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Post-Mining Restoration in Zambia

Screening native tree species for phytoremediation potential

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Post-Mining Restoration in Zambia. Screening native tree species for phytoremediation potential

Abstract

Africa has a long history of mining, which has generated a large amount of wastes (tailings dams and overburden materials) that are hazardous for the environment and human health. Phytoremediation, which involves the use of plants and microorganisms to reduce the toxic effects of heavy metals in the soil environment, is considered an efficient, eco-friendly and cost-effective restoration method. However, little is known about Zambian native tree and shrub species that are suitable for phytoremediation. Thus, the main objective of the studies presented in this thesis was screening candidate tree species for phytoremediation of copper (Cu) mine wastelands. This was achieved first by reviewing the state of knowledge about restoration of mine wastelands in Africa, followed by characterization of mine wastes to get insights into the barriers to restoration, a survey of autochthonous colonization of tailings dams and analysing their biological traits, and finally testing the tolerance mechanism of selected candidate species to elevated Cu concentrations and the potential of biochar and poultry manure as amendments to boost survival and growth. The results showed that (1) research and application of post-mining landscape restoration in Africa is very limited focusing mainly on identifying herbaceous species suitable for phytoremediation; (2) Cu mine wastes were characterized by high soil compaction, poor macro-nutrient availability, high soil acidity, and toxic level of heavy metals, which are the main constraints for successful phytoremediation; (3) there was autochthonous colonization of tailings dams with a floristic composition distinct from nearby natural forests, and the main biological traits of species colonizing tailings dams were light-demanding (93%), moderately tolerant to elevated copper concentration (87%), suitable for erosion control (75%) endomycorrhizal (47%) and nitrogen-fixing (29%); (4) candidate species for phytoremediation tolerated high Cu concentration by excluding its uptake by roots; and (5) amendments of tailings dams substrate with biochar, poultry manure and their combination slightly boosted survival and growth of some selected species. As a whole, the pace of restoration research and practice after mining disturbance in Africa is slow and as mining activities are likely to increase, restoration of mine wastelands will become even more urgent. The know-how to restore those landscapes requires a more mainstreamed approach that can easily be implemented by the mining companies in order to make the mining sector "green".

Keywords: copper mining, tailings dams, restoration, phytoremediation, native tree species, miombo

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Restaurering efter kopparbrytning i Zambia. Potential för fytoremediering med inhemska trädslag

Abstract

Afrika har en lång historia av gruvdrift, vilket har generat stora mängder av slaggprodukter (anrikningsdammar och gråberg) som är farliga för både miljön och för människors hälsa. Fytoremediering, vilket involverar användning av växter och mikroorganismer för att minska de toxiska effekterna av tungmetaller i jordmiljön, anses vara en effektiv, miljövänlig och kostnadseffektiv restaureringsmetod. Lite är dock känt om Zambias inhemska träd- och busksarter lämpliga för fytoremediering. Därför var huvudfokus för studierna i denna avhandling att söka efter potentiella trädslag för fytoremediering på marker efter brytning av koppar (Cu). Detta påbörjades med en omfattande granskning av kunskapen inom restaurering av gruvområden i Afrika, följt av en karakterisering av gruvavfall för att öka förståelsen för vilken problematik som restaureringsförsök står inför. Vidare utfördes en studie om autokton kolonisering på anrikningsdammar och analys av dessa arters biologiska egenskaper, samt slutligen klarlägga utvalda trädslags toleransmekanismer mot förhöjda kopparhalter med eller utan organiska tillsatser såsom biokol och hönsgödsel, samt hur dessa tillsatser kan påverka överlevnad och tillväxt. Resultaten visade att (1) forskning om och tillämpning av resultat från restaurering efter gruvindustrin i Afrika är mycket begränsad och huvudsakligen fokuserad på identifiering av örter och gräs vilka är lämpliga för fytoremediering; (2) anrikningsdammar och gråberg med förhöjda halter av Cu kännetecknades av hög jordkomprimering, dålig tillgänglighet av makro-näringsämnen, lågt pH samt en toxisk nivå av tungmetaller, vilket är de huvudsakliga begränsningarna för en framgångsrik fytoremediering; (3) det fanns autokton kolonisering av anrikningsdammar med en tydlig skillnad i den floristiska sammansättningen jämfört med intilliggande naturskog. De viktigaste biologiska egenskaperna hos arter som koloniserade anrikningsdammarna var att de var ljuskrävande (93%), måttligt toleranta mot förhöjd kopparkoncentration (87%), lämpliga för erosionskontroll (75%) samt hade endo-mykorrhizal symbios (47%) med kvävefixerande mikroorganismer (29%); (4) kandidatarter för fytoremediering tolererade hög Cu-koncentration genom att undvika upptag genom rötterna; och (5) förändring i anrikningsdammarnas substrat genom tillsats av biokol, hönsgödsel och dess kombination ökade till viss del överlevnad och tillväxt hos några av de testade trädarterna. Som helhet är takten i forsknings- och utvecklingsarbetet om restaurering av gruvområden i Afrika långsam. Detta belyser, i motsats till vad många gruvföretag och tillsynsmyndigheter påstår, att vetenskaplig forskning om restaurering samt framtagande av progressiva återställningsrutiner efter gruvdrift i nuläget är alltför begränsad. Av den anledningen måste restaurering av anrikningsdammar och gråberg prioriteras högre i nationella forskningsstrategier, utvecklingsplaner och miljöpolitik, allt för att göra gruvsektorn ”grön”.

Keywords: kopparbrytning, anrikningsdammar, restaurering, fytoremediering, inhemska trädslag, miombo

Dedication

Till männen i mitt liv. Och mamma.

Fail better
Neil Gaiman

Science is not about building a body of known 'facts'. It is a method for asking awkward questions and subjecting them to a reality-check, thus avoiding the human tendency to believe whatever makes us feel good.
Sir Terry Pratchett

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Emma Sandell Festin, Mulualem Tigabu*, Mutale N. Chileshe, Stephen Syampungani, Per Christer Odén (2019). Progress in restoration of post-mining landscape in Africa. *Journal of Forest Research*, 30 (2), pp. 381-396.

II Mutale N. Chileshe, Stephen Syampungani, Emma Sandell Festin, Mulualem Tigabu*, Abolfazl Daneshvar, Per Christer Odén (2019). Physico-chemical characteristics and heavy metal concentrations of copper mine wastes in Zambia: implications for pollution risk and restoration. *Journal of Forest Research*, https://doi.org/10.1007/s11676-019-00921-0

III Emma Sandell Festin*, Carl Salk, Mulualem Tigabu, Stephen Syampungani, Per Christer Odén. (2019) Biological traits of tropical trees suitable for restoration of copper-polluted lands. *Ecological Engineering,* 138, pp 118-125

IV Emma Sandell Festin*, Carl Salk, Mulualem Tigabu, Stephen Syampungani, Liu Aiqin, Henrik Böhlenius, Per Christer Odén (2020). Tolerance of selected native Zambian tree species to elevated copper concentrations with implications for phytoremediation of tailings dams – A greenhouse experiment. *Environmental Science and Engineering* (in review)

V Emma Sandell Festin*, Mulualem Tigabu, Carl Salk, Lui Aiqin. Stephen Syampungani, Per Christer Odén. Is survival and growth performance of native Zambian trees on tailings dam substrate boosted by biochar and chicken manure? (manuscript)

Papers I-III and are reproduced with the permission of the publishers.

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The contribution of Emma Sandell Festin to the papers included in this thesis was as follows:

I. Designed the review study with co-authors, collected information and wrote most of the manuscript.

II. Designed the study with co-authors, and co-wrote the manuscript.

III. Designed the study with co-authors, coordinated and conducted field and laboratory work, managed data analyses and wrote most of the manuscript

IV. Designed the study with co-authors, coordinated and conducted greenhouse experiment and prepared the samples for analysis and wrote most of the manuscript.

V. Designed the study with co-authors, coordinated the field work and wrote the manuscript.

Abbreviations

AMD	Acid mine drainage
As	Arsenic
ANOVA	Analysis of variance
Ba	Barium
BF	Bioaccumulation factor
Ca	Calcium
CD	Degree of contamination
Cd	Cadmium
CEC	Cation exchange capacity
Cif	Contamination factor
Co	Cobalt
Cr	Chromium
Cu	Copper
DRC	Democratic Republic of Congo
GLM	Generalized linear model
K	Potassium
Mg	Magnesium
N	Nitrogen
Na	Sodium
Ni	Nickel
NF	Natural forest
P	Phosphorus
PBS	Percentage base saturation
Pd	Lead
PLI	Pollution load index
RGR	Relative growth rate
TD	Tailings dam
TF	Translocation factor
V	Vanadium
Z	Zinc

1 Introduction

The world we are living in is in a constant state of change, be it from seasonal changes, different weather patterns or anthropogenic activities. Throughout human history we have been, and still are, dependent on natural resources and have altered the landscape, leading to land-use changes. Going back to the evolution and development of the human race, we have used tools, originally made from wood and stones and later from different types of metals. One of the metals that played an important role in shaping our society and is still very much needed is copper.

The demand for copper has gone from small-scale mining to large-scale mining operations with artisanal mining still taking place in some areas in the world. Mining operations have created vast areas of barren wastelands and this has led to a demand for restoration of those landscapes back to a more natural state with beneficial ecosystem services.

Unrestored landscapes, in particular post-mining landscapes, pose a threat to both the environment and human health as they are contaminated with high levels of heavy metals that pollute the surrounding area and can be accumulated into food chains, making agricultural crops unsuitable for consumption. Restoring post-mining landscapes is complicated as there are many different challenges. Not only is the environment a harsh growing medium for most plants, but there also is a lack of knowledge of which plants are suitable for restoration purposes. Identifying native species to that are adaptable and tolerant towards elevated levels of heavy metals is of paramount importance.

Zambia, a country rich in minerals, in particular copper, has seen rapid changes during the past century in terms of mining and land use. Thousands of hectares are covered with some kind of mining waste with no or limited restoration attempts.

1.1 Copper and its mining history

Copper (Cu) is a mineral that is scattered throughout the globe and in some areas is deposited in concentrations that make it economic to mine. In today's society, Cu is a necessity in our daily life. Cu is used in computers, smartphones, for infrastructure and so on (Palacios *et al.*, 2019; Mihaljevic *et al.*, 2018; Elshkaki *et al.*, 2016). It has also played an important role in the development of human technology, first in the Bronze Age and growing quickly during and after the industrial revolution (Tunsu *et al.*, 2019; Ohno *et al.*, 2016). In short, we are and have been, dependent on copper for a very long time (Kinnunen & Kaksonen, 2019; Palacios *et al.*, 2019). In 2016 the global output of Cu mining reached 20.2 million tonnes (Pietrzyk & Tora, 2018) and has increased since then.

1.2 Mining in Africa

Mining has taken place in Africa since the iron age (Miller, 2002) and has a long tradition, mainly as small-scale, artisanal, mines that have been scattered throughout the landscape (Neina *et al.*, 2019; Ashton *et al.*, 2001). Large-scale mining industries started to develop during the colonial era (Ashton *et al.*, 2001; Miller, 2000) throughout sub-Saharan Africa. Because of the long history of mining in Africa, many of the copper and gold mines in countries like the Democratic Republic of Congo (DRC), Zimbabwe and Zambia are located on historical mine sites (Mwitwa *et al.*, 2012; Ashton *et al.*, 2001).

As the mining activities have grown throughout sub-Saharan Africa both local and foreign investors have bought or started new mines. Today, mining is one of the major economic ventures in Africa (Yabe *et al.*, 2010), creating a source of income, mainly through employment, and as the global demand for metal and minerals will increase, mining activities will continue to develop in many African countries (Lindahl, 2014; Sheoran & Sheoran, 2006; Miller, 2002; Limpitlaw, 2000). It is especially expected to increase for countries in the African Copperbelt, such as DRC and Zambia, as these areas have some of the richest deposits of Cu ore in the world (Kaniki & Tumba, 2019; Shutcha *et al.*, 2015) At least 50% of African countries have some kind of mining activity (Kaninga *et al.*, 2019; Kolala & Bwalya Umar, 2019).

Mining creates a destructive impact on the local environment (Gitari *et al.*, 2018) and can also lead to social conflicts in some places (Simutanyi, 2008).

