The impact of nickel and chrome mine tailings on the growth of *Hibiscus cannabinus* and *Linum usitatissimum* and a preliminary assessment of their applicability as economically beneficial phytoremediation species

by

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Declaration

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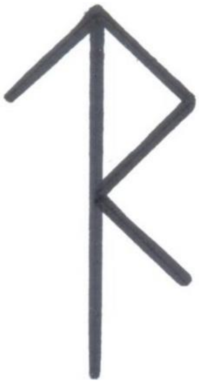
Abstract

Current and previous mining activities in South Africa have caused various environmental, human health and societal impacts. This has led to the formation and enforcement of legislation regarding the rehabilitation of active, closed and abandoned mines in South Africa. The requirements contained in this legislation include rehabilitation, skills transfer, job creation and development of post mine land use regarding active, closed and abandoned mines. A common impact of mining activities is the contamination of soils with various metals. The process of phytoremediation has demonstrated potential in the remediation of metal contaminated soils. Plant species commonly utilised in this process are hyper-accumulators, which can translocate and accumulate high concentrations of various metals from soils into their biomass. However, large areas of previously economically productive land become underutilised when hyper-accumulators are used for phytoremediation. Economically valuable fibrous plant species have demonstrated potential in their use as phytoremediation species. This presents an opportunity in which economically valuable plant species could be utilised in phytoremediation applications on active, closed and abandoned mines in South Africa. Thus, the aim of this research was to assess the ability of *Hibiscus cannabinus* and *Linum usitatissimum* to grow in and extract metals from soil contaminated with nickel and chrome mine tailings. Furthermore, the concurrent use of *H. cannabinus* and *L. usitatissimum* as phytoremediative and economically beneficial plant species was determined. Normal (non-impacted), rehabilitated (previously impacted) and tailings (impacted) soil treatments were collected and used from the Onverwacht tailings storage facility of Nkomati Nickel mine. *Hibiscus cannabinus* and *L. usitatissimum* were cultivated in each soil treatment in greenhouse conditions over a six-month period. Multiple plant growth parameters were recorded at monthly intervals. The amount (mg) and concentration (mg/kg) of Mn, Zn, Ni, Cu, Cr and Co contained within plant tissue samples at the end of the six-month period was determined. The area (ha) of land categories available for *H. cannabinus* and *L. usitatissimum* cultivation onsite was determined using Sentinel 2B satellite imagery and supervised image classification. The measured and expected total yield (t), yield value (R), profit/loss margin (R) and amount (g/ha) of Mn, Zn, Ni, Cu, Cr and Co extracted through cultivation of *H. cannabinus* and *L. usitatissimum* onsite was determined. The growth of *H. cannabinus* and *L. usitatissimum* cultivated in rehabilitated soil was severely impacted. While growth of each species exhibited minimal differences between those cultivated in normal and tailings soil. *Hibiscus cannabinus* consistently exhibited greater growth than *L. usitatissimum*. Both species demonstrated the ability to accumulate varying amounts and concentrations of each of the tested metals in their total, above and below ground components. Both species consistently accumulated increased amounts and concentrations of Mn and Zn. Those cultivated in tailings soil exhibited increased accumulation of Cr. *Linum usitatissimum* generally accumulated metals at higher concentrations than *H. cannabinus*, however, minimal differences in the amount of metal accumulated between species were observed. Based on the measured yield cultivation of each species onsite would result in economic loss and generally low metal extraction. However, based on the expected yield, species cultivation onsite, in normal and tailings soil, would result in economic gain and generally high metal extraction. *Hibiscus cannabinus* and *L. usitatissimum* exhibited phytoremediative and economic potential. Aspects of the current state of mine impacted land in South Africa and the requirements of rehabilitation enforced through South African legislation could possibly be addressed through the application of *H. cannabinus* and *L. usitatissimum* for mine rehabilitation strategies.

Key Words: Phytoremediation, *Hibiscus cannabinus* and *Linum usitatissimum*, Nickel and chrome mine tailings, Supervised image classification, Economic value

All Father

Doctor Alfredo José Pereira de Sampaio



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1 – Introduction

1.1 – General Overview

The emergence of modern civilization as a result of technological advances made during and after the industrial revolutions have catalysed and sustained an increased population and living standards across the globe (Friman 2002). The impacts of prolonged and consistent natural resource extraction have resulted in direct and indirect impacts to the structure and function of the environment, ecosystems and critical biogeochemical cycles (Parmesan and Yohe 2003). Natural resource extraction is conducted globally within the mining sector to supply crucial raw and manufactured materials (Behrens *et al*. 2007). The benefits of mining to the economy and development of first, second and third world countries are apparent. However, the impacts associated with mining processes are evident at all levels of development (Frickel and Freudenburg 1996). The extraction, treatment and disposal stages of mining processes cause various environmental, human health and societal impacts (Davies and Mundalamo 2010, Gouldson and Murphy 2013). Environmental impacts of mining include water pollution, habitat destruction, land degradation and biodiversity decreases (Gouldson and Murphy 2013). These impacts are commonly left untreated before and following mine closure, extending their prevalence and that of subsequent human and societal impacts indefinitely (Gouldson and Murphy 2013). The duality of mining benefits and impacts is evident in South Africa where unregulated mining practices took place for approximately 120 years (Swart 2003). Impacts associated with mining and their exacerbation when left untreated led to the formation of and requirements for mine rehabilitation strategies (Swart 2003, NEMA 2008, MPRDA 2002, US EPA 2008).

Rehabilitation refers to the removal, mitigation and reversal of mining impacts to the environment (Tanner and Möhr-Swart 2007). The process involves procedures which aim to return land impacted by mining processes to a sustainable and usable condition. Rehabilitation of impacted land to what was previously present before mining occurred is currently seen as impossible using current practices. Therefore, the end goals of mine rehabilitation fall into three categories concerning surrounding communities, previous land use capability and biodiversity (McHaina 2001, Tanner and Möhr-Swart 2007). The end product of mine land rehabilitation is either decided by the needs and desires of the surrounding community, the need and potential to use rehabilitated land for economic gain or the need and desire to decrease loses in and maintain biodiversity within the area (Tanner and Möhr-Swart 2007). Rehabilitation procedures used to meet these demands are decided on and planned during the inception and life span of a mine. Rehabilitation plans include stages of soil remediation and re-vegetation which aim to bring soil to a sustainable and usable form with the end goal of re-vegetation (Tanner and Möhr-Swart 2007). Soil remediation is conducted using various physical, chemical and biological processes. Physical and chemical remediation processes are commonly expensive and are associated with further environmental impacts (Bhargava *et al*. 2012). Biological remediation processes involve the use of micro-organisms to break down organic soil contaminants into non-harmful forms while re-establishing the microbiome of impacted soils (Khalid *et al*. 2017). Biological remediation processes also involve the use of plants to immobilise, degrade or extract soil contaminants (Khalid *et al*. 2017, US EPA 2008). Chaney (1983) defined the use of living plants in the immobilisation, degradation and extraction of soil contaminants and subsequent soil remediation as phytoremediation.

Phytoremediation utilises the innate ability of plants to transfer various nutrients, compounds and contaminants from soil into their biomass (Khalid *et al*. 2017). This process involves the mass flow of water containing these substances into plant roots, through the xylem and ultimately through their stomata (Yao *et al*. 2012). Throughout this process substances contained within water are stored in various sections of the plant (Khalid *et al*. 2017 and Yao *et al*. 2012). The transfer and storage of these substances within a plant allows for their utilisation in phytoremediation. Many plant species demonstrate the ability to extract various substances including metals from the soil they inhabit (Khalid *et al*. 2017). Various species have been found to tolerate high concentrations and extract relatively large amounts of metals present in soils (Rascio and Navari-Izzo, 2011). These species are defined as hyper-accumulators and have the ability to tolerate and accumulate metal contaminants within their biomass at higher concentrations than that found in the soil (Rascio and Navari-Izzo, 2011). The use of these species can be applied to impacted sites containing a variety of metal contaminants (Wan *et al*. 2016). *Berkheya coddii* (Roesller, Asterales: Asteraceae) is defined by Morrey *et al*. (1989) as a perennial plant of the Asteraceae family and is a known hyper-accumulator of nickel. *Berkheya coddii* is able to survive in soils containing high concentrations of nickel due to its ability to actively accumulate and store nickel in its above ground biomass (Rascio and Navari-Izzo 2011). This ability has led to the research and application of *B. coddii* in phytoremediation strategies (Keeling *et al*. 2003, Naicker *et al*. 2016).

However, the use of *B. coddii* and other hyper-accumulating plants is limited to the extraction and possible reuse of soil contaminants through the process of phytomining (Mahar *et al*. 2016). This limitation is due to the lack of possible application of hyper-accumulating species to other industrial, medicinal and agricultural practices. The limited use of hyper-accumulators along with the extended period of time taken for contaminant accumulation to occur results in large areas of land remaining economically unused for prolonged periods (Mahar *et al*. 2016). However, species used in phytoremeditaion are not limited to hyper-accumulators. A group of species defined as fibrous plants due to the presence of various economically valuable fibres within their biomass have shown potential for their use as phytoremediation species (Griga and Bjelková 2013, Ramesh *et al*. 2017). Species include, kenaf (*Hibiscus cannabinus* Linnaeus, Malvales: Malvaceae) and flax (*Linum usitatissimum* Linnaeus, Linum: Linaceae).

The fibres are harvested, processed and used in a multitude of different industries from textiles and composite materials to medicinal and nutritional products (Harrison *et al*. 2019). The presence of economically valuable fibres within these species along with their ability to tolerate and remove soil contamination leads to the possibility of their use as both phytoremediation and industrial plants. The use of these species in soil remediation and re-vegetation processes within mine rehabilitation plans and their subsequent harvest and utilisation in the development of a fibre industry could provide a dual service of impact mitigation and economic gain. Furthermore, the cultivation of fibrous plants on mine land could act as a post mining industry following the closure of a mine (Harrison *et al*. 2019). The replacement of one industry with another would serve as a means of protecting the livelihoods of surrounding communities (Harrison *et al*. 2019). This research will act as a feasibility study regarding the use of *H. cannabinus* and *L. usitatissimum* as both phytoremediation and industrial plants through their cultivation in impacted soils associated with nickel and chromium mines.

1.2 – Rationale

The environmental, human and societal impacts associated with mine activities has led to the enactment of legislation which holds mining companies accountable for the rehabilitation of mine land during and after mining activity (Swart 2003, NEMA 2008, MPRDA 2002). Mining in South Africa catalysed and continues to benefit the country's economic development (Wilson 2001, MCSA 2022). However, unregulated mining activities took place over an extended period leading to the proliferation of environmentally impacted areas (Davies and Mundalamo 2010). Implementation of procedures to rehabilitate these impacts has become legal requirement for mining companies currently owning or seeking to create mining operations (MPRDA 2002). The enactment of legislation contained within the Mineral and

Petroleum Resources Development Act (2002) holds mining companies accountable for environmental, human and societal impacts associated with their mining operations. Mining companies are legally required to create funding plans for the rehabilitation of mining impacts during and after the mine's life span (MPRDA 2002). Initial enactment of this legislation resulted in the widespread abandoning of mines to avoid paying for rehabilitation procedures (Limpitlaw and Briel 2014). Mass mine abandonment has further added to the proliferation of areas which remain impacted and unused (Mhlongo and Amponsah-Dacosta 2016).

Mining impacts and mine abandonment result in areas that require rehabilitation which could be further used for different economic or ecological purposes (Harrison *et al*. 2019). Mine rehabilitation plans are dictated by the goals and needs of the mining company and both interested and affected parties (Tanner and Möhr-Swart 2007). Goals of mine rehabilitation include the rehabilitation of mine lands to economically viable post-mine land uses (Tanner and Möhr-Swart 2007). A cost effective and non-impactful rehabilitation method is phytoremediation which utilises plants to extract, degrade, contain or immobilise various soil contaminants (Cunningham and Berti 1993, Khalid *et al*. 2017). Fibrous plant species have demonstrated potential as phytoremediation agents through their ability to extract soil contaminants (Griga and Bjelková 2013). Furthermore, fibrous species are defined by the presence of economically valuable fibres within their biomass. These fibres are harvested, processed and manufactured into various products (Ramesh *et al*. 2017). Multiple potential value chains of products associated with fibrous species have been identified in South Africa (Broadhurst *et al*. 2019).

The legal requirement of rehabilitation and implementation of viable post-mine land uses could be facilitated by the cultivation and subsequent harvest of fibrous species on mine land. This research will determine whether the cultivation of *H. cannabinus* and *L. usitatissimum* in soils impacted by mining activities can be utilised and applied to phytoremediation strategies while providing subsequent and sufficient economic value through their use in fibre industries.

1.3 – Aim and Objectives

Aim

An assessment of the ability of *H. cannabinus* and *L. usitatissimum* to grow in and extract metals from soil contaminated with nickel and chrome mine tailings and to determine if these species can be utilised concurrently as phytoremediative and economically beneficial plant species.

Objectives

1) To determine whether the growth and subsequent yield of *H. cannabinus* and *L. usitatissimum* is impacted when cultivated in soils associated with mining and soils contaminated with nickel and chrome mine tailings.

2) To determine the phytoremediation capacity of *H. cannabinus* and *L. usitatissimum* cultivated in soils associated with mining and soils contaminated with nickel and chrome mine tailings.

3) To determine whether the cultivation of *H. cannabinus* and *L. usitatissimum* in soils associated with mining and soils contaminated with nickel and chrome mine tailings would result in economic gain. Therefore, determining whether each species can be utilised in or developed into a sustainable fibre industry providing profit to both mine and fibre industry stakeholders.

2 – Literature Review

2.1 – Mining

2.1.1 – Overview

Mining is the process through which economically valuable natural resources such as base metals, precious metals, rare earth elements and fossil fuels are extracted from the Earth's crust (Eggert 1994). Extraction of these resources occurs globally in both developed and developing countries. Extracted natural resources are used domestically and traded through the global economy (Behrens *et al*. 2007). Resource extraction and further processing takes place onsite in which raw materials are pulled from the Earth and subsequently processed using various metallurgical techniques. Extraction can take place in the form of surface and/or underground mining (Eggert 1994). Following the extraction process mined materials are further processed in which valuable material is separated from its associated waste material. This separation process involves the use of extractive metallurgy in the form of hydro, pyro, electro and ion metallurgy (Sohn and Wadsworth 2013). Once the separation of valuable material has occurred, waste associated with separation processes are disposed of and stored in the form of mine tailings storage facilities (Blight 2009). Throughout the procedures of mineral extraction and processing various environmental, human health and societal impacts occur (Change 2007, Davies and Mundalamo 2010, Gouldson and Murphy 2013).

2.1.2 – Environmental, Human Health and Societal Impacts

Environmental impacts associated with resource extraction result directly and indirectly through physical and chemical processes conducted during mining activities (Gouldson and Murphy 2013). Surface mining techniques such as strip mining directly impact surrounding environments. Habitat destruction is caused by the removal of large quantities of soil, soil associated organisms, and transformations to the area's topography (Dudka and Adriano 1997). Soils associated with surface mining are also subject to contamination by exposure to toxic substances released through resource extraction and processing. Furthermore, the loosening of soil and removal of vegetation cause increases in soil erosion (Dudka and Adriano 1997). The combination of soil erosion and prevalence of contaminants within soils facilitates the movement of toxic substances through landscapes (Lin *et al*. 2005). The storage of mine waste in the form of tailings, leads to the proliferation of acid mine drainage (AMD) if pyrite and other sulfurous minerals are present in the waste, through the oxidation of metal sulfides via exposure to air and water (Akcil and Koldas 2006). The movement of AMD through soil and water into larger water bodies and surrounding environments threatens terrestrial, aquatic and human health. The presence of contaminants within soils allows for the contamination of ground water as contaminants move through soils and into aquifers. Ground and surface water contamination are directly related to mining associated human health impacts (Ochieng *et* al. 2010, Simate and Ndlovu 2014).

The release of contaminants into surrounding soil, water and air resources can directly impact human health (Blaikie and Brookfield 2015, Fugiel *et al*. 2017). Contaminants released into the air through mining have been observed in soils further from the mines immediate area (Khalil *et al*. 2013). Furthermore, increased air contaminant concentrations have been correlated to an increased prevalence of respiratory diseases in surrounding communities. The aerial deposition of contaminants into soil resources of surrounding communities results in direct contact and health impacts to individual human health (Fugiel *et al*. 2017). Furthermore, the presence of soil contaminants in surrounding communities facilitates the possibility of contaminant accumulation in vegetative food supplies grown in the community (Liu *et al*. 2005). Contamination of water resources through the movement of contaminants in surface and groundwater impacts aquatic food and water supplies of surrounding communities. The presence of contamination in water resources further facilitates direct exposure to toxic substances and the propagation of health impacts in animals and humans (Ochieng *et al*. 2010).

The release of contaminants associated with mining affect immediate and surrounding environments and communities (Dudka and Adriano 1997, Yadav and Jamal 2018). The dependence of various communities on the presence of a mine can cause further societal impacts following mine closure (Heikkinen *et al*. 2008). Resources extracted through mining are finite, the lifespan of a mine is determined based on the desired resource availability in an area. The planning and inception of a mine facilitates the development of a surrounding community that supplies labour to the mine (Heikkinen *et al*. 2008). The mine acts as a central source of economic development while further independent industries develop to accommodate the surrounding community's needs. Mine closure without sufficient planning and consideration for the future of the dependent community results in a collapse of the central economic pillar of that community (Heikkinen *et al*. 2008). The effects of this breakdown include increases in poverty, disease and crime as the employment and health requirements of the community are no longer sustained. The process of mining allows for the development of communities and industries surrounding the mine, and overall, the process of mining facilitates increased economic and industrial development within a country (Limpitlaw and Briel 2014). This process is however, accompanied by various negative environmental, human health and societal impacts. The duality of costs and benefits associated with mining are evident in South Africa.

2.2 – Mining in South Africa

2.2.1 – History and Extracted Resources

The history of commercial mining in South Africa began with the discovery of diamonds in 1867 within the Cape of Good Hope colony (Innes 1984). This was followed by the discovery of gold in the Witwatersrand basin during the late 19th century (Richardson and Van Helten 1984). This discovery led to an influx of global mining practitioners catalysing the development of South Africa's mining industry and subsequent early economic development (Wilson 2001). Global demands, high quantities and high production rates caused South Africa to become the leading global producer and exporter of gold up until 2006 (Neingo and Tholana 2016). Gold ore extraction and processing currently account for 10% of total mining sales and provides 93 841 jobs in South Africa (MCSA 2022). Gold mining in South Africa has facilitated the development of South Africa's economy and multiple mining industries (Wilson 2001). South Africa also contains extensive mineral deposits of other economically valuable minerals such as coal, platinum group metals (PGM's), chrome and nickel (Davies and Mundalamo 2010).

Coal mining in South Africa commenced during the same period of gold's discovery (Hartnady 2010). Coal mining is concentrated in the Mpumalanga province of South Africa. Coal currently accounts for 24% of sales within the mining sector while providing 90 997 jobs (MCSA 2022). A total of 70% of coal mined is utilised domestically in energy generation while the remaining higher quality coal is exported (MCSA 2022). Platinum group metal (PGM) mining occurs within each region of the Bushveld Igneous Complex (BIC) (Hochreiter *et al*. 1985). The BIC is the Earth's largest layered igneous intrusion and contains approximately 80% PGM's and 20% of varying quantities of iron, tin, titanium, vanadium, chrome and nickel (Cawthorn 2015). Platinum mining currently accounts for 35% of sales within South Africa's mining sector (MCSA 2022). As of 2022 a total of 278 tonnes of PGM's are traded, 13 tonnes are traded domestically and 265 tonnes are exported (MCSA 2022). The BIC also contains approximately 72% of the Earth's current viable chromium deposits (Cawthorn 2015). Nickel mining is concentrated in the Western limb of the BIC while deposits and mines exist in Limpopo and Mpumalanga provinces. Nickel production currently accounts for 1% of sales within the mining sector (MCSA 2022).

The presence of vast mineral deposits combined with domestic and global demands for their extraction and processing have facilitated the development of South Africa's current economy. However, the mining sector currently accounts for 7.53% of the total gross domestic profit of South Africa. Furthermore, operations within the entire mining sector have experienced continuous production decreases in all mined minerals over the past decade (Boshoff and Fourie 2020, MCSA 2022).

2.2.2 – Mining Legislation

The development of South Africa's economy through mining activities has led to the proliferation of mine-associated environmental, human health and societal impacts (Davies and Mundalamo 2010). The presence of these impacts has become more prevalent as production throughout the mining sector decreases, leaving multiple areas of land impacted and unused (Limpitlaw and Briel 2014). These impacts include habitat destruction, decreases in biodiversity, water pollution and land degradation. Mine associated impacts to human health and societal wellbeing are also present in South Africa (Davies and Mundalamo 2010). Furthermore, these impacts are exacerbated due to the developing nature of South Africa as a country (Wälde 1992). The presence of historical and current mine associated impacts has led to the enactment and enforcing of legislation concerning the treatment and remediation of such impacts (MPRDA 2002, NEMA 2008). Enactment and enforcement of this legislation culminated in the formation of the Mineral and Petroleum Resources Development Act (MPRDA) which governs the acquisition, use and disposal of mineral rights (MPRDA 2002). The MPRDA also contains legislation which holds mining companies accountable to environmental impacts associated with their mining venture (MPRDA 2002)

Prior to the enactment of legislation contained within the MPRDA (2002) mining companies were not held accountable or required to rehabilitate impacts caused by mining activities (Harrison *et al*. 2019). The enactment of the MPRDA requires mining companies to set aside funds for the development of mine rehabilitation strategies. Through these strategies environmental, human health and societal impacts associated with mining are alleviated. Mining companies are required to develop mine rehabilitation plans prior to mine inception (MPRDA 2002). Procedures outlined within the rehabilitation plan are conducted during and after the mine's lifespan. The official closure of a mine is only legally valid and can only be conducted once a certificate of closure has been issued in terms of section 43 of the MPRDA (2002).

Mining activity prior to enforcement of the MPRDA has therefore allowed for the proliferation of impacted and untreated land areas throughout South Africa (Davies and Mundalamo 2010). Furthermore, enforcement of procedures contained within the MPRDA led to the mass abandonment of various mines across South Africa (Mhlongo and Amponsah-Dacosta 2016). Mass abandonment was due to mining company stakeholders' unwillingness to provide funding for mine impact rehabilitation procedures. The Council for Geosciences of South Africa has currently identified 5906 abandoned mines throughout the country (Harrison *et al*. 2019). Abandoned mines are comprised of varying types and intensities of untreated environmental impacts. Furthermore, the detrimental effects of previous mine activity on human health and communities remain without foreseeable implementation of mitigation procedures (Mhlongo and Amponsah-Dacosta 2016). The presence of active, closed and abandoned mines in South Africa are accompanied by various detrimental environmental, human health and societal impacts. Mitigation and treatment of these impacts are requirements of mining companies as described in the MPRDA (2002). Procedures and strategies regarding the treatment of mining associated impacts are addressed through the process of mine rehabilitation (Heikkinen *et al*. 2008).

