanced species composition. The findings of this study broadly support the work of other studies that show that soil amendments enhance vegetation establishment and growth on tailings dams. Soil amendments could also be responsible for the patchy pattern of vegetation cover observed on the tailings dams as it varied from tailings dam to tailings dams. Santos et al. (2017) observed a similar pattern of vegetation establishment on Nacozari mine tailings in Mexico. Tree species were observed on the walls of the tailings dams which are given priority when amendments programs are planned to reduce environmental contamination. Species abundance shows how well a species functions in an ecosystem in relation to other species. Species with high abundance are well adapted and can reproduce in an area. Verberk et al. (2012) suggested that species abundance shows the common and uncommon species in an area which is key for conservation and management purposes. Abundant species on Copperbelt tailings dams suggest good growth performance and could be considered for application in phytoremediation programs. The abundance of various plant species on Copperbelt tailings dams suggests that such plant species have evolved metal tolerance mechanisms (Mapaure et al 2008).

Tree species occurring on Copperbelt tailings, occur in natural forests (Festin et al. 2018). It can therefore be suggested that dominant species (Table 4.4) on tailings dams could be used in phytoremediation programs as such plant species are readily available on both the TSFs and the natural forests.

4.4.2 Metal concentrations on Copperbelt tailings dams

Tailings are characterized by high heavy metal contents and left-over chemicals, low nutrient and water holding capacity, high salinity or acidity and poor physical structure (Wong 2003; Dumba 2013; Yang et al. 2016a; Festin et al. 2018). The current study observed high concentrations of heavy metals across tailings dams. This confirms the results of other studies (Festin et al. 2018, Chileshe et al. (2019) within the Copperbelt Province of Zambia. High levels of heavy metals were observed on TSFs compared to heavy metal concentrations in local forests (Chileshe et al 2019). Low heavy metal concentration in local forests shows lack of pollution or contamination which could be attributed to the distance between the local forests and the sources of pollution (tailings). Lupupa (2011) reported high metal concentrations in areas near Nkana copper smelter in Kitwe. Studies in Namibia (Mapaure et al. 2008; Dumba 2013), Ghana (Fosu-Mensah et al. 2017) reported high heavy metal concentrations in areas close to wastelands or sources of contamination. Soils contain low levels of heavy metals in their natural state which are essential for plants and other soil organisms (Wuana and Okieimen 2011) hence the observed low levels in natural forest. Dumba (2013) reported a similar trend of high metal concentrations in areas close to Kombat mine tailings dump in Namibia. Similarly, Li and Yang (2008) reported high metal concentrations on a manganese mine wasteland when compared to the "China Environmental Quality Standard for Soils" as the wasteland soils showed high pollution index. Additionally, Sun et al. (2016) reported high heavy metal concentrations on the abandoned mine sites when compared to the Environmental Quality Standard for Soils in China. Due to lack of guidelines for heavy metal concentration limits in soils in Zambia, the screening benchmark and Canadian council of ministers of the environment remediation criteria (CCMERC) were used (Efroymson 1997) (see appendix 10). The mean Cu concentration of the Copperbelt tailings dams exceeded the screening benchmark limit (appendix 10). The Cu concentration on tailings was over 100 times higher than the screening benchmark. The same trend was observed for Mn, Zn, Cr, Co, Ni, B, Ba, Mo and Al. High differences were observed in Al, Cr, B and Co while low differences were observed between Ba and Zn. Cd concentrations on the tailings dams was lower than the screening benchmark. The benchmark does not provide limits for S. The comparison between metal concentrations on tailings dams and the CCMERC limits revealed a different trend. Ba on tailings dams was only 1.25 times more than the CCMERC guideline. Similarly, Ni was 1.3 times more on tailings dams than the CCMERC guideline. Average Cd, Cr and Zn concentrations were lower than the CCMERC guidelines (Efroymson 1997). Cu concentrations on tailings was over 100 times more than the CCMERC guidelines while Mo was 11 times higher on dams than the guidelines. Similarly, Co on dams was over 26 times more than the guideline. Tailings are naturally high in heavy metals and leftover chemicals (Wong 2003, Kossoff et al. 2014) hence the high metal concentrations.

The current study recorded significant differences in heavy metal concentrations between the studied sites (tailings dams). The variations were observed along the different sites and different heavy metals. These results reflect Festin et al. (2018) and Chileshe et al. (2019)'s results who reported high heavy metal concentrations on tailings compared to heavy metal concentrations in local forests. The average Cu, Zn, Ni, Co, Ba and Cr concentrations of this study were relatively higher than the ones reported by Chileshe et al. (2019) except Cd, which was lower, this could be due to site variations. Overall, average Al concentration was the highest with Cd being the lowest. Heavy metal concentrations on Copperbelt tailings dams were in the order Al > Cu > Mn > S > Co > Ba > Cr > B > Zn > Ni > Mo > Cd which is contrary to Chileshe et al. (2019)'s pollution order. Furthermore, significant differences in some metal concentrations between transects of a site were observed. This finding accords with previous observations by Anawar et al. (2013) elsewhere which showed different metal concentrations on various sites. A probable explanation for the variation could be the type and efficiency of the technology employed in the ore processing and the ore grade (Cooke and Johnson 2002). The current study recorded high heavy metal concentrationss when compared to the threshold given by the screening benchmark and CCMERC guidelines (Efroymson 1997) indicating possible effects on species richness and abundance on the tailings dams. The results of this study therefore suggest that heavy metal concentrations affect plant establishment and growth owing to the low species richness and abundance on the dams when compared to species richness in local forests.

4.4.3 Influence of heavy metals on species abundance

The CCA results show variations in responses of tree species to heavy metal concentration across sites. For example, *Terminalia mollis* positively correlated with Cu in low concentration in TD24 (Luanshya) but positively correlated with Cu in high concentration in TD8 (Mufulira). A positive correlation entails a heavy metal's influence on a species while negative correlation means there is no relationship or influence of the heavy metal on a species. In this study, species that showed positive correlations to heavy metals in high concentrations are most likely to perform better in high concentration and vice versa. This trend was observed on all of the tree species with the exception of *Azanza garckeana* that only positively correlated with Cd in TD8 (Mufulira). This may be attributed to variations in metal concentration across sites and other variables that were not considered in this study. Factors such as bioavailability and soil mobility of metals may influence vegetation establishment on contaminated sites (Simon 1978). The interaction between heavy metals and plants depend on heavy metal solubility and reactivity with inorganic and organic molecules of such plant species (Singh and Sinha 2005). Additionally, metal mobility may be "influenced by the soil pH and organic matter" (Šalamún et al. 2015). The variation in these variables may explain the difference in responses of tree species to the same metal in different sites.

High heavy metal concentration in soils have been reported to affect species richness and abundance in different areas (Dumba 2013). Mapaure et al. (2008) reported that heavy metal contamination at Kombat mine influenced vegetation structure and richness on the site. Heavy metals are essential for plant establishment and growth in low concentrations however, they become toxic and inhibit plant growth in high concentrations, due to disruptions in cell functions (Festin et al. 2018). This is line with (Wong 2003; Li 2006)'s suggestion that heavy metals affect plant growth on mine tailings. Lewis et al (2001) reported that excess concentrations of Cu cause stress and plant injuries resulting in chlorosis and plant growth retardation. Similarly, Das et al. (1997) reported that, Cd influences the uptake and transportation of water and vital elements such as Mg, P, K and Ca in low concentrations but when in high concentrations (above 0.05 ug/g or 0.05 mg/kg), Cd causes "chlorosis, root tip browning, growth inhibition and death" (Di Toppi et al. 1999). In high concentrations, it is established that Cr is harmful to plant growth and development due to its effect on enzyme activities, photosynthesis and electron transport (Ugulu 2015). Furthermore, Ni toxicity has been reported to cause chlorosis and necrosis (death of cells due to injury or disease) (Rahman et al. 2005) while Mn toxicity is related to crinkle leaf (plant disease that causes leaf wrinkling and distortion), chlorosis and reduced photosynthetic rates (Kitao et al. 1997).

A notable observation is the correlation of tree species to Cd in low concentrations. The results show that most tree species thrived in lower concentrations of Cd than high concentration. Cd had the lowest metal concentration on all sites with the average in negative. This suggests that, in high concentrations of Cd, the tree species would perform poorly hence affecting species richness and abundance on a site. Most tree species generally showed correlation to Cr in low concentration indicating their optimum performance in low concentration of Cr with species such as *Dombeya rotundifolia*, *Lannea discolor* and *Senegalia polyacantha* showing a correlation in high concentration. Cr being of significant influence on species richness and abundance explains the low richness on tailings dams. Cr concentrations on the sites are high with tree species showing correlation in low concentration, the results suggest a negative influence on species richness and abundance by Cr. The results accord previous observations by Šalamún et al. (2015) which showed a reduction in nematode richness and abundance in high concentrations of Cr. A similar trend was exhibited by species for Cu, Mn, Ni, B, Mo, Al and S. Tree species showed a correlation with metals in low concentration with a few species showing correlation in high concentration. The high concentration of these metals on the tailings dams suggests poor richness and abundance as they significantly influence species richness and abundance.

These results could explain the low species richness (32) on tailings dams compared to local forests (55 species) on the Copperbelt (Festin et al. 2018). A similar observation was made by Nunes (2007) on sites close to Tsumeb Copper Smelter compared to non-contaminated sites species richness. High concentration of Cu inhibits plant growth and reduce biomass development in a number of plant species (Yruela 2005). The results of this study suggest low species richness and abundance in high heavy metal concentration and high species richness and abundance in low concentration of the metals due to the high correlation of tree species with metals in low concentration. Heavy metal concentrations in contaminated soils tend to impact on the population of soil organisms such as nematodes that tend to influence the soil quality (Šalamún et al. 2015). A few plant species have the ability to survive in heavy metal contaminated soils hence the reason for low species richness on Cu contaminated soils (Opaluwa et al. 2012). This is due to Cu's ability to be locked in soil or the environment and its ability to accumulate in plants and animals (Wuana and Okieimen 2011). This was especially the case in the current study where there were high species richness and abundance in TD24(Luanshya) compared to TD26 (Kitwe) which had the lowest species richness and abundance. TD26 had higher Cu, Mn, Co, Ba and S than TD24. These results are in line with Šalamún et al. (2015)'s findings of Cu having a positive impact on species richness and abundance in low concentration and a negative one in high concentration

The study results also suggest that heavy metals influence on species abundance varies from metal to metal. Univariately, some heavy metals (Ni, Co, Cd, B, Mo) did not show significant influence on species abundance (see appendix 12) while in a multivariate analysis, the metals showed significance influence. The site CCA analysis results (Appendix 2-8) show the specific metals that influence species per site.

Species like *Rhus longipes* that was observed to perform well as it had the highest IVI thrives in high heavy metal concentration hence its dominancy.

The results shows high abundance of *Rhus longipes, Dichrostachys cinerea, Albizia antunesiana, Senegalia polyacantha, Dombeya rotundifolia, Albizia versicolor, Albizia amara* and *Phyllanthus guineensis* in sites with high concentration of Cu, Mn, Zn, Ni, Cr, Co, B, Ba, Mo, Al and S while other species exhibited high abundance in low concentration of Cu, Mn, Zn, Ni, Cr, Co, B, Ba, Mo, Al and S. This suggests variations in the tree specie's ability of to tolerate heavy metal concentrations. Dumba (2013) demonstrated the ability of plant species to tolerate heavy metals. "Plant species with the ability to tolerate high concentration of heavy metals will have high diversity and abundance on contaminated sites" (Dumba 2013). Therefore, *Rhus longipes*, *Dichrostachys cinerea*, *Albizia antunesiana* and *Phyllanthus guineensis* can be considered to be more adapted to Cu contaminated sites. *Dichrostachys cinerea* has been reported to tolerate high concentration of Cu, Ni, Cr arsenic and lead (Bako et al. 2004). *Dichrostachys cinerea* was described as an encroacher of contaminated sites due to its ability to adapt and establish vegetation cover over contaminated soils (de Klerk 2004).