1.2.1 Mining in Zambia

Zambia is rich in both cobalt and copper, mainly in the Copperbelt region, a region named after its richness of Cu (Kaniki & Tumba, 2019; Lindahl, 2014; Brems *et al.*, 2009; McGowan *et al.*, 2005) in the north-central part of the country. The mining history dates back to 650 AD (Mwaanga *et al.*, 2019; Mwitwa *et al.*, 2012; Weissenstein & Sinkala, 2011) and large-scale mining started in 1928 (Mwanamuchende *et al.*, 2019; Makondo *et al.*, 2015) only to peak around 1960-1970, making Zambia to one of the top ten producers of Cu in the world (Pietrzyk & Tora, 2018; Ettler *et al.*, 2011; Lillian, 2011; Smithen, 2003) where 30 million tonnes of Cu has been produced (Ettler *et al.*, 2011). On average the ore-grade is above 30 g kg^{-1}, making the Copperbelt region together with DRC one of the richest Cu mining districts in the world (Mwaanga *et al.*, 2019; Ettler *et al.*, 2011; McGowan *et al.*, 2005).

1.3 Mine wastelands

Long-term mining activities result in large areas being covered by different types of mine waste (Ettler *et al.*, 2011). The wastelands are often abandoned open-pit mines, loose soil piles, waste rock, overburden, tailings dams, areas stripped of vegetation and other types of degraded land through mining facilities (Venkateswarlu *et al.*, 2016; Sikaundi, 2010; Wong, 2003). The enrichment process and extraction of metals form byproducts in liquid, gaseous or solid states (Gitari *et al.*, 2018; Pourret *et al.*, 2016). Both the liquid and solid forms can further be divided into overburden, waste rock or tailings dams (Sandell Festin *et al.*, 2019a; Li, 2006).

Mine waste is a global concern. For instance, over 4700 million tonnes of mine waste are stored throughout the European Union. The mining industry creates 70% of China's total solid waste and each year transforms the use of 2000 ha of land (Gitari *et al.*, 2018; Li, 2006). The majority of the waste is deposited in tailings dams and the mining industry creates hundreds of thousands tonnes of tailings each year (Gitari *et al.*, 2018). For instance, depending on the copper content in the rock, to produce one ton of copper, 350-420 tonnes of waste are produced, sometimes even more (Kaninga *et al.*, 2019; Kangwa, 2008).

Due to the long history of mining, thousands of abandoned mines can be found in several countries, for instance, in South Africa alone approximately 6150 mines are left behind with limited, if any, type of restoration done (Venkateswarlu *et al.*, 2016). In the Copperbelt region of Zambia alone, there are 45 tailings dams covering roughly 9125 ha, 20,646 ha of land is covered by overburden, and another 680 ha is covered by waste rock and slag (Sikaundi, 2013; Lindahl, 2014). The characteristics of the two major mine wastes, tailings dams and overburden materials, are described below.

1.3.1 Tailings dams

Tailings dams are hydraulic structures that are created to store large quantities of mine waste (Neina *et al.*, 2019; Gitari *et al.*, 2018; Ginocchio *et al.*, 2017; Pak & Nabipour, 2017; Wang *et al.*, 2014) and can be either a retention dam or raised embankments (Fig. 1). A single tailings dam can cover several square kilometres and the dam walls can be tens of meters high (Schoenberger, 2016). Once the dam is taken into use, the materials disposed into the dam are in a slurry-based form (Wang *et al.*, 2014), consisting of solid sediments and fluid waste water (Shamsai *et al.*, 2007) as well as heavy metals and sulphides (Chileshe *et al.*, 2019; Kaninga *et al.*, 2019; Ngulube *et al.*, 2016).

Tailings dams often consist of fine-grained material ranging from 625 μm to 2 mm (Edraki *et al.*, 2014; Kossoff *et al.*, 2014), depending on the parent rock, and are homogenous. Common characteristics of tailings are high bulk density, compaction due to the fine soil texture and low infiltration rate (Ginocchio *et al.*, 2017; Mensah, 2015; Titshall *et al.*, 2013; Wong, 2003), and a low water holding capacity (Ginocchio *et al.*, 2017; Shi *et al.*, 2016; Ssenku *et al.*, 2014). As tailings are created during extraction of minerals and metals, the soils are young and lack cohesion (Asensio *et al.*, 2018) and have limited organic matter (Titshall *et al.*, 2013; Cooke & Johnson, 2002) and microbial activity (Ginocchio *et al.*, 2017; Shi *et al.*, 2016; Ssenku *et al.*, 2014), can be acidic and contain elevated levels of heavy metals (Chileshe *et al.*, 2019; Shi *et al.*, 2016; Lottermoser, 2010; O'Dell *et al.*, 2007).

1.3.2 Overburden materials

To start any kind of mining activity, the top-layer of soil and rock is removed to gain access to the ore deposits underneath, creating what is called overburden materials (Rankin, 2011; Vela-Almeida *et al.*, 2016). This type of operation can remove material down to a depth of 30 m, sometimes more (Carrick & Krüger, 2007), and the removed layers are stored either in dumps at the mining (Fig. 1) sites or used elsewhere (Franks *et al.*, 2011; Sheoran *et al.*, 2010). Ore with too low mineral concentrations is also part of the overburden as it is not of interest for the industry and is stored at the mining sites (Broda *et al.*, 2015; Rankin, 2011) as rock dumps.

The overburden is nutrient poor and unsuitable for reclamation purposes due to the potential phytotoxicity of the excavated soils without amendments

(Carrick & Krüger, 2007), but can be used for landscape contouring and covered with layers of suitable soil for reclamation attempts (Rankin, 2011).

Overburden is typically coarse-grained rocks that are heterogeneous and a source of acid mine drainage (AMD), which risks polluting the groundwater as the sulphides present in the rocks react with rain water (Broda *et al.*, 2015; Franks *et al.*, 2011; Naicker *et al.*, 2003). The waste rock piles can occupy large areas and are a concern for the local environment through the risk of AMD (Franks *et al.*, 2011).

Figure 1. Photos from different tailings dams. A, aeolian erosion due to a light wind created a dust cloud blowing away from the surface of the dam and over the wall into the natural forest surrounding the dam, TD10, Mufulira. B, a dam wall in the distance, seen from the embarkment of TD8, Mufulira. C, a dead shrub and patches of grass that occasionally sprout on the dam with often low survival rate. TD10, Mufulira. D, an older dam in Kitwe that is also used as a garbage disposal has been colonized at the edges of the dam by trees and parts of the centre have a grass cover. E, shows the start of water erosion that is carving pathways into the surface of the dam during the rainy season, TD10, Mufulira. F, gullies (2-3 meters deep in places) carved into the dam after the rain, TD8, Mufulira. G, former above ground mine with an entrance to an underground mine, Chibuluma. H, Waste rock pile close to the mine, Chibuluma. I, road down to the mine after excavation for the above ground mine, waste rock and overburden can be seen in the distance, Chibuluma. (photos: Emma Sandell Festin).

1.4 Socio-environmental impacts of mining

Mining is by its very nature destructive and causes direct and indirect changes in the landscape (Kolala & Bwalya Umar, 2019; Beckett & Keeling, 2018;

Olobatoke *et al.*, 2016; Schoenberger, 2016; Ettler *et al.*, 2011; Yabe *et al.*, 2010) and the local environment (Gitari *et al.,* 2018) and can lead to social conflicts (Simutanyi, 2008). Although the areas affected by mining are relatively small compared to agricultural conversion or logging, it is one of the main drivers of deforestation (Mwitwa *et al.*, 2012).

The most influential impact of mining is the change of land use and land form due to the clearing of vegetation, the removal of topsoil and the creation of large amounts of mine waste (Beckett & Keeling, 2018; Mwitwa *et al.*, 2012), both during mining operations and ore processing (Kinnunen & Kaksonen, 2019; Nyakudya, 2011; Kangwa, 2008; Remon *et al.*, 2005).

Not only does mining alter the landscape, it also leaves other long-lasting negative environmental impacts, such as air pollution, soil contamination, water pollution and siltation (Mwaanga *et al.*, 2019; Ekta. & Modi, 2018; Ginocchio *et al.*, 2017; Bascetin *et al.*, 2016; Olobatoke *et al.*, 2016; Makondo *et al.*, 2015; Csavina *et al.*, 2014; Lindahl, 2014; Ssenku *et al.*, 2014). Globally, between 5 and 7 billion tonnes of tailings dams are created and over 100 million hectares covered in some type of mining waste each year (Sandell Festin *et al.*, 2019; Bascetin *et al.*, 2016; Edraki *et al.*, 2014; Wei *et al.*, 2013). The increasing amount of land mined over the globe has created an environment that in the absence of adequate mining closure management, poses a serious threat to human health and agricultural productivity. Tailings dams present serious pollution hazards to the environment, human health, agricultural productivity and ground water (Kaninga *et al.,* 2019). The threat to human health is mostly due to the close proximity of the dams to human settlements (Mwaanga *et al.,* 2019; Tutu et al., 2008). The threat consists of several aspects, such as groundwater pollution, bioaccumulation into food chains and contaminated soils (Kaninga *et al.*, 2019; Karaca *et al.*, 2018; Ginocchio *et al.*, 2017; Olobatoke *et al.*, 2016; Lindahl, 2014; Ssenku *et al.*, 2014; Chaturvedi *et al.*, 2012; Juwarkar *et al.*, 2009).

Groundwater pollution is often due to AMD (Tutu el al., 2008) and is a threat to nearby human communities and agriculture practices (Tutu *et al.*, 2008; Sheoran & Sheoran, 2006; von der Heyden & New, 2004) as the low pH and heavy metal content can affect the surrounding landscape (Karaca *et al.*, 2018).

Soil contamination is created by aeolian dispersion and water erosion (Ginocchio *et al.*, 2017) and the concentration is highest up to a distance of 100 m from the edge of the dam walls, but can be spread as far as 2 km from the dam walls (Kuter, 2013). Soil pollution negatively affects the microbial community in terms of diversity, size, composition, activity and survival (Yang *et al.*, 2017; Mensah, 2015; Liao & Xie, 2007; Wang *et al.*, 2007; Wong, 2003). Contaminated soils with elevated levels of heavy metal are often barren as the metal concentrations are toxic to most plants; only a few exceptionally tolerant species can grow on these types of soils (Ssenku *et al.,* 2014)

1.5 Restoration of mine wastelands

The Society for Ecological Restoration (2004) defined ecological restoration as "*the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed*." In mining context, restoration is often used interchangeably with rehabilitation, reclamation and remediation (Sandell Festin *et al.,* 2019a; Seabrook *et al.,* 2011), thus the term restoration is being used in this thesis.

Restoration of mine wastelands poses serious challenges (Jutsz & Gnida, 2015), as both the abiotic and biotic changes have been too extreme to be able to restore the landscape back to its original state (Pietrzykowski, 2015; Hobbs *et al.,* 2009). In the end a novel ecosystem will be created (Hobbs *et al.,* 2009). One of the biggest challenges of post-mining restoration is that remediation of soils polluted by heavy metals is more complex and complicated than other types of restoration as heavy metals cannot be destroyed. They can only be transformed from an organic compound or oxidized to another form (Jutsz & Gnida, 2015), meaning that for a successful restoration, human assistance is often required (Holl & Aide, 2011; McIver, 2001).

1.5.1 Restoration approaches and methods

There are two major types of restoration: passive and active restoration approaches. Passive restoration involves an effort to minimise further anthropogenic disturbances, thereby allowing natural or unassisted recovery of the disturbed site, whereas active restoration entails an active human intervention in efforts to accelerate and/or influence the successional trajectory (Mensah, 2015; Weiersbye *et al.,* 2006; Leteinturier *et al.,* 2001; Bradshaw, 2000).The active approach includes physical, chemical and biological methods to aid in the restoration attempts (see Paper **I** for further details).

One of the biological methods that has gained increasing attention in both research and application is phytoremediation. Phytoremediation involves the use vegetation to reclaim and cover mine wastelands in order to minimize the toxic effects of contamination in the surrounding environment (Mendez & Maier, 2007) and is described in detail below.

1.5.2 Phytoremediation

In recent decades, phytoremediation has been frequently used to restore mine wastelands and is considered to be efficient, eco-friendly and cost-effective (Mendez & Maier, 2007). Species ideal for phytoremediation should either translocate and accumulate heavy metals in the shoots (accumulators) or exclude uptake of heavy metals by the root (excluders) and survive high acidity and other harsh post-mining conditions, and grow quickly (Anning & Akoto, 2018). This technique is particularly beneficial on soils where the biotic and/or abiotic changes have been too extreme for restoration back to its original state (Hobbs *et al.*, 2009) and can also be used on sites for removal of heavy metal and other pollutants (Ekta. & Modi, 2018; Chaney & Baklanov, 2017; Ali *et al.*, 2013; Chehregani *et al.*, 2009). Another reason why using plants is preferable for post-mining restoration is the high cost of chemical and/or physical restoration methods, and several other technical limitations (Ekta & Modi, 2018; Mahar *et al.*, 2016; Mokgalaka-Matlala *et al.*, 2010).