2.3 – Mine Rehabilitation

2.3.1 – Overview

Mine rehabilitation is a concept and procedure which addresses the presence of and aims to mitigate, minimise and reverse impacts associated with mining during and after a mine's lifespan (McHaina 2001). The process of rehabilitation involves strategies and techniques which aim to return impacted land areas to their former state. However, the goals of mine rehabilitation will differ between countries and mining sites based on the demands or requirements of the surrounding community and environment (McHaina 2001). Currently rehabilitation techniques are unable to completely return impacted land to its previous state. Therefore, the end goals of rehabilitation, specifically for South Africa, fall into categories regarding communities surrounding the mine, the previous land use capability of the area and concerns of biodiversity management (Tanner and Möhr-Swart 2007). Planning and implementation of rehabilitation plans are a requirement of mine inception and life span (MPRDA 2002). Rehabilitation plans are developed and decided on during the prospecting stage of a new mine (Tanner and Möhr-Swart 2007).

The procedure and goals of mine rehabilitation plans are developed in conjunction with the economic goals of the mine. Baseline environmental studies regarding the state of the area before commencement of mining activities are conducted. The expected impacts on variables investigated within baseline environmental studies are identified (Tanner and Möhr-Swart 2007). Activities proposed during mining are evaluated and their expected effect on land use capabilities and ecological effect are determined. The mine plan is re-evaluated in accordance with recommendations made by baseline environmental studies to minimise and mitigate expected impacts of mining activity (Tanner and Möhr-Swart 2007). The agreed upon procedures of mining and rehabilitation are incorporated into the mines environmental management programme which is submitted to the authorities and interested and affected parties. Procedures of mining and rehabilitation are then amended to accomodate the concerns of mining authorities and interested and affected parties. This process continues until an agreement on the proceeding of mining and rehabilitation is established. Plans concerning the generation and procurement of funds required for agreed upon rehabilitation plans are created (Tanner and Möhr-Swart 2007). Establishment of an agreed upon rehabilitation plan and budgeting of funds required for rehabilitation provide mining companies legal rights to commence mining procedures (Mostert *et al*. 2019, MPRDA 2002).

2.3.2 – Procedures of Rehabilitation

Prior to the commencement of mining procedures, the construction and development of mining infrastructure is designed based on agreements set out in the rehabilitation plan. Infrastructure is designed and constructed to minimise the amount of area impacted by construction procedures and subsequent presence of infrastructure (Tanner and Möhr-Swart 2007). Furthermore, the removal and stockpiling of top soils from construction areas proceeds as construction takes place. Soil removed from these areas and other areas facilitating mining activities are stockpiled in demarcated areas conducive to efficient execution of rehabilitation procedures following mine closure (Tanner and Möhr-Swart 2007). The process of removing soil commences in accordance with soil stripping guidelines specific to the mine. Soil stripping is a key procedure in the success of subsequent rehabilitation as it allows for the swift commencement of soil amelioration and re-vegetation procedures (Tanner and Möhr-Swart 2007). During the planning stages of rehabilitation, soil surveys are conducted which identify the type and state of soils throughout the site. Soils are stockpiled according to their type and requirements for successful storage (Tanner and Möhr-Swart 2007).

Soil stripping removes soil horizons suitable for supporting plant growth as mining proceeds (Keipert *et al*. 2006). Mining activity commences in the stripped area while soils are replaced where ever possible as mining concludes (Tanner and Möhr-Swart 2007). The success of soil replacement, remediation and subsequent re-vegetation depends on the effective stockpiling of different soil types (Strohmayer 1999). Soil stockpiles must be located in areas which minimise the chance of compaction and soil structure damage. Furthermore, stockpiles should be in locations where water can freely drain from the soil to avoid waterlogging (Tanner and Möhr-Swart 2007). Stockpile management should aim to minimise any additional damage to the physical, chemical and biotic make up of stockpiled soils (Keipert *et al*. 2006). Finally, soil stockpiles must remain unused unless for their intended purpose set out in the rehabilitation plan. Processes of soil stripping and stockpiling continue throughout the mine's life span (Tanner and Möhr-Swart 2007).

The conclusion of mining activity results in the presence of abandoned and unused infrastructure previously associated with mining (Mhlongo and Amponsah-Dacosta 2016). Mine related infrastructure can either be removed or utilised depending on the agreed end land use type of the mine (Tanner and Möhr-Swart 2007). The process of infrastructure removal or reutilisation includes the removal of reusable and hazardous materials associated with infrastructure construction or removal. Conclusion of infrastructure removal or reutilisation allows for remediation of land changes that occurred during mining (Tanner and Möhr-Swart 2007). Land form re-creation is the process of using excess mine materials and overburden to alter the topography of the site (Cook 1976). The final desired upon topography is determined during the rehabilitation planning process and is dependent on the end land use goal outlined in the rehabilitation plan (Tanner and Möhr-Swart 2007). Rehabilitation plans stating an end land use of re-vegetation to promote biodiversity would use land form recreation procedures to create a topography similar to that present before mining. End land use goals regarding crop cultivation would utilise land re-creation procedures to create forms conducive to crop cultivation, reduced erosion and water runoff (Tanner and Möhr-Swart 2007). Once the desired topography is created soil replacement procedures can commence.

Soil replacement commences utilising previously stockpiled soils to recreate various soil profiles based on soil surveys conducted during the rehabilitation planning phase (Tanner and Möhr-Swart 2007). Recreation of soil profiles throughout the rehabilitation site are then subjected to various amelioration and remediation techniques. Determination of procedures used to ameliorate and/or remediate soils are decided based on the soil's current physical, chemical and biological structure (Heikkinen *et al*. 2008, US EPA 2008). Stockpiling and replacement procedures affect these soil structures. Increased soil compaction mandates soil ripping to facilitate plant growth while mixing of top and subsoil horizons dilutes soil fertility creating requirements for fertilizer addition (Tanner and Möhr-Swart 2007). Furthermore, replacement soils are commonly subjected to detrimental impacts caused through the accumulation of mine associated contaminants (Tordoff *et al*. 2000). Various physical, chemical and biological procedures are utilised to remove contaminants from soils (Khalid *et al*. 2017). The removal of contaminants is vital to the success of subsequent re-vegetation procedures (Tordoff *et al*. 2000).

Re-vegetation procedures are decided on during the rehabilitation planning stage. The end land use goal of the site determines the type of vegetation cultivated on remediated soils within the site (Tanner and Möhr-Swart 2007, Tordoff *et al*. 2000). Post mine land use re-vegetation goals can include re-establishment of native vegetation and promotion of biodiversity, erosion control for the protection of water resources, creation and establishment of high-quality grazing lands and land preparation for subsequent crop cultivation (Tordoff *et al*. 2000, Kuter 2013). The implementation of all procedures outlined in the rehabilitation plan is followed by prolonged rehabilitation monitoring and maintenance programmes. This is to ensure that objectives and processes determined in the rehabilitation remain on track towards success (Heikkinen *et al*. 2008, Kuter 2013, Tanner and Möhr-Swart 2007). Soil remediation and re-vegetation procedures commonly determine the success of rehabilitation plans as they dictate the final land use capability of the rehabilitated site (Banning *et al*. 2011).

The establishment of native species or cultivated crops on rehabilitated land areas is dependent on the success of soil remediation procedures (Tordoff *et al*. 2000). Soil remediation can be conducted using various physical, chemical and biological techniques. These techniques are commonly used to extract mine associated contaminants and promote development of a micro-biome in impacted soils used in soil replacement rehabilitation procedures (Liu *et al*. 2018). Soil remediation techniques vary in financial cost, human involvement, subsequent environmental effects and applicability to different types and intensities of soil contamination (Liu *et al*. 2018). Physical remediation techniques include: soil isolation, vitrification and electrokinetic remediation. These techniques are applied to the remediation of heavily contaminated sites (Khalid *et al*. 2017). However, the use of these techniques are limited by increased financial costs and manpower requirements. Therefore, physical remediation techniques are best applied to smaller and highly contaminated sites (Khalid *et al*. 2017). Contrastingly, chemical remediation techniques are most efficient at remediating moderately contaminated sites (Khalid *et al*. 2017).

Chemical remediation techniques include: contaminant immobilisation, encapsulation and soil washing (Yao *et al*. 2012). The addition of foreign substances such as immobilisation agents, concrete, lime, asphalt, leaching reagents and extractants alter naturally occurring soil physical, biological and chemical compositions (Yao *et al*. 2012). This along with the specificity of soil contamination and type to which chemical remediation can be applied lowers the viability of these techniques in various scenarios (Liu *et al*. 2018). Extreme alterations to natural soil conditions are somewhat avoided by the use of biological remediation techniques (Khalid *et al*. 2017). Biological remediation techniques are divided into two types, namely bioremediation and phytoremediation. These techniques are relatively cost effective and require minimal human involvement throughout the remediation process. Furthermore, these techniques can be applied to large areas subjected to low and moderate levels of contamination (Marques *et al*. 2011 and Khalid *et al*. 2017). Bioremediation takes advantage of the ability of micro-organisms to alter the physical characteristics and chemical species type of soil contaminants. These alterations decrease the rate at which contaminants migrate through soils and their bioavailability (Dada *et al*. 2015). Bioremediation has been documented to operate efficiently when used in conjunction with phytoremediation (Khalid *et al*. 2017).

2.4 – Phytoremediation

Phytoremediation involves the use of living plants in order to extract, degrade, contain or immobilise various contaminants in soil, groundwater or other contaminated media (Cunningham and Berti 1993). Phytoremediation is divided into four processes: rhizodegradation, phytostabilisation, phytovolatilisation and phytoextraction (Mahar *et al*. 2016). Rhizodegradation can be defined as plant-assisted bioremediation in which the presence of a rhizosphere enhances surrounding micro-organism activity. Enhanced micro-organism activity reduces the migration rate and bioavailability of contaminants (Mahar *et al*. 2016). The success of this technique is site specific as micro-organisms tested in laboratory conditions may not operate in the same manner in field conditions (Khalid *et al*. 2017, US EPA 2008).

Phytostabilisation utilises the plant’s rhizosphere to immobilise contaminants. This is done through the adsorption of contaminants to plant roots, the formation of metal complexes and the precipitation of metal ions (Sarwar *et al*. 2017). Sites utilising this technique need to be monitored and require consistent removal of dead and senescing plants (Sarwar *et al*. 2017). Phytovolatilisation involves the transpiration of contaminants into the atmosphere through their uptake by plants (Sarwar *et al*. 2017). Therefore, use of this remediation procedure is limited to sites contaminated by substances with the ability to enter a volatile state (Sarwar *et al*. 2017). Lastly phytoextraction utilises tolerant and accumulating species to absorb and subsequently store contaminants within their above ground mass (Khalid *et al*. 2017, Sarwar *et al*. 2017).

Phytoextraction results in the permanent removal of contaminants from soils (Khalid *et al*. 2017, Van Nevel *et al*. 2007). Furthermore, the treatment, removal, oxidation and recycling of contaminants stored within above ground plant biomass requires less economic and energy input than those stored directly within soil (Khalid *et al*. 2017). This process can be applied to large and moderately contaminated sites (Khalid *et al*. 2017, Yao *et al*. 2012). Economic efficiency, low soil and environmental disturbance, acceptance by the general public, no need for soil excavation or transport and the possibility of its use in multi-metal contaminated sites are further advantages offered by phytoextraction (US EPA 2008). Plant species commonly used in phytoextraction procedures are hyper-accumulators (Rascio and Navari-Izzo 2011).

Hyper-accumulators are capable of accumulating contaminants, specifically metals, in their above ground biomass at levels greater than those present in contaminated soils or other non-accumulating species (Rascio and Navari-Izzo 2011). The use of hyper-accumulators in phytoextraction depends on their ability to accumulate high amounts of contaminants within their above ground biomass, tolerance to high concentrations of contaminants, growth rate, total biomass and the extent of their root system. Currently no known plant species fulfils all requirements of a viable phytoextraction species (Khalid *et al*. 2017, Rascio and Navari-Izzo 2011). However, the use of hyper-accumulating plant species in phytoextraction strategies has been successful in multiple studies (Bhargava *et al*. 2012, Mahar *et al*. 2016, Raskin *et al*. 1997, Schwitzguébel 2002). Species used in phytoextraction strategies are primarily used to extract metal contaminants which can possibly be regathered and recycled through incineration of plant biomass. This process is known as phytomining (Rascio and Navari-Izzo 2011).

The ability of hyper-accumulators to extract increased amounts of soil contaminants is overshadowed by the time period required for extraction. Furthermore, the extent of many hyper-accumulating species' root systems only allows for contaminant extraction in shallow soils (Khalid *et al*. 2017). The time period required for and extent of contaminant extraction by hyper-accumulating species combined with their limited economic use in phytomining decreases their viability in efficient, effective and profitable remediation strategies. Furthermore, cultivation of these species in re-vegetation procedures would not serve as a viable post mine land use in rehabilitation plans. However, a group of species defined as fibrous plants have demonstrated potential use in phytoextraction strategies and provide economic benefit through their utilisation in existing fibre industries (Harrison *et al*. 2019).

2.5 – Fibrous Plants

2.5.1 – Definition and Species

Fibrous plants are defined by the presence of economically valuable fibres contained within their above ground biomass (Ramesh *et al*. 2017). Fibres produced in fibrous species are associated with different components of their anatomy resulting in the occurrence of different fibre types, namely bast, leaf, seed, stalk, grass and wood (Ramesh *et al*. 2017). Cultivation and subsequent harvest of fibres are utilised to manufacture various fibre products (Ramesh *et al*. 2017). Products associated with fibrous species cultivation, harvesting and processing include biofuel, textiles and composite materials (Harrison *et al*. 2019). Stems of bast fibre species contain an outer fibre and inner woody layer. Bast fibres are present as bundles located within the phloem of these fibre species (Ramesh *et al*. 2017). Bast fibre from species such as kenaf (*Hibiscus cannabinus* Linnaeus, Malvales: Malvaceae) and flax (*Linum usitatissimum* Linnaeus, Linum: Linaceae) can be processed and utilised in the production of: non-woven and technical-use textiles, composite motor vehicle parts, paper and pulp, bioethanol and various other wood products (Broadhurst *et al*. 2019).

2.5.2 – Cultivation Parameters, Yield and Economic Value Estimations

2.5.2.1 – Climate

Hibiscus cannabinus cultivation can be accomplished within tropical, sub-tropical and temperate climate zones ranging from 30° North to 30° South (Dempsey 1975). However, optimal total dry mass and fibre mass yields are achieved within either tropical or sub-tropical climate zones (Alexopoulou *et al*. 2007, Kozlowski *et al*. 2005, Saba *et al*. 2015). *Hibiscus cannabinus* is able to tolerate a temperature range of 10 °C to 30 °C (Saba *et al*. 2015), while a range of 15 °C to 28 °C will result in optimal total dry mass and fibre mass yields (Harrison *et al*. 2019). Dempsey (1975) stated that *H. cannabinus* cultivation required 100-125 mm of rainfall each month over a five to six month growing period. Crane (1947) recommended a total of 500-625 mm of rainfall over the same growing period. These recommendations are congruent with *H. cannabinus* cultivation parameters in Alexopoulou *et al.* (2007) who stated that a range of 90-275 mm each month would produce optimal total dry mass and fibre mass yields. Furthermore, *H. cannabinus* remains in a vegetative growth state under conditions of mean day lengths greater than 12.5 hours (Kozlowski *et al*. 2005). Therefore, mean day lengths greater than 12.5 hours are recommended for optimal fibre mass production (Harrison *et al*. 2019, Kozlowski *et al*. 2005).

Linum usitatissimum cultivation can be accomplished within tropical and temperate climate zones (Harrison *et al*. 2019). However, cultivation in temperate climate zones is recommended for optimal total dry biomass, fibre mass and seed mass production (Arslanoglu *et al*. 2022, Kozlowski *et al*. 2005, Sultana 1983). Currently the cool, moderate and coastal climates are recommended for *L. usitatissimum* cultivation in South Africa (Jacobsz and Van der Merwe 2012). *Linum usitatissimum* can tolerate temperature ranges of -4 °C to 32 °C after seedlings have established (Jacobsz and Van der Merwe 2012). However, a range of 16 °C to 24 °C is recommended for optimal total dry mass and fibre yields (Harrison *et al*. 2019). *Linum usitatissimum* can be cultivated in areas that have a mean annual rainfall range of 600-800 mm (Kozlowski *et al*. 2005). However, *L. usitatissimum* cultivation in South Africa can be achieved in areas which have a mean annual rainfall ranging between 450-700 mm (Jacobsz and Van der Merwe 2012, Harrison *et al*. 2019). Furthermore, optimal total dry mass and fibre mass have been recorded for *L. usitatissimum* cultivated under conditions with a mean day length greater than 12 hours (Lloveras *et al*. 2006, Oelke *et al*. 1992).

2.5.2.2 – Soil

Hibiscus cannabinus can adapt to and be successfully cultivated in a wide range of soils, from organic peat soils to sandy desert soils (Dempsey 1975, Harrison *et al*. 2019). However, optimal total dry mass and fibre mass yields have been consistently achieved by cultivating *H. cannabinus* in well-draining sandy loam soils (Alexopoulou *et al*. 2007, Oelke *et al*. 1992, Saba *et al*. 2015). *Hibiscus cannabinus* can tolerate a soil pH range of 4.3-8.2 (Harrison *et al*. 2019). However, optimal production of total dry biomass and fibre mass occurs in soils with a pH range of 6-7.5 (Harrison *et al*. 2019, Panoutsou and Alexopoulou 2020). Furthermore, optimal production is achieved in soil with a salinity level that is less than 4 dS/m (Harrison *et al*. 2019). *Hibiscus cannabinus* cultivation requirements of nitrogen, phosphorus and potassium vary depending on existing soil conditions within the cultivation area (Dempsey 1975, Xu *et al*. 2020). General requirements for cultivation in South African sandy soils are: 35-70 kg/ha nitrogen, 40-60 kg/ha phosphorus and 45-65 kg/ha potassium (Coetzee 2004).

Linum usitatissimum can be successfully cultivated in well-draining sandy loam soils and tolerate soils with a pH range of 5-7 (Easson and Molloy 2000, Kozlowski *et al*. 2005, Sultana 1983). However, optimal production can be achieved in soils with a pH range of 6-6.5 and a salinity level less than 4 dS/m (Jacobsz and Van der Merwe 2012, Harrison *et al*. 2019). *Linum usitatissimum* cultivation requirements of nitrogen, phosphorus and potassium vary depending on existing soil conditions within the cultivation area (Duguid *et al*. 2007). *Linum* *usitatissimum* cultivation guidelines in South Africa recommend the addition of 35-80 kg/ha nitrogen and 35 kg/ha phosphorus to soils within the cultivation area (Jacobsz and Van der Merwe 2012, Gomez-Campos *et al*. 2021, Oelke *et al*. 1992). Recommendations for the addition of potassium are not provided within the South African guideline for *L. usitatissimum* cultivation. However, Oelke *et al*. (1992), Arslanoglu *et al*. (2022) and Mańkowski *et al*. (2013) applied additional potassium of 22-90 kg/ha, 50 kg/ha and 120 kg/ha respectively to soils used for *L. usitatissimum* cultivation trials for optimal total dry biomass and fibre mass production.

2.5.2.3 – Cultivation Practices

Hibiscus cannabinus completes its growth cycle within a minimum of 100 days and a maximum of 240 days (Harrison *et al*. 2019). Seeding rates vary depending on the plant density and what the cultivator wishes to achieve (Kozlowski *et al*. 2005). Furthermore, plant density directly impacts the final total dry biomass and fibre mass of cultivated *H. cannabinus* (Kozlowski *et al*. 2005). Xu *et al*. (2020) stated that *H. cannabinus* plant density for optimal production can range between 200 000 to 500 000 plants/ha while Alexopoulou *et al*. (2007) gave a general recommendation of 200 000 to 250 000 plants/ha. However, successful fibre production in South Africa can be achieved at a density of 270 000 to 300 000 plants/ha (Harrison *et al*. 2019). This plant density can be achieved by sowing *H. cannabinus* seeds at a rate of 20-35 kg/ha and by cultivating *H. cannabinus* using row spacing of 25-35 cm (Harrison *et al*. 2019 and Saba *et al*. 2015). Panoutsou and Alexopoulou (2020) showed that the mean total production cost of *H. cannabinus* cultivation across producers within the European Union was 417.00 €/ha (R 6872.16/ha) in low quality farmland and 499.00 €/ha (R 8223.52/ha) in average quality farming land at an exchange rate of 1 € = R 16.48 (15 June 2022).

Linum usitatissimum completes its growth cycle within a minimum of 80 days and a maximum of 180 days (Harrison *et al*. 2019). As with *H. cannabinus*, seeding rates of *L. usitatissimum* depend on the desired final plant density within a cultivation area and impacts the final total dry biomass and fibre mass (Arslanoglu *et al*. 2022). Sultana (1983) proposed a seeding rate of 100-140 kg/ha resulting in a density of 1800 plants/m^2. However, guidelines for the cultivation of *L. usitatissimum* in South Africa recommend a seeding rate of 65 kg/ha (Jacobsz and Van der Merwe 2012). While according to Arslanoglu *et al*. (2022) a final plant density of 2000 plants/ha will produce optimal total dry biomass and fibre mass. Therefore, the seeding density of *L. usitatissimum* should aim to result in a final plant density of 2000 plants/ha. Based on *L. usitatissimum* production in the European Union Bran *et al*. (2017)

estimated the cultivation cost of *L. usitatissimum* to be 500-600 €/ha (R 8239.29 – R 9887.15/ha) at an exchange rate of 1 € = R 16.48 (15 June 2022).

2.5.2.4 – Yield and Economic Value Estimation

Hibiscus cannabinus total dry biomass and fibre mass yields vary depending on climatic, soil, and cultivation factors (Dempsey 1975). Xu *et al*. (2020) stated that the total dry biomass yields of *H. cannabinus* range from 23-25 t/ha while Saba *et al*. (2015) estimated total dry biomass yields of 6-25 t/ha. Furthermore, total dry biomass yields of *H. cannabinus* that range between 15-25 t/ha, 9-22 t/ha and 6-25 t/ha have been produced (Alexopoulou *et al*. 2007, Sen and Reddy 2011, Singh 2010). Kozlowski *et al*. (2005) estimated that the total dry biomass of *H. cannabinus* contains 18-20 percent of fibre mass. However, the range of fibre mass yields produced from *H. cannabinus* varies as Singh (2010) estimated a fibre yield of 5-10 t/ha while Saba *et al*. (2015) estimated yields of 1-2.5 t/ha. Furthermore, Kozlowski *et al*. (2005) found that *H. cannabinus* produced a mean fibre yield of 1-2 t/ha that increased to 3-3.5 t/ha in favourable conditions. Although the total dry matter and fibre yield of *H. cannabinus* vary, the value of the yield may be estimated based on the existing market price of the produced mass. Panoutsou and Alexopoulou (2020) found the mean market selling price of *H. cannabinus* total dry mass to be 80 €/t (R 1318.29/t) within the European Union (exchange rate of 1 € = R 16.48 - 15 June 2022).

Linum usitatissimum total dry biomass and fibre mass yields are also determined by climatic, soil and cultivation factors (Sultana 1983). However, the ranges of these yields are not as wide compared to those of *H. cannabinus*. Total dry mass yields of *L. usitatissimum* range from lower yields of 3-4.5 t/ha, 4.2-4.7 t/ha and 3.15-5.6 t/ha to higher yields of 7 t/ha, 8.92 t/ha and 11 t/ha (Couture *et al*. 2002, Lloveras *et al*. 2006, Bran *et al*. 2017, Gomez-Campos *et al*. 2021, Mańkowski *et al*. 2013, Easson and Molloy 2000). However, the ranges of fibre mass yields of *L. usitatissimum* appear to be more consistent. The estimated total dry biomass yield found in Couture *et al*. (2002) of 3-4.5 t/ha produced an estimated fibre mass yield of 1-2 t/ha, while the estimated total dry biomass yield of 11 t/ha was found to produce a fibre mass yield of 2.2 t/ha (Easson and Molloy 2002). Furthermore, Panoutsou and Alexopoulou (2020) showed that the fibre mass yield of *L. usitatissimum* cultivated in average farming land was 1.4 t/ha while Kozwolski *et al*. (2005) estimated the mean global average to be 1.27 t/ha. As with *H. cannabinus*, the value of the *L. usitatissimum* yield may be estimated using the existing market price of the produced mass. According to Bran *et al*. (2017) a minimum selling price of 160 €/t (R 2636.57/t) may be used to calculate the economic value of *L. usitatissimum* total dry biomass yield (exchange rate of 1 € = R 16.48 - 15 June 2022).