4.5 Conclusion

Understanding heavy metal influence on species richness and abundance on Copperbelt tailings dams is key to successful implementation of phytoremediation of contaminated soils. The study has provided information important for undertaking phytoremediation of Copper tailings dams. It has categorized plant species based on their relationship with varying concentrations of heavy metals across sites. Two groups of plants based on their diversity and abundance are observed from this study namely, those that thrive well in lower concentrations of heavy metals and those that thrive well in high concentrations of heavy metals. The study has demonstrated that some plant species tolerate low concentrations of heavy metals while other species are more adapted to high concentration of some heavy metals. Therefore, this study gives us an indication of the type of plant species to use in soils with varying heavy metal concentrations. The study also suggests the need to understand the influence of heavy metals on plant species diversity together with other factors such as soil pH and organic matter.

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CHAPTER FIVE: SYNTHESIS OF THE THESIS

Abstract

Mine tailings dam present major environmental problems globally. Restoration of these metalliferous sites via phytostabilization presents a cost effective and efficient way of restoring the sites. Screening native tree species occurring on copper (Cu) tailings dams of the Copperbelt province in Zambia is vital for the restoration of copper tailings dams. The current study evaluated the phytostabilization potential of 32 native tree species from 13 families occurring on the TSFs for phytostabilization potential of Cu, Co, Zn, Al, S, B, Cd, Cr, Ni, Mn and Mo. The study employed the use of the bioconcentration factor (BF) and translocation factor (TF) to determine heavy metal accumulation strategies of species growing on Copperbelt tailings dams. *Rhus longipes* was the most dominant specie based on the importance value index (IVI) (15.985 ± 3.428) followed by *Syzygium guineense* (13.316 ± 5.093) > *Senegalia polyacantha* (8.642 ± 3.822) > *Ficus craterostoma* (8.201 ± 2.069) > *Albizia adianthifolia* (6.477 ± 0.925) > *Bauhinia thonningii* (6.451 ± 3.032) > *Combretum molle* (5.106 ± 2.334). Heavy metal concentrations as well as the BF and TF varied from specie to specie with most species having BF and TF < 1 for various heavy metals. Significant differences were observed in heavy metal accumulation by roots and shoots. The results suggest that *Albizia adianthifolia, Albizia antunesiana, Albizia versicolor, Azanza garckeana, Bauhinia petersiana, Bauhinia thonningii, Brysocarpus orientalis, Combretum apiculatum, Combretum molle, Combretum microphyllum, Dichrostachys cinereal, Diospyros mespiliformis, Dodonaea viscosa, Ficus capensis, Ficus craterostoma, Ficus sycomorus, Peltophorum africanum, Phyllanthus guineensis, Rhus longipes, Senegalia polyacantha, Senna singueana, Syzygium guineense, Terminalia mollis, Terminalia stenostachya* and *Vachelia sieberiana* have potential for phytostabilizing Cu contaminated sites.

Keywords: Phytostabilization, Heavy metals, Indigenous tree species, Tailings dams, Ecological restoration.

5.1 Introduction

Mining of copper and other mineral in Zambia has led to the generation of mine wastelands. The increase in demand for copper and other minerals at global level will drive the increase in mine wasteland generation. Mine wastelands are associated with risks of water, land and air pollution. They, therefore, present major environmental concerns as wasteland toxic elements are easily transported into local environments through water erosion and aeolian dispersion (Mendez and Maier 2008). The effects of mine wastelands on the environment necessitated the need for mine wasteland restoration (Nirola et al. 2015).

Recently, an increasing interest in mine wasteland restoration has been observed. Several studies have from various regions namely Central and Southern Africa (Shutcha et al. 2015; Festin et al. 2018), Western Africa (Mensah 2015), India (Seenivasan et al. 2015) and China (Li 2006; Yang et al. 2017) documented various restoration methods with phytoremediation receiving more and more attention among researchers. "Phytoremediation is the use of plants and related microorganisms to reduce effects of toxic contaminants in the environment" (Mendez and Maier 2007). It is well established from literature that phytoremediation reduces contamination of the environment by tailings and stabilizes mine wastelands (Santos et al. 2017, Festin et al. 2018). Phytoremediation is a comprehensive method as it combines techniques from the physical and chemical methods making it ideal for wasteland restoration . Phytoremediation is composed of two techniques; phytoextraction and phytostabilization. Phytoextraction uses hyperaccumulator plants that uptake metals from the soil, translocate and stores them in aboveground parts of a plant while phytostabilization uses excluders that inhibit metal translocation to aboveground parts of the plant and reduces metal bioavailability hence reducing environmental contamination (Sun et al. 2016). Phytoextraction is suitable for the rehabilitation of sites with low metal concentrations as accumulated metals could be dried and extracted (Sainger et al. 2011), while phytostabilization creates a vegetation structure that stabilizes the site thereby reducing contamination of the environment. Phytostabilization is the ideal technique for abandoned mine wastelands and tailings dam restoration (Gujarathi et al. 2015; Sun et al. 2016; Santos et al. 2017). Selection of suitable plant species is critical for implementing a successful phytostabilization program. Several researchers screened the phytostabilization potential of plant species that naturally colonize contaminated sites (Pandey 2015; Del Río- Celstino et al. 2006; Nirola et al. 2015; Nirola et al. 2016; Sun et al. 2016; Santos et al. 2017; Schachtschneider et al. 2017). Plants that do not naturally colonize mine wastelands have also been screened for phytostabilization potential (Pandey 2013). Literature shows that there are a number of indigenous species with metal excluding abilities associated with mine wastelands in many mining regions (Sun et al. 2016; Santos et al. 2017) other than

Zambia. These plant species easily adapted to the mine wasteland site thereby ensuring the acceleration of ecological restoration of the mine wastelands (Sun et al. 2016).

In Zambia, restoration attempts using plants on tailings dams have been made. However, more failures than success have been recorded (Kambinga and Syampungani 2012). This could be due to lack of understanding of the nutrient dynamics of the dumps and the functional diversity of the species used in the re-vegetation of these wastelands. To date, few studies (e.g. Shutcha et al 2015; Mwangi 2017) have evaluated the “phytostabilization potential of plant species growing on Copper tailings dams”. Most studies on Copper mine wastelands have concentrated on understanding the physical and chemical properties of the wastelands, concentration of heavy metals and the contamination of nearby areas (Ikenaka et al. 2010; Kríbek et al. 2014; Volk and Yerokun 2016; M’kandawrire et al. 2017; Chileshe et al. 2019). Little is known of the phytoremediation potential of tree species that colonize mine wastelands particularly tailings dams on the Copperbelt. This indicates the need to understand the metal accumulation strategies of the tree species growing on Copperbelt tailings dams for ecological restoration purposes. Classification of species based on their accumulation strategies will provide an understanding of the suitable species for re-vegetating tailings dams. The dissertation therefore was designed to evaluate the phytoremediation potential of indigenous tree species growing on Copperbelt tailings dams by identifying the tree species metal accumulation strategies for wasteland restoration. Furthermore, the study was also intended to develop an understanding of the influence of heavy metals on species abundance across contaminated sites as this is also key for implementing sustainable restoration programmes.

5.2 Research process and methodology

Existing literature on the ecological restoration of mine wastelands through re-vegetation and the application of phytoremediation techniques was reviewed. Evidence from literature was used to develop the methodology adopted in this research. The studies reviewed collectively suggest that successful restoration of vegetation on mine wastelands via phytoremediation depends on the plant species used (Li 2006; Mendez and Maier 2008; Bolan et al. 2011; Zou et al. 2012; Santos et al. 2017, Yang et al. 2017; Festin et al. 2018). Suitable species for phytoremediation have high metal tolerance, either as excluders or accumulators (Dongmei and Changqun 2008; Parra et al. 2016).

5.3 Species richness and abundance

32 indigenous tree species from 13 families and 22 genera recorded were observed to colonize and adapt to Copperbelt tailings dams (Chapter 3). These species have varying richness and abundance across the studied mine wastelands (Chapter 3). At family level, Fabaceae and Combretaceae dominated the tailings dams with 11 and 6 species, respectively. However, based on the species IVI's, *Rhus longipes* and *Syzygium guineense* showed dominancy of the TSFs (Chapter 3). The high IVI's attest to the species dominance of the area, high density and good growth performance which indicate high adaptability to the sites. Species suitable for phytostabilization, should have attributes such as good growth performance and production of large biomass besides their heavy immobilization potential (Sun et al. 2016). The tree species recorded on mining generated wastelands also occur in natural forests and local environments (Jew et al. 2016). The species adaptability to both contaminated and non-contaminated sites makes their application in the remediation of contaminated sites for accelerated ecological restoration viable (Luo et al. 2015). Other species that showed dominance of the tailings dams and possess potential to grow and establish vegetation cover include, *Senegalia polyacantha, Ficus craterostoma, Albizia adianthifolia, Bauhinia thonningii* and *Combretum molle* while the other species that showed the exclusion strategy of accumulation with poor growth performance could be considered for application in phytostabilization after soil amendments.

5.4 Phytostabilization potential of indigenous trees of Copperbelt tailings dams

The phytoremediation potential of tree species colonizing Copperbelt tailings dams was determined using the species BF and TF of Mn, Ni, Cr, Co, Cu, Cd, B, Ba, Mo, Al, Zn and S. Variations in heavy metal accumulation were observed across species. Generally, heavy metal concentration in soils were higher than roots so were the concentration in shoots when compared to root concentration. Cd averages showed a different trend as the mean concentration in roots was higher than in soils for some species. Species with low metal concentrations in roots than soil show the exclusion strategy of heavy metal accumulation while the ones with high concentration in roots show the accumulation strategy (Yang et al. 2014). All the tree species accumulated low root metal concentrations for all the elements except *Diospyros mespiliformis* for Ba and *Albizia adianthifolia, Combretum microphyllum, Bauhinia thonningii* and *Lannea discolor* for S indicating their accumulation ability of these elements. Significant differences between heavy metal concentrations in tailing soils, roots and shoots among species across heavy metals were observed. Likewise, most species accumulated high concentrations of metals in shoots compared to the roots for the various elements except for a few species for certain metals. For example, *Albizia amara, Annona senegalensis, Combretum zeyheri, Dombeya rotundifolia, Ozoroa insignis* and *Parinari curatellifolia* accumulated more Cu in the shoots than the roots suggesting a Cu accumulation strategy. Tree species that accumulate higher concentration of metals in their shoots as compared to roots have the accumulation strategy of those metals (Yang et al 2014). Accumulator species are not ideal for application in phytostabilization, therefore, all the identified Cu accumulators are not ideal for phytostabilizing Cu contaminated sites but could be applied in the phytomining of Cu. High concentrations of S, Mn, Zn and B in shoots of most the species occurring on Copperbelt TSFs were observed, therefore the tree species application in phytostabilization of sites contaminated with these elements should be done cautiously. Variations in heavy metal accumulation was observed across species as they showed exclusion strategy for some metals and accumulation for other metals, therefore, caution is advised when deciding to employ certain species in phytostabilization of contaminated sites.

The study classified 26 tree species as excluders and 6 identified as accumulators for Cu. All the species were identified as Mn accumulators while 4 were identified as excluders and 28 as accumulators for Zn. 14 accumulators and 18 excluders for Ni, 9 accumulators and 23 excluders for Cr and 12 accumulators and 20 excluders for Co were also identified. Furthermore, 8 accumulators and 24 excluders for Cd, 2 excluders, 1 hyperaccumulator and 29 accumulators for Ba, 11 accumulators and 21 excluders for Mo, 7 accumulators and 25 excluders for Al and 2 excluders, 27 accumulators and 3 hyperaccumulators for S were identified. The results suggest that all the studied tree species accumulate Mn hence their application in the phytostabilization of Cu contaminated sites with high concentrations of Mn should be done cautiously, otherwise their application in the phytomining of Mn would be viable.

5.5 Influence of heavy metals on species richness and abundance

Tailings are characterized by high heavy metal contents and left-over chemicals, low nutrient and water holding capacity, high salinity or acidity and poor physical structure (Wong 2003; Dumba 2013; Yang et al. 2016a; Festin et al. 2018). Heavy metal concentrations on Copperbelt tailings dams were higher than those reported in local forests (Festin et al. 2018, Chileshe et al. (2019) and with great variation (Chileshe et al. 2019). The average Cu, Zn, Ni, Co, Ba, Cr concentrations of this study were relatively higher than the ones reported by Chileshe et al.