Metalophytes, plants adapted to grow on soils with naturally elevated levels of heavy metals, are preferable for phytoremediation as they have evolved to grow and reproduce under harsh conditions (Titshall *et al.*, 2013; Weiersbye, 2006). This is due to the two major heavy metal resistance strategies: exclusion which restricts the transport of metals into the aboveground biomass, and accumulation which translocates metals into the aboveground biomass (Mahar *et al.*, 2016; Jutsz & Gnida, 2015; Baker, 2008).

Successful phytoremediation of mine wastelands depends on the plants' traits. These include an ability to tolerate the harsh growth environment, have a high growth rate (Silva *et al.*, 2015; Souza *et al.*, 2013), extensive root system (Ssenku *et al.*, 2014), nitrogen fixation, mycorrhizal symbiosis (Blaser *et al.*, 2014; Ssenku *et al.*, 2014; Gathuru, 2011; Singh *et al.*, 2004a), drought tolerance, a high light requirement, general stress tolerance (Sandell Festin *et al.*, 2019b; Ilunga wa Ilunga *et al.*, 2015; Nirola *et al.*, 2015; de Souza *et al.*, 2013) and a capability to compete with less tolerant plants (Dadea *et al.*, 2017; Mahar *et al.*, 2016; Ilunga wa Ilunga *et al.*, 2015; Baker, 2008; Brooks, 1989). Native species are preferable as non-native species have the potential to become invasive and impact biodiversity (Ekta. & Modi, 2018). The species that spontaneously colonize, establish and grow prior to restoration are of interest (Ginocchio *et al.*, 2017). as they have a higher chance of surviving and growing on land polluted by heavy metals (Delhaye *et al.*, 2016; Faucon *et al.*, 2015; Ilunga wa Ilunga *et al.*, 2015)

1.5.3 Using trees for phytoremediation

Previous studies of post-mining restoration have mainly focused on evaluating the phytoremediation potential of grasses, herbs and to some extent shrubs (Laghlimi *et al.*, 2015). Only recently has the focus shifted towards trees (Sandell Festin *et al.*, 2019a). Grasses have been extensively studied as they have several beneficial characteristics for phytoremediation (Karaca *et al.*, 2018; Laghlimi *et al.*, 2015). Grasses are often pioneer species and quickly create a ground cover. Shrubs have been studied as well, and often in terms of a secondary successor after grasses to help, in combination with trees, create nutrients through litter fall, provide shade and lower the water stress and improve soil properties (Laghlimi *et al.*, 2015; Hamzah & Priyadarshini, 2014; Sinha *et al.*, 2013).

There are several benefits of using trees for phytoremediation. Planting a mixture of tree species can create long-term vegetation cover, increase the available nutrients and organic matter in the soil through litter fall (Sandell Festin *et al.*, 2019a; Osei-Tutu *et al.*, 2018; Ssenku *et al.*, 2014) and forms a less homogeneous landscape (Singh *et al.*, 2004b). Some tree species have a capacity to immobilise metals in their tissues, mainly the roots, and can help decrease the amount of heavy metals in the surface soil (Karaca *et al.*, 2018; Silva *et al.*, 2015). As trees have a larger and deeper root system (Karaca *et al.*, 2018) it can lead to less compacted soils and help decrease bulk densities (Osei-Tutu *et al.*, 2018; Ilunga wa Ilunga *et al.*, 2015; Laghlimi *et al.*, 2015; Mensah, 2015; Singh *et al.*, 2004b). The survival rate can be increased by using native species with less risk of dying from local pests and pathogens (Osei-Tutu *et al.*, 2018; Bozzano, 2014).

1.5.4 Phytoremediation with organic amendments

To assist restoration of degraded lands, organic amendments can be used, such as biochar, green waste and animal manure in combination with other biological and physical methods (Asensio *et al.*, 2013; Karami *et al.*, 2011; Beesley *et al.*, 2010). Each amendment has its own benefits and is suited for different degraded soils. Biochar, a low-density material of up to 90% carbon content (Tang *et al.*, 2013) is produced through pyrolysis of different types of organic residues (Beesley *et al.*, 2011). Addition of biochar helps create a more favourable growing environment for most plants (Sovu, 2011) as it contains nutrients essential for plant growth: nitrogen, phosphorus, calcium, iron, zinc and potassium (Forján *et al.*, 2017). It also boosts the pH, increases water holding capacity (Fellet *et al.*, 2011; Beesley *et al.*, 2010) and helps immobilize pollutants in the soil (Tang *et al.*, 2013; Beesley & Marmiroli, 2011).

Biochar is a useful amendment with some limitations, mainly that only adding biochar to soils with a low nutrient value can increase arsenic mobility and cationic nutrient immobilization (Nandillon *et al.*, 2019). For aided restoration on poor soils, biochar together with other types of organic amendments create a better environment for plant establishment (Tang *et al.*, 2013; Fellet *et al.*, 2011; Karami *et al.*, 2011; Beesley *et al.*, 2010). Organic residue, such as compost or animal manure can be used (Asensio *et al.*, 2013) and can increase microbial activity and decrease the concentration of contamination within the soil (Carlson *et al.*, 2015; Ruttens *et al.*, 2006). Apart from increasing nutrient availability, organic residues also increase the water holding capacity of the soil (Shutcha *et al.*, 2015).

2 Objectives

As the demand for metals such as Cu is unlikely to decrease, the need for restoration of mine wastelands is necessary to minimize the environmental impacts and to prevent potential threats to human health. However, there is still a lack of scientifically-proven methods for restoration of mine wastelands, such as tailings dams. Phytoremediation technology is considered as efficient, eco-friendly and cost-effective. Nonetheless, there is little knowledge about Zambian native tree and shrub species that are suitable for phytoremediation. Thus, the main objective of the studies presented in this thesis was screening candidate tree species for phytoremediation of Cu mine wastelands. This was achieved first by reviewing the state-of-knowledge about restoration of mine wastelands in Africa; second through characterization of the mine wastes to get insights into the barriers to restoration, third by conducting a vegetation survey of spontaneous colonization of tailings dams and analysing their biological traits, and finally testing the tolerance mechanism of selected candidate species to elevated Cu concentrations and the potential of biochar and poultry manure as amendments to boost survival and growth.

The specific research questions:

- I What is the state-of-knowledge about post mining landscape restoration in Africa? What are the gaps in research and application of restoration?
- II How do mine wastelands' physico-chemical characteristics differ from natural forest soil in terms of heavy metal concentrations? Can the mine wastelands be a source of pollution?
- III Does the floristic composition of species that naturally colonized copper mine tailings dams in Zambia differ from the nearby natural forests? What biological traits can indicate that a woody species can be useful for phytoremediation?

IV Do survival and early seedling growth of potential phytoremediation species differ with respect to elevated Cu concentration? What are the coping mechanisms for elevated Cu concentration?

V Are survival and growth of selected native trees boosted by biochar and poultry manure amendment of tailings substrate?

3 Materials and Methods

3.1 The state-of-knowledge about restoration of mining wastelands (**Paper I**)

An extensive literature review was conducted to synthesize the current knowledge and practice of mining restoration in Africa to identify potential knowledge gaps. The review focused on research that has been done to restore mine wastelands and large-scale practices to restore post-mining landscapes. This was done by searching in Scopus, Web of Science and Google Scholar for phytoremediation, reclamation of mine wastelands, and restoration following mining disturbance in Africa.

3.2 Study areas in Zambia

The study areas for Papers II and III were situated in Chingola, Kitwe, Mufulira and Mwekera in the Copperbelt region in Zambia (Fig. 2). In the three latter sites, a vegetation survey was conducted on tailings dams and natural forests. In Chingola, the physico-chemical characteristics and heavy metal concentrations of tailings dams and overburden materials were studied.

The study areas receives an average of 1000 mm of rainfall annually (Mwaanga *et al.*, 2019) and has three distinct seasons: rainy season (November – April), cool dry season (May – August) and the hot dry season (August – November). Due to the country's elevation between 900-1500 meters above sea level, most areas have a subtropical climate with mean monthly temperatures between 16°C in June and July and 26°C in October (Chileshe *et al.*, 2019).

The vegetation is mainly miombo woodland, which covers around 2.7 million km^2 in south, central and eastern parts of Africa (Gumbo *et al.*, 2018; Kalaba, 2012; Backéus *et al.*, 2006; Frost, 1996). The dominant species are in the genera *Brachystegia*, *Julbernardia* and *Isoberlinia* (Gumbo *et al.*, 2018; Giliba *et al.*, 2011; Syampungani, 2008; Malmer, 2007) and a mature miombo consists of an upper canopy layer with trees between 10-20 m high and few sub-layer trees scattered between (Ribeiro *et al.*, 2015; Desanker & Prentice, 1994).

Despite a nutrient-poor topsoil layer that often is acidic (Malmer, 2007), the miombo is rich in biodiversity with 8500 plant species, half of them endemic, and around 300 tree species (Chirwa, 2015; Ribeiro *et al.*, 2015). Miombo forest supports over 100 million people directly and 50 million indirectly for their daily life. The biome also controls soil erosion, modifies hydrological cycles, maintains soil fertility and is a carbon sink (Gumbo *et al.*, 2018). The high biodiversity is due to nitrogen fixation of many trees, not all of them legumes, and mycorrhizal symbiosis (Sandell Festin *et al.*, 2019b; Malmer, 2007; Högberg & Alexander, 1995).

Figure 2. Map of Zambia. The Copperbelt region is marked in the stripped grey and the sampled areas are within the black dot.

3.3 Characterisation of mine wastes (**Paper II**)

This study was done in Chingola, one of the oldest mining towns in Zambia. A total of 12 overburdens, 12 tailings dams and 6 natural forest sites were randomly selected and 12 soil/substrate samples were collected from each site. At each site, transects were laid out and at every 100 m interval five soil samples within a 20 m radius from the centre were collected with a closed soil auger down to 30 cm depth, mixed together and 2 kilos of composite sample were brought back to the laboratory for analysis.

The physico-chemical characteristics (particle size, bulk density, total organic carbon, total N content, available P, exchangeable cation capacity (CEC) and pH) of the soil/substrate were analysed in the laboratory at Copperbelt University while heavy metal concentrations were analysed in a commercial laboratory in Sweden (Eurofins Environment Sweden AB).

One-way ANOVA was used for analysing the differences in heavy metal concentration and physico-chemical characteristics between the collected substrates. The extent of contamination of sediments by heavy metal was analysed using the following indices: contaminant factor (Cif), the degree of contamination (CD) and pollution load index.

3.4 Autochthonous colonization of tailings dams and biological traits of trees (**Paper III**)

A vegetation survey was conducted on the four tailings dams near the towns of Mufulira (TD8 and TD10) and Kitwe (TD25 and TD29) and four corresponding unpolluted forest stands in the Copperbelt Province of Zambia (Fig. 2) to characterize the species composition and diversity of naturally-regenerated trees. The dams were in use until the mid-20th century except for TD10 which was decommissioned in 1988. The dams in Mufulira have had some restoration efforts, mainly by planting seedlings of *Senagalia polyacantha* close to the dam walls, while little restoration action has been made on the dams in Kitwe.

In each site, 15 plots (5 × 5 m) were laid out along transect lines. The distance between plots was 100-150 m while the distance between transect lines was 200-300 m; the exact distance varied as the sampled areas were of different sizes. As the tailings dams were partly covered by vegetation, the transects lines were placed as far away from each other as possible, but some of the dams had less tree coverage and therefore the distance between the transects was adapted to the local conditions. Similarly, 15 plots were established in each natural forest stand. In each plot, all tree species were counted and identified to species level by local experts with the aid of published references (Storrs, 1979; Palgrave & Palgrave, 2002; van Wyk & van Wyk, 2013).

Soil and leaf samples were collected during the inventory on each plot (Fig. 3). Leaf samples (200 g) were immediately frozen and transported to the laboratory. Five soil samples were taken in each plot, one at each corner and one at the centre of the plot to a depth of 30 cm and then mixed to make a composite sample (2 kg).

Data were analysed by using several complementary analyses to examine patterns of species composition and diversity on tailings dams (TD) and natural forest sites (NF). These analyses included dominant species in TD and NF, species similarity and rarefaction to assess site-specific patterns of species diversity. To identify species suitable for phytoremediation of heavy-metal laden sites, we compiled a database of biological traits for each species encountered in our field sites. We examined whether these traits differed systematically between plants growing on tailings dams and natural forests using χ^2 tests or Mann-Whitney-Wilcoxon tests.