2.5.3 – Processing and Manufactured Products

Following cultivation, the harvested bast fibres are separated from within plant stems through the process of retting (Sponner *et al*. 2005). This process utilises the natural occurrence of micro-organisms and moisture to break down cellular tissue and pectin surrounding bast fibre bundles (Sponner *et al*. 2005). Retted and subsequently dried stems are further processed by being fed through a sequence of mechanical processes which separate fibres from woody tissue. This process is known as decortication (Amaducci and Gusovius 2010, Sponner *et al*. 2005). Decortication consists of breaking, milling and scutching procedures. These procedures allow for the separation of long, short and woody fibres which can be further converted into various final products (Amaducci and Gusovius 2010, Chen and Liu 2010). Long bast fibres are utilised in the production of high-end materials such as textiles and polymer composites. Short bast fibre are manufactured into medium value material such as paper or cordage. Woody tissue and core fibres can be utilised to manufacture low end or bulk materials such as hempcrete and insulation boards (Deyholos and Potter 2014).

Allen *et al*. (2019) investigated the potential formation and development of a South African fibre industry through the use of economic complexity and network analytics. The study revealed that South Africa has relatively under invested in the development of a fibre industry. However, South Africa was also found to have sufficient capabilities in regard to the development, sustaining and diversification of a fibre industry (Allen *et al*. 2019). The use of economic complexity analytics allowed for the identification of "fibrous frontier products" which would allow for the initial development of a fibre industry (Allen *et al*. 2019). These products include non-woven and textile use textiles, motor vehicle parts, paper and pulp products, bioethanol and various wood products (Allen *et al*. 2019). The economic viability of fibrous species is accompanied by their potential use in phytoremediation strategies (Harrison *et al*. 2019).

2.5.4 – Phytoremediative Capability

Fibrous species have been recorded to extract varying amounts of metal contamination from soil (Griga and Bjelková 2013). The ability of fibrous species to extract contamination and contain it within their above ground biomass along with high growth rates, total biomass and proliferous root systems of certain species further supports their use in phytoremediation

(Khalid *et al*. 2017). Ludvíková and Griga (2019) proposed the use of *H. cannabinus* and *L. usitatissimum* in phytoextraction strategies. *H. cannabinus* demonstrated phytoextractive capabilities through accumulation of lead, cadmium and zinc in its roots, shoots, leaves and seed capsules (Angelova *et al*. 2004, Bada and Raji 2010, Meera and Agamuthu 2012). While Hosman *et al*. (2017) demonstrated that *L. usitatissimum* had the ability to accumulate lead, zinc and copper within its roots and seed capsules.

The use of fibrous species in phytoextraction may be limited by their low tolerance to high levels of soil contamination (Khalid *et al*. 2017). Furthermore, the presence of contaminants may hinder plant growth and fibre production while contaminant accumulation in fibres may degrade their overall quality. Therefore, application of fibrous plants to phytoextraction strategies will need to be conducted on a site-specific basis (Khalid *et al*. 2017, Yao *et al*. 2012). However, the economic value chain associated with fibrous plant products and their demonstration of phytoremediative properties leads to the possibility of their dual use as phytoremediation and industrial crops.

In the application of fibrous plants to soil remediation and re-vegetation stages of mine rehabilitation, plants would provide economic gain through environmental impact mitigation and fibre product manufacture. The development of a fibre industry using mine land during and after a mines lifespan could provide mine owners and surrounding communities with a viable post mine land use opportunity (Harrison *et al*. 2019). The multifaceted nature of a fibre industry containing various cultivation, harvesting, processing and manufacturing procedures could aid an increase in employment opportunities to surrounding communities (Harrison *et al*. 2019). Skills required in each of these processes could be taught to previous mine workers to fulfil the need for the development of transferable skills outlined in the MPRDA (2002). Fibre industry development could potentially fulfil mining legislative requirements of rehabilitation, transferable skills development and viable post mine land use.

The environmental, human health and societal impacts associated with historical and current mining practices in South Africa have led to the formation and enforcement of mine rehabilitation commitments (Swart 2003). The abandonment of mines and continuation of mining has caused the proliferation of impacted land areas which remain economically unused (Limpitlaw and Briel 2014). A cost effective, non-polluting, low disturbance and perhaps sustainable solution to the treatment of impacted areas may be the application of phytoremediation strategies. The use of fibrous species could serve as both phytoremediation and industrial crops due to their phytoextractive capabilities and economic value (Harrison *et al*. 2019, Griga and Bjelková 2013). Development of a fibre industry using mine land may fulfil the requirements of rehabilitation, skills transfer, job creation and post mine land use to various active, closed and abandoned mines throughout South Africa (Harrison *et al*. 2019, Mostert *et al*. 2019). This research investigated the phytoremediative potential and economic value of *H. cannabinus* and *L. usitatissimum* through a site-specific greenhouse trial. Measurements of yield and phytoextractive capability of each species were recorded and scaled to infer the total phytoextractive potential and economic value of each species cultivated on available areas at a specific mine site. Through this investigation the viability and success of fibrous species' application to phytoremediation, economic gain and post mine land use was determined.

3 – Methods

3.1 – Soil Collection and Analysis

3.1.1 – Soil Collection

Soils were collected from the Onverwacht tailings storage facility (TSF), located approximately 10 km South of Nkomati Nickel Mine, for subsequent analysis and use in the germination and greenhouse growth trial. A total of 1080 kg of soil consisting of two soil types and tailings dust were collected. All soils were collected within a depth range of 0 to 30 cm. Approximately 510 kg of normal soil (non-impacted), 360 kg of rehabilitated soil (previously impacted) and 210 kg of tailings dust were collected from the Onverwacht TSF (Figure 1). Collection locations were chosen based on recommendations from the TSF resident technician and onsite observations (Figure 1). Collected material was transported to and stored at the Oppenheimer Life Sciences greenhouse located at the University of the Witwatersrand.

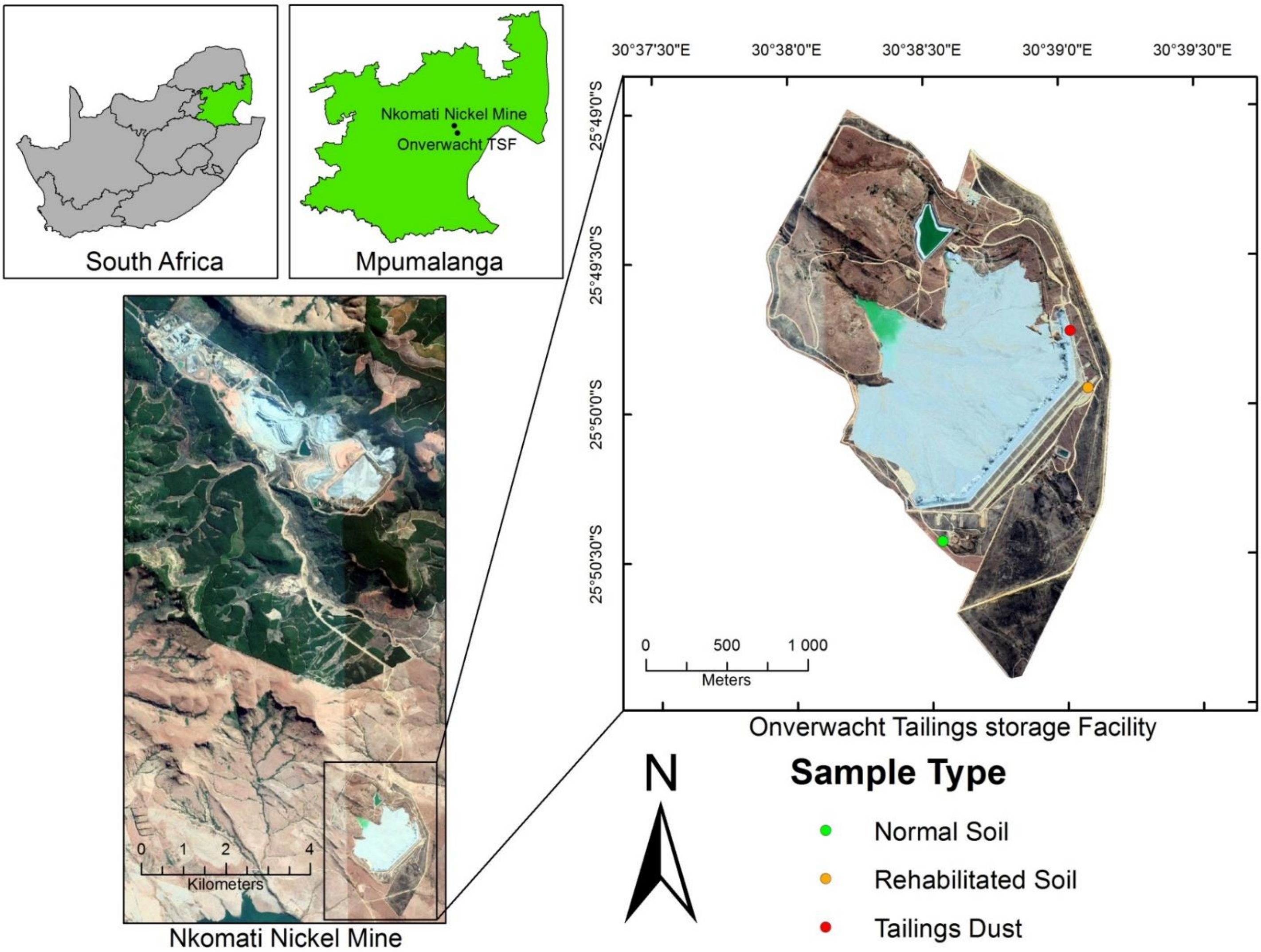


Figure 1: Site map and soil collection points at the Onverwacht tailings storage facility, Mpumalanga, South Africa.

3.1.2 – Soil Analysis

Results obtained from the germination trial, presented in chapter 4.2, indicated that a mix of 75 % normal soil and 25 % tailings dust, prepared as stated in chapter 3.2.1, was optimal for *H. cannabinus* and *L. usitatissimum* germination and growth. Therefore, soil analysis was conducted on this normal soil and tailings dust mix, defined as tailings soil. Soil analysis was also conducted on normal and rehabilitated soil treatments. Rehabilitated soil was defined based on recommendations from the resident tailings technician at the Onverwacht TSF. The source of the rehabilitated soil is unknown and it is unclear whether this soil was collected from a previous stockpile, naturally occurring within the TSF's vicinity or imported from a different area. However, Nkomati Nickel Mine may have purposefully used this type of soil, with its specific physicochemical variables, to contain possible leakage from the TSF. Furthermore, it is unclear what amendments were added and what processes were applied to the soil in order to define it as a rehabilitated soil. Details of the processes and methods used in the soils rehabilitation should be accessible to the public. However, access to these details could not be obtained even after multiple attempts to acquire them from Nkomati Nickel Mine and the Department of Mineral Resources and Energy of South Africa. A single sample, approximately 500 g, of each soil treatment was sent to Bemlab for analysis of soil physicochemical variables.

These variables included a soil texture analysis and classification based on the stone volume, sand, silt and clay percentages of each soil treatment. Furthermore, the pH (KCl), electrical conductivity (µS/cm) and electrical resistance (Ω) were determined. Further analyses included the total nitrogen (%), phosphorus (mg/kg), potassium (mg/kg) and carbon (%) content of each soil treatment. The available phosphorus (mg/kg) and organic carbon (%) content was determined using the Bray II and Walkley-Black method respectively. Finally, the soluble sulfur content (mg/kg), cation exchange capacity (CEC) and base saturation (BS) of each soil treatment were determined.

Single samples, approximately 500 g, of each soil treatment were sent to the Central Analytical Facilities, Stellenbosch University for metal content analysis. Inductively coupled plasma mass spectrometry (ICP-MS) was utilised to determine the concentration (mg/kg) of arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), vanadium (V) and zinc (Zn) of each soil treatment.

3.2 – Germination Trial

3.2.1 – Soil and Seedling Tray Preparation

The germination trial utilised six different soil treatments to analyse the germination and growth success of *H. cannabinus* and *L. usitatissimum*. Normal and rehabilitated soils were sieved through mesh with a diameter of 15 mm to remove large organic debris and stone material. Four normal soil and tailings dust mixes were used to represent a contaminated soil medium. These mixes were created by mixing normal soil and tailings dust samples in the following percentage weight ratios – 90:10, 75:25, 50:50, and 25:70, constituting the tailings one to four soil treatments. These mixes were made by adding the relative amounts (kg) of normal soil and tailings dust to create a total mix of 5 kg for each of the required normal soil to tailings dust mix ratios. Seedling trays consisting of 30 seedling compartments each of 60 mm height and 45 mm aerial diameter were used. For each soil treatment 30 seedling compartments were filled with 100 g of the respective soil treatment. Therefore, a total of 180 seedling compartments were used.

3.2.2 – Seed Preparation, Sowing and Water Regime

A total of 90 *H. cannabinus* and 90 *L. usitatissimum* seeds were submerged and soaked in distilled water for 24 hours prior to sowing. 15 seeds of each species were placed in separate seedling compartments for each soil treatment. All seeds were sown at a depth of approximately one cm. Seedling trays were kept in conditions that had a mean minimum and maximum temperature of 16 °C and 24 °C, respectively. The germination trial was conducted over a two-week period. The watering regime of each soil treatment was determined by calculating the amount (g) of water drained from each soil treatment after a 24-hour period. This was determined by placing 100 g of a soil treatment into a pot of equal dimension to the seedling compartment. 50 ml of water, enough to induce drainage, was added to the 100 g soil sample. Once water drainage had ceased the initial soil and pot mass (g) was recorded. The final soil and pot mass was recorded after a 24-hour period. The initial soil and pot mass was subtracted from the final mass. This difference revealed the amount of water drained from each soil treatment over the 24-hour period. This procedure was conducted five times for each soil treatment, allowing for the mean amount of water drained over a 24-hour period to be calculated. This mean amount of water was added to each seedling tray compartment daily throughout the germination trial. The amount of water added daily to each seedling compartment for each soil treatment is presented in Table 1.

Table 1: Daily amount of water (mL) added to each seedling compartment for each soil treatment during the two-week germination trial of *H. cannabinus* and *L. usitatissimum*.

Soil Treatment	**Mean Daily Water Amount (mL)**
Normal	9
Rehabilitated	8
Tailings One (90:10)	9
Tailings Two (75:25)	8
Tailings Three (50:50)	7
Tailings Four (25:70)	4

3.2.3 – Data Collection and Statistical Analysis

At the end of the two-week period the total germination percentage of *H. cannabinus* and *L. usitatissimum* sown in each soil treatment was calculated. Furthermore, the mean above ground height (cm) of *H. cannabinus* and *L. usitatissimum* seedlings grown in each soil treatment was calculated. A Shapiro-Wilk test was conducted to confirm normality of data. A two-way ANOVA and subsequent Tukey's HSD test was conducted to compare the mean above ground height within and between species and soil treatment. The normal soil to tailings dust mix that indicated the highest total germination percentage and mean above ground height of each species was then used for the subsequent greenhouse growth trial.

3.3 – Greenhouse Growth Trial

3.3.1 – Soil and Pot Preparation

The greenhouse growth trial utilised three soil treatments, normal, rehabilitated and tailings soil, to analyse the growth of *H. cannabinus* and *L. usitatissimum* over a six-month period. Prior to exposure to treatment soils seedlings of *H. cannabinus* and *L. usitatissimum* were allowed to establish in pots of 65 mm height and 68 mm diameter containing Culterra potting soil. 500 g of Culterra potting soil was added to each of 210 pots which were placed in the Oppenheimer Life Sciences greenhouse located at the University of the Witwatersrand.

Normal and rehabilitated soils were sieved through mesh with a diameter of 15 mm to remove large organic debris and stone material. Tailings (75% normal soil: 25% tailings dust) was made by adding the relative amounts of normal soil and tailings dust to create a total mix of 10 kg at a time until the required number of pots were filled. Cultivation pots of 4 L volume were used for *H. cannabinus* and *L. usitatissimum* cultivation in treatment soils after seedling establishment. 60 cultivation pots were filled with 3 kg of soil for each soil treatment. Thus, a total of 180 cultivation pots were used and placed in the Oppenheimer Life Sciences greenhouse located at the University of the Witwatersrand.

3.3.2 – Seed preparation, Sowing, Water Regime and Seedling Establishment

500 mL of water was added to each pot containing Culterra potting soil prior to seed sowing. A total of 525 *H. cannabinus* and 1050 *L. usitatissimum* seeds were submerged and soaked in distilled water for 24 hours prior to sowing. Five *H. cannabinus* and ten *L. usitatissimum* seeds were sown in each pot containing Culterra potting soil at a depth of approximately one cm. The higher number of *L. usitatissimum* sown in each pot was done to account for the inherent size differences between the two species. Therefore, a higher number of *L. usitatissimum* was sown in each pot to produce sufficient plant mass for use in future chemical analyses. The watering regime of pots containing Culterra potting soil was determined using the same method conducted in the germination trial. However, 500 g of Culterra potting soil in the same pots used for seedling establishment and 300 mL of water was used for each of five replicates to determine the mean amount of 50 mL added to each pot daily. Seeds were left to germinate and seedlings establish for one month. The watering regime of pots containing normal, rehabilitated and tailings soil treatments was also determined using the same method conducted in the germination trial. However, three kg of each soil treatment in a 4 L cultivation pot and 1 L of water was used for each of the five replicates, per soil treatment, to determine the mean amount of water (mL) added to each pot daily, these amounts would keep soils in each cultivation pot at approximately 100% field capacity (Table 2)

Table 2: Daily amount of water (mL) added to each cultivation pot for each soil treatment during the six-month greenhouse growth trial of *H. cannabinus* and *L. usitatissimum*.

Soil Treatment	**Mean Daily Water Amount (mL)**
Normal	100
Rehabilitated	150
Tailings	100

3.3.3 – Seedling Transplantation and Plant Growth

After one month of growth in pots containing Culterra potting soil, seedlings were transplanted into cultivation pots containing the three soil treatments. Thirty cultivation pots were used for each species and each soil treatment. Cultivation pots were saturated with 1 L of water prior to the transplanting of five *H. cannabinus* and ten *L. usitatissimum* seedlings into each pot. Plants were left to grow in soil treatments over a six-month period from January 2021 to June 2021. Plants were watered with the calculated mean daily water amount (mL) relative to each soil treatment (Table 2).

3.3.4 – Plant Harvesting and Data Collection

Data were collected at the end of each month, thus a total of seven data sets were recorded. A single set of data after the seedling establishment phase in Culterra potting soil and six data sets during plant growth in soil treatments. At the end of each growing month five cultivation pots of each soil treatment were randomly selected for each species. Random selection was done using a random number generator (RNG). The RNG generated a number between one and the number of available cultivation pots for each species and soil treatment. The relative cultivation pot was selected based on the RNG generated number. The above ground height (from soil surface to shoot apex), basal diameter (~0.5 cm from soil surface) and leaf number of each plant in each cultivation pot was measured or counted. The basal circumference of each plant was calculated by multiplying the basal diameter with π. For harvesting and analysis, cultivation pots were submerged and left to soak for approximately 15 minutes in a container of water to loosen soils within the pot. Plant matter and soils were then removed from the cultivation pot and left to soak in the container for a further 15 minutes. The majority of soil was separated from plant roots during this time. The remaining soil was separated by rinsing each soil clod in a container of water and manually removing any visible soil matter. Each bundle of plant roots was then thoroughly rinsed under running water and finally soaked in a container of distilled water to ensure no soil remained. Removal of soil was conducted in separate containers for each soil treatment to avoid cross contamination. Roots of each plant were then separated from one another and measured from the beginning of the plant shoot to the root apex, thus constituting the below ground root length of each plant. The above ground height and below ground root length of each plant were summed to calculate the total length of each plant.

All plant materials were placed into separate paper bags and placed in a convection oven at 70 °C for 24 hours. The dry masses of the above (shoot) and below (root) ground component of plants from each cultivation pot were weighed. These two masses were summed to calculate the total plant mass within each cultivation pot. All plant material was stored in bags for future chemical analyses.

3.3.5 – Statistical Analysis

The mean total length, above ground height, below ground root length, basal circumference and leaf number was calculated for each monthly data set using individual plants of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment. The mean total, above and below ground mass was calculated for each monthly data set using the collective masses of plants in each cultivation pot for *H. cannabinus* and *L. usitatissimum* grown in each soil treatment. The means of each plant growth variable were plotted against time and a linear trend line was applied in Microsoft Excel. These linear trend lines were compared within species and between soil treatments and between species within soil treatments. These comparisons were done using the emtrends function in Rstudio (12.0) which conducted pairwise comparisons, utilising a Tukey adjustment, between the observed trend of each variable for each species and soil treatments. The relative percent difference of the mean above ground mass between soil treatments at the end of the six-month period was calculated for each species using the following equation:

$$(V_1 – V_2) / [(V_1 + V_2) / 2] \times 100$$

Where V_1 is the mean above ground mass within a species grown in a specific soil treatment and V_2 is that of the same species grown in a different soil treatment. These relative percent differences were used in calculations for the yield (t/ha) and economic value (R) of each species, presented in chapter 3.6.

3.4 – Metals in Plant Tissue Analysis

3.4.1 – Metal Content Analysis

Results obtained from the soil analysis, presented in chapter 4.1, indicated that concentrations (mg/kg) of Mn, Zn, Ni, Cu, Cr and Co were higher than other metals across soil treatments. Therefore, these six metals were selected to be tested for within plant tissue samples. Five samples of the above ground component and five samples of the below ground component, were obtained and oven dried during the greenhouse growth trial, of both *H. cannabinus* and *L. usitatissimum* grown in Culterra potting soil prior to exposure to treatment soils and grown in each soil treatment at the end of the six-month growth period were used. Each sample of *H. cannabinus* and *L. usitatissimum* consisted of plant matter from five and ten plants respectively. These samples of *H. cannabinus* and *L. usitatissimum* were sent to the Central Analytical Facilities, Stellenbosch University for metal content analysis. Inductively coupled plasma mass spectrometry (ICP-MS) was utilised to determine the concentration (mg/kg) of Mn, Zn, Ni, Cu, Cr and Co in plant tissue samples.

The mean amount (mg) of each metal in each plant component of each species was calculated using the concentration (mg/kg) of each metal accumulated and the mass (kg) of each plant component. The mean amount of each metal accumulated in the above and below ground plant component of *H. cannabinus* was calculated by multiplying the concentration of each metal by the above or below ground plant mass of five plants grown in a single cultivation pot for five cultivation pots. The initial amount of metal within *H. cannabinus*, within plants grown before exposure to each treatment was negligible. The same was true for the concentration values. The amount of each metal within the total plant component of plants grown in each cultivation pot was calculated by summing the metal amount within the above and below ground plant component. The concentration of each metal within the total plant component was calculated by summing the concentration within the above and below ground component and dividing the value by two. Thus, resulting in the mean metal amount and concentration accumulated within the total, above and below ground plant component of 25 *H. cannabinus* plants. The same calculations were done with *L. usitatissimum*, however, ten *L. usitatissimum* plants were grown in each of the five cultivation pots resulting in the mean metal amount and concentration accumulated within 50 *L. usitatissimum* plants.