(2019) except Cd, which was lower. Site variations could be due to the technology used in ore processing and the quality of the ore (Cooke and Johnson 2002). Low heavy metal concentration in local forests was attributed to lack of contamination which is due to distance between the tailings and local forests (Lupupa 2011; Mapaure et al. 2008; Dumba 2013), Ghana (Fosu-Mensah et al. 2017). Heavy metal concentrations of Copperbelt tailings dams were compared with the screening benchmark and Canadian Council of Ministers Council remediation criteria (CCMERC) (Efroymson 1997). Heavy metal concentration on Copperbelt tailings dams were generally higher than the recommended limits for the soil parameter (Efroymson 1997). Tailings are naturally high in heavy metals and leftover chemicals (Wong 2003, Kossoff et al. 2014) hence the high metal concentrations.

Generally, tree species positively correlated with metals in low concentrations with a few species (*Rhus longipes, Dichrostachys cinerea, Albizia antunesiana, Senegalia polyacantha, Dombeya rotundifolia, Albizia versicolor, Albizia amara* and *Phyllanthus guineensis)* positively correlating with Cu, Mn, Zn, Ni, Cr, Co, B, Ba, Mo, Al and S in high concentrations. Species that correlate with heavy metals in low concentration suggest optimum growth and establishment in low concentration while those that correlate with metals in high concentration show an affiliation to metals in high concentration. Plant species that can tolerate high metal concentrations would have high diversity and abundance on contaminated sites (Dumba 2013). Species such as *Rhus longipes* and *Syzygium guineense* showed good growth performance even in areas where soil amendments were not done. This suggests their strong affiliation to highly contaminated sites hence their probable application in phytostabilization. Low species richness on the tailings dams is due to high heavy metal concentrations as tree species positively correlated with metals in low concentrations. This explains the low concentration on the tailings dams as tree species do not thrive in high heavy metal concentrations. A dominant species (due to high IVI) *Rhus longipes* was observed to perform well as it thrives in high heavy metal concentration hence its dominancy of the dams. *Dichrostachys cinerea* also showed positive correlations to metals in high concentration indicating its ability to colonize contaminated sites. It has been reported to encroach and establish vegetation cover on contaminated sites (de Klerk 2004; Bako et al. 2004; Dumba 2013). This study determined that Ni, Cu, S, Cr, Al, Co, Mn, B, Mo and Cd influence species richness and abundance on the Copperbelt tailings dams.

5.6 Research implications on mine wasteland restoration

Pollution arising from mine wasteland has been of global concern for the past two decades owing to their associated effects. Ecological restoration of mine tailings dams via phytostabilization is dependent on the plant species used and the soil ameliorations applied to the soil. The study classified 32 indigenous tree species into excluders and accumulators of Cu, Mn, Zn, Ni, Cr, Co, B, Ba, Cd, Mo, Al and S for possible application in phytostabilization for sustainable vegetation restoration of Copperbelt TSFs.

The research sheds new light on the mechanism behind plant succession on TSFs and offers an opportunity for indigenous tree species to be used in phytostabilization. The findings make an important contribution to the field of ecological restoration by advancing the knowledge of excluders and accumulators of various heavy metals. The findings of this study make original contributions to mine wasteland restoration by classifying indigenous tree species into excluders and accumulators thereby identifying tree species suitable for phytostabilizing Cu, Zn, Ni, Cr, Co, B, Ba, Al, Cd and S contaminated sites. Furthermore, the determination of heavy metals that influence species richness and abundance on Copperbelt tailings dams provide insights on how to use species to remove contaminants from tailings, guide decision making with regards to soil ameliorations to be applied to tailings, inform rehabilitation (restoration) programs and plans. This will enhance vegetation establishment on TSFs thereby enhancing species richness and abundance which in turn reduce heavy metals on tailings dams, hence reducing environmental contamination. The findings provide information which could be applied in phytostabilization programs to enhance the establishment and growth of a forest ecosystem that would support human livelihood. The findings also provide information on diversity conservation which could be applied in phytostabilization programs to enhance the establishment and growth of an ecosystem that would support human livelihood.

5.7 Conclusion

The study set out to determine the heavy metal accumulation strategies of indigenous tree species growing on Copperbelt tailings dams for possible application in phytostabilization. The study classified tree species into excluders, accumulators and hyperaccumulators for Cu, Mn, Zn, Ni, Cr, Co, B, Ba, Cd, Mo, Al and S. The study has demonstrated the variation in survival strategies of plants on mining generated wastelands. Additionally, the study identified several species with phytostabilization potential for stabilizing the 12 studied heavy metals and elements. However, 9 species (*Rhus longipes, Syzygium guineense, Senegalia polyacantha, Ficus craterostoma, Bauhinia thonningii, Albizia adianthifolia, Combretum molle, Peltophorum africanum* and *Ficus sycomorus)* proved to have great potential for phytostabilizing Copperbelt tailings dams. This is due to their heavy metal exclusion abilities and good growth performance. The tree species are well adapted to the sites and seeds could be collected from the tailings dams for re-vegetation of tailings dams. The study also suggested species that could be used in phytoextraction and phytostabilization of sites contaminated with metals such as Al, Ba, Zn, Cd, Co, Cr, S, Ni, Mn, Mo and B. The results of this study provide a new understanding of the heavy metal accumulation strategy of indigenous tree species growing on Copperbelt tailings dams thereby contributing to the existing knowledge of species with phytoremediation potential for mine wasteland restoration. Conclusively, indigenous tree species growing on Copperbelt tailings dams could be used in the phytostabilization of Cu, Ni, Cd, Co, and Al contaminated sites thereby providing a cost-effective solution for the remediation of mine wastelands. This solution would eventually result in a self-sustaining ecosystem that promotes carbon sequestration, water conservation, clean air and enhanced soil quality for food production thereby promoting sustainable development.

The study also categorized tree species based on their correlations with heavy metal concentrations across sites. Two groups of tree species were observed, one group thrived in low metal concentrations while the other thrived in high metal concentrations. Therefore, giving an indication of the type of trees to use in soils with varying concentrations of heavy metals. The results show that species that correlate with metals in low concentrations are best suited for sites with low concentration while those that correlate with metals in high concentration would be ideal for re-vegetating the Copperbelt tailings dams which are high in metal concentration. The study identified species that could be used to remediate sites with both low and high concentrations of heavy metals. The results of this study could help mining companies in Zambia and other mining regions to develop ecological restoration of mine wastelands programs. The study recommends empowering local farmers to engage in the production of seedlings for the identified tree species for the remediation of Copperbelt TSFs. Policy changes that recognize the use of phytoremediation by mining houses would enhance its adoption and implementation. This will reduce contamination and stabilize TSFs. Additionally, promoting the use of phytoremediation products in bioenergy, biochar etc, would equally enhance the adoption of phytoremediation by mining houses.

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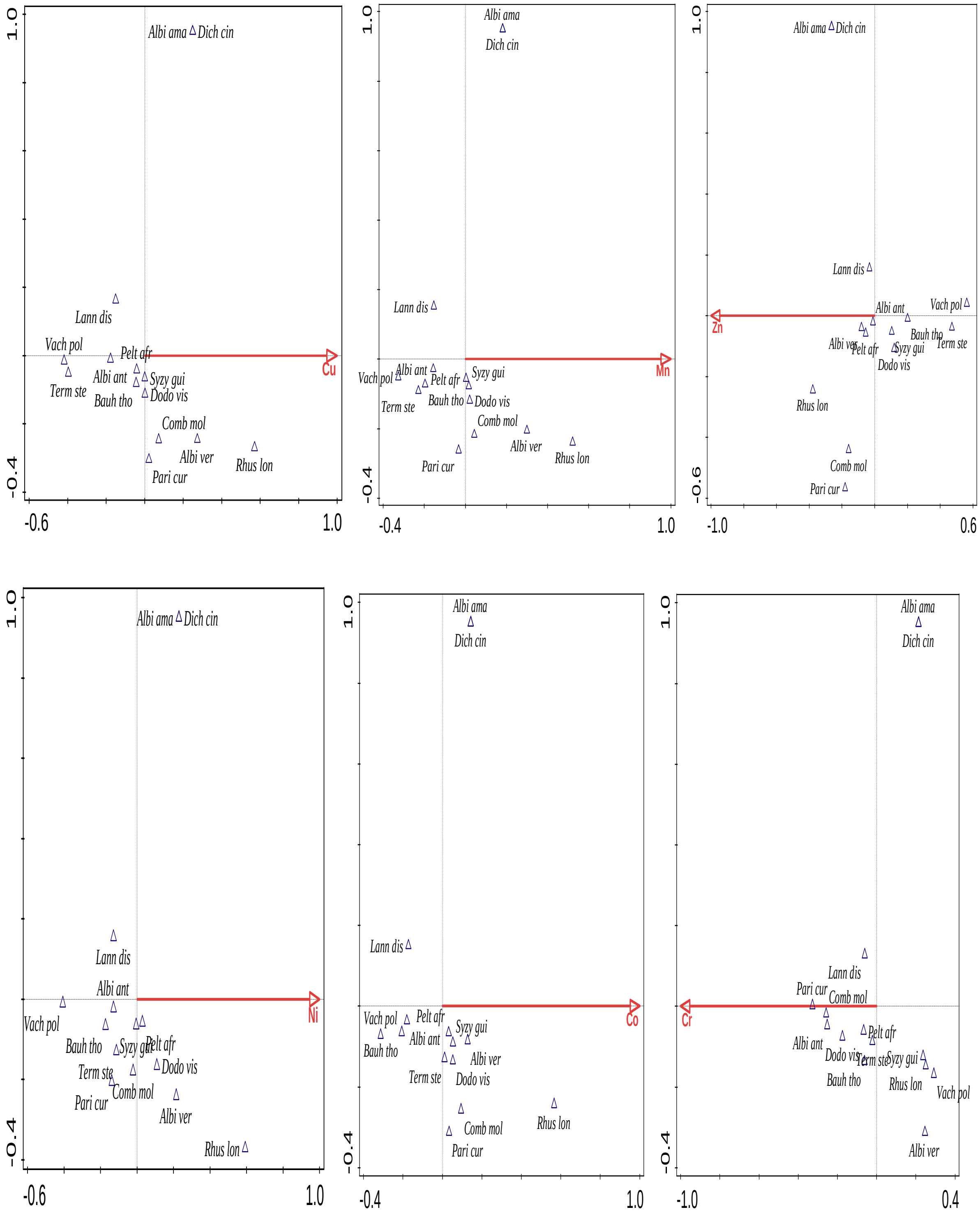
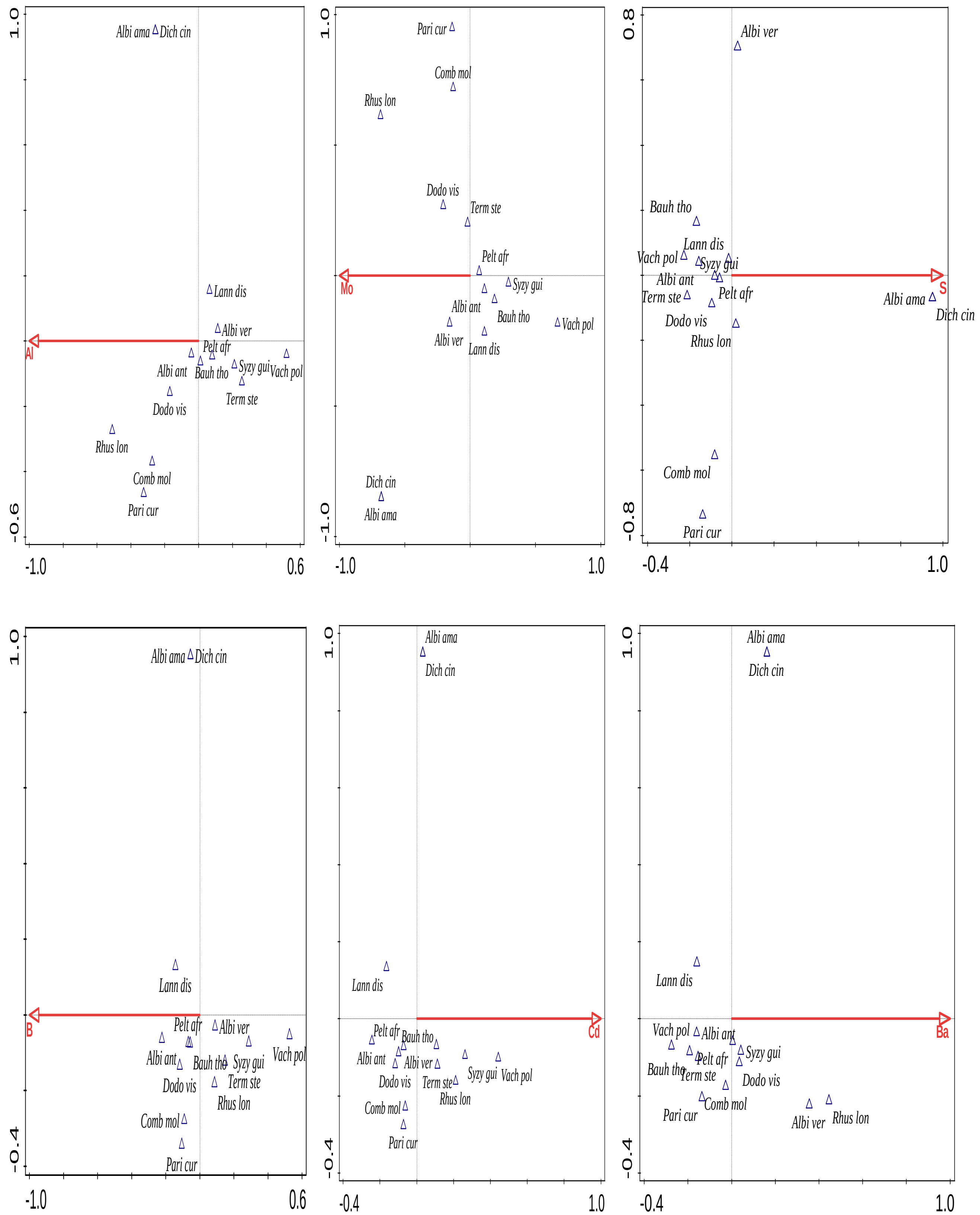
6.0 Appendices