3.5 Survival and growth of seedlings in response to contamination by elevated Cu concentration (**Paper IV)**

A greenhouse experiment was carried out at the Swedish University of Agricultural Science, Alnarp, Sweden for 6 months to examine the tolerance of selected Zambian native species to elevated Cu concentration so as to evaluate their potential for phytoremediation of Cu mining wasteland. Based on our field study, 17 tree and shrub species were selected. The majority of the species had been found on tailings dams or growing in close proximity. An analysis of functional traits had shown that the species were legumes, nitrogen fixing, light demanding or having other attributes that had potential for phytoremediation. The experiment was set up by germinating the selected species from seeds that were pre-treated to promote germination (Appendix 1). However, the overall germination was poor for most of the species and many of the species had a germination success of less than 10% (Appendix 1), with some species lacking any germination at all. Some species had a satisfactory germination but were sensitive to transplanting and were therefore excluded from the experiment as they had a poor survival rate. As a result, only five out of 17 selected species, *Albizia amara*, *Brachystegia bohemii*, *Cassia abbreviata*, *Combretum collinum* and *Senna singueana*, were used for studying survival and growth in response to contamination by elevated Cu concentrations in the growing media.

Figure 3. Photos from the fieldwork done on four tailings dams and corresponding natural forest sites. A, TD10 in Mufulira, at the edge of the vegetation zone. B, Mwekera, soil sampling and leaf sampling in one of the plots. C, Mwekera, leaf sampling from one of the bigger trees within one plot. D, natural forest site, Mufulira, secondary forest after charcoal production. E, TD10, Mufulira, walking along one of the transects towards a new plot. F, TD8, Mufulira, walking along one of the transects through gullies in the centre of the dam. G and H, TD10, Mufulira, sampling two of the plots at the border of the vegetated zone (photos: Emma Sandell Festin).

Figure 4. Photos from the greenhouse experiment. Top row of photos (A-C) is from the start of the experiment and photos D-H are three months after contamination. A, a control seedling of *Senna singuena* at the start of the experiment. B, *Combretum collinum* contaminated with 100 ppm of copper. C, *Senna singuena* contaminated with 750 ppm of copper. D, *Combretum collinum* contaminated with 3000 ppm of Cu. E, *Combretum collinum* contaminated with 1500 ppm of Cu. F, *Albizia amara* contaminated with 3000 ppm of Cu. G, *Cassia abbreviata* contaminated with 3000 ppm of Cu. H, *Brachystegia boehmii* at 100 ppm (photos: Emma Sandell Festin).

Once 65 seedlings had sprouted in the germination tray and when the first non-cotyledon leaf had emerged, the seedlings were transplanted into individual circular plastic pots (18 cm in diameter and 15 cm high) with five drainage holes at the bottom (Fig. 4). Each pot had its own tray to prevent transfer of Cu among pots and potential leaching after irrigation. The pot was filled with 1.5 l of sand and 30 g of slow-releasing fertilizer (Basacote). This fertilizer contained all the required nutrients for healthy growth of most plants, including an amount of Cu equal to 15 ppm in this volume of sand in the pots. The plants were left to grow for 7 days to acclimatize to the growing conditions. Thereafter, each seedling was randomly assigned to one of the following Cu concentrations: 0.75, 5.626, 11.25- and 22.5 g copper chloride (97% $CuCl_2$) per litre distilled water applied as a solution, a total of 20 ml per pot. These concentrations of correspond to 100, 750, 1500 and 3000 ppm Cu, which represented the range of copper concentrations found in the natural forests and tailings dams in the region. After applying the treatments, the pots were randomly placed on the tables, and re-arranged weekly to mitigate any effects of spatially-biased light availability. The temperature in the greenhouse was maintained between 22-28 ˚C and 15-25% relative humidity. Each pot was connected to a separate irrigation tube that was individual for each seedling. Tap water was used for irrigation which contained 0.028 mg/l of Cu. Initially the seedlings received 75 ml of water once per day, which was later increased to twice a day. Height, root collar diameter and stress level were measured and recorded after 30, 60, 120 and 190 days of the treatment.

Seedlings were harvested after 190 days and shoot and root dry mass were determined after oven-drying for 72 hours at 60 ˚C to constant mass. Shoot and root samples were taken from each seedling for analysis of Cu concentration. For this purpose, samples were treated with nitric-perchloric acid and the Cu concentration was determined by ICP-MS (mass spectrometry) or ICP-OES (optical emission spectrometry) at Eurofins laboratory in Sweden. Data on survival rate and shoot to root ratio were analysed using a generalized linear model (GLM) with a binomial error model and a Gaussian error model, respectively. The relative growth rate (RGR) in height and root collar diameter was calculated and analysed. In addition, the bioaccumulation factor (BF) was calculated as the ratio of Cu concentration in the shoot to Cu concentration in the soil (Asensio *et al.*, 2018), and the translocation factor (TF) was calculated as the ratio of Cu concentration in the shoot to Cu concentration in the root (Baker & Brooks, 1989).

3.6 Growth performance of native Zambian tree species on biochar- and manure-amended tailings substrate (**Paper V)**

A nursery experiment was conducted to investigate the potential of biochar and poultry manure as amendments to tailings dams' substrate to boost growth performance of selected tree species suitable for phytoremediation. Based on our floristic study done on tailings dams and corresponding natural forest, five native tree species were selected: *Rhus longipes*, *Terminalia stenostachya*, *Vachelia polyacantha*, *Kigelia africana* and *Phyllanthus muellerianus*. The selection of species for this trial was different from the previous greenhouse study due to a paucity of seeds during the establishment of the nursery trial.

Biochar was produced from wood shaving as feedstocks using a self-designed pyrolysis unit (Fig. 5). The pyrolysis unit was made of a barrel (200 l capacity) with several small holes underneath. The feedstocks were put into the barrel, torched and covered with a lid. The limited air supply came from below through the holes. After approximately one hour of pyrolysis, there was no smoke coming out of the unit, and the fire was put out by spraying water and letting the biochar cool down for 24 hours.

Figure 5. *Production of biochar. A, wood shavings from local species. B, pyrolysis units in the background and wood shavings in the foreground. C, finished biochar (photos: Stephen Syampungani).*

The experiment involved addition of different amounts of biochar, poultry manure or a combination of biochar and manure to tailings dam substrate (Table 1). Each growing pot was filled with treatment-specific amounts of biochar and poultry manure and the tailing substrate. Seeds of the selected species were directly sown in each pot and left to grow under nursery condition for seven months. Seedlings were watered every day, and height and root collar diameter were measured every month.

Table 1. *The different amendments and concentrations added in each pot (g/ 3kg of substrate). Biochar, poultry manure or a combination.*

Treatment name	Amendments	
	Biochar	Chicken manure
C	0	0
B1	25	0
B2	50	0
B3	75	0
M1	0	25
M2	0	50
M3	0	75
BM1	25	25
BM2	50	50
BM3	75	75

At the end of the experiment, seedlings were harvested and shoot and root dry mass measured. Two-way analyses of variance were performed to detect significant differences in relative growth rate in height and root collar diameter, and dry matter content in shoot and roots among different amendments and species. Each treatment had six seedlings of each species.

4 Main results and discussion

4.1 Review of post-mining restoration in Africa (**Paper I)**

The literature review revealed that research on post-mining restoration in Africa is very rare, a total of 24 published studies, compared to elsewhere in the global south. For instance, Asian countries, such as India, the Philippines and China, have a large number of post-mining restoration studies, particularly concentrating relatively more research in phytoremediation (Koelmel *et al*., 2015). Most of the studies in Africa have been focused on identifying species that are accumulators or hyperaccumulators of copper, cobalt and nickel, mainly in D.R Congo (Reeves, 2003) and on species suitable for phytoremediation (Leteinturier *et al.*, 2001).

For most of the studied sites, passive restoration has been reported, with slow processes of natural revegetation and succession. Amendments of mine waste substrates using compost, lime, inorganic fertilizer, topsoil and sewage sludge were evaluated at an experimental scale in the province of Katanga, D.R Congo (Shutcha *et al.*, 2010, 2015), Rwanda (Cao Diogo *et al.*, 2017), South Africa (Titshall *et al.*, 2013) and Zimbabwe (Nyakudya *et al.*, 2011). With regard to large-scale post-mining restoration practice in Africa, there are very few notable cases from Kenya, South Africa and Ghana. In Kenya, 2 km^2 areas of open quarries have been successfully restored using various tree species (Siachoono, 2010). In South Africa, 400 ha of sand mining wasteland has been reclaimed since 1978 using topsoil application methods and natural regeneration of trees from the soil seed bank (Cooke & Johnson 2002). In Ghana, AngloGold Ashanti restored 110 hectares of its concession at the Iduapriem mine using a combination of physical, chemical and biological methods (Tetteh *et al*., 2015; Mensah 2014).

4.2 Characteristics of mine wastes (**Paper II)**

The soil texture in tailings dams and overburden materials was dominated by silt while the natural forest was mostly dominated by sand. The mean bulk density was 1.49, 1.44 and 1.24 g cm^{-3} for tailings dams, overburden and natural forest, respectively. The pH value differed between sites; the natural forest had an average of pH 5.05, the overburdens pH 5.63 and the tailings dams pH 8.4. The contents of total organic carbon and nitrogen were significantly lower in mine waste substrates while the contents of calcium and magnesium were significantly higher in tailings dam substrate than the forest soil. There were no significant differences in concentrations of available P, K and Na between mine waste substrates and natural forest soil.

In terms of heavy metal concentrations, both the tailings dams and overburden substrates had significantly ($p < 0.01$) higher concentrations compared to the natural forest soil with Cu being the most dominant followed by Co. Pollution load index (PLI) for both mine wastes were generally high at 5.84 for the overburden and 8.97 for the tailings dams.

As a whole, the results show that soil compaction, poor macro-nutrient availability and soil acidity (particularly on overburden sites), coupled with toxic levels of heavy metals, are major barriers for revegetation of mine waste sites.

4.3 Floristic composition of tree species naturally colonizing tailings dams and their biological traits (**Paper III**)

A total of 56 tree species were identified, of which 42 were recorded in the natural forest stands and 30 on the tailings dams. While 16 species were found in both natural forests and tailings dams, 14 species were only found on the tailings dams. The dominant species on tailings dams was *Rhus longipes* while *Brachystegia boehmii* was the dominant species in the natural forest stands. Only two species (*Albizia antunesiana* and *Syzygium guineense*) were among the ten species with the highest stem density in both types of sites. No pattern was observed in the rarefied diversity of a 15-stem sample; i.e., in some sites, the natural forest was more diverse, while in others the tailings dam was more diverse. The overall pattern between TD and NF was non-significant (paired t-test, $t_3 = 1.4528$, $p = 0.2422$).

The species naturally colonizing the tailings dam sites differed from the natural forest assemblages in some key biological traits. More N-fixing species were found on the tailings dams than on natural forests and species growing on tailings dams were more light demanding than in natural forest ($\chi^2 = 18.815$, df

= 2, $p < 0.0001$). Mycorrhizal association type differed greatly between the tailings dams and natural forest species, with more ectomycorrhizal species in the natural forest than on the tailings dams (χ^2 test, $\chi^2 = 18.815$, df = 2, $p < .0001$). Species in natural forests were more likely to grow on sandy soil than those on tailings dams (χ^2 test, $\chi^2 = 14.216$, df = 1, $p = 0.0002$) while species growing on tailings dams were more likely tolerant of moderate concentration of metals than species in the natural forest (χ^2 test, $\chi^2 = 46.867$, df = 2, $p < 0.0001$). As a whole, the results show a remarkable recovery of tree species diversity on naturally-colonized tailings dams, although the species composition is quite different from nearby forests. This may in part be explained by the age difference of the stands and their successional statues. Dispersal limitation may also play a role in this pattern, as evidenced from increasing similarity in species composition between plot pairs that were closer to each other. The results also suggest that there is a pool of species that may be adapted to these extreme environments, and hence particularly suited for restoration of degraded mine wastelands. In particular, our results show that the trees growing on the tailings dams are more adaptable to the local growing conditions and were to a large extent N-fixers and/or had symbiosis with mycorrhiza.

Studies made on heavy metal-contaminated sites have shown that trees that are either pioneer species or legumes are more likely to survive (Mensah, 2015; Greipsson, 2011; Singh *et al.,* 2004a) as they are fast-growing in early-successional environments and have a higher survival rate. Some species growing on the tailings dams, such as *Dodonaea viscosa*, *Peltophorum africanum*, *Pericopsis angolensis*, *Rhus longipes*, *Senna singueana*, *Syzygium guineense*, *Terminalia* sp., and *Vachellia polyacantha*, were tolerant to elevated Cu concentration. Thus, they are candidate species for phytoremediation of tailings dams from copper mining. The species that colonized tailings dams in our study tended to have endomycorrhizal and vesicular-arbuscular mycorrhizal associations, which could be an adaptation to extract chemically-bound nutrients, such as phosphorus.