3.4.2 – Statistical Analysis

A comparison of the mean amount and concentration of each metal between soil treatments within each species and within each plant component was conducted. A comparison of the mean amount and concentration of each metal within soil treatments within each species and between plant components was conducted. A comparison of the mean amount and concentration of each metal within soil treatments between each species and within each plant component was conducted. Furthermore, comparisons between the mean amounts and concentrations of each metal were conducted within soil treatments within each species and within plant components. Comparisons of the mean amount and concentration accumulated within the total plant component were conducted between soil treatments within each species and within soil treatments between each species. Furthermore, the mean amounts and concentrations of each metal accumulated within the total plant component were compared within each species and soil treatment. A Shapiro-Wilk test was conducted to confirm normality of data. All comparisons were analysed using two- and three-way ANOVA's and subsequent Tukey's HSD tests of metal amount, concentration and type between soil treatment, species and plant component.

3.5 – Remote sensing and Site Classification

Supervised image classification (SIC) was conducted in ArcGis (10.4) to determine an area (ha) estimate of different land categories present at the Onverwacht tailings storage facility (TSF) available for the cultivation of *H. cannabinus* and *L. usitatissimum*. Atmospherically corrected scenes presenting surface reflectance for Landsat 8 and Sentinel 2B were obtained from the United States Geological Survey (USGS) and Copernicus Open Access Hub respectively. Scenes obtained from both satellites were from the mid-Winter period of 2020, the same time period when soil samples were collected from the Onverwacht TSF for use in the germination and greenhouse growth trials. Furthermore, this allowed for the minimisation of the effect of vegetative cover in order to maximise the soil spectral signature. Therefore, SIC of the site could be determined based on soil spectral signatures present onsite.

Multiple composite images (Table 3), utilising various combinations of individual bands, which measure different wavelength (nm) regions of the electromagnetic spectrum, were created using Landsat 8 and Sentinel 2B scenes and the composite bands tool in ArcGIS (10.4). Landsat 8 composite images were pan sharpened utilising the panchromatic band to a resolution of 15 m. Bands of lower resolution in the Sentinel 2B composite images were resampled using the cubic convolution function to a resolution of 10 m. Image classification of each composite image was conducted using the supervised image classification tool in ArcGis (10.4). Classification training samples were initially defined using the natural colour composite images as a reference for deciding the placement of training samples. Based on the natural colour composite image, onsite observations and recommendations of the onsite TSF resident technician, seven land categories were defined: water, tailings sludge, tailings dust, rehabilitated soil, grassland, burnt grassland and dirt road.

Table 3: Composite image name and band combination order used in the creation of composite images using Sentinel 2B and Landsat 8 scenes of the Onverwacht tailings storage facility for the mid-Winter period of 2020.

Composite Image Name	**Satellite and Band Combination Order**	
	Sentinel 2B	**Landsat 8**
Natural Colour	4-3-2	4-3-2
Colour Infrared	8-4-3	5-4-3
Short Wave Infrared	12-8A-4	7-6-4
Agriculture	11-8-2	6-5-2
Geology	12-11-2	7-6-2

A single composite image for each satellite scene was created using all bands used in the creation of the various composite images (Table 3). A minimum of 30 representative training samples, as recommended by the Environmental Systems Research Institute (ESRI), were drawn in each of the defined land categories for these composite images. Training samples drawn for each composite image were then evaluated to determine whether they were representative of each of the defined land categories. This was done by examining the spectral profile of training samples from each composite image. Data regarding the mean scaled surface reflectance relative to each band used for each composite image was extracted using ArcGis (10.4). The mean scaled surface reflectance of each land category's training samples were plotted against the central wavelength of each band using Microsoft Excel.

The decision of which satellite and respective composite image (Table 3) to use for estimations of land category areas (ha) onsite was made based on which presented a clearer image and which bands exhibited the greatest degree of spectral separation between land categories. This composite image and training samples defined using the natural colour composite image were utilised for the final SIC. The resultant raster image created using the SIC tool was converted into a polygon shape file. The area (ha) of each land category was extracted from this polygon shape file.

3.6 – Economic Estimations and Analysis

Five of the seven identified land categories present at the Onverwacht TSF were defined as available for *H. cannabinus* and *L. usitatissimum,* presently or in the future following the site's remediation. These land categories were grassland, burnt grassland, rehabilitated soil, tailings sludge and tailings dust. The sum of the grassland and burnt grassland land categories areas was used to represent the area of normal soil present onsite. The area of the rehabilitated land category was used to represent the area of rehabilitated soil present onsite. The sum of the tailings sludge and tailings dust land categories areas was used to represent the area of tailings soil present onsite. Therefore, the areas of normal, rehabilitated and tailings soil present at the Onverwacht TSF were determined.

The measured yield (t/ha) of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment was calculated using the recommended plant density (plants/ha) and the mean above ground mass (kg) of each species after six months of growth in each soil treatment (Arslanoglu *et al*. 2022, Harrison *et al*. 2019). Initially, the recommended plant density, 300 000 and 20 000 000 for *H. cannabinus* and *L. usitatissimum* respectively, was divided by the number of plants harvested at the end of the six-month growth trial, 25 and 50 for *H. cannabinus* and *L. usitatissimum* respectively. These values were then multiplied by the mean above ground mass recorded for each soil treatment. Therefore, this calculation produced the measured yield (t/ha) for each species grown in each soil treatment. The expected yield of *H. cannabinus* (10 t/ha) and *L. usitatissimum* (7.64 t/ha) was obtained from relevant literature (Arslanoglu *et al*. 2022, Harrison *et al*. 2019). These values were used as the expected yield for the soil treatment in which *H. cannabinus* and *L. usitatissimum* exhibited the greatest amount of growth at the end of the six-month growth period. These treatments were normal and tailings soil for *H. cannabinus* and *L. usitatissimum* respectively. Therefore, calculations of the expected yield for the remaining soil treatments of each species were made using the relative percent differences of mean above ground mass between plants grown in the remaining soil treatments and that which produced the greatest amount of growth. These relative percent difference values were multiplied by the expected yield of each species, this value was then subtracted from the initial expected amount. Therefore, this produced expected yield values of each species grown in each soil treatment.

The measured and expected total yield (t) of *H. cannabinus* and *L. usitatissimum* cultivated in available areas at the Onverwacht TSF was calculated by multiplying the relative yield (t/ha) by the available area (ha) for each soil treatment. These values were then multiplied by the economic value (R/t) of each species (R 1318.29/t for *H. cannabinus* and R 2636.57/t for *L. usitatissimum*) to calculate the measured and expected total yield value of each species cultivated onsite (Bran *et al*. 2017, Panoutsou and Alexopoulou 2020). The estimated total cultivation cost of each species (R 6872.16/ha for *H. cannabinus* and R 9887.15/ha for *L. usitatissimum*) cultivated in each soil treatment onsite was calculated by multiplying the cultivation cost (R/ha) by the relative area (ha) available onsite (Bran *et al*. 2017, Panoutsou and Alexopoulou 2020). Finally, the measured and expected profit/loss margin was calculated by subtracting the estimated cultivation cost (R) from the relative total yield value (R) of each species for each soil treatment.

The estimated measured and expected amount (g/ha) of Mn, Zn, Ni, Cu, Cr and Co extracted by *H. cannabinus* and *L. usitatissimum* cultivated in available land areas onsite was calculated. The measured amount was calculated by dividing the recommended plant density (plants/ha) of each species by 25 and 50 for *H. cannabinus* and *L. usitatissimum* respectively (Arslanoglu *et al*. 2022, Harrison *et al*. 2019). These values were then multiplied by the mean amount (g) of each metal present in the above ground mass of 25 *H. cannabinus* and 50 *L. usitatissimum* plants at the end of the six-month growth period. Therefore, producing the measured amount (g/ha) of each metal extracted by each species cultivated in each soil treatment. The expected amount (g/ha) extracted was calculated by dividing 1000 kg by the mean above ground mass (kg) of each species grown in each soil treatment at the end of the six-month growth period. These values were then multiplied by the mean amount (g) of each metal present in the above ground mass of 25 *H. cannabinus* and 50 *L. usitatissimum* plants at the end of the six-month growth period. These values were then multiplied by the expected yield (t/ha) of each species cultivated in each soil treatment. Therefore, producing the expected amount (g/ha) of each metal extracted by each species cultivated in each soil treatment.

4 – Results

The data and analyses presented in this section aim to address the three primary objectives of this research from a bottom-up approach. Beginning with an analysis of multiple soil physicochemical variables of soils collected from the Onverwacht tailings storage facility (TSF). The soil analysis is followed by the germination trial which presents an analysis of the germination and growth of *H. cannabinus* and *L. usitatissimum* in normal, rehabilitated and four tailings (varying ratios of normal soil to tailings dust mixes) soil treatments. This analysis was conducted to determine which of the four tailings soil treatments would be used in the subsequent greenhouse growth trial. The greenhouse growth trial presents data and analyses of *H. cannabinus* and *L. usitatissimum* growth over a six-month period. Plant tissue samples of both species at the end of the six-month growth trial were analysed for metal content. This section presents data and analyses of the amount (mg) and concentration (mg/kg) of Mn, Zn, Ni, Cu, Cr and Co accumulated in the total, above and below ground plant component of *H. cannabinus* and *L. usitatissimum*. Following this is the remote sensing and site classification section. This section presents the product of the supervised image classification (SIC) remote sensing technique of the Onverwacht TSF. This section allowed for determination of the area (ha) of the different available land categories for *H. cannabinus* and *L. usitatissimum* cultivation at the Onverwacht TSF. Finally, the economic estimations and analysis section is presented. This section utilises data from the greenhouse growth trial, metals in plant tissue analysis and remote sensing and SIC sections. Data and analyses of the measured and estimated yield (t/ha), value (R), profit/loss margin (R) and amount (g/ha) of the tested metals extracted by *H. cannabinus* and *L. usitatissimum* if cultivated at the Onverwacht TSF are presented.

4.1 – Soil Analysis

The results presented are a comparison of the physical and chemical variables of normal, rehabilitated and tailings soil treatments. Only a single sample of each soil treatment was analysed. Rehabilitated soil had a noticeably greater stone volume percentage than normal and tailings soil, while the sand and clay percentage were slightly lower and higher respectively (Table 4). However, all three soils were classified as fine sandy loams based on their particle size distribution (Table 4). Tailings soil was classified as a moderately acidic soil with a higher pH than normal and rehabilitated soil (Table 4). Normal and rehabilitated soil were defined as acidic soils while rehabilitated soil had a lower pH than normal soil (Table 4). All soil treatments were defined as non-saline soils based on their electrical conductivity (EC) (Table

4). Tailings soil had a noticeably higher EC than normal and rehabilitated soil, while rehabilitated soil had a lower EC than normal soil (Table 4). The electrical resistance of rehabilitated soil was substantially higher than the similar electrical resistance values of normal and tailings soil (Table 4).

Table 4: Particle size distribution (stone volume, sand, silt and clay percentage), texture classification, pH (KCl), electrical conductivity and electrical resistance of normal, rehabilitated and tailings soil treatments.

Soil Variable	**Soil Treatment**		
	Normal	**Rehabilitated**	**Tailings**
Stone Volume (%)	3.05	19.31	3.86
Sand (%)	81	75	81
Silt (%)	6	8	8
Clay (%)	13	17	11
Classification	Fine Sandy Loam	Fine Sandy Loam	Fine Sandy Loam
pH (KCl)	5.00	4.77	5.9
Electrical Conductivity (µS/cm)	420	272	1240
Resistance (Ω)	390	2130	280

The total nitrogen (N), potassium (K) and carbon (C) concentrations in rehabilitated soil were lower than normal and tailings soil, while normal soil had higher concentrations than tailings soil (Table 5). However, tailings soil had a greater total phosphorus (P) concentration than normal and rehabilitated soil, while this concentration was higher in rehabilitated soil than normal soil (Table 5).

Table 5: Total N, P, K and C concentrations and available P, organic C and soluble S concentrations of normal, rehabilitated and tailings soil treatments.

Soil Variable	**Soil Treatment**		
	Normal	**Rehabilitated**	**Tailings**
Total N (%)	0.12	0.03	0.09
Total P (mg/kg)	132	154	193
Total K (mg/kg)	178	44.20	156
Total C (%)	2.91	1.01	1.98
Available P (mg/kg)	10.90	9.40	14.4
Organic C (%)	2.13	0.45	1.70
Soluble S (mg/kg)	305	39.80	714

Although the total P concentration in rehabilitated soil was higher than normal soil, the available P concentration was lower than normal soil (Table 5). Furthermore, the available P concentration in tailings soil was greater than normal and rehabilitated soil (Table 5). The concentration of organic C and soluble sulfur (S) in rehabilitated soil were noticeably lower than normal and tailings soil (Table 5). Furthermore, the concentration of soluble S in tailings soil was more than twice the concentration in normal soil (Table 5).

The cation exchange capacity (CEC) of tailings soil was greater than normal and rehabilitated soil which had similar CEC's (Table 6). The amount of hydrogen (H^+) and calcium (Ca^{2+}) cations were highest in tailings soil followed by normal soil and lastly rehabilitated soil (Table 6). The amount of magnesium cations (Mg^{2+}) was greatest in rehabilitated soil followed by tailings soil and lastly normal soil (Table 6). The amount of K^+ cations was highest in normal soil and similar to that of tailings soil while both were higher than rehabilitated soil (Table 6). The amount of sodium cations (Na^+) in normal and rehabilitated soil was similar while both were higher than tailings soil (Table 6).

Table 6: Cation exchange capacity (CEC) and amounts of individual cations (hydrogen, calcium, magnesium, potassium and sodium) in normal, rehabilitated and tailings soil treatments.

Soil Treatment	**Cation (cmol/kg)**					**CEC (cmol/kg)**
	H^+	**Ca^{2+}**	**Mg^{2+}**	**K^+**	**Na^+**	
Normal	0.64	4.60	1.50	0.46	0.08	7.28
Rehabilitated	0.43	3.60	2.10	0.11	0.07	6.31
Tailings	0.89	7.50	2.00	0.40	0.02	10.99

The base saturation (BS) of rehabilitated soil was higher than normal and tailings soil which had similar base saturations (Table 7). The saturation of H^+, Ca^{2+}, K^+ and Na^+ in rehabilitated soil was lower than normal and tailings soil (Table 7). However, the saturation of Mg^{2+} in rehabilitated soil was higher than normal and tailings soil (Table 7). The saturation of H^+, Mg^{2+} and K^+ in normal soil was higher than tailings soil (Table 7). While the saturation of Ca^{2+} and Na^+ were higher in tailings soil (Table 7).

Table 7: Base saturation percentage (BS) and percent saturation of individual cations (hydrogen, calcium, magnesium, potassium and sodium) in normal, rehabilitated and tailings soil treatments.

Soil Treatment	**Saturation (%)**					**BS (%)**
	H^+	**Ca^{2+}**	**Mg^{2+}**	**K^+**	**Na^+**	
Normal	8.83	63.16	20.60	6.32	1.10	91.21
Rehabilitated	6.84	57.03	33.27	1.74	1.11	93.19
Tailings	8.12	68.23	18.19	3.64	1.82	91.90

The concentration (mg/kg) of As, $Cr^{3+/6+}$, Co, Cu, Hg and Ni was highest in tailings soil followed by rehabilitated soil and lastly normal soil (Table 8). One exception was that the concentration of Hg was below the detection limit (BDL) for normal and rehabilitated soil (Table 8). The concentration of Pb, Mn, V and Zn was highest in rehabilitated soil followed by tailings soil and lastly normal soil (Table 8). Soil screening values level one (SSV1) indicate the maximum allowable metal concentration in soils that is protective of human and ecosystem health, including the possibility of migration into a water source, as defined by the Department of Environmental Affairs (DEA 2010) South Africa. The concentration of Cu in rehabilitated and tailings soil and the concentration of Ni in tailings soil was higher than the SSV1 (Table 8). Out of the 12 metal concentrations the six highest concentrations in all soil treatments were Mn, $Cr^{3+/6+}$, Ni, Zn, Cu and Co (Table 8). Therefore, due to the high concentrations, these six metals were used in further analysis of metals accumulated within the biomass of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment during the greenhouse growth trial.

Table 8: Selected metal concentrations in normal, rehabilitated and tailings soil treatments compared to soil screening values level one (SSV1) recommended by the Department of Environmental Affairs South Africa. BDL = below detection limit.

Metal	**Soil Treatment and Metal Concentration (mg/kg)**			**SSV1 (mg/kg)**
	Normal	**Rehabilitated**	**Tailings**	
As	1.20	1.22	6.60	5.80
Cd	0.02	0.02	0.02	7.50
Cr^{3+}	53.96	103.60	785.70	46000
Cr^{6+}				6.50
Co	5.18	13.90	19.90	300.00
Cu	7.61	21.90	40.80	16.00
Pb	3.10	3.51	3.47	20.00
Mn	88.25	324.50	205.80	740.00
Hg	BDL	BDL	0.04	0.93
Ni	26.49	69.40	422.20	91.00
V	2.20	4.10	3.00	150.00
Zn	16.00	51.40	23.50	240.00

Overall, rehabilitated soil exhibited the lowest values for multiple soil properties. This included lower total N, K and C values, lower available P, organic C, soluble S, pH and CEC compared to normal and tailings soil. However, the electrical resistance value of rehabilitated soil was substantially higher than normal and tailings soil. The combination of lower elemental concentrations, pH and CEC would negatively impact plant growth. This would be due to a decrease in nutrient bioavailability and efficiency of fundamental cation exchange from soil colloids to soil solution. Furthermore, the lower pH value would increase the bioavailability of metals present, while the increased soil electrical resistance would impede water uptake of plants grown in rehabilitated soil. The combination of these factors would lead to an overall decrease in plant growth.

Selected metal concentrations in normal soil were consistently lower than rehabilitated and tailings soil. Whilst tailings soil exhibited substantially higher concentrations of $Cr^{3+/6+}$ and Ni compared to normal and rehabilitated soil, rehabilitated soil exhibited higher concentrations of Mn and Zn compared to normal and tailings soil. The lower metal concentrations present in normal soil would possibly lead to a decreased impact on plant growth, while the higher metal concentrations in rehabilitated and tailings soil could lead to an increased impact on plant growth. However, the other stated lower values of soil properties of rehabilitated soil may have greater impact on plant growth than the present metal concentrations. Therefore, although higher metal concentrations were present in tailings soil, the increased values of other soil properties may override the detrimental effects of increased metal concentrations. Thus, resulting in a growth pattern in which increased growth is exhibited by plants grown in normal soil followed by tailings soil and lastly rehabilitated soil.

4.2 – Germination Trial

The total germination percentage of *H. cannabinus* was 80% or above in all soil treatments excluding tailings four (Figure 2). Furthermore, *H. cannabinus* germinated in normal and tailings two soil treatments reached 100% germination (Figure 2). The total germination percentage of *L. usitatissimum* was 70% or above in all soil treatments excluding rehabilitated soil which exhibited the lowest total germination percentage (Figure 2).

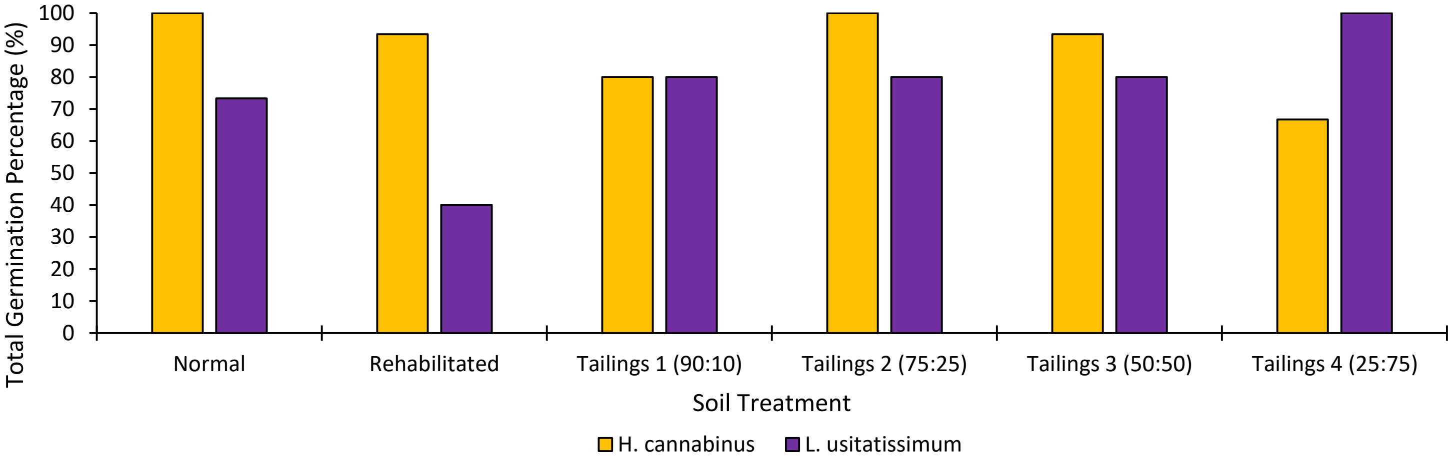


Figure 2: Total germination percentage of 15 *H. cannabinus* and 15 *L. usitatissimum* seeds at the end of the 14 day growth period in normal, rehabilitated and tailings, one (90:10), two (75:25), three (75:25) and four (25:75), soil treatments.

The highest *L. usitatissimum* total germination percentage was observed in tailings four (Figure 2). The total germination percentage of *H. cannabinus* was higher than *L. usitatissimum* in each soil treatment excluding tailings one and four (Figure 2). The 100% total germination percentage of *H. cannabinus* and 80% of *L. usitatissimum* in tailings two added to the justification of its use as the tailings soil treatment used in the greenhouse growth trial (Figure 2).

Comparisons of the mean above ground height within and between species and soil treatment showed no statistical (Df = 5, F = 2, $p > 0.05$) difference (Figure 3). The lack of significance may have been due to the small sample size and high variability around the mean above ground height of *H. cannabinus* and *L. usitatissimum* grown in most soil treatments (Figure 3). *Hibiscus cannabinus* exhibited higher mean above ground heights than *L. usitatissimum* across soil treatments excluding tailings four (Figure 3).Furthermore, *H. cannabinus* exhibited similar mean above ground heights across soil treatments excluding tailings four (Figure 3). The mean above ground height of *L. usitatissimum* was also similar across soil treatments, the lowest of which was observed in rehabilitated soil (Figure 3).