Appendix 1: Tree species on Copperbelt Tailings dams' IVI

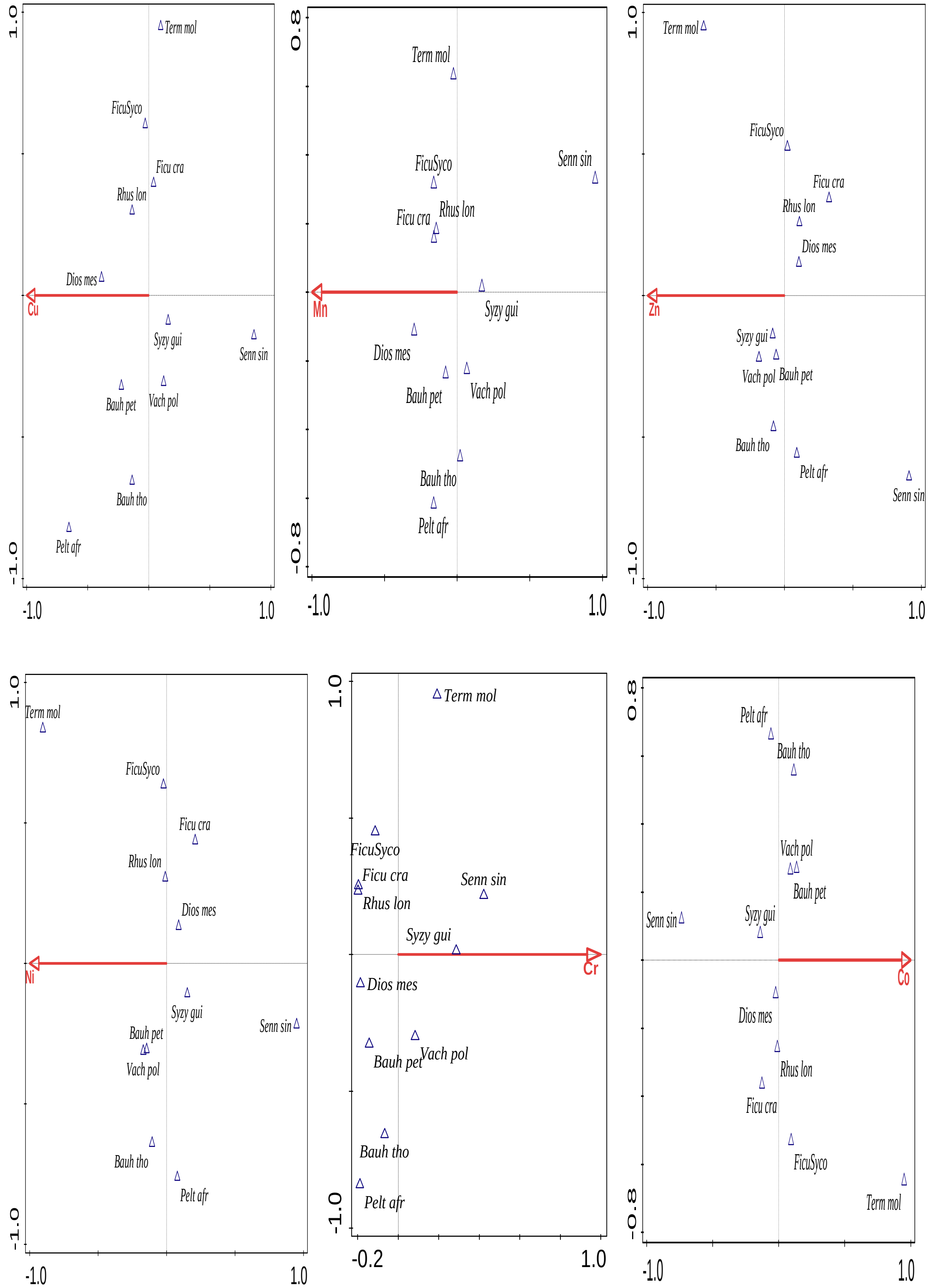
No	Species	Family	IVI
1	*Albizia adianthifolia*		6,477 ± 0,925
2	*Albizia amara*	Fabaceae	1,506 ± 0,717
3	*Albizia antunesiana*	Fabaceae	2,436 ± 1,126
4	*Albizia versicolor*	Fabaceae	3,061 ± 1,748
5	*Annona senegalensis*	Annonaceae	0,701 ± 0,350
6	*Azanza garckeana*	Malvaceae	0,846 ± 0,846
7	*Bauhinia petersiana*	Fabaceae	3,803 ± 2,081
8	*Bauhinia thonningii*	Fabaceae	6,451 ± 3,032
9	*Brysocarpus orientalis*	Connaraceae	1,358± 0,193
10	*Combretum apiculatum*	Combretaceae	0,200 ± 0,200
11	*Combretum microphyllum*	Combretaceae	0,698 ± 0,338
12	*Combretum mole*	Combretaceae	5,106± 2,334
13	*Combretum zeyheri*	Combretaceae	1,134 ± 0,948
14	*Dichrostachys cinerea*	Fabaceae	1,002 ± 0,387
15	*Diospyros mespiliformis*	Ebenaceae	0,794 ± 0,794
16	*Dodonaea viscosa*	Sapindaceae	1,987± 0,664
17	*Dombeya rotundifolia*	Sterculiaceae	0,398 ± 0,258
18	*Ficus capensis*	Moraceae	0,284 ± 0,284
19	*Ficus craterostoma*	Moraceae	8,201 ± 2,069
20	*Ficus sycomorus*	Moraceae	4,809 ± 3,237
21	*Lannea discolor*	Anacardiaceae	2,191 ± 1,235
22	*Ozoroa insignis*	Anacardiaceae	0,225 ± 0,225
23	*Parinari curatellifolia*	Chrysobalanaceae	0,307 ± 0,307
24	*Peltophorum africanum*	Fabaceae	4,156 ± 2,653
25	*Phyllanthus guineensis*	Phyllanthaceae	0,872 ± 0,456
26	*Rhus longipes*	Anacardiaceae	15,985 ± 3,428
27	*Senegalia polyacantha*	Fabaceae	8,642 ± 3,822
28	*Senna singueana*	Fabaceae	3,043 ± 1,314
29	*Syzigium guineense*	Myrtaceae	13,316 ± 5,093
30	*Terminalia mollis*	Combretaceae	3,430 ± 1,083
31	*Terminalia stenostachya*	Combretaceae	2,483 ± 1,967
32	*Vachellia sieberiana*	Fabaceae	1,311 ± 0,890

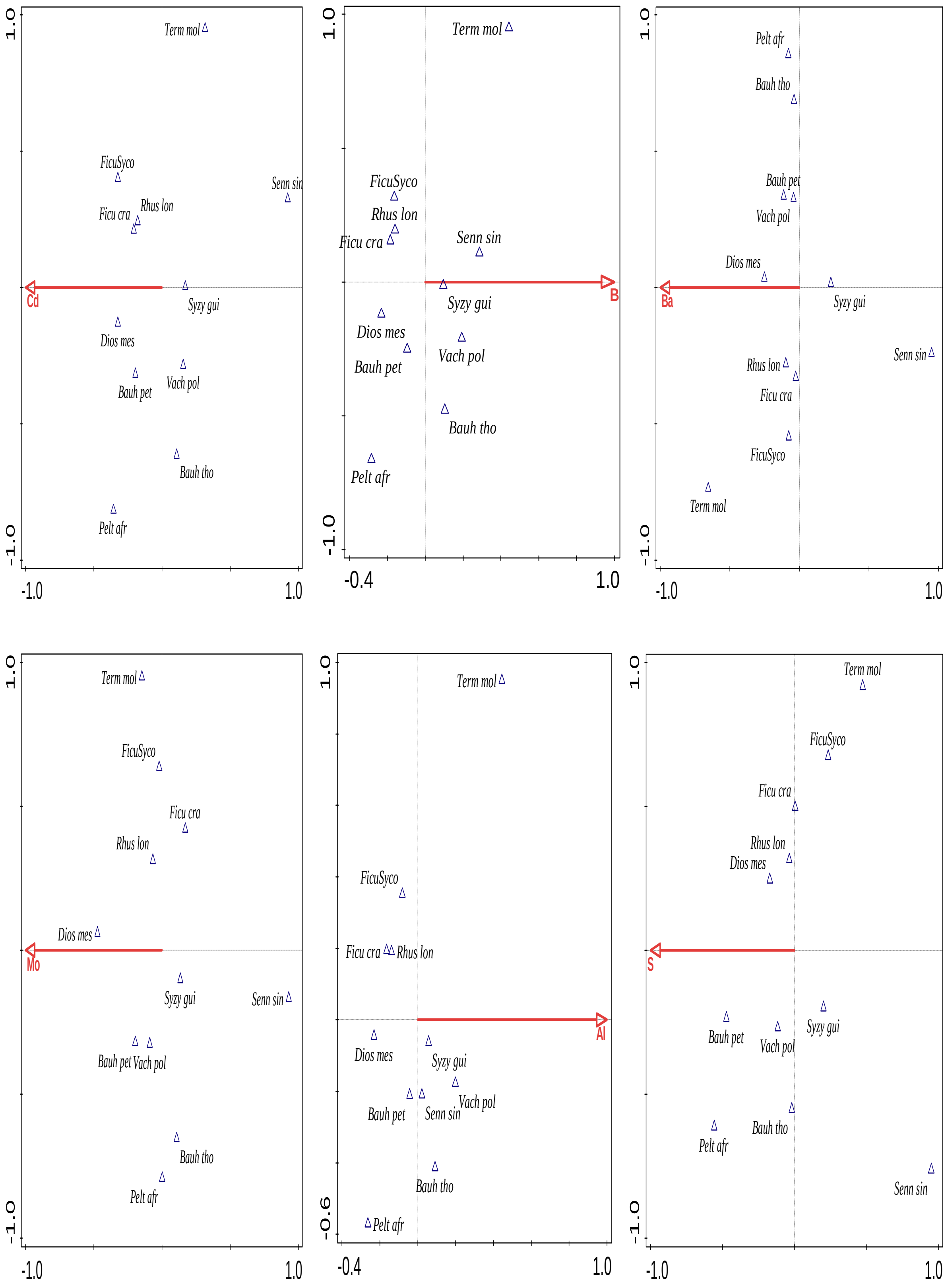
Appendix 2: CCA analysis showing influence of heavy metals on species richness and abundance on TD25 (Kitwe)