Species encountered on tailings dams had higher leaf copper concentrations than those growing on natural forest stands (Mann-Whitney-Wilcoxon test, W = 16395, $p = 0.00121$). The bioaccumulation factor was significantly lower for species growing on the tailings dams for the most commonly found species. Furthermore, there was a substantial within-site variation in the bioaccumulation factor due to differences in copper concentration in the soil and tailings substrates. As a whole, species grown on tailings dams had bioaccumulation factors much smaller than 1, suggesting that the species regulate their uptake of Cu, similar to excluders (Bothe & Slomka, 2017; Baker & Brooks, 1989) and/or facultative metalophytes as many of these species were also found in the natural forest (Bothe & Slomka, 2017). The elevated Cu concentrations in the tailings dam substrates and low Cu accumulation in the aboveground tissues in all species colonizing the tailings dams also suggest that they are capable of a well-balanced uptake and translocation of metals under heavily Cu-contaminated conditions, consistent with previous research (Nouri *et al.*, 2009). Based on the biological traits analysed, the results showed that there are certain biological traits that can be used to guide selection of tree species for phytoremediation of mine wasteland. The candidate species should have the following attributes: N-fixation, high metal tolerance, mycorrhizal symbiosis (endo or VA), diverse tolerance for soil grain size, drought tolerance, pioneer species and high sunlight requirement.

4.4 Tolerance of selected tree species to elevated copper concentration (**Paper IV**)

Seed germination of species to be used for restoration purposes is crucial, and species with poor germination should not be considered due to the increased cost of raising seedlings. The greenhouse experiment showed only five out of the 17 tested species had a germination rate over 60% and could be used for the trial, despite being treated to increase germination. Post-mining soils with their elevated levels of heavy metals are a harsh environment for plant establishment and limit growth and development of seedlings in several ways (Karaca *et al.*, 2018; Shi *et al.*, 2016).

The survival rate varied both among species and between Cu concentrations (Fig. 6). The highest survival rate was in the control and the lower concentration treatments. The two lowest copper concentrations, the control and the 100 ppm, had the same range of Cu that can be found in the natural forest, indicating that the species tolerate low levels of Cu as they didn't show any clear signs of copper toxicity. On treatments with higher concentrations (750-3000 ppm) similar to tailings dams, the majority of the seedlings either died or showed clear signs of copper toxicity.

The relative growth rate (RGR) for the different species varied; the highest RGR was found in the lowest Cu concentration treatment and the control. Several previous studies have shown that with an increased level of copper, growth and development is reduced (Marques *et al.*, 2018; Silva *et al.*, 2015, Kupper *et al.*, 2009), which corresponds well with our findings.

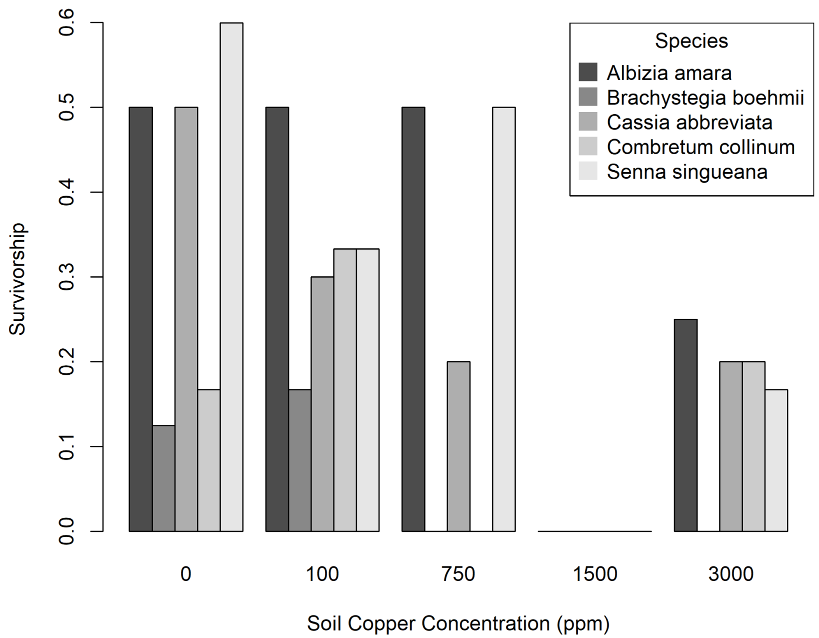

Figure 6. Survival rate (%) of the tested species, at different soil Cu concentrations.

There was a differential trend in root/shoot ratio; the lower Cu concentration treatments resulted in higher shoot development than root development, while on the higher concentration treatments the root/shoot ratios were more equal and on the highest concentration treatment the ratio was skewed in favour of root development. Other studies have shown similar results, that when concentrations of heavy metals in the soil are above natural levels, the root development increases (Cicatelli & Castiglione, 2016) to prevent increasing uptake (Pollard *et al.*, 2014; Boojar & Goodarzi, 2007) and potential damage to the above ground biomass (Jutsz & Gnida, 2015). This in turn indicates that the species are excluders (Lange *et al.*, 2016a; Gan *et al.*, 2013).

To further understand the mechanism by which the species tolerate elevated Cu concentration, the bioaccumulation factor (BF) and translocation factor (TF) were examined. The results showed that the studied species had an average BF around 0.5 with one species reaching up to 2.0 at 100 ppm Cu concentration treatment (Fig. 7). The translocation factor (TF) was between 0.2 and 0.7 depending on the Cu concentration in the soil (Fig. 8).

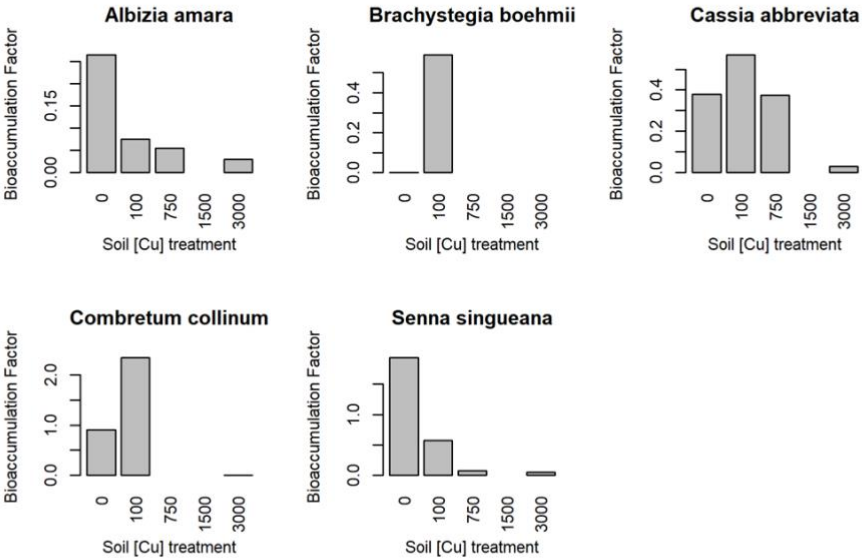

Figure 7. Bioaccumulation factor (BF) for the tested species at different soil Cu concentrations. Missing bars indicate that no plants in a treatment survived. Note the different y-axis scales.

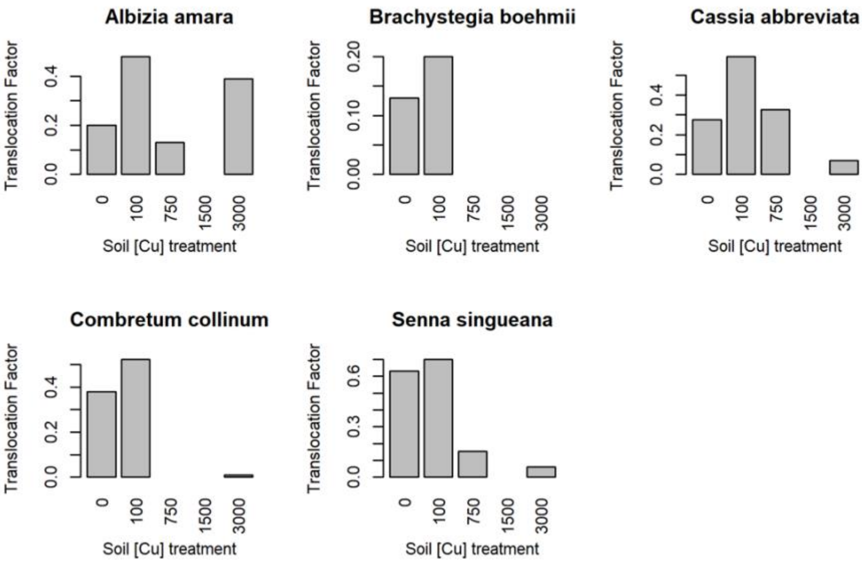

Figure 8. Translocation factor (TF) for the tested species at different soil Cu concentrations. Missing bars indicate that no plants in a treatment survived. Note the different y-axis scales.

The highest TF was found in the control and in the lowest Cu concentration. This correlates well with other studies, which demonstrated that BF and TF increase with lower Cu concentrations (Marco *et al.*, 2016; Nirola *et al.*, 2015). The BF is similar to previous studies showing that even Cu-tolerant species respond as excluders with limited translocation to the shoot (Lange *et al.*, 2016b). As the natural Cu concentration in soils ranges between 30-60 ppm worldwide (Printz *et al.*, 2016; Ghaderian & Ghotbi Ravandi, 2012), it indicates that the trees growing on both tailings dams and in the natural forest can also be facultative metallophytes and not just excluders (Delhaye *et al.*, 2018; Wójcik *et al.*, 2017; Pollard *et al.*, 2014).

4.5 Growth performance of selected tree species on biochar- and manure-amended tailings dam substrates (**Paper V**)

The survival rate of species differed across all treatments (Table 2). *R. longipes* and *T. stenostachya* had a good survival in all treatments except 75 g manure and 75 g biochar + manure amendments at 1 and 7 months after establishment. While *V. polyacantha* had good survival in biochar or manure-amended substrate alone or in combination, *K. africana* and *P. muellerianus* had relatively lower survival rates than the other species across most of the treatments.

Table 2. *Survival rate (%) of seedlings for five tree species grown on biochar and/or poultry manure-amended tailings dam substrate after 30 and 210 days of development.*

Species	*Rhus longipes*		*Terminalia stenostachya*		*Vachelia polyacantha*		*Kigelia africana*		*Phyllanthus muellerianus*	
Time (days)	30	210	30	210	30	210	30	210	30	210
Treatment										
Control	100	100	83	83	67	67	33	50	67	50
B1	100	100	100	83	67	67	50	67	0	0
B2	100	100	67	67	100	100	0	33	17	33
B3	83	83	100	83	83	83	33	67	50	33
M1	100	100	83	83	83	67	33	50	33	17
M2	67	67	50	67	50	50	67	67	50	0
M3	0	0	50	33	83	67	50	67	NA	17
BM1	100	100	100	83	83	83	50	83	50	33
BM2	83	83	67	50	100	67	67	33	50	17
BM3	33	0	17	17	50	50	67	67	NA	33

The relative growth rate in root collar diameter relative growth rate varied significantly ($p < 0.05$) among species, treatments and their interaction. Averaged over all treatments, *R. longipes* and *V. polyacantha* had better root collar diameter growth than *K. africana* and *P. muellerianus* (Fig. 9). Amendment with 75 g biochar resulted in bigger diameters than the untreated control for *R. longipes* while diameter growth was higher in the untreated control for *T. stenostachya.* For *K. africana*, amendments with either biochar or manure or their combination didn't bring comparative advantages in diameter growth compared to the untreated control. Amendments with 50 g biochar and 75 g manure tended to enhance diameter growth of *V. polyacantha.*

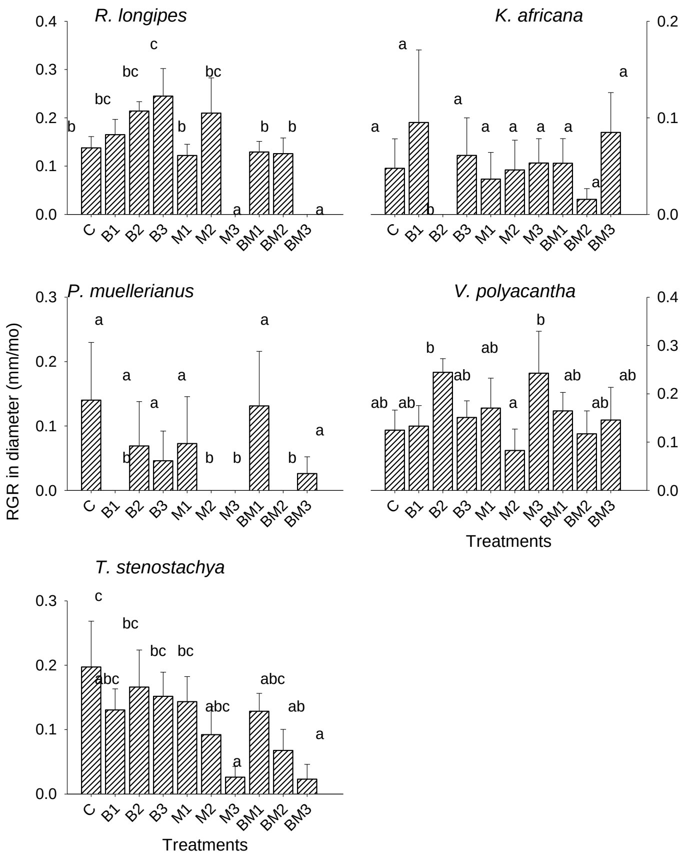

Figure 9. Diameter relative growth rate (RGR) of five tree species grown on biochar and poultry manure amended tailings dam substrate. Bars with different letters show significant differences among treatments at the p<.05 level. Treatment C is the control, B1, B2 and B3 biochar, M1, M2 and M3 are poultry manure, and BM1, BM2 and BM3 are mixed biochar and manure.