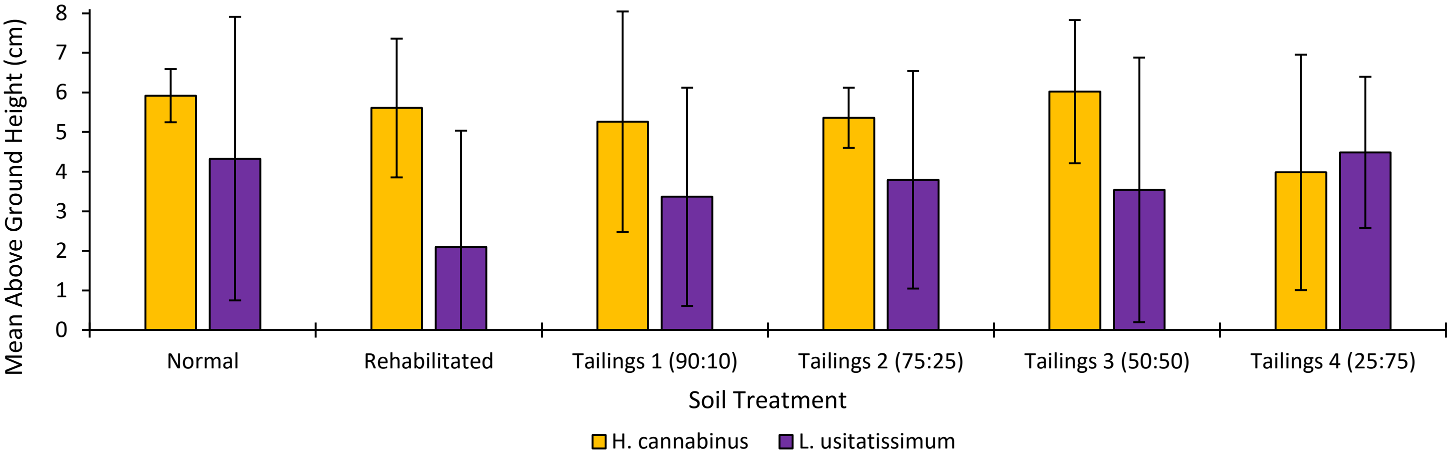


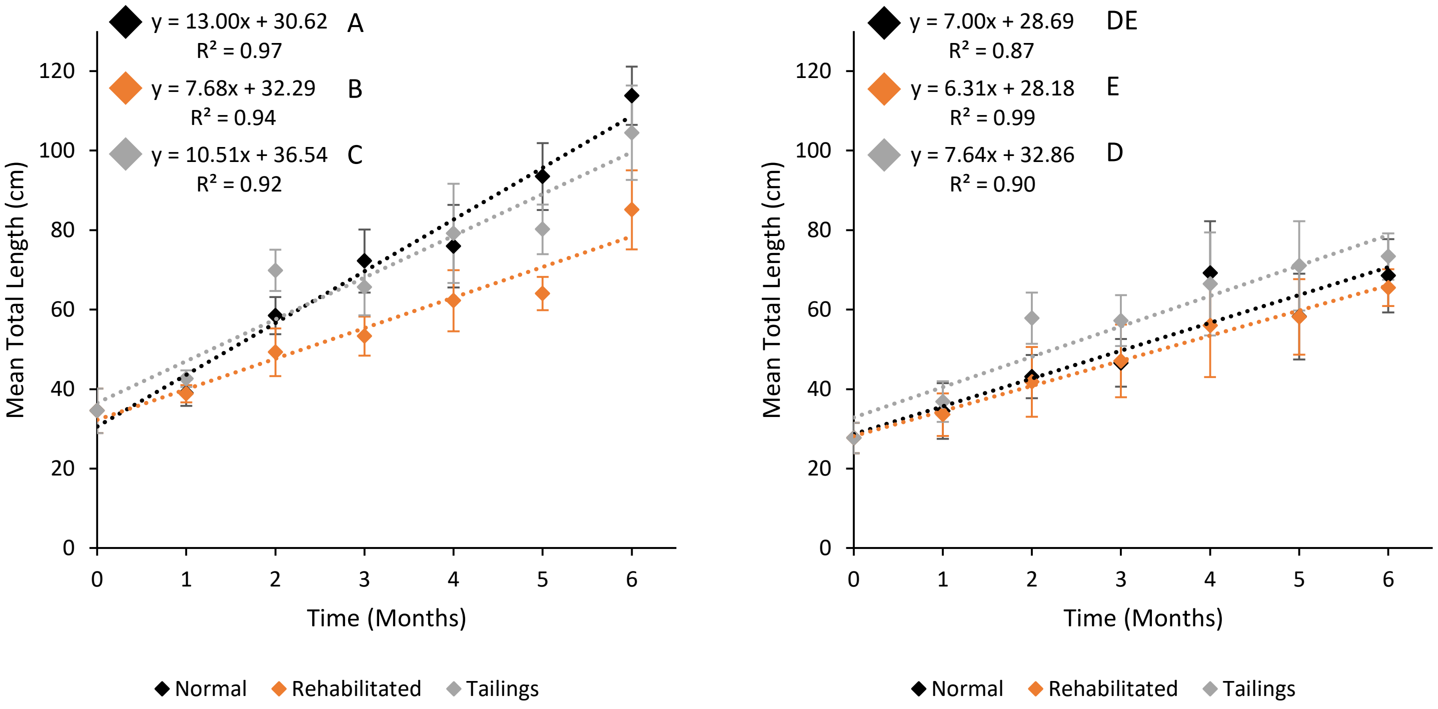
Figure 3: Mean above ground height of *H. cannabinus* and *L. usitatissimum* at the end of a 14 day growth period in normal, rehabilitated and tailings, one (90:10), two (75:25), three (75:25) and four (25:75) soil treatments. Error bars represent standard deviations of each mean, n = 15.

The total germination percentage and mean above ground height of *H. cannabinus* and *L. usitatissimum* was generally consistent across soil treatments. However, lower values of each variable were present in *L. usitatissimum* grown in rehabilitated soil. While variables for both species were high in normal soil. Variables for both species were high in the tailings two soil treatment. The use of this normal soil to tailings dust mix (75:25) as a third soil treatment alongside normal and rehabilitated soil was used in the subsequent greenhouse growth trial. The use of this tailings soil treatment allowed for comparisons between contaminated and noncontaminated soils to be made. Furthermore, accounting for the possibility that a normal soil and tailings dust mix that was either too low or high would either show no effect on plant growth or impact plant growth to a point where plants would not complete the six-month greenhouse growth trial.

4.3 – Greenhouse Growth Trial

Total Length:

The mean total length of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment exhibited a positive linear relationship with time over the six-month growth period (Figure 4). The rate of increase of mean total length in *H. cannabinus* grown in rehabilitated soil was significantly lower than *H. cannabinus* grown in normal (Df = 1563, T ratio = 11, $p < 0.05$) and tailings (Df = 1563, T ratio = -5.84, $p < 0.05$) soil (Figure 4A). Furthermore, this rate of increase was significantly (Df = 1563, T ratio = 5.16, $p < 0.05$) higher in *H. cannabinus* grown in normal soil compared to *H. cannabinus* grown in tailings soil (Figure 4A). Comparisons of the rate of increase of mean total length in *L. usitatissimum* revealed that this rate of increase was significantly (Df = 1563, T ratio = -3.87, $p < 0.05$) higher in *L. usitatissimum* grown in tailings soil compared to *L. usitatissimum* grown in rehabilitated soil (Figure 4B).



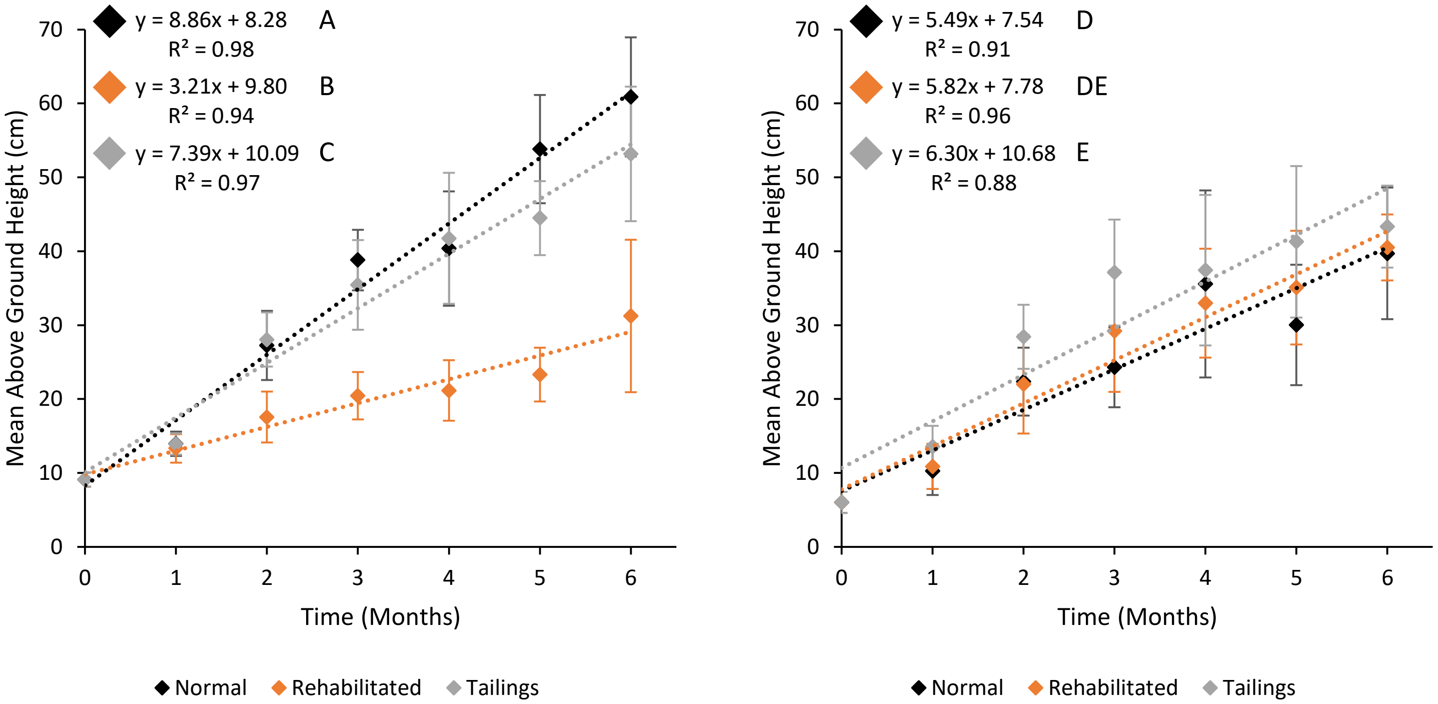
(A) (B)

Figure 4: Mean total length of *H. cannabinus* (A) and *L. usitatissimum* (B) grown in normal, rehabilitated and tailings soil treatments over six months. Each point represents the mean total length of 25 *H. cannabinus* and 50 *L. usitatissimum* plants grown in each soil treatment. Line equations and R^2 values from top to bottom relate to the slope of each line for normal, rehabilitated and tailings soil for each species. Letters next to each line equation indicate significance. Line equations with different letters indicate a significant difference, those that share the same letters indicate no significant difference.

However, no statistical difference was found in the rate of increase between *L. usitatissimum* grown in tailings and normal soil and *L. usitatissimum* grown in normal and rehabilitated soil (Figure 4B). The rate of increase in mean total length of *H. cannabinus* was significantly higher than *L. usitatissimum* grown in normal (Df = 1563, T ratio = -14.38, $p < 0.05$), rehabilitated (Df = 1563, T ratio = -3.32, $p < 0.05$) and tailings (Df = 1563, T ratio = -6.90, $p < 0.05$) soil (Figure 4).

Above Ground Height:

The mean above ground height of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment exhibited a positive linear relationship with time over the six-month growth period (Figure 5). The rate of increase in mean above ground height of *H. cannabinus* grown in rehabilitated soil was significantly lower than *H. cannabinus* grown in normal (Df = 1560, T ratio = 15.02, $p < 0.05$) and tailings (Df = 1560, T ratio = -11.11, $p < 0.05$) soil (Figure 5A). Furthermore, this rate of increase was significantly (Df = 1560, T ratio = 3.91, $p < 0.05$) higher in *H. cannabinus* grown in normal soil compared to *H. cannabinus* grown in tailings soil (Figure 5A).



(A) (B)

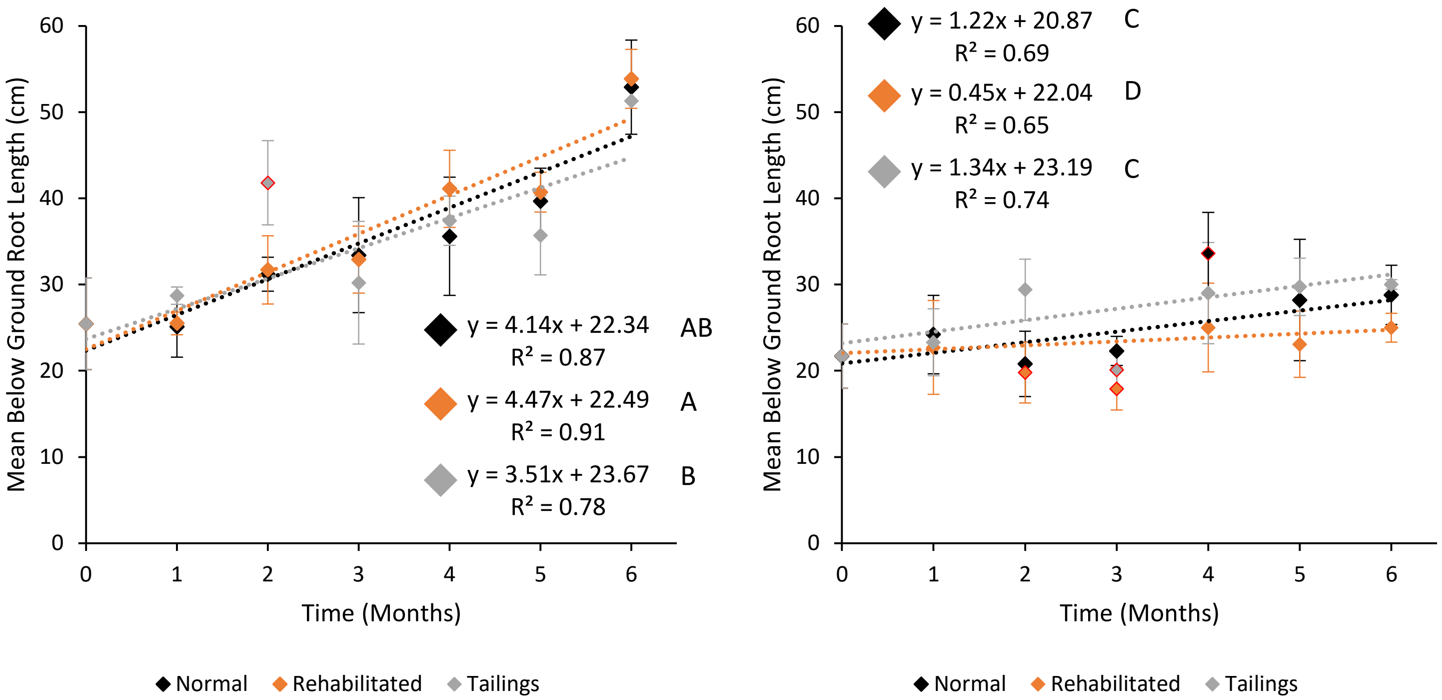
Figure 5: Mean above ground height of *H. cannabinus* (A) and *L. usitatissimum* (B) grown in normal, rehabilitated and tailings soil treatments over six months. Each point represents the mean above ground height of 25 *H. cannabinus* and 50 *L. usitatissimum* plants grown in each soil treatment. Line equations and R^2 values from top to bottom relate to the slope of each line for normal, rehabilitated and tailings soil for each species. Letters next to each line equation indicate significance. Line equations with different letters indicate a significant difference, those that share the same letters indicate no significant difference.

Comparisons of the rate of increase in mean above ground height of *L. usitatissimum* revealed that this rate of increase was significantly (Df = 1560, T ratio = -3.04, p < 0.05) higher in *L. usitatissimum* grown in tailings soil compared to *L. usitatissimum* grown in normal soil (Figure 5B). However, this rate of increase did not statistically differ between *L. usitatissimum* grown in normal and rehabilitated soil and *L. usitatissimum* grown in tailings and rehabilitated soil (Figure 5B). The rate of increase in mean above ground height of *H. cannabinus* was significantly higher than *L. usitatissimum* grown in normal (Df = 1560, T ratio = -10.42, p < 0.05) and tailings (Df = 1560, T ratio = -3.42, p < 0.05) soil (Figure 5). However, this rate of increase in *H. cannabinus* grown in rehabilitated soil was significantly (Df = 1560, T ratio = 7.90, p < 0.05) lower than that of *L. usitatissimum* (Figure 5).

Below Ground Root Length:

The mean below ground root length of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment exhibited a positive linear relationship with time over the six-month growth period (Figure 6). However, these positive linear relationships were only evident once multiple outliers had been removed from the data set. These outliers included month two of *H. cannabinus* grown in tailings soil, month four of *L. usitatissimum* grown in normal soil, months two and three of *L. usitatissimum* grown in rehabilitated soil and month three of *L. usitatissimum* grown in tailings soil (Figure 6). These outliers may have been caused by measuring broken off root pieces that appeared to be connected to the root mass but had slid down the root mass therefore increasing the measured root length. Lower values may have been caused by a loss of the longest root piece during plant processing.

The rate of increase in mean below ground root length of *H. cannabinus* grown in tailings soil was significantly (Df = 1338, T ratio = 3.64, p < 0.05) lower than that of *H. cannabinus* grown in rehabilitated soil (Figure 6A). However, this rate of increase did not statistically differ between *H. cannabinus* grown in normal and rehabilitated soil and *H. cannabinus* grown in normal and tailings soil (Figure 6A). Comparisons of the rate of increase in mean below ground root length of *L. usitatissimum* revealed that this rate of increase was significantly lower in *L. usitatissimum* grown in rehabilitated soil compared to *L. usitatissimum* grown in normal (Df = 1338, T ratio = 4.10, p < 0.05) and tailings (Df = 1338, T ratio = -4.74, p < 0.05) soil (Figure 6B). The rate of increase in mean below ground root length of *H. cannabinus* was significantly higher than *L. usitatissimum* grown in normal (Df = 1338, T ratio = -12.87, p < 0.05), rehabilitated (Df = 1338, T ratio = -17.70, p < 0.05) and tailings (Df = 1338. T ratio = -9.53, p < 0.05) soil (Figure 6).



(A) (B)

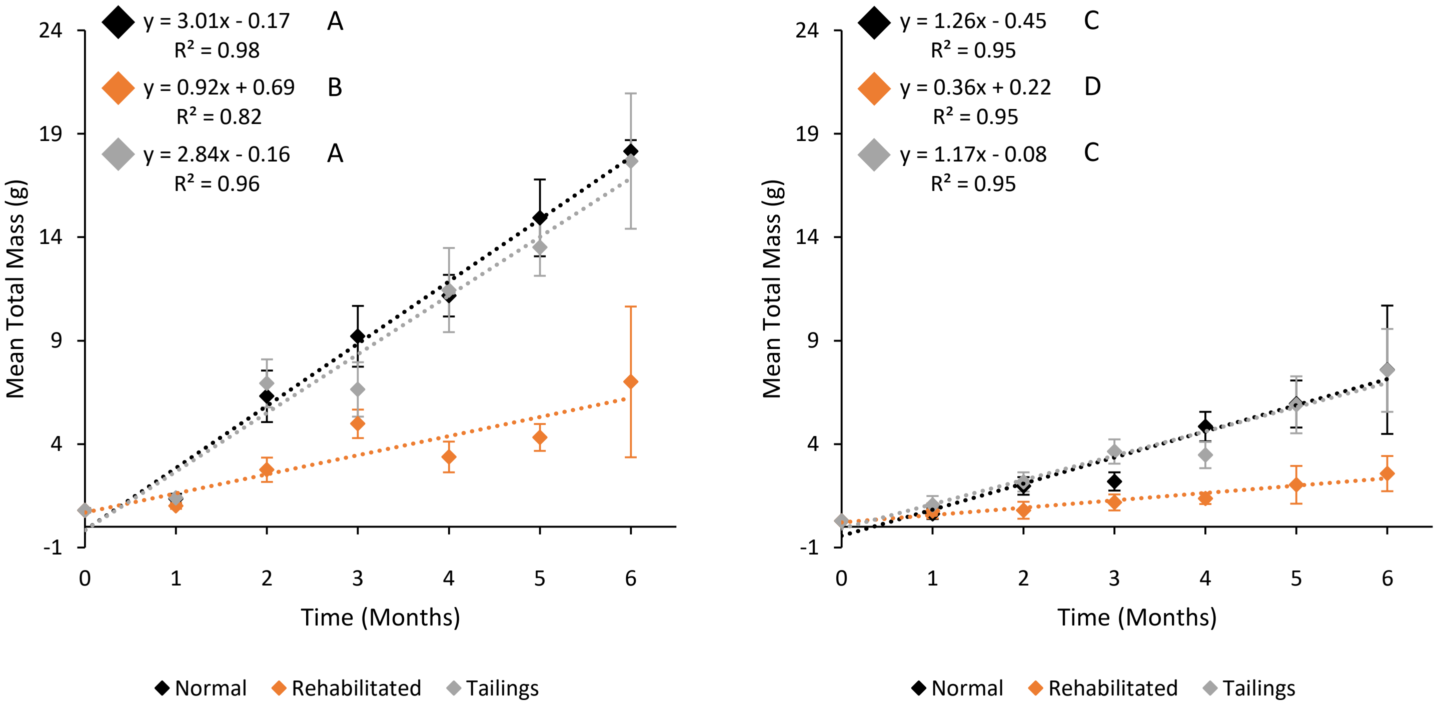
<u>Figure 6:</u> Mean below ground root length of (A) *H. cannabinus* and (B) *L. usitatissimum* grown in normal, rehabilitated and tailings soil treatments over six months. Each point represents the mean below ground root length of 25 *H. cannabinus* and 50 *L. usitatissimum* plants grown in each treatment. Line equations and R^2 values from top to bottom relate to the slope of each line for normal, rehabilitated and tailings soil for each species. Letters next to each line equation indicate significance. Line equations with different letters indicate a significant difference, those that share the same letters indicate no significant difference. Outliers are represented as points with a red border.

The effects of soil treatment on mean total length and above ground height were more evident in *H. cannabinus*. Furthermore, these effects on the mean below ground root length were less evident in both *H. cannabinus* and *L. usitatissimum*. Within *H. cannabinus* lower rates of increase in mean total length and above ground height were present in *H. cannabinus* grown in rehabilitated soil. However, the rate of increase in mean below ground root length was highest in *H. cannabinus* grown in rehabilitated soil. Within *L. usitatissimum* the rate of increase in all three variables was highest in *L. usitatissimum* grown in tailings soil. Overall, *H. cannabinus* exhibited higher rates of increase than *L. usitatissimum* within each soil treatment, excluding the mean below ground root length within the rehabilitated soil treatment.

<u>Total Mass:</u>

The mean total mass of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment exhibited a positive linear relationship with time over the six-month growth period (Figure 7). The rate of increase in mean total mass of *H. cannabinus* grown in rehabilitated soil was significantly lower than *H. cannabinus* grown in normal (Df = 198, T ratio = 16.97, p <

0.05) and tailings (Df = 198, T ratio = -12.25, $p < 0.05$) soil (Figure 7A). However, this rate of increase did not statistically differ between *H. cannabinus* grown in normal and tailings soil (Figure 7A). A similar pattern of differences in this rate of increase were present in *L. usitatissimum* (Figure 7B). The rate of increase in mean total mass of *L. usitatissimum* grown in rehabilitated soil was significantly lower than *L. usitatissimum* grown in normal (Df = 198, T ratio = 5.82, $p < 0.05$) and tailings (Df = 198, T ratio = -5.23, $p < 0.05$) soil (Figure 7B). Furthermore, this rate of increase did not statistically differ between *L. usitatissimum* grown in normal and tailings soil (Figure 7B). The rate of increase in mean total mass of *H. cannabinus* was significantly higher than *L. usitatissimum* grown in normal (Df = 198, T ratio = -11.15, $p < 0.05$), rehabilitated (Df = 198, T ratio = -3.64, $p < 0.05$) and tailings (Df = 198, T ratio = -10.66, $p < 0.05$) soil (Figure 7).