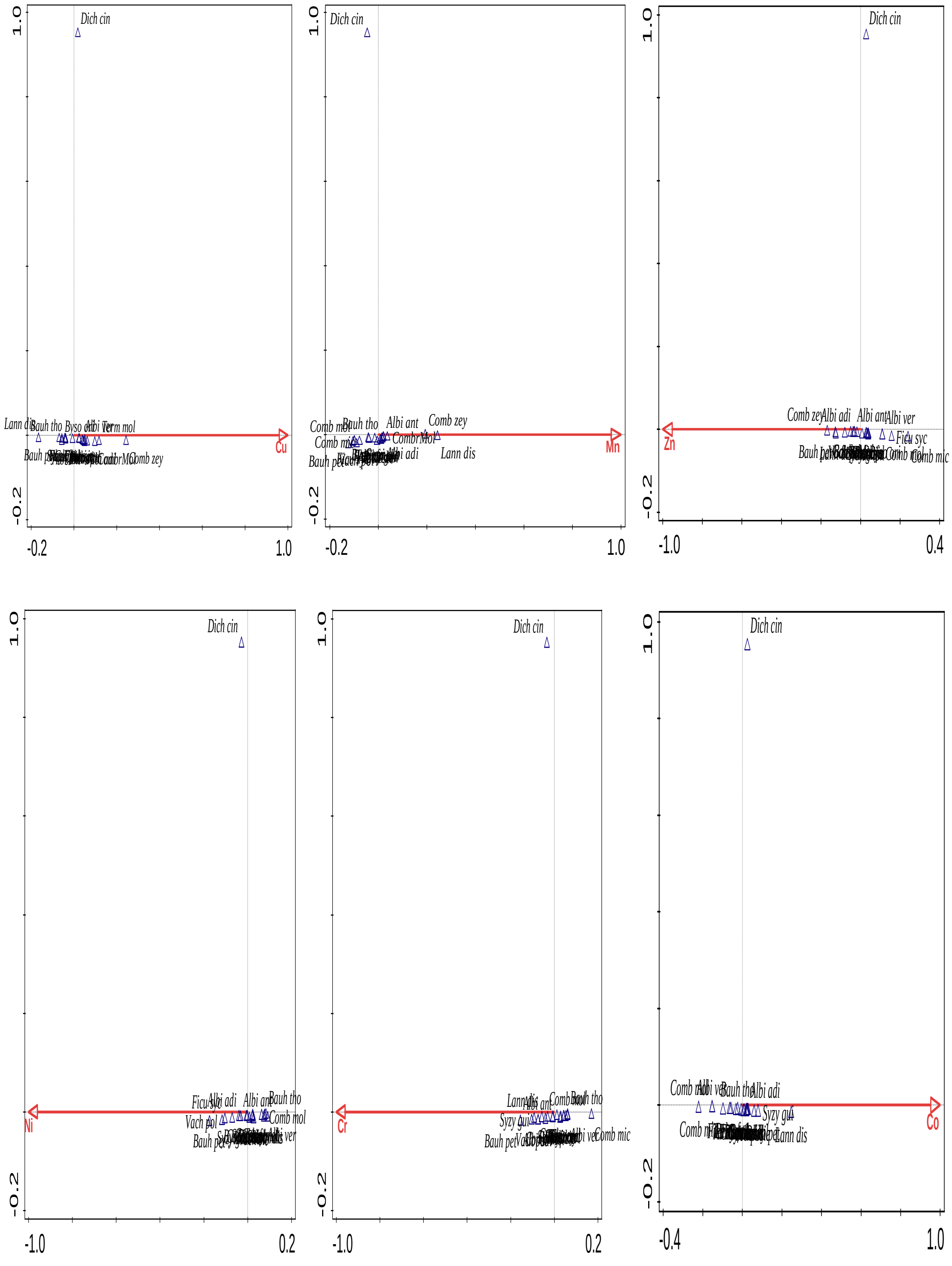

1.0
-0.6
-1.0
0.6
Albi ama
Dich cin
Lann dis
Albi ver
Pelt afr
Albi ant
Bauh tho
Syzy gui
Vach pol
Term ste
Dodo vis
Rhus lon
Comb mol
Pari cur
Al
1.0
-1.0
-1.0
1.0
Pari cur
Comb mol
Rhus lon
Dodo vis
Term ste
Pelt afr
Syzy gui
Albi ant
Bauh tho
Vach pol
Albi ver
Lann dis
Dich cin
Albi ama
Mo
0.8
-0.8
-0.4
1.0
Albi ver
Bauh tho
Lann dis
Vach pol
Syzy gui
Albi ant
Term ste
Pelt afr
Albi ama
Dich cin
Dodo vis
Rhus lon
Comb mol
Pari cur
S
1.0
-0.4
-1.0
0.6
Albi ama
Dich cin
Lann dis
Pelt afr
Albi ver
Albi ant
Bauh tho
Syzy gui
Vach pol
Dodo vis
Term ste
Rhus lon
Comb mol
Pari cur
B
1.0
-0.4
-0.4
1.0
Albi ama
Dich cin
Lann dis
Pelt afr
Bauh tho
Albi ant
Albi ver
Dodo vis
Term ste
Syzy gui
Vach pol
Rhus lon
Comb mol
Pari cur
Cd
1.0
-0.4
-0.4
1.0
Albi ama
Dich cin
Lann dis
Vach pol
Albi ant
Bauh tho
Pelt afr
Syzy gui
Term ste
Dodo vis
Comb mol
Pari cur
Albi ver
Rhus lon
Ba

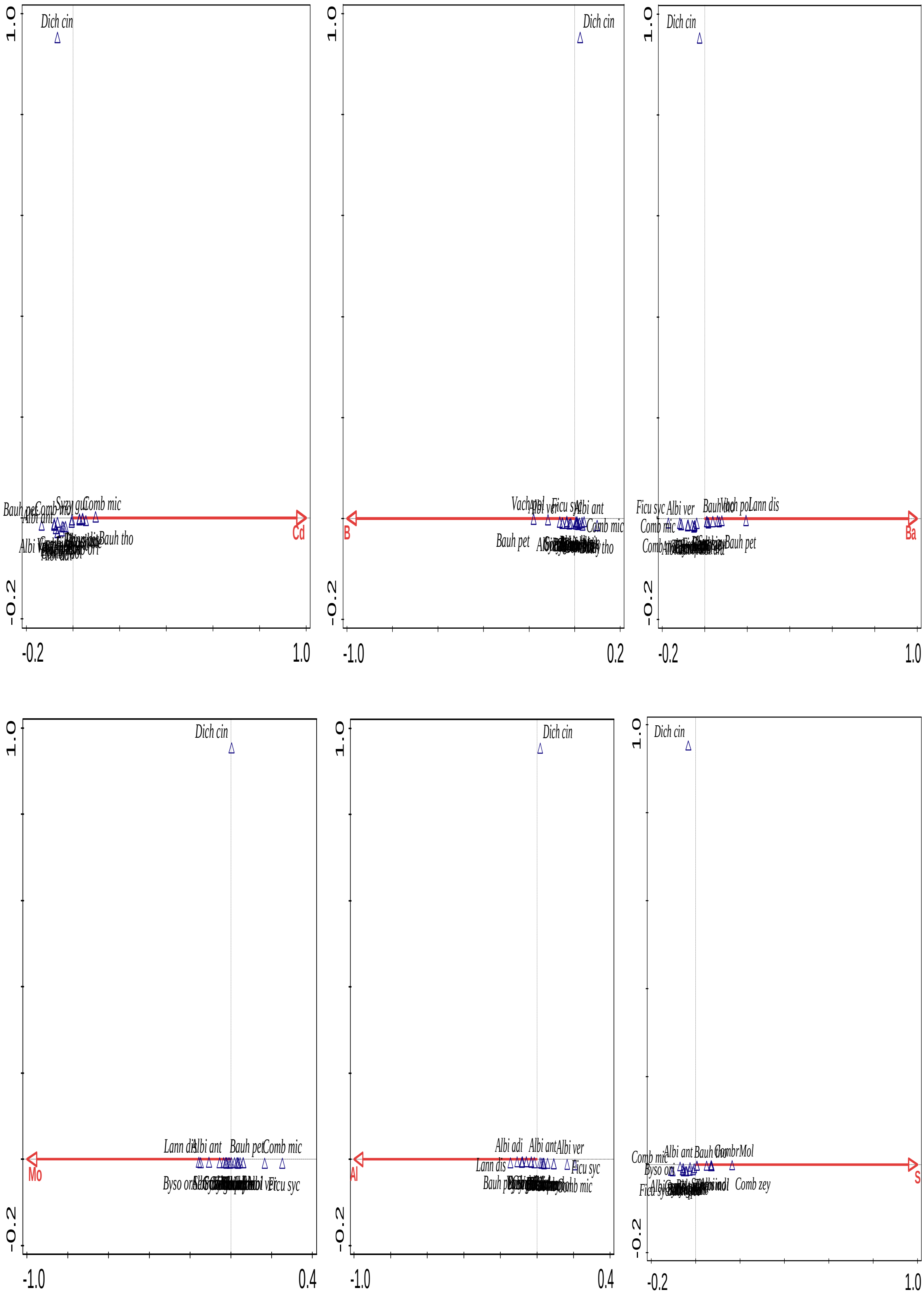
Appendix 3: CCA analysis showing influence of heavy metals on species richness and abundance on TD26 (Kitwe)