Similarly, relative growth rate in height varied significantly ($p < 0.05$) among species, treatments and their interaction. Averaged across all treatments. Height growth was faster for *R. longipes* and *V. polyacantha* than *K. africana* and *P. muellerianus* while height growth of *T. stenostachya* was in between this extreme (Fig. 10). Height growth of *R. longipes* was higher in 75 g biochar amended substrate than 75 g manure alone or 75 g biochar + manure amended substrate while that of *K. africana* was higher in 25 g biochar amended substrate than the untreated control. Height growth of *P. muellerianus* was suppressed by treatments involving 25 g and 75 g biochar and 75 g manure and 50 g biochar + 50 g manure compared to the control, which in turn didn't differ significantly from other treatments. While no significant difference in height growth among treatments was detected for *V. polyacantha*, height growth of *T. stenostachya* was higher in untreated substrates than amended substrates, except the 50 g biochar (B2) treatment.

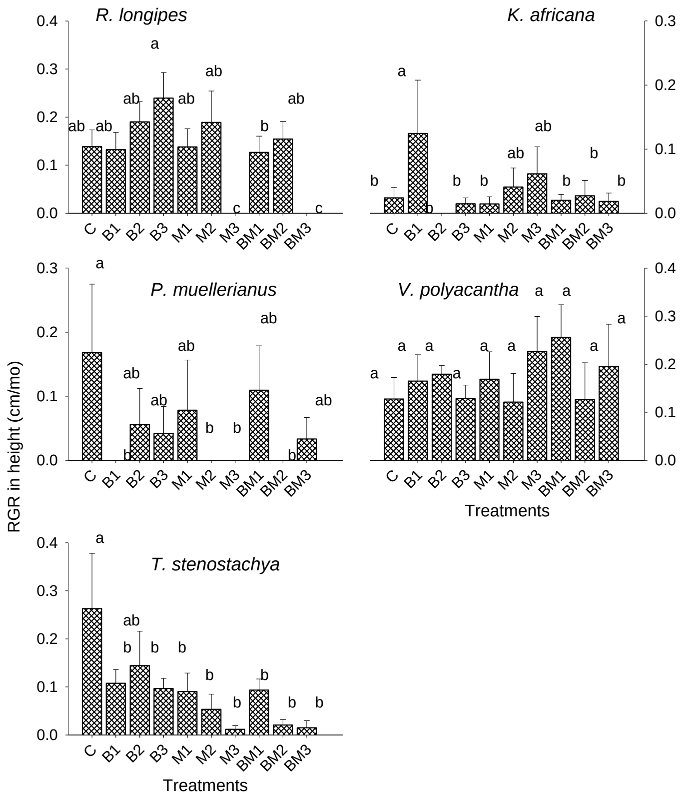

Figure 10. Height relative growth rate (RGR) of five tree species grown on biochar and poultry manure amended tailings dam substrate. Bars with different letters show significant differences

among treatments at $p<.05$. Treatment C is the control, B1, B2 and B3 are biochar, M1, M2 and M3 are poultry manure, and BM1, BM2 and BM3, mixed biochar and manure, respectively.

Generally, addition of biochar to poor soil creates a more favourable growing environment for most plants (Sovu, 2011) as it contains nutrients essential for plants, such as nitrogen, phosphorus, calcium, iron, zinc and potassium (Forján *et al.,* 2017). It also boosts the pH, increases water holding capacity (Fellet *et al.,* 2011; Beesley *et al.,* 2010) and has the ability to immobilize pollutants in the soil (Tang *et al.,* 2013; Beesley & Marmiroli, 2011). Organic residues, such as compost or animal manure can be used to improve nutrient-poor sites (Asensio *et al.,* 2013) and can increase microbial activity and decrease the concentration of contamination within the soil (Carlson *et al.,* 2015; Ruttens *et al.,* 2006). In addition to increasing nutrient availability in the soil, addition of organic residues to the growing medium also increases its water holding capacity (Shutcha *et al.,* 2015). The limited success in the present study might be related to the application rate, which might be small. As this is the first attempt to use biochar and manure to amend mine waste substrate, other dose-response studies should be conducted, which will hopefully give a conclusive result.

5 General discussion and practical implications

5.1 Potential to defuse man-made environmental bombs?

There is no argument that the need for restoring these types of land is of importance (Mahar *et al.*, 2016). However, several issues complicate the matter, such as the ownership of the dams (Nirola *et al.*, 2016; Nirola *et al.*, 2015), environmental commitments of the mining industry (Lèbre *et al.*, 2017), type of mining operation, mine wasteland characteristics, lack of knowledge of how to do it, which method is most cost effective to restore hundreds of hectares of mine wasteland and so on (Lèbre *et al.*, 2017; Nirola *et al.*, 2015; Ruiz-Jaen & Aide, 2005).

The most efficient restoration attempts in Africa have been with some kind of assisted restoration, either through adding amendments and/or establishment of a vegetation cover (Neina *et al.*, 2019). The biological approach with organic amendments, such as compost, biochar, animal manure combined with planting of selected species seems to be the most promising method (Madejon *et al.*, 2018; Sun *et al.*, 2018a; Sun *et al.*, 2018b).

Based on the restoration done on post-mining sand dunes in South Africa (Cooke & Johnson, 2002), there is a possibility to restore mining wastelands, if not back to their original ecosystem, towards a novel ecosystem (Sun *et al.*, 2018a; Hobbs *et al.*, 2009) that can provide other ecosystem services and that might in time become a functional self-sustaining biome that prevents leakage of heavy metals and chemicals into the surrounding areas.

5.2 Selection of tree species for restoration, which species have potential for phytoremediation?

Native tree species should be used (Sun *et al.*, 2018a; Dutta & Agrawal, 2003) to avoid introducing exotic species, as invasive species affect the local flora (Mahar *et al.*, 2016). Seeds for seedling production for restoration of tailings dams should be collected either on the dams or in close proximity (Delhaye *et al.*, 2016) as trees already growing on polluted soils are more likely to have a higher germination success (Gan *et al.*, 2013). Another benefit with using local species is that they are more likely to already have some kind of metal tolerance adaptation (Delhaye *et al.*, 2016; Nirola *et al.*, 2015).

Based on the biological traits tested in both **Paper III** and **Paper IV,** it is clear that tolerance for elevated levels of copper is crucial as well as a high light requirement, adaptable soil preferences and N-fixing ability (Sandell Festin *et al.*, 2020; Karaca *et al.*, 2018). High biomass production is also a benefit (Mahar *et al.*, 2016; Dutta & Agrawal, 2003) as well as drought tolerance (Madejón *et al.*, 2018).

The germination success and early seedling growth is crucial for selecting species for phytoremediation as species with a high germination (Karaca *et al.*, 2018; Gan *et al.*, 2013) rate are of more interest to reduce the cost of seedling production. Complicated germination and low germination rates should be avoided as it will be too work-intensive to use those species for phytoremediation (Sandell Festin *et al.*, 2020). It is also beneficial to use seedlings for restoration of tailings dams as the germination of many of the miombo forest species have different requirements to germinate; some require fire, others to be digested by animals while some have less complex germination. And as mine wastelands have unique soil characteristics (Dutta & Agrawal, 2003), few seeds can germinate on bare tailings dams substrate.

6 Conclusion

Restoration of post-mining landscapes is urgently needed due to the threat to human health and the environment. However, restoring those lands is challenging as each type of wasteland has its own physio-chemical characteristics that require a basic knowledge of the properties, such as soil grain type and potential heavy metal contamination before any restoration attempts can be made. Addition of organic amendments seems to boost establishment and survival in some species, but to what extent is still unclear.

Overall, restoration efforts in Africa have been limited compared to other continents, despite the urgency of restoring degraded areas to prevent further land degradation and land-use changes. Several countries have pledged to restore several thousands of hectares in the near future as part of the African Forest Landscape Restoration Initiative. One of the biggest challenges in restoration is how to restore as it requires knowledge of both the degraded landscape and of the local flora as well as setting the goal of what kind of restoration should be done at the actual location.

Using native flora is important, not only because of the decreased risk of invasive species, but because of local adaptations to biotic and abiotic factors that can increase the survival rate. Especially in natural copper-rich soils, native species are already adapted to some extent to elevated levels of copper and have some kind of coping mechanism that regulates copper uptake.

When using trees for restoration by assisted phytoremediation, selecting which species to use should focus on biological traits that can increase both their survival rate and growth. Pioneer species that can fix nitrogen, are drought tolerant, are copper tolerant and have a fast growth, can increase the chance of successful restoration of mine wastelands. Establishing a mixture of species is also beneficial as different species can contribute differently in terms of root depth, litter production, growth pattern and different crown shape.

Another important factor for selecting species is germination rate. Species with poor germination or that require complicated treatments to germinate should be excluded as the germination process should be as simple as possible to minimize labour costs. Ideally, the seeds should be collected from trees that are growing either on tailings dams or in close proximity as it might help boosting the copper tolerance of the seedlings if the parent trees already have been exposed to elevated levels of copper.

As a whole, the pace of restoration research and practice after mining disturbance in Africa has been slow compared to other continents. That the practice of abandoning mines after extracting the desired material has been common since the Iron Age highlights the need for a more proactive approach in terms of restoration attempts and research. Today mining companies are required to restore the land after mining, however, a “mainstreamed” technique that can be implemented is lacking and this should be addressed on several levels. National strategies for each country with a development plan and environmental policy that can assist companies in their attempts and help make the mining sector “green”.

Some of the key findings from these studies point out why characterizing mine waste substrates, particularly tailings dams and overburden materials, is vital as it assists with planning restoration activities. By having knowledge of the total heavy metal content in the soil, estimation can be done of how the concentrations may hinder establishment and growth of many plant species. As soil compaction is common on most mine wastelands, in particular on tailings dams, to boost plant establishment, soil scarification and addition of organic amendments should be considered to increase the survival rate.

Biochar seems to be one of the most promising amendments that can help plants to grow and develop on mine wastelands as it increases the amount of carbon and nutrients in the soil for plants to establish. Biochar in combination with other organic amendments together with native species is one potential way towards restoration of mine wastelands and to help prevent further leakage from tailings dams.

7 Future research

As the review study demonstrated, there are several knowledge gaps when it comes to restoration of the post-mining landscape in Africa, which accentuates the need for more research. Based on the study, the most important issues are:

1) An inventory of mine wastelands should be carried out for developing appropriate restoration plans as the size, number and status of abandoned (dormant) mine lands are not well known.

2) Further screening of candidate species, particularly tree species, should be undertaken as trees might be more beneficial for long-term restoration. As the Miombo forest hosts 300 different tree species, finding native species suitable for phytoremediation should be possible. A breeding program to promote promising species that have shown tolerance for elevated levels of heavy metals should be initiated. Parent trees should be selected from either close proximity to mine wastelands or individuals already established and growing on those types of substrate.

3) The use of organic amendments should continue to be evaluated, in particular biochar and other easily accessible amendments, such as poultry or cow manure. Biochar has proven to be a resource that is easily available and cheap to manufacture and has several benefits as a soil amendment. Further trials in terms of dose-response in selected species for phytoremediation should be done.

4) As the mining industry strives to become "greener" and lessen its negative environmental footprint, an inventory of species composition prior to any mining activity should be integrated into mining concessions as the regional biodiversity loss is poorly documented. If there is also a solid scientific knowledge base of species suitable for phytoremediation for the companies to lean on, they can collect and preserve species for phytoremediation for future use.

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Popular science summary

Humans have always changed and shaped nature to support and suit our needs and demands for us to survive and for our society to develop. This had created land-use changes, some are easily restored back to their natural state and others need assistance to not pose a threat to human health and the environment.