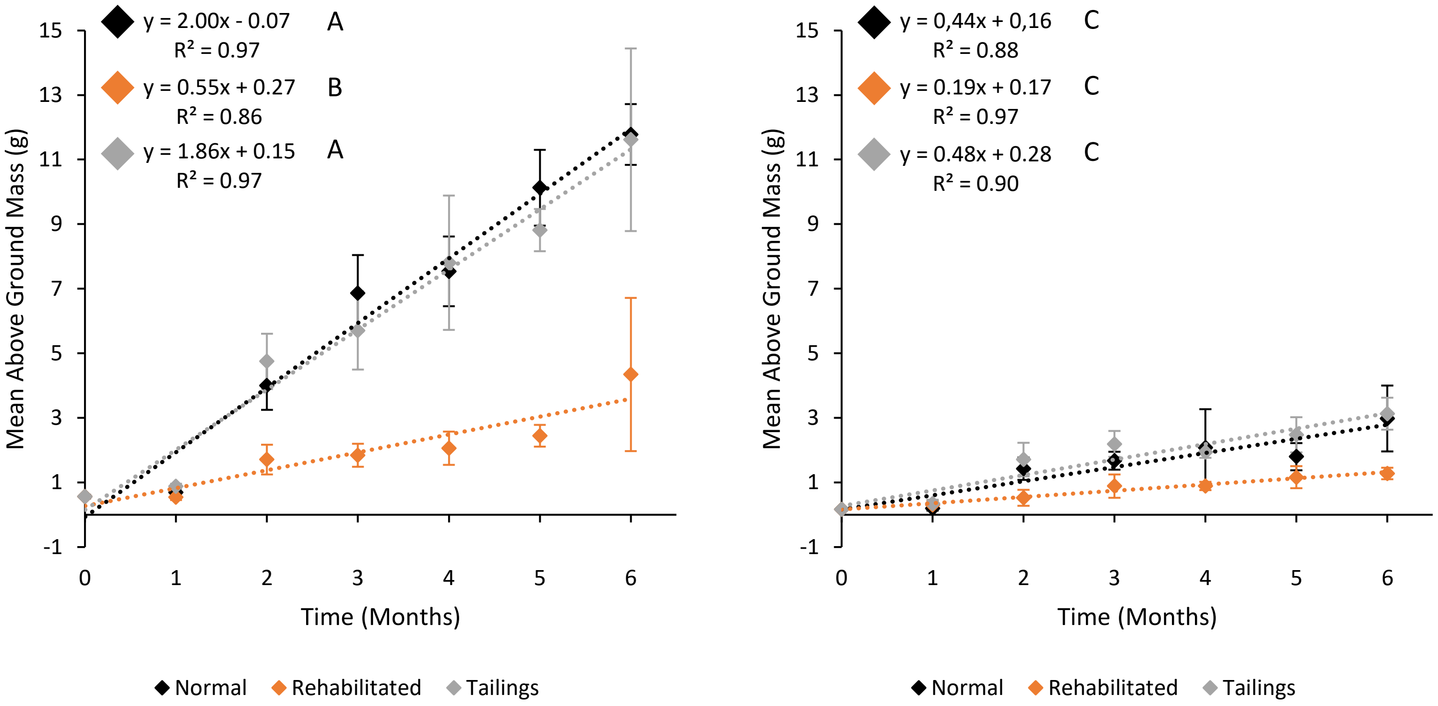


(A) (B)

Figure 7: Mean total mass of *H. cannabinus* (A) and *L. usitatissimum* (B) grown in normal, rehabilitated and tailings soil treatments over six months. Each point represents the mean total mass of 25 *H. cannabinus* and 50 *L. usitatissimum* plants grown in each soil treatment. Line equations and R^2 values from top to bottom relate to the slope of each line for normal, rehabilitated and tailings soil for each species. Letters next to each line equation indicate significance. Line equations with different letters indicate a significant difference, those that share the same letters indicate no significant difference.

Above Ground Mass:

The mean above ground mass of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment exhibited a positive linear relationship with time over the six-month growth period (Figure 8). The rate of increase in mean above ground mass of *H. cannabinus* grown in rehabilitated soil was significantly lower than *H. cannabinus* grown in normal (Df = 198, T ratio = 16.47, $p < 0.05$) and tailings (Df = 198, T ratio = -11.89, $p < 0.05$) soil (Figure 8A). However, this rate of increase did not statistically differ between *H. cannabinus* grown in normal and tailings soil (Figure 8A). The rate of increase in mean above ground mass of *L. usitatissimum* did not statistically differ between soil treatments (Figure 8B). However, this rate of increase was lower in *L. usitatissimum* grown in rehabilitated soil compared to *L. usitatissimum* grown in normal and tailings soil (Figure 8B). The rate of increase in mean above ground mass of *H. cannabinus* was significantly higher than *L. usitatissimum* grown in normal (Df = 198, T ratio = -14.23, $p < 0.05$), rehabilitated (Df = 198, T ratio = -3.28, $p < 0.05$) and tailings (Df = 198, T ratio = -12.58, $p < 0.05$) soil (Figure 8).



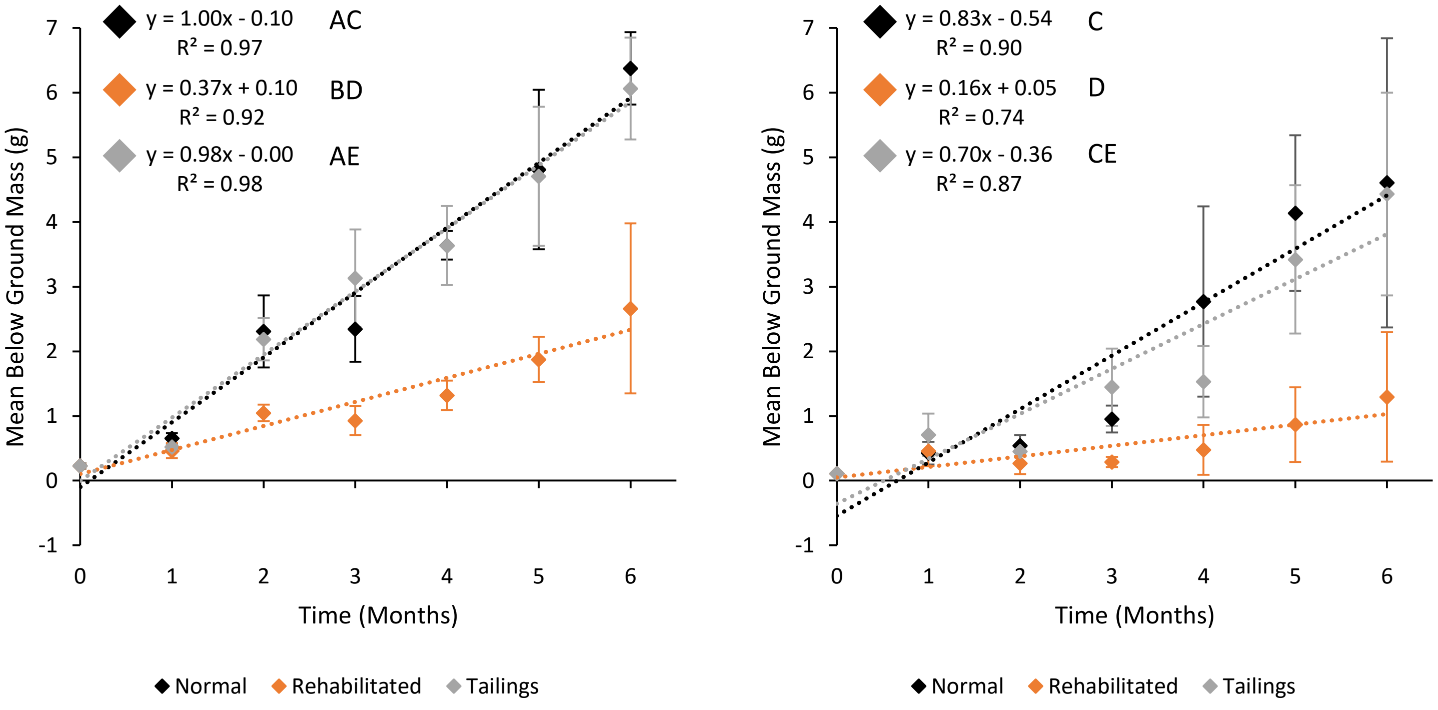
(A) (B)

Figure 8: Mean above ground mass of *H. cannabinus* (A) and *L. usitatissimum* (B) grown in normal, rehabilitated and tailings soil treatments over six months. Each point represents the mean above ground mass of 25 *H. cannabinus* and 50 *L. usitatissimum* plants grown in each treatment. Line equations and R^2 values from top to bottom relate to the slope of each line for normal, rehabilitated and tailings soil for each species. Letters next to each line equation indicate significance. Line equations with different letters indicate a significant difference, those that share the same letters indicate no significant difference.

At the end of the six-month growth period the highest mean above ground mass was observed in normal and tailings soil for *H. cannabinus* and *L. usitatissimum* respectively (Figure 8). The relative percent difference in mean above ground mass between soil treatments for *H. cannabinus* were, 92.00% (normal versus rehabilitated), 91.09% (rehabilitated versus tailings) and 1.40% (normal versus tailings). The relative percent differences observed in *L. usitatissimum* were, 79.81% (normal versus rehabilitated), 84.00% (rehabilitated versus tailings) and 4.90% (normal versus tailings).

Below Ground Mass:

The mean below ground mass of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment exhibited a positive linear relationship with time over the six-month growth period (Figure 9). The rate of increase in mean below ground mass of *H. cannabinus* grown in rehabilitated soil was significantly lower than *H. cannabinus* grown in normal (Df = 198, T ratio = 6.09, $p < 0.05$) and tailings (Df = 198, T ratio = -5.82, $p < 0.05$) soil (Figure 9A). However, this rate of increase did not statistically differ between *H. cannabinus* grown in normal and tailings soil (Figure 9A). A similar pattern in these differences was also present in *L. usitatissimum* (Figure 9B). The rate of increase in mean below ground mass of *L. usitatissimum* grown in rehabilitated soil was significantly lower than *L. usitatissimum* grown in normal (Df = 198, T ratio = 6.39, $p < 0.05$) and tailings (Df = 198, T ratio = -5.13, $p < 0.05$) soil (Figure 9A). However, this rate of increase did not statistically differ between *L. usitatissimum* grown in normal and tailings soil (Figure 9B). The rate of increase in mean below ground mass did not statistically differ between *H. cannabinus* and *L. usitatissimum* grown in each soil treatment (Figure 9).



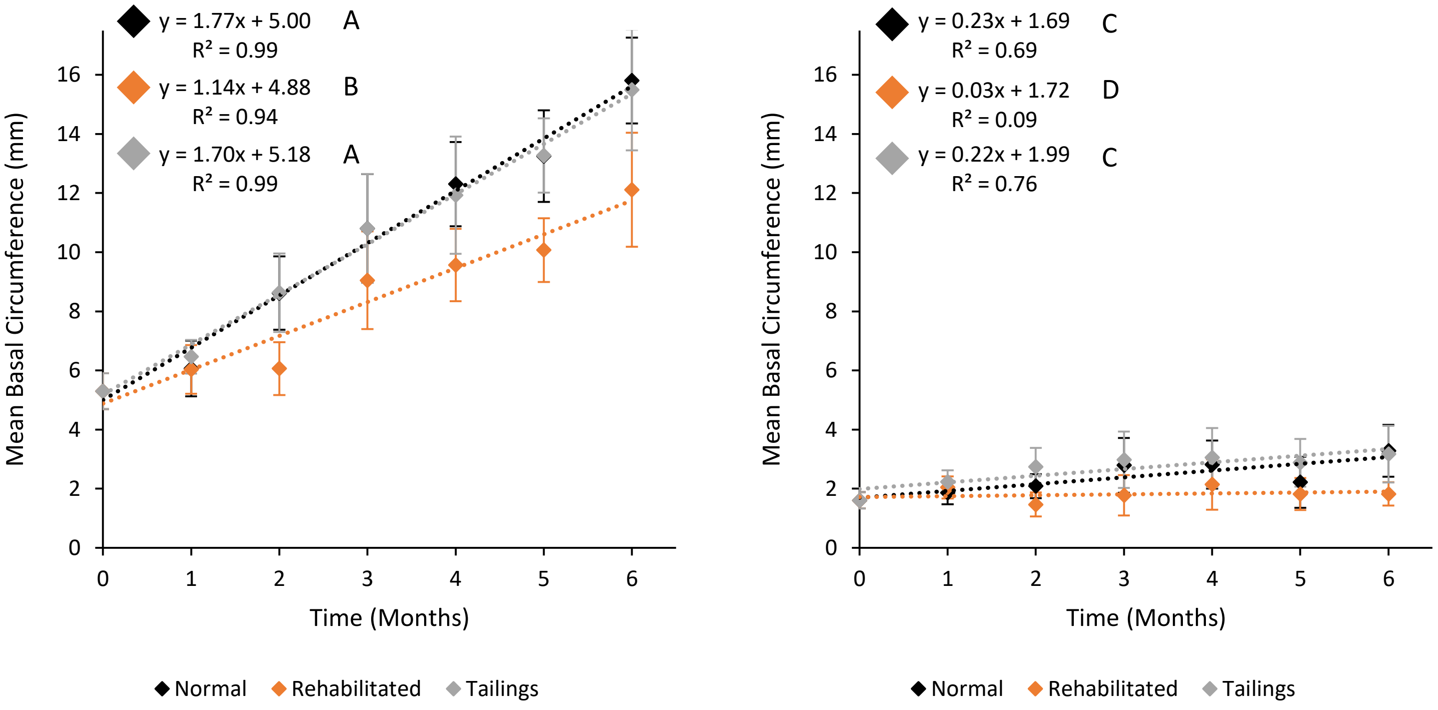
(A) (B)

Figure 9: Mean below ground mass of *H. cannabinus* (A) and *L. usitatissimum* (B) grown in normal, rehabilitated and tailings soil treatments over six months. Each point represents the mean below ground mass of 25 *H. cannabinus* and 50 *L. usitatissimum* plants grown in each treatment. Line equations and R^2 values from top to bottom relate to the slope of each line for normal, rehabilitated and tailings soil for each species. Letters next to each line equation indicate significance. Line equations with different letters indicate a significant difference, those that share the same letters indicate no significant difference.

The effects of soil treatment on mean total, above and below ground mass were evident in both *H. cannabinus* and *L. usitatissimum*. However, these effects were less evident regarding the mean above ground mass of *L. usitatissimum*. Within *H. cannabinus* and *L. usitatissimum* the lowest rates of increase in each variable were present within the rehabilitated soil treatment. Overall, *H. cannabinus* exhibited higher rates of increase in mean total and above ground mass than *L. usitatissimum* within each soil treatment. However, no difference was found in the rate of increase in mean below ground mass between *H. cannabinus* and *L. usitatissimum* within each soil treatment.

Basal Circumference:

The mean basal circumference of *H. cannabinus* and *L. usitatissimum* grown in each soil treatment exhibited a positive linear relationship with time over the six-month growth period (Figure 10). The reduced R^2 value of *L. usitatissimum* grown in rehabilitated soil may be accounted for by the high variance within the data set (Figure 10B). The rate of increase in mean basal circumference of *H. cannabinus* grown in rehabilitated soil was significantly lower than *H. cannabinus* grown in normal (Df = 1560, T ratio = 37.28, $p < 0.05$) and tailings (Df = 1560, T ratio = -10.29, $p < 0.05$) soil (Figure 10A).



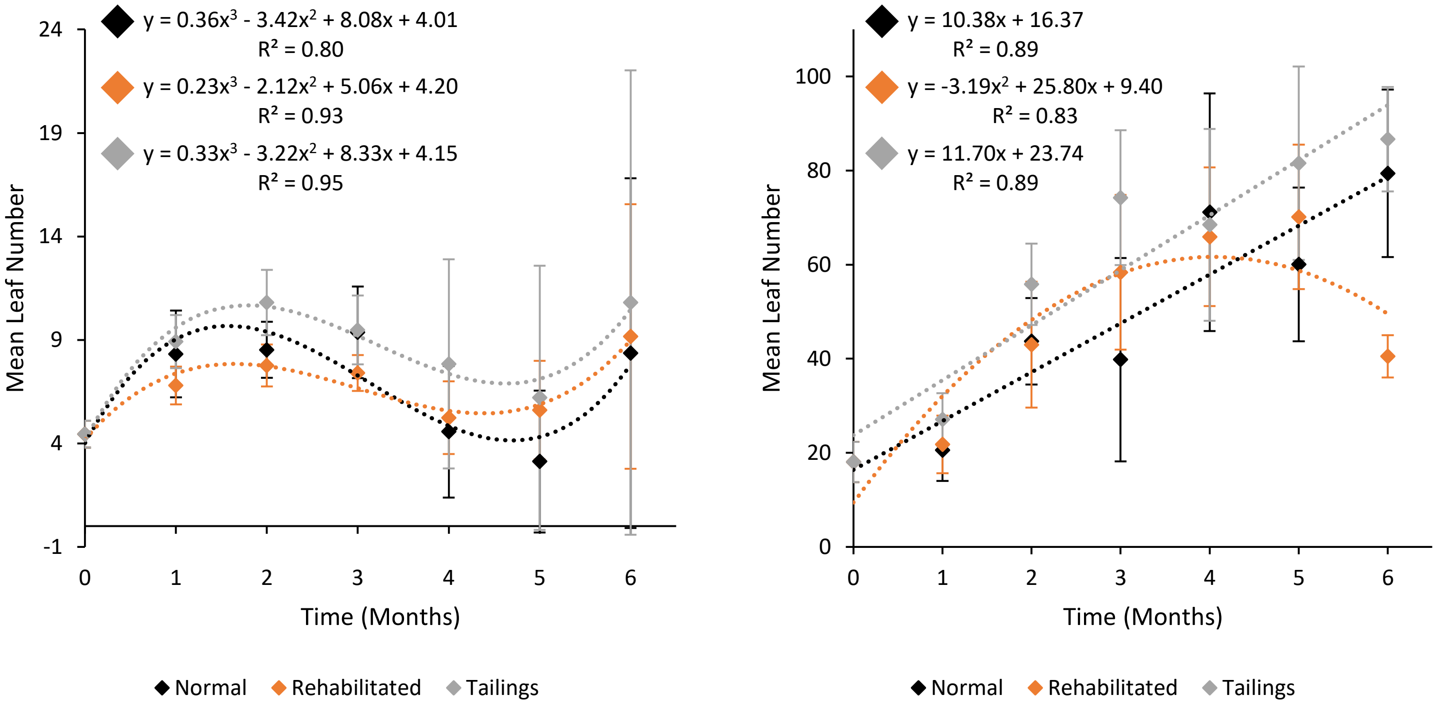
(A) (B)

Figure 10: Mean basal circumference of *H. cannabinus* (A) and *L. usitatissimum* (B) grown in normal, rehabilitated and tailings soil treatments over six months. Each point represents the mean basal circumference of 25 *H. cannabinus* and 50 *L. usitatissimum* plants grown in each treatment. Line equations and R^2 values from top to bottom relate to the slope of each line for normal, rehabilitated and tailings soil for each species. Letters next to each line equation indicate significance. Line equations with different letters indicate a significant difference, those that share the same letters indicate no significant difference.

However, this rate of increase did not statistically differ between *H. cannabinus* grown in normal and tailings soil (Figure 10A). A similar pattern in these differences was also present in *L. usitatissimum* (Figure 10B). The rate of increase in mean basal circumference of *L. usitatissimum* grown in rehabilitated soil was significantly lower than *L. usitatissimum* grown in normal (Df = 1560, T ratio = 5.12, $p < 0.05$) and tailings (Df = 1560, T ratio = -4.98, $p < 0.05$) soil (Figure 10B). However, this rate of increase did not statistically differ between *L. usitatissimum* grown in normal and tailings soil (Figure 10B). The rate of increase in mean basal circumference of *H. cannabinus* was significantly higher than *L. usitatissimum* grown in normal (Df = 1560, T ratio = -33.11, $p < 0.05$), rehabilitated (Df = 1560, T ratio = -23.81, $p < 0.05$) and tailings (Df = 1560, T ratio = -31.64, $p < 0.05$) soil (Figure 10).

Leaf Number:

The mean leaf number of *H. cannabinus* grown in each soil treatment exhibited a oscillating growth pattern over the six-month growth period (Figure 11A). During the first two months of growth the mean leaf number of *H. cannabinus* increased within each soil treatment (Figure 11A). During month two to four the mean leaf number of *H. cannabinus* decreased within in each soil treatment (Figure 11A). During month four to six the mean leaf number of *H. cannabinus* increased within each soil treatment (Figure 11A).



(A) (B)

Figure 11: Mean leaf number of *H. cannabinus* (A) and *L. usitatissimum* (B) grown in normal, rehabilitated and tailings soil treatments over six months. Each point represents the mean leaf number of 25 *H. cannabinus* and 50 *L. usitatissimum* plants grown in each treatment. Line equations and R^2 values from top to bottom relate to the slope of each line for normal, rehabilitated and tailings soil for each species.

After the first two months of growth the mean leaf number of *H. cannabinus* grown in each soil treatment exhibited high variability (Figure 11A). This high variability may have been due to leaf senescence and new leaf production differences between individual plants (Figure 11A). However, at the end of the six-month growth period the highest mean leaf number was observed in *H. cannabinus* grown in tailings soil. Followed by *H. cannabinus* grown in rehabilitated soil and lastly in *H. cannabinus* grown in normal soil (Figure 11A).

The mean leaf number of *L. usitatissimum* grown in normal and tailings soils exhibited a positive linear relationship with time over the six-month growth period (Figure 11B). However, the mean leaf number of *L. usitatissimum* grown in rehabilitated soil exhibited a hyperbolic growth pattern over the six-month growth period (Figure 11B). Similarly to *H. cannabinus* the mean leaf number of *L. usitatissimum* grown in each soil treatment exhibited high variance from month two to six (Figure 11B). However, at the end of the six-month growth period the highest mean leaf number was observed in *L. usitatissimum* grown in tailings soil, followed by *L. usitatissimum* grown in normal soil and lastly *L. usitatissimum* grown in rehabilitated soil (Figure 11B). Overall *L. usitatissimum* exhibited substantially higher values of mean leaf number than *H. cannabinus* within each soil treatment over the six-month growth period (Figure 11). Furthermore, the rate of increase in mean leaf number of *L. usitatissimum* was higher than *H. cannabinus* within each soil treatment (Figure 11).

The effects of soil treatment on mean basal circumference were evident in both *H. cannabinus* and *L. usitatissimum*. The lowest rate of increase in mean basal circumference was found within the rehabilitated soil treatment for both species. *Hibiscus cannabinus* exhibited a higher rate of increase in mean basal circumference than *L. usitatissimum* within each soil treatment. The effects of soil treatment on mean leaf number appeared to be present within both species. The highest rates of increase in mean leaf number were found in the tailings soil treatment for both species. However, the high variance found within this data sets caused the effects of soil treatment and time to become confounded. Therefore, the effects of soil treatment and time on mean leaf number could not be reliably concluded.