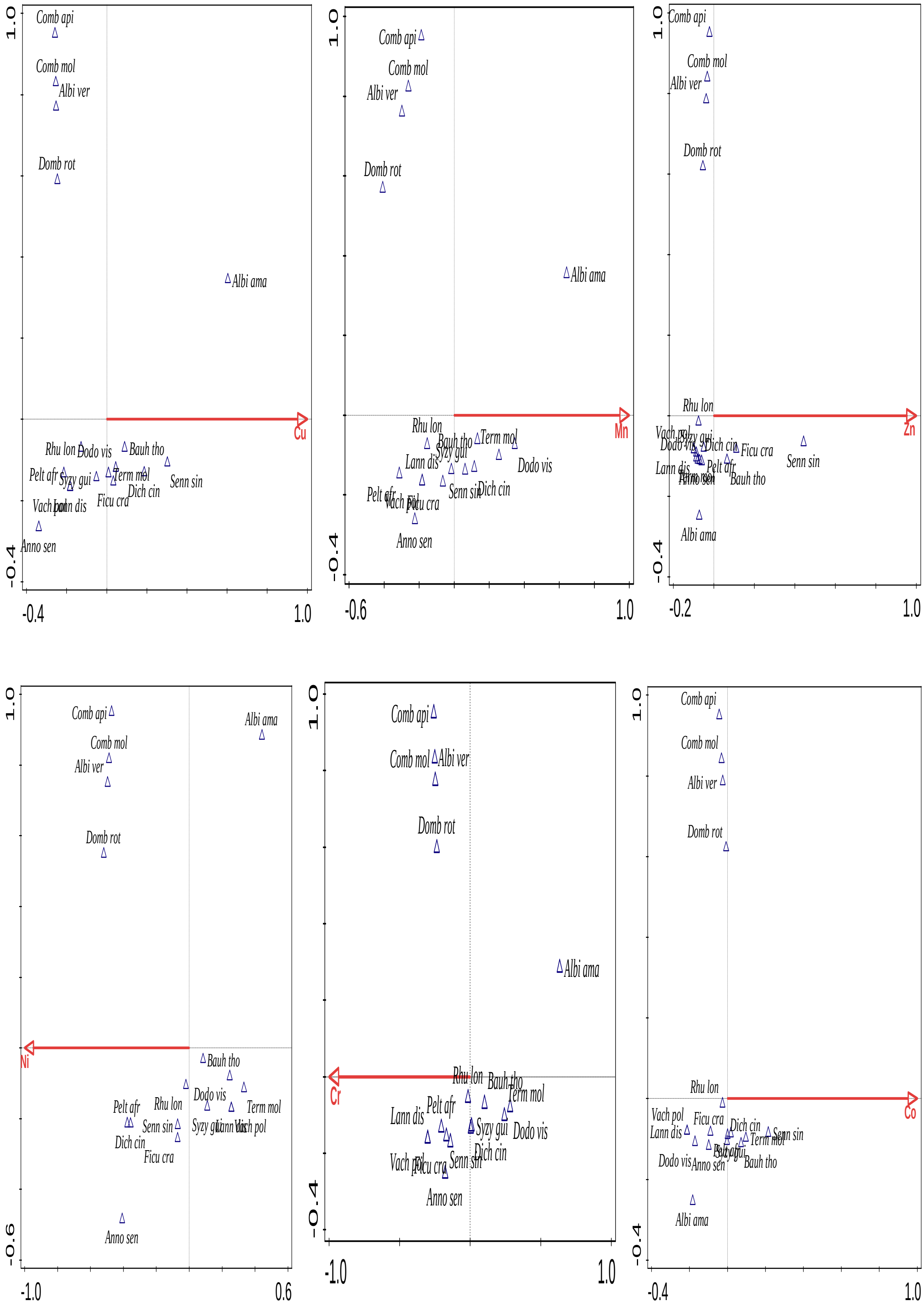

1.0
-1.0
-1.0
1.0
Term mol
FicuSyco
Senn sin
Ficu cra
Rhus lon
Cd
Syzy gui
Dios mes
Vach pol
Bauh pet
Bauh tho
Pelt afr
-0.4
B
Ba
Mo
Al
S
-0.6

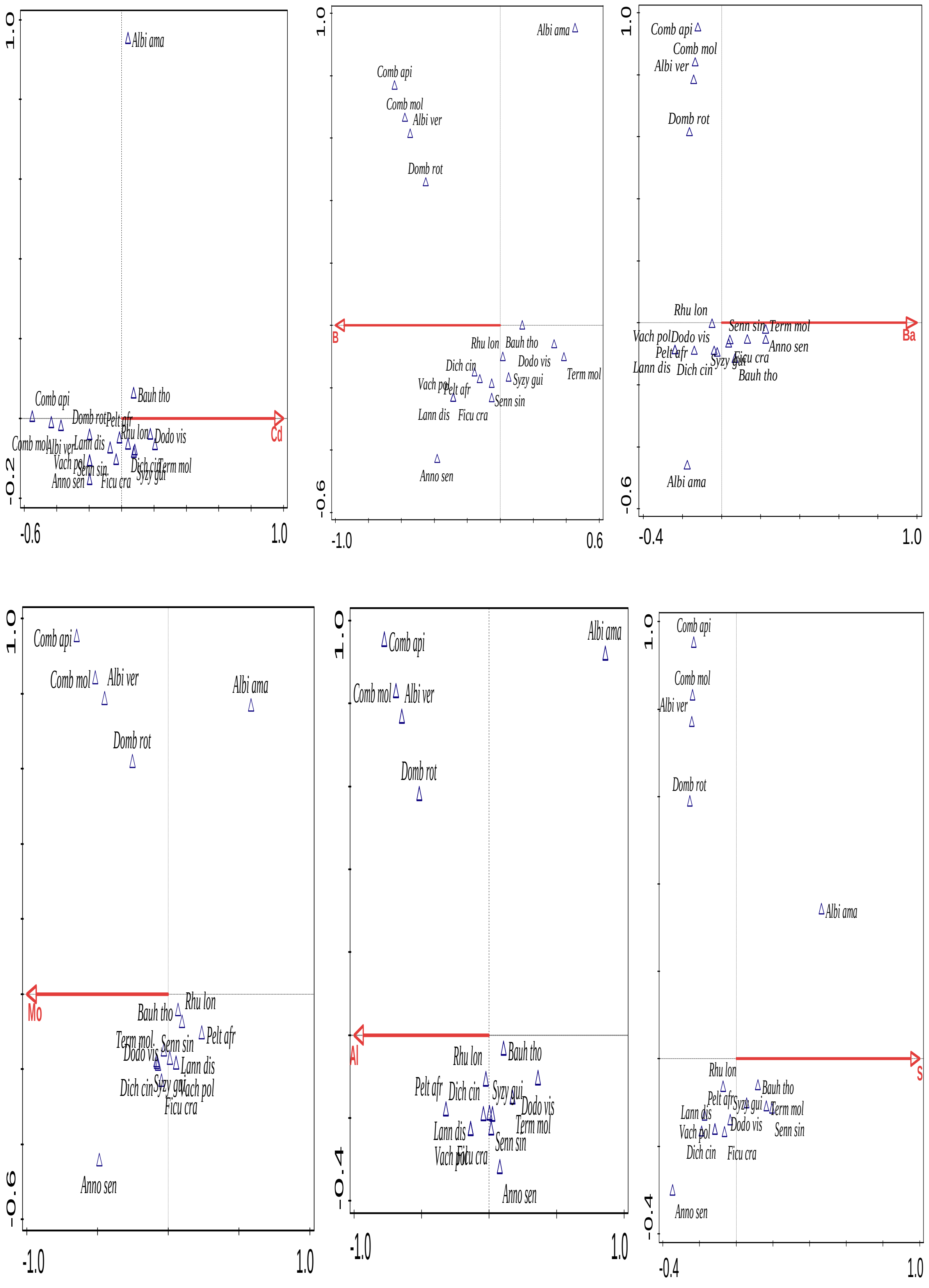
Appendix 4: CCA analysis showing influence of heavy metals on species richness and abundance on TD24 (Luanshya)



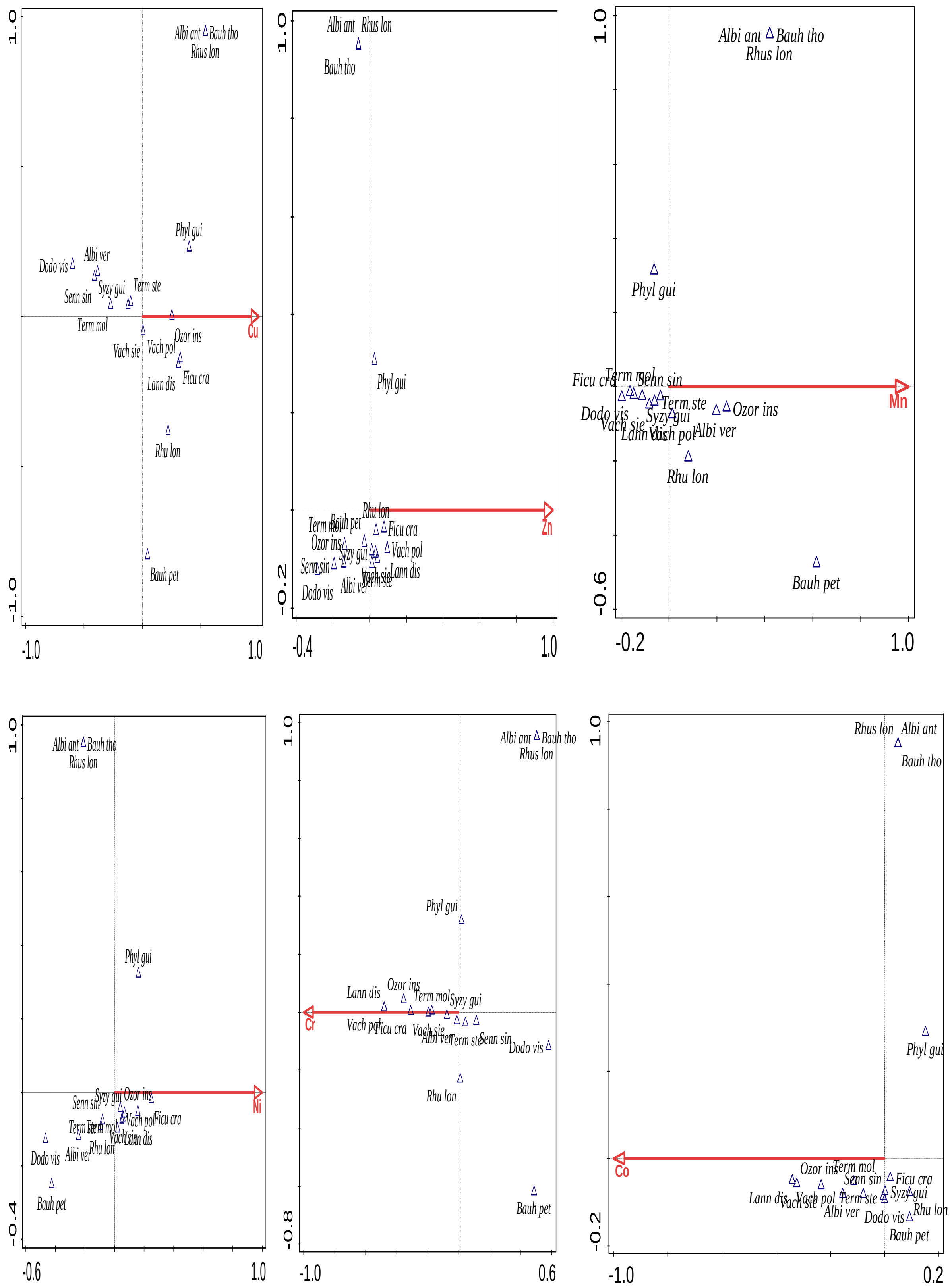

Dich cin
Comb mic
Bauh tho
Cd
1.0
-0.2
-0.2
1.0
Dich cin
Albi ant
Comb mic
Bauh pet
B
1.0
-0.2
-1.0
0.2
Dich cin
Ficu syc
Albi ver
Comb mic
Lann dis
Bauh pet
Ba
1.0
-0.2
-0.2
1.0
Dich cin
Albi ant
Comb mic
Ficu syc
Mo
1.0
-0.2
-1.0
0.4
Dich cin
Albi adi
Albi ant
Albi ver
Lann dis
Ficu syc
Comb mic
Al
1.0
-0.2
-1.0
0.4
Dich cin
Comb mic
Albi ant
Comb zey
S
1.0
-0.2
-0.2
1.0