Since the Bronze Age, copper has played an important role in our society, such as decoration, weapons, tools and means of payment, up to today's use in electronics, power lines, mobile phones and more. In today's society, we depend on copper in our daily lives. However, demand for copper has changed the landscapes where it is mined. Beginning with the industrial revolution, copper mining shifted from small-scale mines scattered across the landscape to larger-scale mines with more efficient methods of mining and extracting the ore. This led to major changes in the landscape and conflicts over land use. One of the pressing environmental problems with the mining industry is, besides the change in the landscape, the slag products containing elevated levels of heavy metals and chemicals exposed to the weather. They can thus easily spread to the surrounding landscape. Some landscapes easily fall back to their original state after mining ends, while others require assistance to return to their original state.

Some of the slag products come from tailings dams, also called enrichment ponds. These are artificially created dams near the smelter. Sediments settle out of the liquid-based slurry and the excess water is pumped out for reuse. The slurry consists of finely crushed sand, called enrichment sand, which has been extracted from its copper content and rejected from the smelter. The slurry is what is known as tailings.

These dams can be several hundred hectares in size and have walls up to 20 meters high. To extract one ton of copper, between 320-450 tons of tailings is created. As several thousand tons of copper are extracted globally each year, the amount of tailings is substantial. The tailings dams are not always constructed properly and dust collapses occur from time to time, the last two in Brazil having a major impact on both human life and the environment.

The tailings dams are an inhospitable environment for most plants with their homogeneous sand, elevated heavy metal contents, low water availability and often low pH. They are somewhat reminiscent of a toxic desert. Few plants are able to establish and survive on these types of land. The dams pose a threat to human health through leaching of heavy metals and sometimes even chemicals, which spread in the soil, air and water. They can be absorbed by crops, and are a threat to the surrounding environment. Therefore, restoration of the dams is important. And with restoration, the main aim is to create a vegetation cover over the former pond that can reduce and prevent leaching and erosion.

A relatively new method that is gaining more and more attention is phytoremediation. It involves using plants to stabilize contaminated soil through vegetation cover. The goal is to create or recreate an ecosystem that can sustain itself without human impact. The big question is which plants can establish on tailings dams and develop into a self-sufficient ecosystem?

In this work, we have focused on tailings dams after copper mining in Zambia. Our first step was to find out what type of restoration efforts had been done in Africa. Through a literature study we identified what types of restoration techniques are available and how they can be used on heavily-polluted soil (study **I**). We also took soil sampled from several different dams to see what properties tailings dams have when it comes to concentrations of heavy metals and soil grain size (study **II**). We also looked closely at the species of trees and shrubs naturally established on or near abandoned dams and identified their characteristics (study **III**). Based on these findings, we selected a number of tree species to grow from seed and then subjected the plants to high copper concentrations to study survival and growth (study **IV**). Because the plants did not survive at high concentrations, we investigated whether it was possible to boost growth performance by adding biochar and chicken manure (study **V**). The overall goal was to find native tree species that can survive on these dams, with or without human assistance, to promote phytoremediation and to guide future research about how to re-establish vegetation on tailings dams.

The natural forest consists of a forested savanna, called miombo. It is dominated by three different trees, *Brachystegia*, *Julbernardia* and *Isoberlinia* which grow up to 20 meters high. In total, miombo has over 300 shrub and tree species and covers approximately 2.7 million square kilometres of land. Around 150 million people directly or indirectly depend on this forest for their livelihoods. The biodiversity of the miombo is largely due to the nitrogen-fixing capabilities of many tree species. The trees also help to bind the soil and prevent erosion. In addition, they improve the fertility of the soil by retaining soil water and creating a humus layer by shedding their leaves before the dry season.

In study **I**, we synthesized the state-of-knowledge about post-mining restoration in Africa. We identified how different restoration attempts can be assisted by biological, chemical or physical methods, and mapped which countries have restored or attempted to restore mining wastelands. Today, over 700 million hectares are in need of restoration in Africa. Several countries have signed agreements to reforest large areas within the next 10 years, but much knowledge on how to implement them is lacking. Compared to other parts of the world, both research and experiments are far behind in Africa. The research that has been done has primarily focused on native species of grasses, herbs, shrubs and to some extent trees. We found a few published examples of successful post-mining restoration practices. In South Africa, forests have been regrown on sand dunes after aluminum mining. In Kenya, a two-square-kilometer area has been reforested after limestone quarrying and in 30 years more than 300 native species have returned. In Ghana, cooperation between the local population and a gold mine has led to several places being reforested with indigenous species through active phytoremediation.

In study **II**, we mapped the chemical and physical properties of tailings dams, overburden and in the natural forest to compare the differences in soil properties. The substrates in both the tailings dams and the overburdens were dominated by silt while the natural forest was dominated by sand. Soil compaction, poor macro-nutrient availability and soil acidity (particularly on overburden sites) are typical features of mine waste sediments. The tailings dams had the highest content of copper and other heavy metals and a pH of 8.4. The overburden had a pH value of 5.63 and the natural forest had a pH value of 5.05. The copper content was between 1,000-15,000 ppm for the tailings dams, between 1,000-8,000 ppm for the overburden, and between 50-1,000 ppm for the natural forest. Copper levels above 60 ppm are considered hazardous to human health.

In study **III**, we investigated the copper content of tailings dams and the nearby natural forest. We also investigated the different biological properties of trees and shrubs that regenerated naturally on the tailings dams and in the nearby forest. Here, too, the copper content was significantly higher on the dams than in the natural forest, between 750-7,000 ppm compared to 41-950 ppm. The copper content in the natural forest is affected by the proximity to the dams and the proximity to the smelters and the supply of the dams. One of the areas with natural forest had a copper content between 41-73 ppm, just above the limit for it to be hazardous to health. One explanation for this is that the area was further away from any mining industry activities.

We studied the species composition between the dams and the natural forest and found a total of 55 different tree species, 42 species in the natural forest and 30 species on the dams, 16 of which were found in both areas. There was a clear difference between both species composition and dominance; there was also a difference between the biological characteristics of the tree species. The species on the tailings dams were to a greater extent nitrogen-fixing and sun-demanding tree species, while the trees in the natural forest were more shade tolerant species and less likely to be nitrogen-fixing. The conclusion we could draw from our study was that trees and shrubs most suitable for phytoremediation are sun-loving, tolerant to elevated levels of copper (and other heavy metals), resistant to drought, adaptable to different soil grain sizes, have some form of mycorrhizal symbiosis and nitrogen-fixing.

In studies **III** and **IV**, we looked at trees' ability to regulate copper uptake. Copper is an important nutrient for trees and plants and is used, among other things, for photosynthesis and cell membrane structure, but at high levels it becomes toxic and damages the tissue of the cells. Therefore, plants regulate their uptake of copper either by binding it on or in the roots so that it is not transported further up the tissue or by uptake of copper to the whole plant. The different techniques are called exclusion, accumulation or hyperaccumulation depending on the amount of copper taken up. In our studies, we found that all tested tree species excluded copper and prevent uptake and transport further up the plant.

In study **IV**, we examined the germination and survival rate of 17 Zambian tree species in a greenhouse trial. A clear result was the poor germination of the majority of the seeds. Only five species had sufficient germination to be used in the experiment. After the plants became big enough and grew their first leaves, they were transplanted in fine sand to mimic enrichment sand along with slow-release fertilizers. The seedlings had seven days to acclimatize to their new growing environment before they were subjected to different concentrations of copper: 0, 100, 750, 1500 and 3000 ppm. Overall, mortality was high at the two higher concentrations and lower at low concentrations. Growth was also affected and the plants that were contaminated at 100 ppm were the ones that developed best together with the control. The result was not so surprising since this part of Zambia has naturally high levels of copper in the soil and vegetation is adapted to it.

In study **V**, we studied the growth performance of five native tree species on tailings dam's substrates amended with biochar, poultry manure and their combination. Biochar is created by organic matter undergoing oxygen-poor combustion and creating carbon that can be used to stabilize the soil. Five different tree species had sufficient germination to be included in the experiment.

We used three different concentrations of biochar, chicken manure and a combination of biochar and chicken manure, 25, 50 and 75 grams per kilo of substrate. We could see that the combination of biocarbon and chicken manure created the most favourable environment for both establishment and growth. Here, too, we could see that trees that are nitrogen fixers had higher survival rates.

Our results indicate that restoration of tailings dams is complicated, partly because of the physical and chemical properties of the dams, but also because there is limited knowledge of the native tree species. The conclusions we can draw are that trees that are pioneers, nitrogen fixing and resistant to elevated copper levels are preferred. The combination of biochar and chicken manure or biocarbon alone can help the trees establish themselves. And although it is costlier to plant seedlings instead of seeds, seedlings are a better option because of the uncertain germination rate of many species. When choosing parent trees, it is best to pick seeds on or adjacent to the dams as the trees may have adapted to some extent to the elevated levels of copper and other heavy metals in the soil. Many trees also propagate vegetatively, so cuttings are an alternative to raising seedlings from seeds.

By using scientifically proven methods for restorations, selecting the species with suitable biological traits in combination with organic amendments, the mining industry can, in time, achieve a more “green” approach to mining.

Populärvetenskaplig sammanfattning

Människan har förändrat landskapet för att passa våra behov och krav för överlevnad. Det har skapat landskapsändringar, en del kan enkelt falla tillbaka till sitt ursprungsstadie medan andra förändringar kräver assistans för att återgå till sitt ursprungliga skick.

Sedan bronsåldern har koppar spelat en viktig roll i vårt samhälle, som utsmyckning, vapen, redskap och betalningsmedel till dagens användning i elektronik, el-ledningar, mobiltelefoner m.m. I dagens samhälle är vi beroende av koppar i vårt dagliga liv.

Starten av den storskaliga brytningen av koppar var i samband med den industriella revolutionen. Från att ha varit mindre gruvor spridda över landskapet började stora gruvor etableras med effektivare metoder att bryta och extrahera malmen. Det ledde till större förändringar i landskapet och konflikter om markanvändandet. Det största problemet med gruvindustrin är, förutom förändringen i landskapet, slaggprodukterna som innehåller förhöjda halter av tungmetaller och kemikalier som är exponerade för vädrets makter. De kan därmed lätt spridas till det omkringliggande landskapet.

En del av slaggprodukterna kommer från anrikningsdammar, även kallat sedimenteringsdammar. Det är artificiellt skapade dammar i närheten av smältverken. I dessa pumpas det ut en vätskebaserad, trögflytande blandning för att sedimenteras varefter överskottet av vattnet pumpas tillbaka igen. Den trögflytande blandningen består av fint krossad sand, kallad anrikningssand, som utvunnits på sin koppar. Dessa dammar kan vara flera hundra hektar stora och ha upp mot 20 meter höga väggar. För att utvinna ett ton koppar erhålls mellan 320–450 ton av anrikningssand. Eftersom det globalt utvinns flera tusen ton koppar per år handlar det om väldiga mängder. Anrikningsdammarna är inte alltid konstruerade på rätt sätt och dammkollapser sker då och då, de två senaste i Brasilien med stor påverkan på både människors liv och miljöpåverkan.

Dessa anrikningsdammar utgör en ogästvänlig miljö för de flesta växter med sin homogena sand, förhöjda halter av tungmetaller, låg tillgång på vatten och ofta lågt pH. Det påminner lite om en giftig öken. Det är få växter som klarar av att etablera sig och överleva på dessa typer av mark.

Dammarna utgör ett hot mot människors hälsa genom urlakning av tungmetaller och ibland även kemikalier, som sprids i både marken, luften och i vatten. De kan tas upp av grödor, samt är ett hot mot den omkringliggande miljön. Därför är restaurering av dammarna viktig. Och med restaurering åsyftas främst att skapa ett vegetationstäcke som täcker dammen och skapar ett slags organiskt lock som kan minska och förhindra urlakning och erosion.

En relativt ny metod som får mer och mer uppmärksamhet kallas för fytoremediering. Den går ut på att använda växter för att stabilisera förorenade marker genom ett vegetationstäcke. Målet är att skapa eller återskapa ett ekosystem som utan mänsklig inverkan kan växa och utvecklas. Den stora frågan är vilka växter som kan etablera sig på anrikningsdammar och utvecklas till ett självförsörjande ekosystem?