4.4 – Metals in Plant Tissue Analysis

H. cannabinus - metal amount between or within soil treatments and within or between plant components:

The mean amount of Mn, Ni, Cr and Co did not statistically differ in the above or below ground component of *H. cannabinus* between soil treatments (Figure 12). However, two exceptions to this were the significantly ($p < 0.05$) lower amount of Mn in the above ground component of *H. cannabinus* grown in tailings soil compared to that of *H. cannabinus* grown in normal soil (Figure 12). The other exception was the significantly ($p < 0.05$) lower amount of Cr in the below ground component of *H. cannabinus* grown in rehabilitated soil compared to *H. cannabinus* grown in normal and tailings soil (Figure 12). The mean amount of Zn and Cu in the above and below ground component of *H. cannabinus* grown in rehabilitated soil were significantly ($p < 0.05$) lower than *H. cannabinus* grown in normal and tailings soil (Figure 12). Generally, *H. cannabinus* exhibited increased metal accumulation within the below ground plant component, excluding the amounts of Mn in *H. cannabinus* grown in normal and rehabilitated soil (Figure 12). The mean amount of Ni, Cu, Cr and Co were significantly ($p < 0.05$) higher in the below ground component of *H. cannabinus* grown in each soil treatment (Figure 12). One exception was that the mean amount of Cr did not statistically differ between components of *H. cannabinus* grown in rehabilitated soil (Figure 12). Mn and Zn did not statistically differ between plant components of *H. cannabinus* grown in each soil treatment (Figure 12).

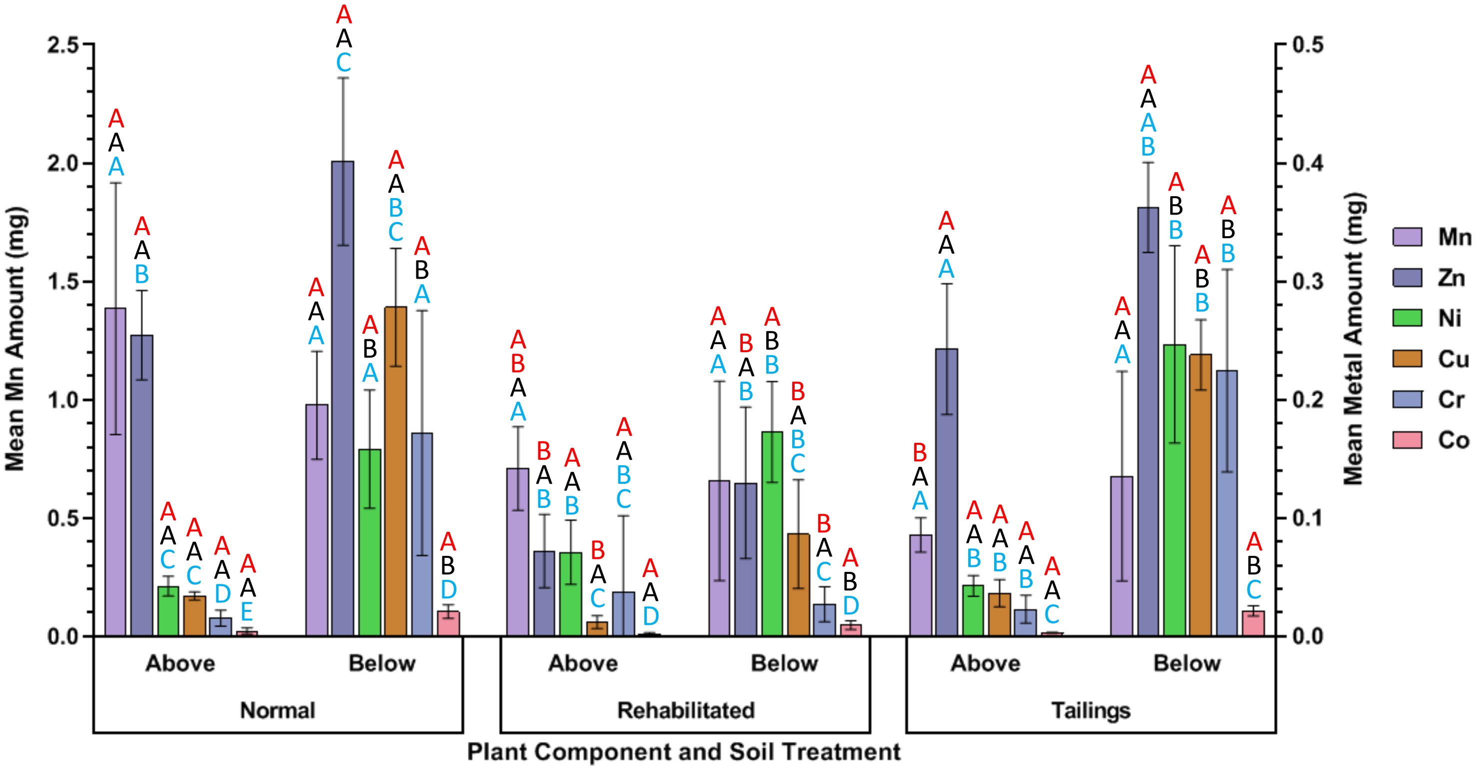


Figure 12: Mean amount of Mn, Zn, Ni, Cu, Cr and Co in the above and below ground plant component of *H. cannabinus* after six months of exposure to normal, rehabilitated and tailings soil treatments. Different letters above each bar represent significant differences of the measured variable. Bars with the same letters represent no statistical difference. Red letters compare metal amounts between soil treatments within the above and below ground plant component. Black letters compare metal amounts within soil treatments between the above and below ground plant component. Blue letters compare different metal amounts within soil treatments within the above and below ground plant component. Error bars represent standard deviations of each mean, n = 5.

The mean amount of Co in the above and below ground component of *H. cannabinus* was significantly ($p < 0.05$) lower than all other metals across soil treatments (Figure 12). The mean amount of Mn in the above and below ground component of *H. cannabinus* grown in normal and rehabilitated soil was significantly ($p < 0.05$) higher than all other metals (Figure 12). The mean amount of all metals in the above ground component of *H. cannabinus* grown in normal soil were significantly ($p < 0.05$) different following the order, from highest to lowest, of Mn > Zn > Ni = Cu > Cr > Co (Figure 12). The mean amount of Ni and Cu did not statistically differ (Figure 12). The mean amount of Zn in the below ground component of *H. cannabinus* grown in normal soil was significantly ($p < 0.05$) higher than Ni and Cr (Figure 12). The mean amount of Cu did not statistically differ from Zn, Ni and Cr (Figure 12). The same is true for the mean amount of Ni and Cr (Figure 12).

The mean amount of Cr in the above ground component of *H. cannabinus* grown in rehabilitated soil did not statistically differ from Zn, Ni and Cu (Figure 12). The same is true for Zn and Ni while both were significantly ($p < 0.05$) higher than Cu (Figure 12). The mean amount of Cu in the below ground component of *H. cannabinus* grown in rehabilitated soil did not statistically differ from Zn, Ni and Cr (Figure 12). The same is true for Zn and Ni while both were significantly ($p < 0.05$) higher than Cr (Figure 12). The mean amount of Mn and Zn in the above ground component of *H. cannabinus* grown in tailings soil did not statistically differ and were significantly ($p < 0.05$) higher than Ni, Cu and Cr which did not statistically differ (Figure 12). The mean amount of Mn in the below ground component of *H. cannabinus* grown in tailings soil was significantly ($p < 0.05$) higher than Ni, Cu and Cr yet did not statistically differ from Zn (Figure 12). The mean amount of Zn, Ni, Cu and Cr did not statistically differ (Figure 12).

Overall, *H. cannabinus* exhibited higher metal accumulation in the below ground component. The general pattern of accumulation in *H. cannabinus* showed that the accumulation of Mn and Zn was higher than other metals in the above and below ground plant component across soil treatments. However, the accumulation of Ni was greater than Zn in the below ground component of *H. cannabinus* grown in rehabilitated soil while the accumulation of Zn and Cu were lower than *H. cannabinus* grown in normal and tailings soil. The accumulation of Mn and Zn did not differ between the above and below ground components of *H. cannabinus* in each soil treatment. However, the accumulation of Ni, Cu, Cr and Co was higher in the below ground component of *H. cannabinus*. An unexpected result was that the mean amount of Mn and Zn in the above ground component and Mn, Zn, Ni, Cu and Cr in the below ground component of *H. cannabinus* grown in normal soil were so high. In some cases, these mean amounts were higher than those in the rehabilitated and tailings soil treatments.

H. cannabinus - Metal amount in the total plant component between soil treatments and different metal amounts within soil treatments:

The mean amount of Mn in *H. cannabinus* grown in tailings soil was significantly ($p < 0.05$) lower than *H. cannabinus* grown in normal soil, while the mean amount of Cr was significantly ($p < 0.05$) higher than *H. cannabinus* grown in rehabilitated soil (Figure 13). The mean amount of Zn and Cu in *H. cannabinus* grown in rehabilitated soil were significantly ($p < 0.05$) lower than *H. cannabinus* grown in normal and tailings soil (Figure 13). The mean amount of Ni and Co in *H. cannabinus* did not statistically differ between soil treatments (Figure 13). The mean amount of Mn in *H. cannabinus* was significantly ($p < 0.05$) higher than all other metals, while the amount of Co was significantly ($p < 0.05$) lower than all other metals, across soil treatments (Figure 13). The mean amount of Zn in *H. cannabinus* grown in normal and tailings soil was significantly ($p < 0.05$) higher than Ni, Cu and Cr (Figure 13). The mean amount of Ni in *H. cannabinus* grown in normal soil did not statistically differ with the amount of Cu and Cr, while the amount of Cu was significantly ($p < 0.05$) higher than Cr (Figure 13). The mean amount of Zn, Ni and Cu in *H. cannabinus* grown in rehabilitated soil did not statistically differ, while the amount of Zn and Ni were significantly ($p < 0.05$) higher than Cr which did not statistically differ from Cu (Figure 13). The mean amount of Ni, Cu and Cr in *H. cannabinus* grown in tailings soil did not statistically differ (Figure 13).

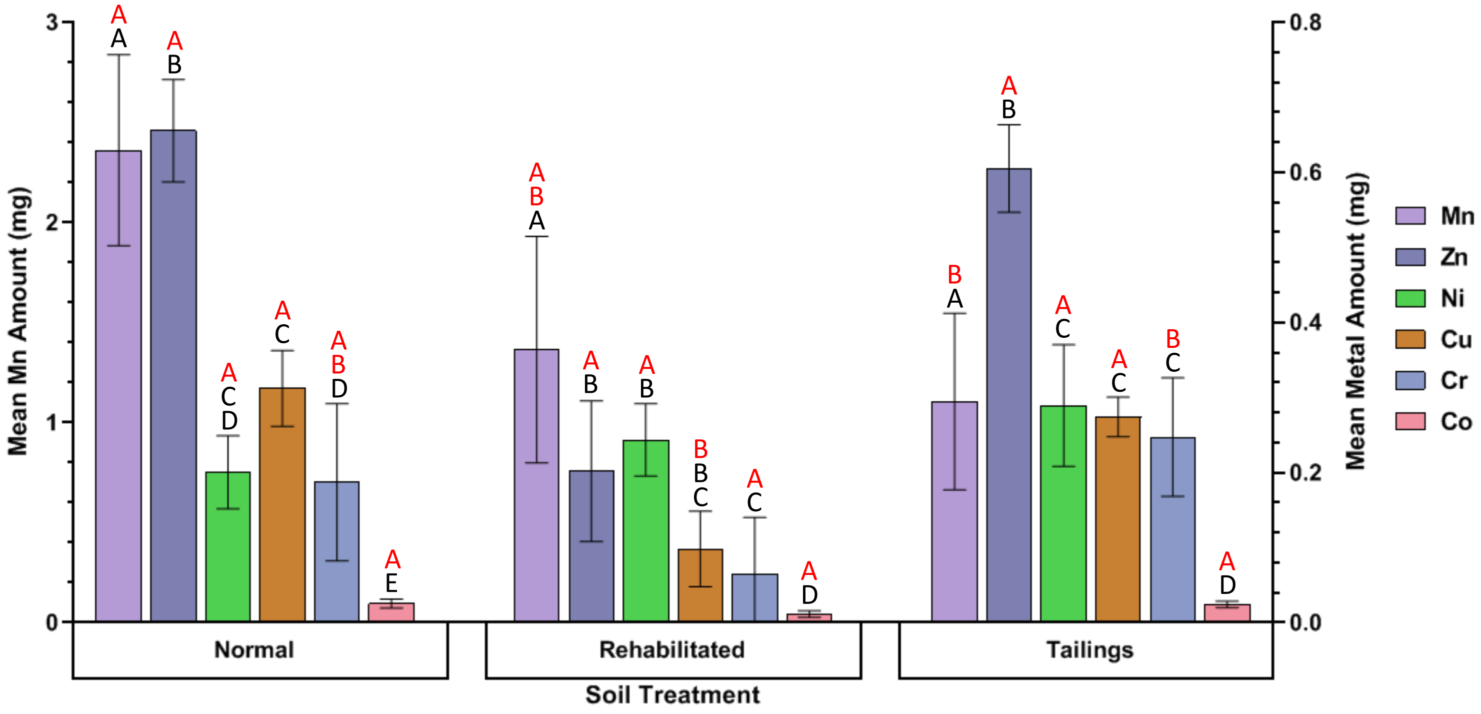


Figure 13: Mean amount of Mn, Zn, Ni, Cu, Cr and Co in the total plant component of *H. cannabinus* after six months of exposure to normal, rehabilitated and tailings soil treatments. Different letters above each bar represent significant differences of the measured variable. Bars with the same letters represent no statistical difference. Red letters compare metal amounts between soil treatments. Black letters compare different metal amounts within soil treatments. Error bars represent standard deviations of each mean, n = 5.

Overall, Mn was the highest accumulated metal while Co was the lowest accumulated metal in each soil treatment. The amount of Zn, Cu and Cr accumulated in *H. cannabinus* grown in rehabilitated soil was noticeably lower than those grown in normal and tailings soil. As with metal accumulation in the above and below ground component of *H. cannabinus* an unexpected result were the high metal accumulation values observed in *H. cannabinus* grown in normal soil. Generally, all metal accumulation values, excluding Mn, in normal and tailings soil were similar and higher than those of rehabilitated soil.

<u>*L. usitatissimum* - metal amount between or within soil treatments and within or between plant components:</u>

The mean amount of Mn was significantly ($p < 0.05$) higher in the above ground component of *L. usitatissimum* grown in normal soil compared to *L. usitatissimum* grown in rehabilitated soil (Figure 14). This amount was also significantly ($p < 0.05$) higher within the below ground component of *L. usitatissimum* grown in tailings compared to *L. usitatissimum* grown in rehabilitated soil (Figure 14). The mean amount of Zn in the above and below ground component of *L. usitatissimum* grown in rehabilitated soil was significantly ($p < 0.05$) lower than *L. usitatissimum* grown in normal and tailings soil (Figure 14). The mean amount of Cu in the above and below ground component of *L. usitatissimum* did not statistically differ between soil treatments (Figure 14). One exception was that the mean amount of Cu in the above ground component of *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in rehabilitated and tailings soil (Figure 14). The mean amount of Ni, Cr and Co in the above ground component of *L. usitatissimum* did not statistically differ between soil treatments (Figure 14). The mean amount of Ni and Cr were significantly ($p < 0.05$) higher in the below ground component of *L. usitatissimum* grown in tailings soil compared to *L. usitatissimum* grown in normal and rehabilitated soil (Figure 14). The mean amount of Co was significantly ($p < 0.05$) higher in the below ground component of *L. usitatissimum* grown in tailings soil compared to *L. usitatissimum* grown in rehabilitated soil (Figure 14).

Overall, *L. usitatissimum* also exhibited increased metal accumulation within the below ground component (Figure 14). The mean amount of Zn did not statistically differ between the above and below ground components of *L. usitatissimum* across soil treatments (Figure 14). The mean amount of Ni, Cr and Co were significantly ($p < 0.05$) higher in the below ground component of *L. usitatissimum* grown in normal soil, while the amount of Mn and Cu did not statistically differ between components (Figure 14). The mean amount of Cu and Cr were significantly ($p < 0.05$) higher in the below ground component of *L. usitatissimum* grown in rehabilitated soil while the amount of Mn, Ni and Co did not statistically differ between components (Figure 14). The mean amount of all metals, excluding Zn, were significantly ($p < 0.05$) higher within the below ground component of *L. usitatissimum* grown in tailings soil (Figure 14).

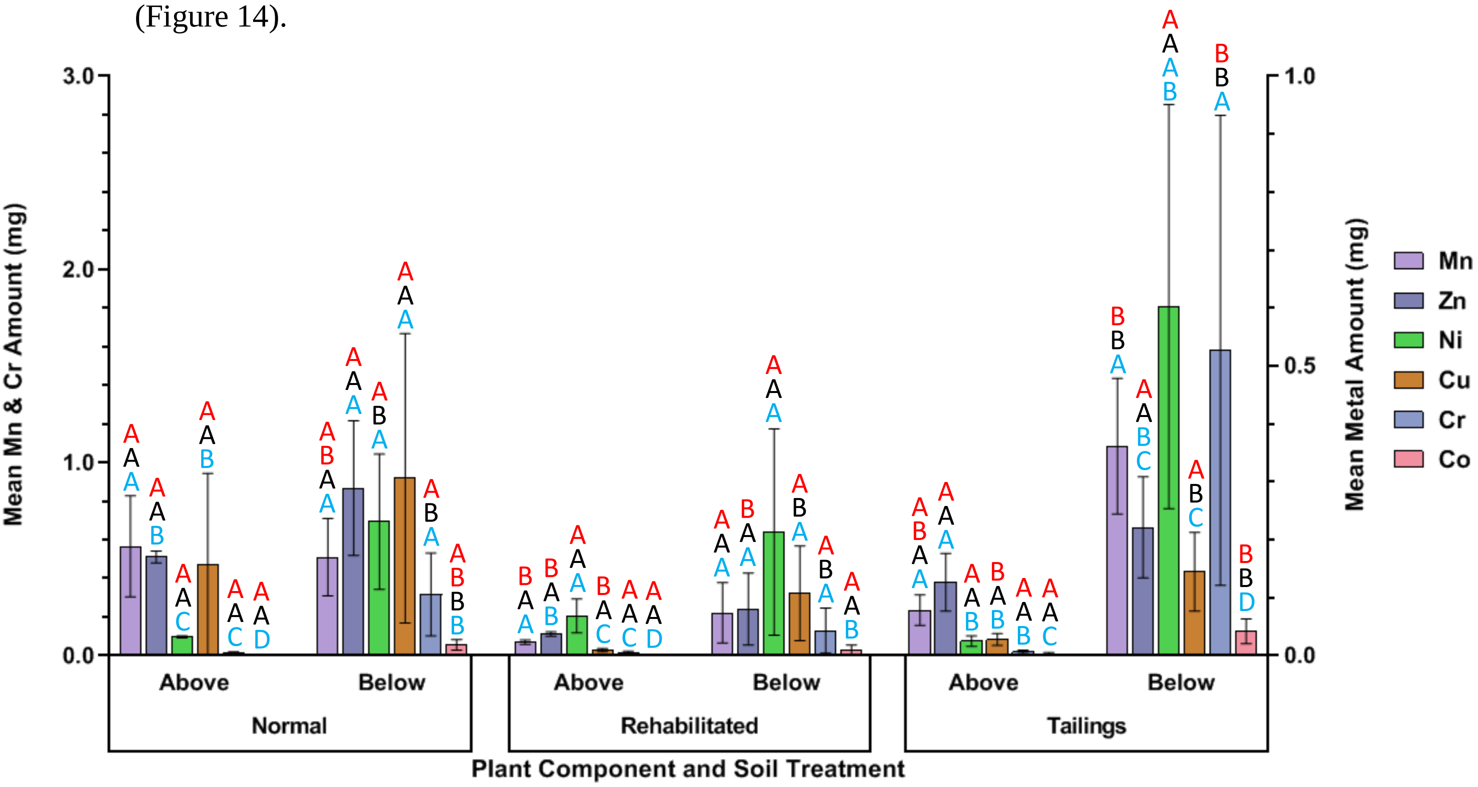


Figure 14: Mean amount of Mn, Zn, Ni, Cu, Cr and Co in the above and below ground plant component of *L. usitatissimum* after six months of exposure to normal, rehabilitated and tailings soil treatments. Different letters above each bar represent significant differences of the measured variable. Bars with the same letters represent no statistical difference. Red letters compare metal amounts between soil treatments within the above and below ground plant component. Black letters compare metal amounts within soil treatments between the above and below ground plant component. Blue letters compare different metal amounts within soil treatments within the above and below ground plant component. Error bars represent standard deviations of each mean, n = 5.

The mean amount of Co in the above and below ground component of *L. usitatissimum* was significantly ($p < 0.05$) lower than all other metals across soil treatments (Figure 14). The mean amount of Mn in the above ground component of *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than all other metals (Figure 14). The mean amount of Zn and Cu did not statistically differ and were significantly ($p < 0.05$) higher than Ni and Cr which did not statistically differ (Figure 14). The mean amount of Mn, Zn, Ni, Cu and Cr in the below ground component of *L. usitatissimum* grown in normal and rehabilitated soil did not statistically differ (Figure 14). The mean amount of Mn and Ni in the above ground component of *L. usitatissimum* grown in rehabilitated soil was significantly ($p < 0.05$) higher than all other metals (Figure 14). The mean amount of Cu and Cr did not statistically differ and were significantly ($p < 0.05$) lower than Zn (Figure 14). The mean amount of Mn and Zn in the above ground component of *L. usitatissimum* grown in tailings soil did not statistically differ and were significantly ($p < 0.05$) higher than Ni, Cu and Cr which did not statistically differ (Figure 14). The mean amount of Mn, Ni and Cr in the below ground component of *L. usitatissimum* grown in tailings soil did not statistically differ and were significantly ($p < 0.05$) higher than Cu (Figure 14). Furthermore, the mean amount of Mn and Cr were significantly ($p < 0.05$) higher than Zn which did not statistically differ from Ni and Cu (Figure 14).

Overall, *L. usitatissimum* exhibited increased metal accumulation in the below ground component. The accumulation of Mn was higher than all other metals in the above and below ground component of *L. usitatissimum* grown in normal and tailings soil. Only the accumulation of Cr was higher than Mn in the tailings soil treatment. *Linum usitatissimum* grown in rehabilitated soil exhibited a higher and similar accumulation of Mn and Ni in both plant components compared to other metals. The accumulation of Mn, Ni and Cr was also noticeable in the below ground component of *L. usitatissimum* grown in tailings soil. *Linum usitatissimum* grown in normal and rehabilitated soil exhibited less difference in metal accumulation amounts between the above and below ground component. However, accumulation of all metals, excluding Zn, in the below ground component of *L. usitatissimum* grown in tailings soil were higher than in the above ground component. Generally, *L. usitatissimum* exhibited higher metal accumulation in the below ground component, Mn and Zn was consistently high across soil treatments, *L. usitatissimum* grown in normal soil exhibited high values of Cu in both components and a high Ni value in the below ground component, *L. usitatissimum* grown in rehabilitated soil exhibited a higher and similar accumulation of Mn and Ni in both components and *L. usitatissimum* grown in tailings exhibited a noticeably high accumulation of Mn, Ni and Cr in the below ground component.