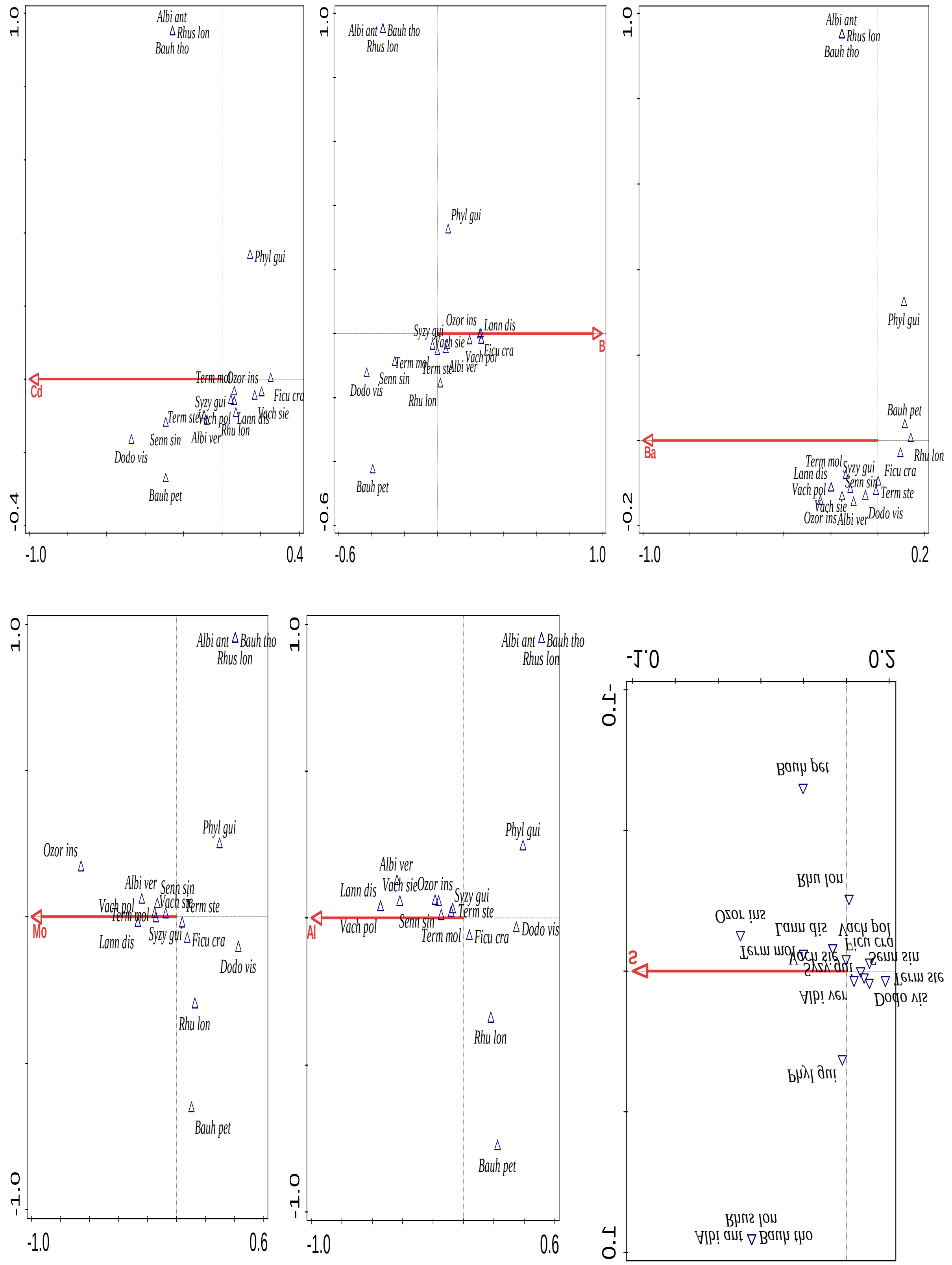
Appendix 5: CCA analysis showing influence of heavy metals on species richness and abundance on TD25 (Luanshya)




Cd
B
Ba
Mo
Al
S
Albi ama
Comb api
Comb mol
Albi ver
Domb rot
Rhu lon
Bauh tho
Dodo vis
Term mol
Senn sin
Pelt afr
Lann dis
Dich cin
Syzy gui
Vach pol
Ficu cra
Anno sen
1.0
-0.2
-0.6
-0.4
0.6

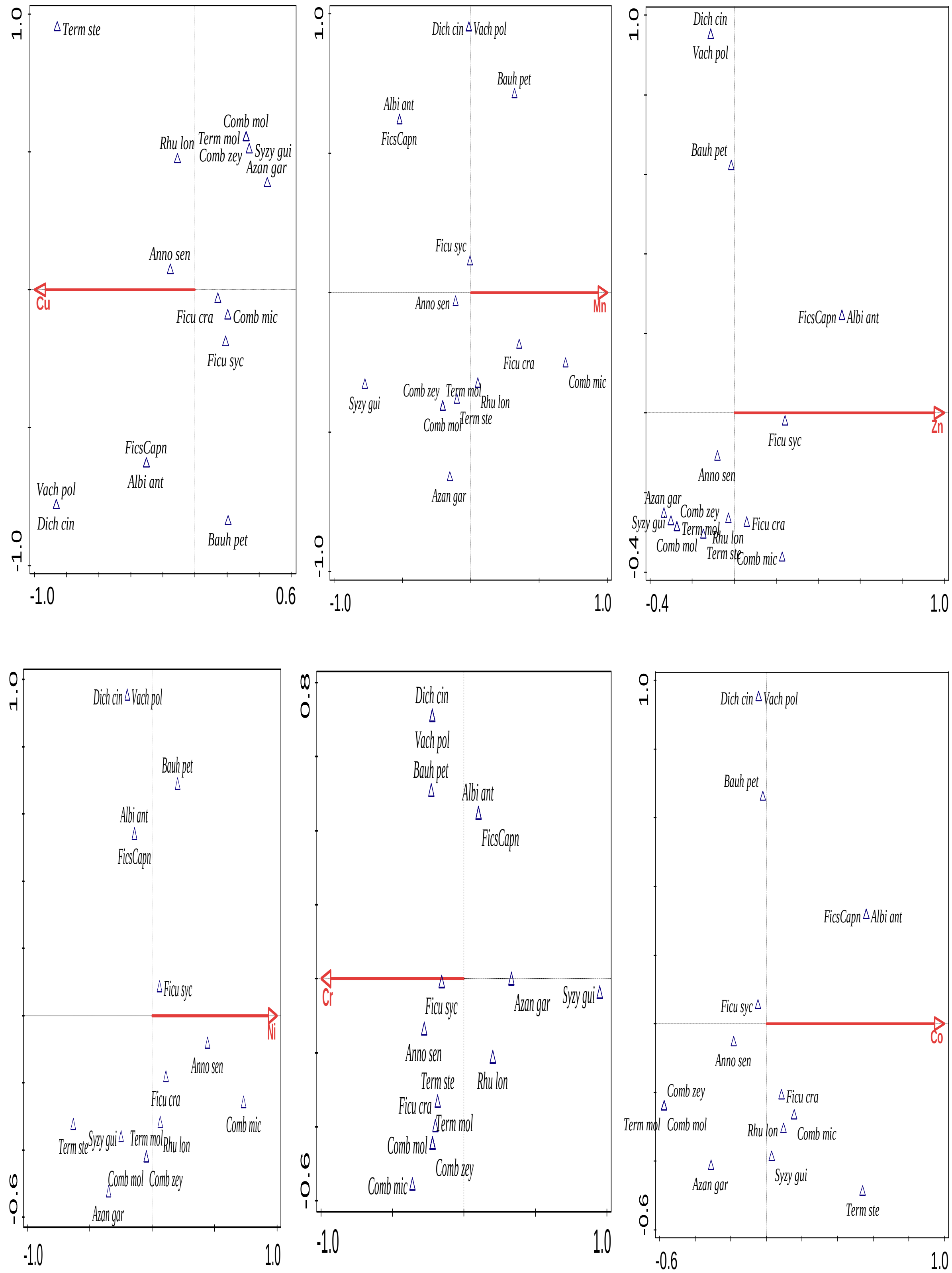
Appendix 6: CCA analysis showing influence of heavy metals on species richness and abundance on TD26 (Luanshya)