I det här arbetet har vi genom omfattande litteraturstudier undersökt vilka typer av restaurering som finns och hur de kan användas på kraftigt förorenad mark (studie **I**), vilka egenskaper som anrikningsdammar har när det kommer till halter av tungmetaller och jordkornsstorlek (studie **II**). Vi har också tittat närmare på vilka trädslag och buskar som etablerats på dammarna eller i deras närhet och identifierat deras egenskaper (studie **III**). Baserat på de fynd vi gjorde, valde vi ut ett antal trädslag för att driva upp från frön och sedan utsätta plantorna för höga koppar koncentrationer för att studera överlevnad och tillväxt (studie **IV**). Och eftersom plantorna inte överlevde på höga koncentrationer undersökte vi om det gick att påverka överlevnaden genom att tillsätta biokol och hönsgödsel (studie **V**). Målet med forskningen var att hitta trädslag som kan klara av att kolonisera dessa dammar, med eller utan assistans från människor i form av biokol och hönsgödsel, för att kunna använda oss av fytoremediering. Samt att kunna komma med förslag för kommande forskning i framtiden för hur anrikningsdammar ska kunna återbeskogas.

Fokus för vår forskning var på anrikningsdammar efter kopparbrytning i Zambia, ett land i södra centrala Afrika som är rikt på koppar och kobolt och som har en lång historik av gruvdrift. Skogen består av en beskogad savann, kallad miombo. Den domineras av tre olika trädslag, *Brachystegia, Julbernardia* and *Isoberlinia* som blir upp till 20 meter höga.

Totalt finns det 300 busk- och trädarter och den här typen av ekosystem täcker ungefär 2.7 miljoner kvadratkilometer land med 150 miljoner människor som direkt eller indirekt är beroende av skogen för sitt dagliga liv. Att miomboskogen är så artrik beror till stor del av de kvävefixerande förmågorna hos många träd, antingen genom symbios med blågröna bakterier eller genom mykorrhizal symbios. Träden hjälper också till att binda jorden och förhindra erosion. Dessutom förbättrar de fertiliteten i jorden genom att binda markvatten och skapa ett humuslager genom att de fäller sina löv inför torrperioden.

I det här arbetet började vi med att undersöka vilka typer av restaurering som gjorts i Afrika sedan gruvindustrin tog fart fram tills idag. Vi kartlade de kemiska och fysikaliska egenskaperna som olika anrikningsdammar har i jämförelse med den naturliga skogen och en annan slaggprodukt kallad gråberg. Vi undersökte vilka biologiska egenskaper som är nödvändiga för fytoremediering samt undersökte olika inhemska trädslags förmåga att överleva och växa på kopparförorenad jord med eller utan tillsatt organiskt kol och/eller hönsgödsel.

I studie **I** studerade vi vilka olika typer av restaurering som finns, hur de delas in och vad som är målet med varje typ. Vi identifierade hur olika typer av restaurering eventuellt kan nå större framgång genom olika metoder, biologisk, kemisk eller fysisk och kartlade vilka länder som restaurerat eller försökt efter gruvdrift.

Idag är över 700 miljoner hektar i behov av restaurering i Afrika. Flera länder har skrivit på avtal att återbeskoga stora arealer inom 10 år, men mycket kunskap om hur det ska kunna genomföras saknas. I vår studie fann vi studier från nio olika länder där det publicerats vetenskapliga artiklar om restaureringsförsök på gruvmark, totalt fann vi 24 studier. Jämfört med andra delar av världen ligger både forskningen och försöken långt efter i Afrika. Den forskning som har gjorts har i första hand fokuserat på inhemska arter av gräs, örter, buskar och träd. Det finns tre exempel på framgångsrik återbeskogning. I Sydafrika har skog kunnat återplanteras på sanddyner efter aluminiumbrytning. I Kenya har ett två kvadratkilometer stort område återbeskogats efter kalkbrytning och på 30 år har mer än 300 inhemska arter kommit tillbaka. I Ghana har ett samarbete mellan lokalbefolkningen och en guldgruva medfört att flera platser börjar återbeskogas av inhemska arter genom aktiv fytoremediering.

I studie **II** kartlade vi de kemiska och fysikaliska egenskaperna hos anrikningsdammar, gråberg och i den naturlig skogen för att jämför skillnaderna i kornstorlek i jorden, halten av föroreningar och pH värden. Anrikningsdammarna hade högst halter av koppar och andra tungmetaller och hade en föroreningsgrad på närmare 9 och ett pH-värde på 8.4. Gråberget hade en föroreningsgrad på närmare 6 och ett pH- värde på 5.63 och den naturliga skogen hade en föroreningsgrad på 0 och ett pH-värde på 5.05. Kopparhalten låg mellan 1.000-15.000 ppm för anrikningsdammarna, mellan 1.000-8.000 ppm för gråberget och 50-1.000 ppm för den naturliga skogen. Kopparhalter över 60 ppm anses vara hälsofarligt. Både dammarna och gråberget hade finare kornstorlek, mjäla, och den naturliga skogen domineras av sand.

I studie **III** undersökte vi kopparhalten på fyra dammar och i den naturliga skogen. Vi undersökte också de olika biologiska egenskaperna hos träd och buskar som växte på dammarna och i den närliggande skogen. Även här var kopparhalten betydligt högre på dammarna än i den naturliga skogen, den låg mellan 750-7.000 ppm jämfört med 41–950 ppm i skogen. Kopparhalten i den naturliga skogen påverkas av närheten till dammarna och av närheten till smältverken och matning av dammarna. Ett av områdena med naturlig skog hade en kopparhalt mellan 41–73 ppm, precis över gränsvärdena för att det skulle vara hälsofarligt. En förklaring till detta är att området låg längre bort från någon dam eller form av gruvindustri.

Vi studerade artsammansättningen mellan dammarna och den naturliga skogen och hittade sammanlagt 55 olika trädslag, 42 arter i den naturliga skogen och 30 arter på dammarna, 16 av dessa arter återfanns på båda områdena. Det var en tydlig skillnad både mellan artsammansättningen och vilka arter som dominerade, det var också en skillnad mellan de biologiska egenskaperna. Arterna på dammarna var i större utsträckning kvävefixerande och tydliga pionjärträdslag medan träden i den naturliga skogen var mer sekundärträdslag och var i mindre utsträckning kvävefixerande.

Den slutsats vi kunde dra av vår studie var att egenskaper som gynnar fytoremediering är om träden eller buskarna är pionjärer, solälskande, toleranta mot förhöjda halter av koppar (och även andra tungmetaller), tåliga mot torka, anpassningsbara vad gäller jordstorlek, ha någon form av mykorrhiza symbios och vara kvävefixerande.

I studie **III** och **IV** tittade vi på trädens förmåga att reglera sitt upptag av koppar. Koppar är ett viktigt näringsämne för träd och växter och används bland annat till fotosyntesen och cellmembranets uppbyggnad, men i för höga halter blir den giftig och skadar cellernas vävnad. Därför reglerar växter sitt upptag av koppar antingen genom att binda kopparen vid rötterna eller i rötterna för att inte koppar ska transporteras vidare upp i vävnaden eller genom upptag av koppar till hela växten. De olika teknikerna kallas exkludering, ackumulering eller hyperackumulering beroende på mängden koppar som tas upp. I våra studier fann vi att alla testa träden exkluderade koppar och förhindrar upptag och transportering vidare upp i plantan.

I studie **IV** undersökte vi grobarheten och överlevnadsgrad av 17 zambiska trädslag i ett växthusförsök. Ett tydligt resultat var den dåliga grobarheten hos majoriteten av fröna, endast en handfull trädslag hade tillräckligt bra grobarhet för att kunna användas i experimentet. Till slut återstod bara fem arter att använda. Efter att plantorna blivit tillräckligt stora och satt sina första löv omplanterades de i fin sand för att efterlikna anrikningssand tillsammans med långsamt lösande gödsel. När plantorna var sju dagar förgiftades de med olika koncentrationer av koppar, 100, 750, 1500 och 3000 ppm. Överlag var dödligheten hög på de två högre koncentrationerna och lägre på låga koncentrationer. Tillväxten påverkades också och plantorna som förorenats med 100 ppm var de som utvecklades bäst tillsammans med kontrollplantorna. Resultatet var inte så förvånade eftersom Zambia har höga halter av koppar naturligt i marken och växtligheten är anpassad efter det.

I studie **V** studerade vi hur grobarheten och överlevnaden av frön från inhemska trädslag påverkades av direkt plantering på anrikningsdamssubstrat med olika koncentrationer av biokol och hönsgödsel. Biokol skapas genom att organiskt material genomgår en syrefattig förbränning och skapar kol som kan användas för att stabilisera jorden. Fem olika trädslag hade tillräckligt bra grobarhet för att vara med i experimentet och vi hade tre olika koncentrationer av biokol, hönsgödsel och en kombination av biokol och hönsgödsel, 25, 50 och 75 gram per kilo substrat. Vi kunde se att kombinationen av biokol och hönsgödsel skapade den gynnsammaste miljön för både etablering och grobarhet. Även här kunde vi se att träd som är kvävefixerare hade högre överlevnad.

Baserat på våra resultat indikerar det att återbeskoga anrikningsdammar är komplicerat, dels på grund av dammarna fysikaliska och kemiska egenskaper, men också för att det finns så begränsat med kunskap om de inhemska trädslagen. De slutsatser som vi kan dra är att träd som är pionjärer, kvävefixerande och tåliga mot förhöjda halter av koppar är att föredra. Kombinationen av biokol och hönsgödsel eller enbart biokol kan hjälpa träden att etablera sig. Och även om det är mer kostsamt att plantera plantor istället för frön, är det bättre med plantor eftersom grobarheten av fröna hos de flesta arter är så pass låg att det krävs stora mängder frön. Vid val av föräldra-träd är det bäst att plocka frön på eller i anslutning till dammarna då träden kan ha anpassat sig till viss del till de förhöjda halterna av koppar och andra tungmetaller i marken. Många träd förökar sig också vegetativt, att ta sticklingar är därmed också en möjlighet.

Gruvindustrin kan genom att använda sig av vetenskapligt bevisade metoder och genom val av trädslag med rätt biologiska egenskaper i kombination med organiska gödsel, som biokol, fortsätta sin strävan mot att bli en ”grön” industri med mindre negativ miljöpåverkan.

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Appendix 1. Germination treatment and germination rate

Species	Seed source	Treatment	Germination rate
Albizia amara	Lusaka	Nicked the seeds, soaking in hot water for 10 minutes, then soaked in cold water for 24 hours.	75%
Brachystegia bohemii	Lusaka	Nicked the seeds, then soaked in cold water for 24 hours.	85%
Cassia abbreviata	Lusaka	Nicked the seeds, soaking in hot water for 10 minutes, then soaked in cold water for 24 hours.	90%
Combretum collinum	Lusaka	Nicked the seeds, then soaked in cold water for 24 hours.	90%
Combretum molle	TD25, Kitwe	Nicked the seeds, then soaked in cold water for 24 hours.	75%
Combretum mycrophyllum	TD8, Mufulira	Nicked the seeds, then soaked in cold water for 24 hours.	65%
Combretum zeyheri	Lusaka	Nicked the seeds, then soaked in cold water for 24 hours.	55%
Dombeya rotundifolia	Lusaka	Removed the wings, crushed the seeds in order to release the small black seeds within the pod.	5%
Ficus sycomorus	Kitwe	Crushed the outer coating and soaked in cold water for 24 hours.	0%
Kigelia africana	Lusaka	Nicked the seeds, then soaked in cold water for 24 hours.	50%
Parinari curatellifolia	Lusaka	Nicked the seeds, then soaked in cold water for 24 hours.	0%
Piliostigma thoningii	TD8, Mufulira	Nicked the seeds, then soaked in cold water for 24 hours.	5%
Pterocarpus angolensis	TD8, Mufulira	Nicked the seeds, then soaked in cold water for 24 hours.	0%
Senna singueana	TD25, Kitwe	Nicked the seeds, then soaked in cold water for 24 hours.	95%
Terminalia mollis	TD8, Mufulira	Removed the wings, nicked the seeds, soaked in cold water for 24 hours.	0%

Species	Seed source	Treatment	Germination rate
Terminalia spp	TD8, Mufulira	Removed the wings, nicked the seeds, soaked in cold water for 24 hours.	0%
Vachellia erioloba	TD5, Mufulira	Nicked the seeds, soaking in hot water for 10 minutes, then soaked in cold water for 24 hours.	1%
Vachellia polyacantha	TD29, Kitwe	Nicked the seeds, soaking in hot water for 10 minutes, then soaked in cold water for 24 hours.	1%
Vachellia sieberana	TD8, Mufulira	Nicked the seeds, soaking in hot water for 10 minutes, then soaked in cold water for 24 hours.	1%

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Vast areas of land are in need of restoration, in particular post-mining landscapes. In Zambia, tailings dams are left barren after decommission and pose a threat to both human health and the environment. A solution is to use native tree species to create a vegetation cover, using the phytoremediation technique. The aim of this thesis was to find suitable species with biological traits that can promote successful restoration with the aid of organic amendments.

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