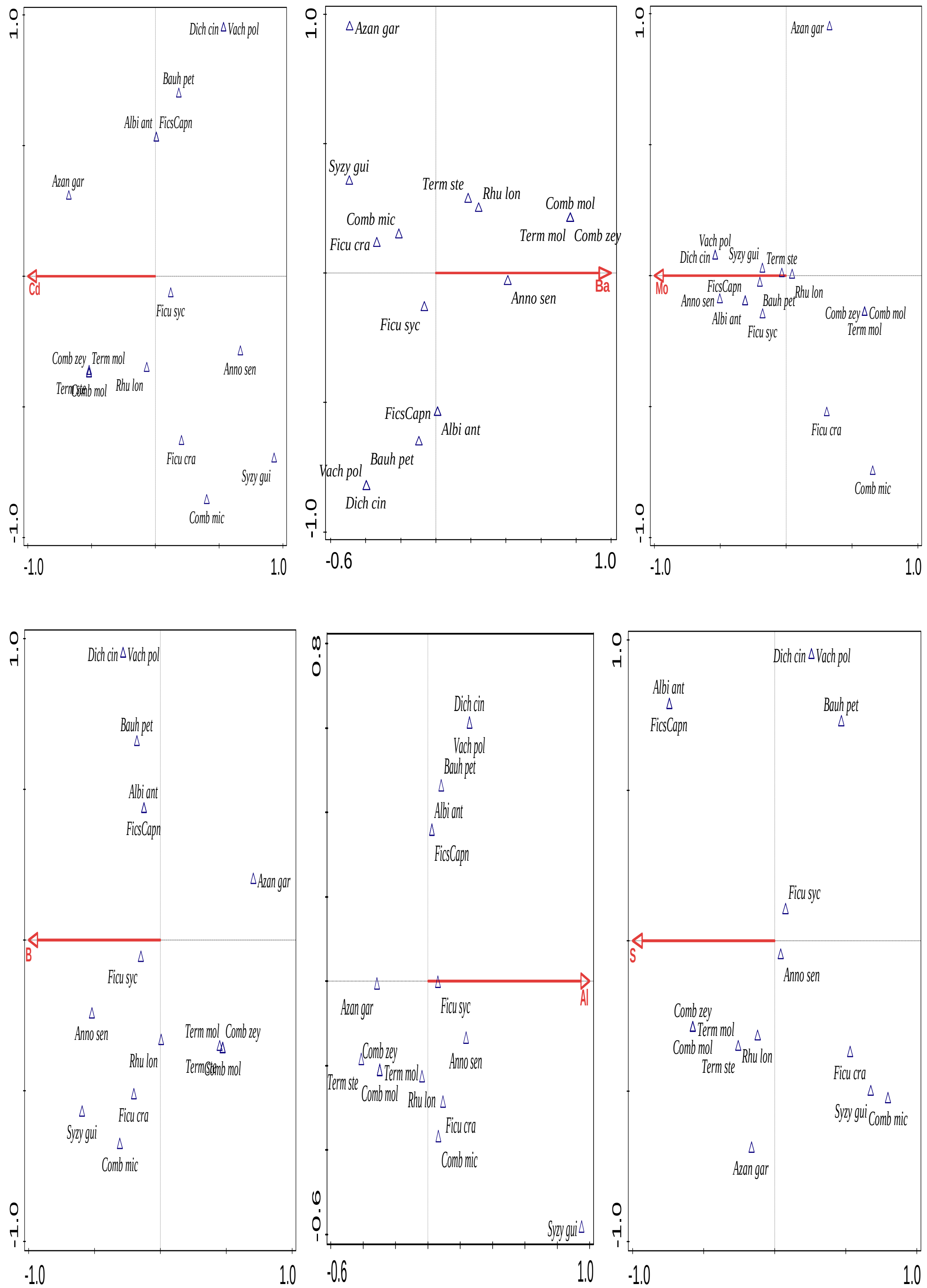




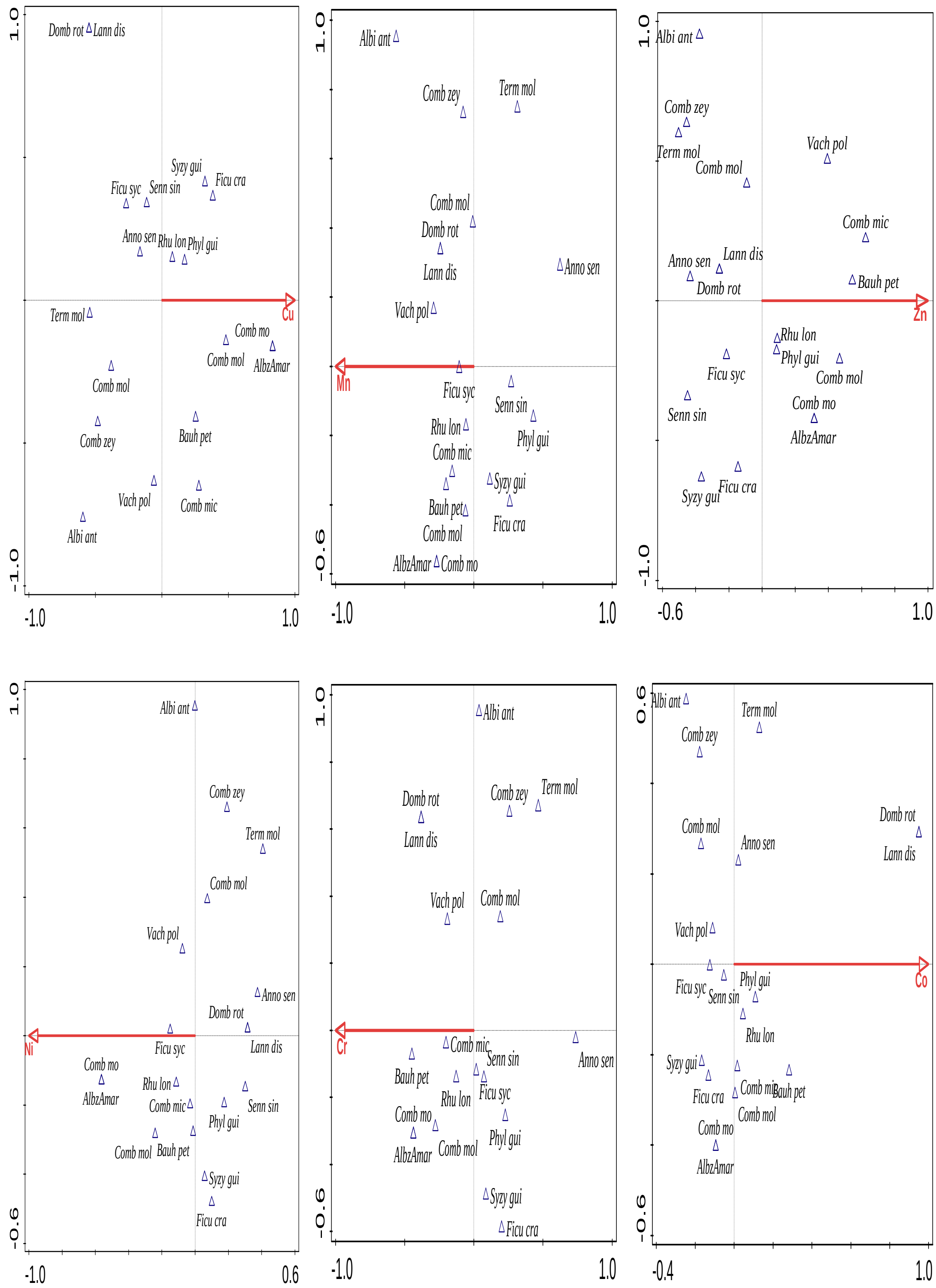
Albi ant
Rhus lon
Bauh tho
Phyl gui
Term mol
Ozor ins
Cd
Ficu cra
Syzy gui
Term ste
Vach pol
Lann dis
Vach sie
Rhu lon
Senn sin
Albi ver
Dodo vis
Bauh pet
B
Ba
Mo
Al
S
1.0
-0.4
-0.6
-0.2
-1.0
0.4
0.6
0.2

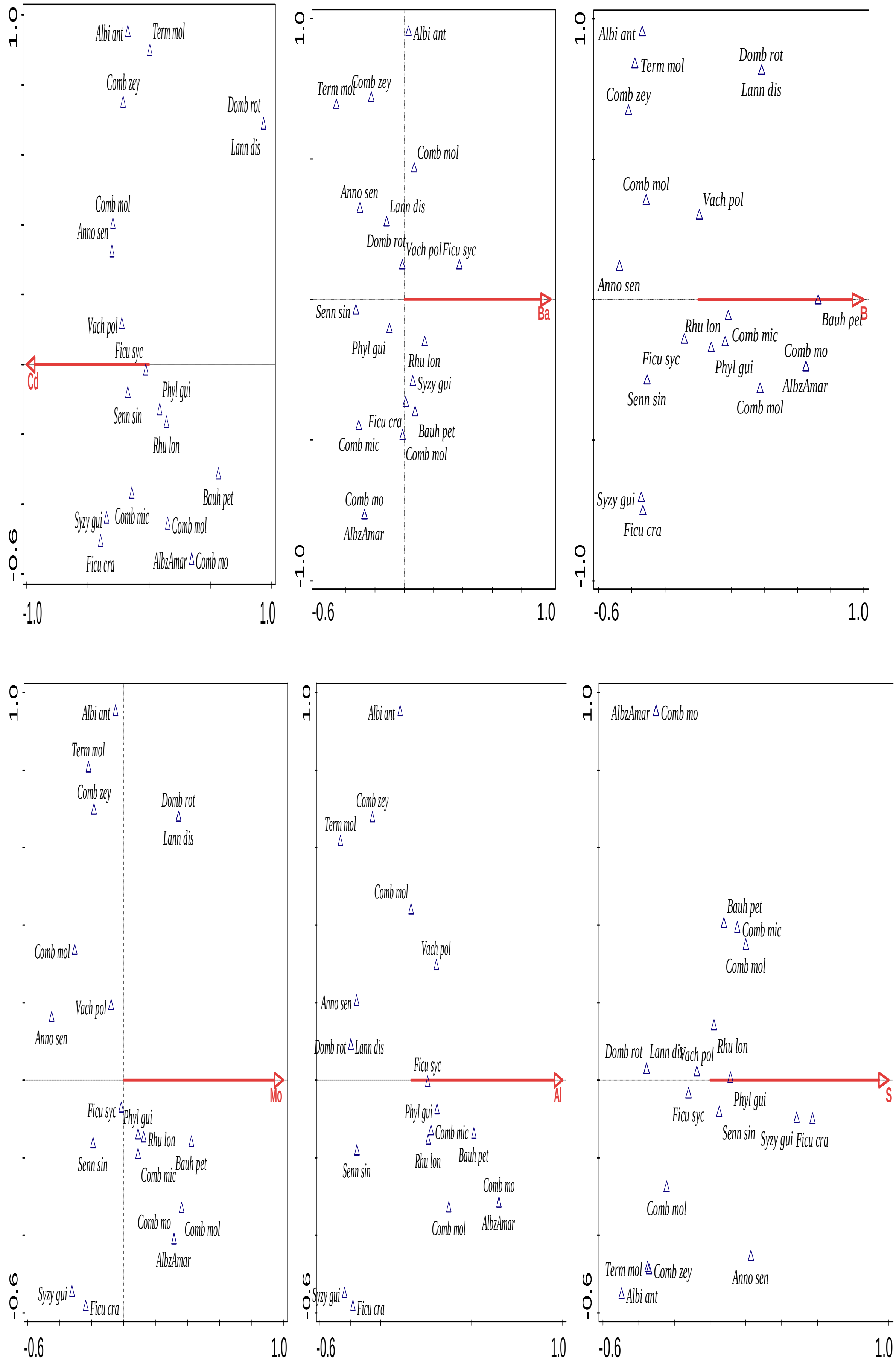
Appendix 7: CCA analysis showing influence of heavy metals on species richness and abundance on TD8 (Mufulira)




Dich cin
Vach pol
Bauh pet
Albi ant
FicsCapn
Azan gar
Cd
Ficu syc
Comb zey
Term mol
Term ste
Comb mol
Rhu lon
Anno sen
Ficu cra
Syzy gui
Comb mic
1.0
-1.0
Azan gar
Syzy gui
Term ste
Rhu lon
Comb mol
Comb mic
Ficu cra
Term mol
Comb zey
Ba
Anno sen
Ficu syc
FicsCapn
Albi ant
Bauh pet
Vach pol
Dich cin
-0.6
Azan gar
Vach pol
Dich cin
Syzy gui
Term ste
Mo
FicsCapn
Anno sen
Bauh pet
Rhu lon
Albi ant
Ficu syc
Comb zey
Comb mol
Term mol
Ficu cra
Comb mic
Dich cin
Vach pol
Bauh pet
Albi ant
FicsCapn
Azan gar
B
Ficu syc
Anno sen
Term mol
Comb zey
Rhu lon
Term ste
Comb mol
Ficu cra
Syzy gui
Comb mic
0.8
-0.6
Dich cin
Vach pol
Bauh pet
Albi ant
FicsCapn
Azan gar
Ficu syc
Al
Anno sen
Comb zey
Term mol
Term ste
Comb mol
Rhu lon
Ficu cra
Comb mic
Syzy gui
Dich cin
Vach pol
Albi ant
FicsCapn
Bauh pet
Ficu syc
S
Anno sen
Comb zey
Term mol
Comb mol
Rhu lon
Term ste
Ficu cra
Syzy gui
Comb mic
Azan gar

Appendix 8: CCA analysis showing influence of heavy metals on species richness and abundance on TD10 (Mufulira)




Albi ant
Term mol
Comb zey
Domb rot
Lann dis
Comb mol
Anno sen
Vach pol
Ficu syc
Cd
Phyl gui
Senn sin
Rhu lon
Bauh pet
Syzy gui
Comb mic
Comb mol
Ficu cra
AlbzAmar
Comb mo
1.0
-0.6
-1.0
1.0
Ba
B
Mo
Al
S
-0.6
1.0

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Appendix 9: Monte Carlo permutation tests results per site

	TD25 (Kitwe)		TD26 (Kitwe)		TD24 (Luanshya)		TD25 (Luanshya)		TD26 (Luanshya)		TD8 (Mufulira)		TD10 (Mufulira)	
Heavy metal	F	P-Value	F	P-Value	F	P-Value	F	P-Value	F	P-Value	F	P-Value	F	P-Value
Cu	0.9	0.644	0.9	0.608	0.7	0.768	1.5	0.13	1.6	0.064	1.0	0.482	2.2	0.002
Mn	0.8	0.746	1.7	0.064	0.6	0.95	1.9	0.018	2.1	0.074	0.6	0.85	0.9	0.628
Zn	0.8	0.518	1.2	0.369	0.7	0.862	0.8	0.538	0.2	1	0.7	0.662	1.8	0.018
Ni	0.8	0.758	1.0	0.46	0.7	0.854	1.3	0.246	1.2	0.266	0.5	0.974	0.9	0.6
Cr	1.0	0.472	0.9	0.48	0.7	0.878	1.7	0.048	2.0	0.018	1.1	0.396	1.4	0.118
Co	0.6	0.862	1.1	0.306	0.6	0.916	0.3	0.958	0.4	0.936	0.8	0.68	1.6	0.088
Cd	0.8	0.676	1.3	0.17	0.7	0.844	1.2	0.292	1.0	0.404	1.1	0.264	1.2	0.286
B	0.9	0.525	1.1	0.408	0.7	0.85	1.6	0.086	1.3	0.198	1.0	0.458	1.5	0.102
Ba	0.8	0.686	0.9	0.548	0.7	0.918	0.4	0.93	0.4	0.952	1.1	0.302	0.6	0.906
Mo	1.4	0.208	0.6	0.872	0.9	0.662	1.2	0.236	1.0	0.422	1.8	0.004	1.0	0.512
Al	1.1	0.38	0.9	0.55	0.7	0.896	2.2	0.008	1.5	0.146	1.1	0.384	1.3	0.134
S	2.2	0.072	1.1	0.432	0.5	0.972	1.0	0.442	0.8	0.608	1.2	0.222	1.7	0.02

Appendix 10: Mean heavy metal concentration of Copperbelt tailings dams, Screening Benchmark and CCMERC soil metal guideline limits

Heavy metal	Mean (mg/kg) ± SE	Screening Benchmark (mg/kg)	CCMERC (mg/kg)
Cu	17425,591 ± 2158,161	200	150
Mn	11369,180 ± 1038,886	500	-
Zn	227,153 ± 31,576	50	600
Ni	197,099 ± 44,998	30	150
Cr	628,053 ± 31,926	1	750
Co	1073,598 ± 405,718	20	40
Cd	-9,831± 1,434	4	3
B	407,506 ± 62,286	0,5	2
Ba	938,800 ± 232,376	500	750
Mo	55,843 ± 7,706	2	5
Al	147083,033 ± 34367,468	50	-
S	3385,845 ± 806,626	-	-

The – shows that the metal limit is not provided.