Metal amount within soil treatment within plant component between species:

The mean amount of all metals did not statistically differ in the above or below ground components between *H. cannabinus* and *L. usitatissimum* grown in each soil treatment (Figure 12&14). However, in each soil treatment, one exception was present. The mean amount of Mn in the above ground component of *H. cannabinus* grown in normal and rehabilitated soil was significantly ($p < 0.05$) higher than *L. usitatissimum* (Figure 12&14). While the mean amount of Cr in the below ground component of *L. usitatissimum* grown in tailings soil was significantly ($p < 0.05$) higher than *H. cannabinus* (Figure 12&14).

Comparisons of the accumulation of specific metals in the above and below ground components between *H. cannabinus* and *L. usitatissimum* did not differ for all soil treatments.

Only the accumulation of Mn in the above ground component of *H. cannabinus* grown in normal and rehabilitated soil were higher than *L. usitatissimum*, while the accumulation of Cr in the below ground component of *L. usitatissimum* grown in tailings soil was higher than *H. cannabinus*. Overall, the accumulation of specific metals within the above and below ground component did not differ between *H. cannabinus* and *L. usitatissimum* grown in each soil treatment and the accumulation of Mn and Zn were consistently high for all soil treatments. *Hibiscus cannabinus* and *L. usitatissimum* grown in rehabilitated soil exhibited increased Ni accumulation in the above ground component while *L. usitatissimum* grown in tailings soil exhibited increased Mn, Ni and Cr accumulation in the below ground component. The accumulation of Co was consistently low in both components for each species across soil treatments.

L. usitatissimum - Metal amount in the total plant component between soil treatments and different metal amounts within soil treatments:

The mean amount of Mn and Zn in *L. usitatissimum* grown in rehabilitated soil was significantly ($p < 0.05$) lower than *L. usitatissimum* grown in normal and tailings soil (Figure 15). The mean amount of Ni in *L. usitatissimum* did not statistically differ between soil treatments (Figure 15). The mean amount of Cu in *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in rehabilitated soil (Figure 15). The mean amount of Cr in *L. usitatissimum* grown in tailings soil was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in normal and rehabilitated soil, while the mean amount of Co was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in rehabilitated soil (Figure 15). The mean amount of Co in *L. usitatissimum* was significantly ($p < 0.05$) lower than all other metals across soil treatments (Figure 15). The mean amount of Mn in *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than Ni and Cr, while not statistically differing from Zn and Cu (Figure 15). The mean amount of Zn, Ni, Cu and Cr in *L. usitatissimum* grown in normal soil did not statistically differ (Figure 15). The mean amount of Mn, Zn, Ni, Cu and Cr in *L. usitatissimum* grown in rehabilitated soil did not statistically differ (Figure 15). The mean amount of Mn in *L. usitatissimum* grown in tailings soil was significantly ($p < 0.05$) higher than Zn and Cu, while not statistically differing from Ni and Cr (Figure 15). The mean amount of Ni and Cr in *L. usitatissimum* grown in tailings soil did not statistically differ and were significantly ($p < 0.05$) higher than Cu (Figure 15). The mean amount of Cr in *L. usitatissimum* grown in tailings soil was significantly ($p < 0.05$) higher than Zn which did not statistically differ from Ni and Cu (Figure 15).

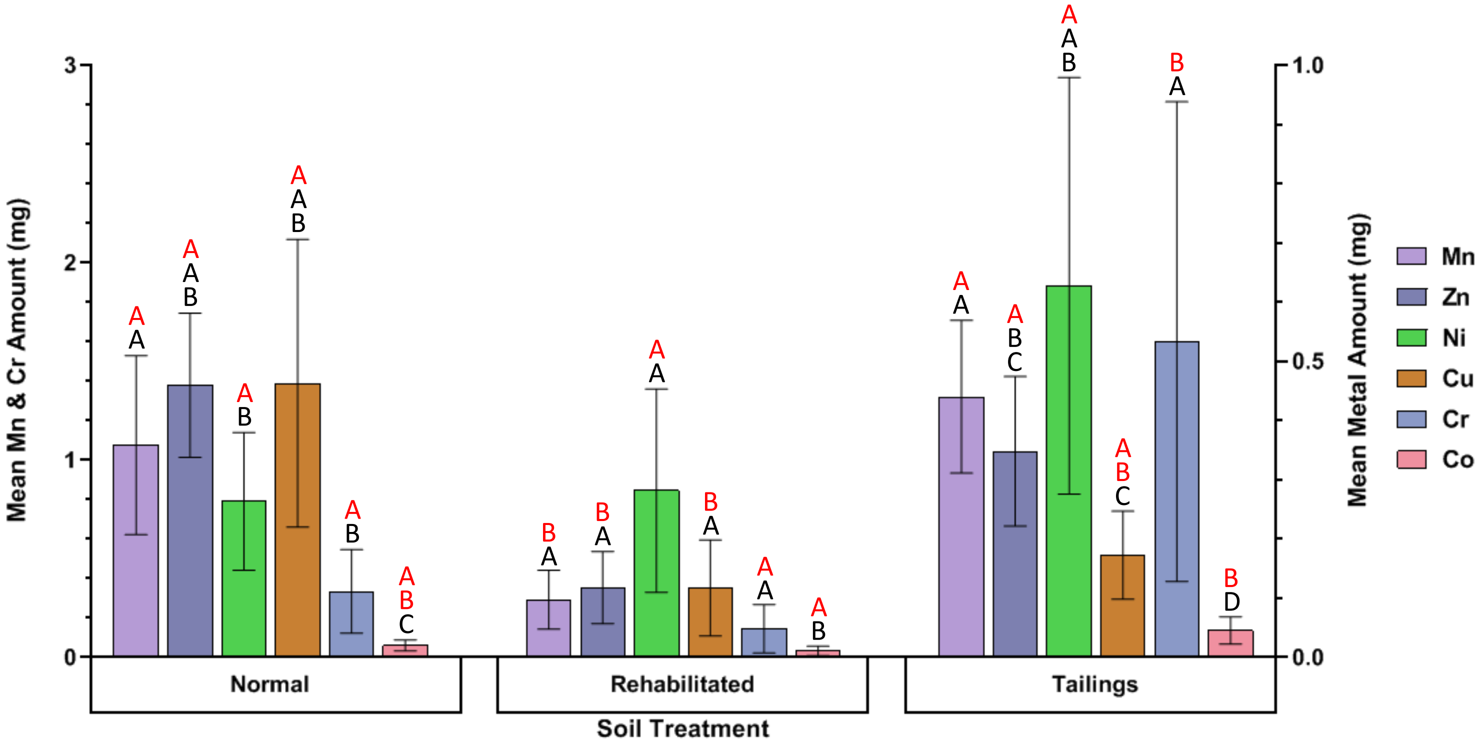


Figure 15: Mean amount of Mn, Zn, Ni, Cu, Cr and Co in the total plant component of *L. usitatissimum* after six months of exposure to normal, rehabilitated and tailings soil treatments. Different letters above each bar represent significant differences of the measured variable. Bars with the same letters represent no statistical difference. Red letters compare metal amounts between soil treatments. Black letters compare different metal amounts within soil treatments. Error bars represent standard deviations of each mean, n = 5.

Metal amount in the total plant component within soil treatment between species:

The mean amount of all metals did not statistically differ between *H. cannabinus* and *L. usitatissimum* grown in each soil treatment (Figure 13&15). Exceptions to this were the significantly ($p < 0.05$) higher amounts of Mn in *H. cannabinus* grown in normal and rehabilitated soil (Figure 13&15). Further exceptions were the significantly ($p < 0.05$) lower amount of Zn and significantly ($p < 0.05$) higher amount of Cr in *L. usitatissimum* grown in tailings soil (Figure 13&15).

Comparisons of specific metal accumulation in the total plant component between *H. cannabinus* and *L. usitatissimum* grown in each soil treatment generally showed no difference. Exceptions were the higher amounts of Mn in *H. cannabinus* grown in normal and rehabilitated soil, the lower amount of Zn and higher amount of Cr in *L. usitatissimum* grown in tailings soil. The accumulation of Zn and Cu in *H. cannabinus* grown in rehabilitated soil was lower than *H. cannabinus* grown in normal and tailings soil while the same is true for the accumulation of Mn and Zn in *L. usitatissimum* grown in rehabilitated soil. Other noticeable differences in accumulation were the higher amounts of Mn and Cu in *H. cannabinus* grown in normal soil compared to *H. cannabinus* grown in tailings and rehabilitated soil respectively. Another noticeable difference in accumulation was the higher amount of Cr in *L. usitatissimum* grown in tailings soil compared to *L. usitatissimum* grown in normal and rehabilitated soil. The accumulation of Co was lower than all other metals for both species grown in each soil treatment, while the accumulation of Mn was higher than all other metals, excluding those in *L. usitatissimum* grown in rehabilitated soil.

H. cannabinus - metal concentration between or within soil treatments and within or between plant components:

The mean concentration of Zn, Cu and Co in the above and below ground components of *H. cannabinus* did not statistically differ between soil treatments (Figure 16). The same is true for the mean concentration of Mn, in the below ground component, and Cr in the above ground component (Figure 16). The mean concentration of Mn in the above ground component of *H. cannabinus* grown in tailings soil was significantly ($p < 0.05$) lower than *H. cannabinus* grown in normal and rehabilitated soil (Figure 16). The mean concentration of Cr in the below ground component of *H. cannabinus* grown in tailings soil was significantly ($p < 0.05$) higher than *H. cannabinus* grown in rehabilitated soil (Figure 16). The mean concentration of Ni in the above ground component of *H. cannabinus* grown in rehabilitated soil was significantly ($p < 0.05$) higher than *H. cannabinus* grown in normal and tailings soil (Figure 16). While this concentration in the below ground component was only significantly ($p < 0.05$) higher than *H. cannabinus* grown in normal soil (Figure 16). The concentration of all metals in the below ground component of *H. cannabinus* were significantly ($p < 0.05$) higher than those in the above ground component (Figure 16). The only exceptions to this were the concentration of Mn in *H. cannabinus* grown in normal soil and the concentration of Mn and Cr in *H. cannabinus* grown in rehabilitated soil which did not statistically differ between components (Figure 16).

The mean concentration of Co in the above and below ground component of *H. cannabinus* was significantly ($p < 0.05$) lower than all other metals across soil treatments (Figure 16). The mean concentration of Mn in the above and below ground component of *H. cannabinus* grown in normal and rehabilitated soil was significantly ($p < 0.05$) higher than all other metals (Figure 16). The mean concentration of Zn in the above ground component of *H. cannabinus* grown in normal soil was significantly ($p < 0.05$) higher than Ni, Cu and Cr (Figure 16). The mean concentration of Ni and Cu did not statistically differ and were significantly ($p < 0.05$) higher than Cr (Figure 16). The mean concentration of Zn in the below ground component of *H. cannabinus* grown in normal soil did not statistically differ from Cu and was significantly ($p < 0.05$) higher than Ni and Cr (Figure 16). The mean concentration of Ni, Cu and Cr did not statistically differ (Figure 16).

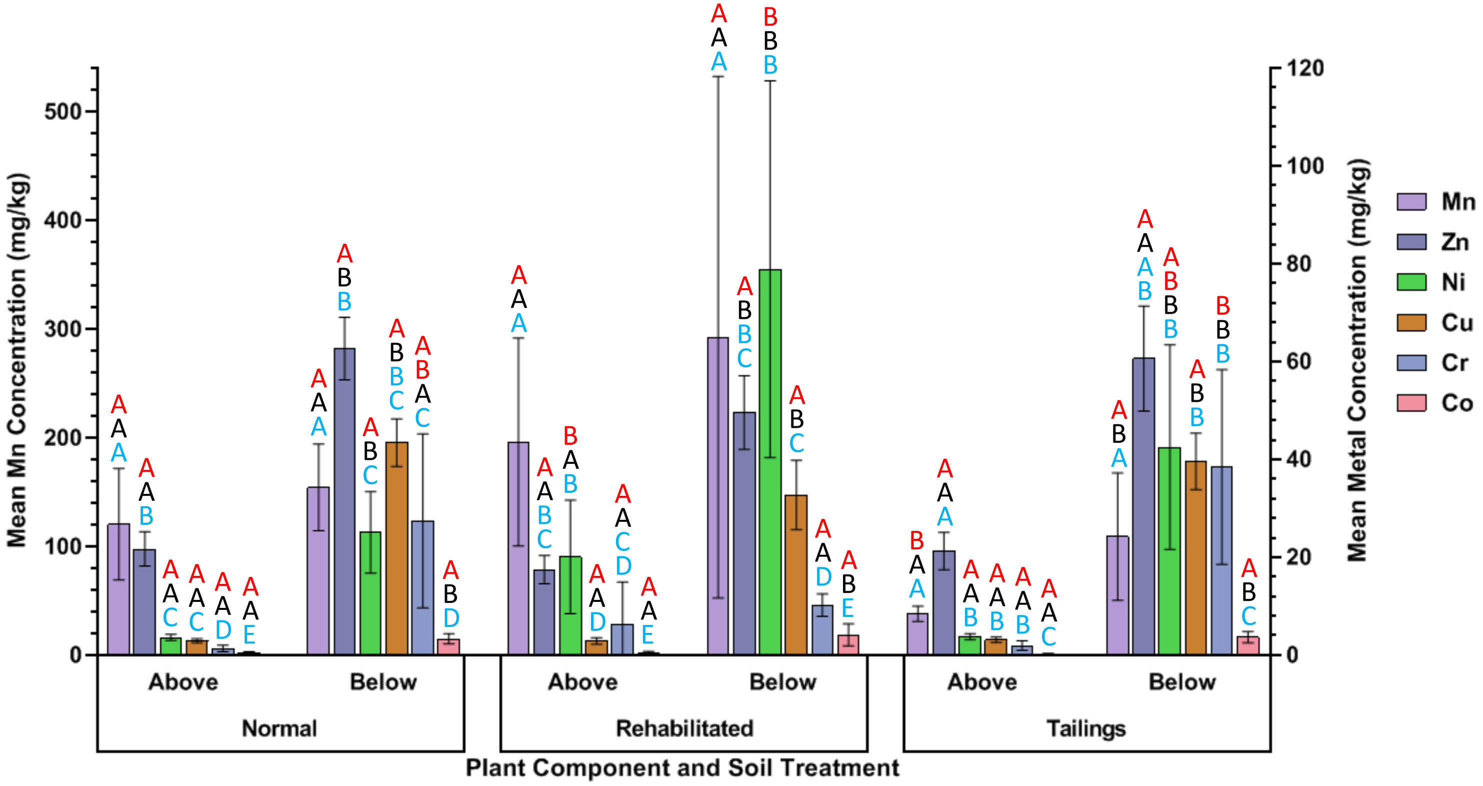


Figure 16: Mean concentration of Mn, Zn, Ni, Cu, Cr and Co in the above and below ground plant component of *H. cannabinus* after six months of exposure to normal, rehabilitated and tailings soil treatments. Different letters above each bar represent significant differences of the measured variable. Bars with the same letters represent no statistical difference. Red letters compare metal concentrations between soil treatments within the above and below ground plant component. Black letters compare metal concentrations within soil treatments between the above and below ground plant component. Blue letters compare different metal concentrations within soil treatments within the above and below ground plant component. Error bars represent standard deviations of each mean, n = 5.

The mean concentration of Cu in the above ground component of *H. cannabinus* grown in rehabilitated soil was significantly ($p < 0.05$) lower than Zn and Ni (Figure 16). The mean concentration of Cr in the above ground component of *H. cannabinus* grown in rehabilitated soil did not statistically differ with Cu and Zn while being significantly ($p < 0.05$) lower than Ni (Figure 16). The mean concentration of Zn and Ni in the above and below ground component did not statistically differ (Figure 16). The mean concentration of Cr in the below ground component of *H. cannabinus* grown in rehabilitated soil was significantly ($p < 0.05$) lower than Zn, Ni and Cu (Figure 16). The mean concentration of Cu in the below ground component of *H. cannabinus* grown in rehabilitated soil was significantly ($p < 0.05$) lower than Ni, while not statistically differing from Zn (Figure 16). The mean concentration of Mn and Zn in the above ground component of *H. cannabinus* grown in tailings soil did not statistically differ and were significantly ($p < 0.05$) higher than Ni, Cu and Cr which did not statistically differ (Figure 16). The mean concentration of Mn in the below ground component of *H.*

cannabinus grown in tailings soil did not statistically differ with Zn, and was significantly ($p < 0.05$) higher than Ni, Cu and Cr (Figure 16). The mean concentration of Zn, Ni, Cu and Cr did not statistically differ (Figure 16).

Overall, higher concentrations of all metals were present in the below ground component of *H. cannabinus*. However, the concentration of Mn did not differ between components of *H. cannabinus* grown in normal and rehabilitated soil, the same was true for Cr in *H. cannabinus* grown rehabilitated soil. The Ni concentration in the above ground component of *H. cannabinus* grown in rehabilitated soil was higher than those grown in normal and tailings soil. While the Ni concentration in the below ground component of *H. cannabinus* grown in normal soil was lower than those grown in rehabilitated and tailings soil. Generally, differences in metal concentration were similar to those of metal amount, higher concentrations were present in the below ground component of *H. cannabinus* across soil treatments. Concentrations of Mn and Zn in both components were consistently high across soil treatments. The concentration of Ni in both components was noticeably high in *H. cannabinus* grown in rehabilitated soil.

H. cannabinus - Metal concentration in the total plant component between soil treatments and different metal concentrations within soil treatments:

The mean concentration of Zn, Cu, Cr and Co in *H. cannabinus* did not statistically differ between soil treatments (Figure 17). The mean concentration of Mn in *H. cannabinus* grown in tailings soil was significantly ($p < 0.05$) lower than *H. cannabinus* grown in normal and rehabilitated soil (Figure 17). The mean concentration of Ni in *H. cannabinus* grown in rehabilitated soil was significantly ($p < 0.05$) higher than *H. cannabinus* grown in normal and tailings soil (Figure 17). The mean concentration of Co in *H. cannabinus* was significantly ($p < 0.05$) lower than all other metals across soil treatments (Figure 17). The mean concentration of Mn in *H. cannabinus* grown in normal and rehabilitated soil was significantly ($p < 0.05$) higher than Zn, Ni, Cu and Cr (Figure 17). The mean concentration of Zn in *H. cannabinus* grown in normal soil was significantly ($p < 0.05$) higher than Ni, Cu and Cr, which did not statistically differ (Figure 17). The mean concentration of Cr and Cu in *H. cannabinus* grown in rehabilitated soil did not statistically differ, while the concentration of Cr was significantly ($p < 0.05$) lower than Zn and Ni (Figure 17). The mean concentration of Ni in *H. cannabinus* grown in rehabilitated soil was significantly ($p < 0.05$) higher than Cu, while the concentration of Zn did not statistically differ from Ni and Cu (Figure 17). The mean concentration of Mn and Zn in *H. cannabinus* grown in tailings soil did not statistically differ, Mn was significantly higher than Ni, Cu and Cr, while Zn was significantly ($p < 0.05$) higher than Cu and Cr (Figure 17). Furthermore, the mean concentrations of Ni, Cu and Cr did not statistically differ (Figure 17).

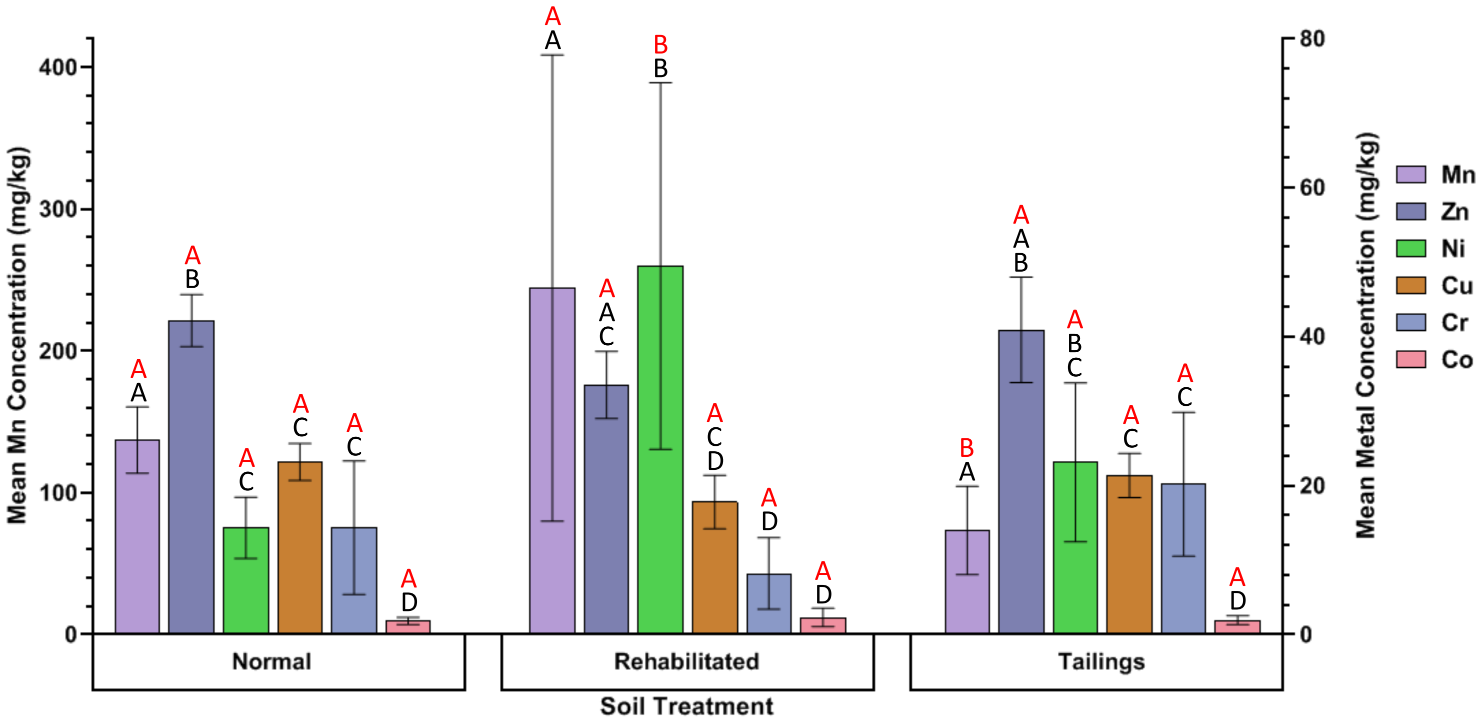


Figure 17: Mean concentration of Mn, Zn, Ni, Cu, Cr and Co in the total plant component of *H. cannabinus* after six months of exposure to normal, rehabilitated and tailings soil treatments. Different letters above each bar represent significant differences of the measured variable. Bars with the same letters represent no statistical difference. Red letters compare metal concentrations between soil treatments. Black letters compare different metal concentrations within soil treatments. Error bars represent standard deviations of each mean, n = 5.

L. usitatissimum - metal concentration between or within soil treatments and within or between plant components:

The mean concentration of Co in the above and below ground components of *L. usitatissimum* did not statistically differ across soil treatments (Figure 18). The same is true for the concentrations of Zn and Cr in the below and above ground component, respectively (Figure 18). The mean concentration of Mn and Zn in the above ground component of *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in rehabilitated and tailings soil, while the concentration of Mn in *L. usitatissimum* grown in tailings soil was higher than *L. usitatissimum* grown in rehabilitated soil (Figure 18). The concentration of Mn and Cr in the below ground component of *L. usitatissimum* grown in tailings soil was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in normal and rehabilitated soil (Figure 18). The mean concentration of Ni in the above ground component of *L. usitatissimum* grown in rehabilitated soil was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in normal and tailings soil (Figure 18). While the Ni concentration in the below ground component of *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) lower than *L. usitatissimum* grown in rehabilitated and tailings soil (Figure 18).

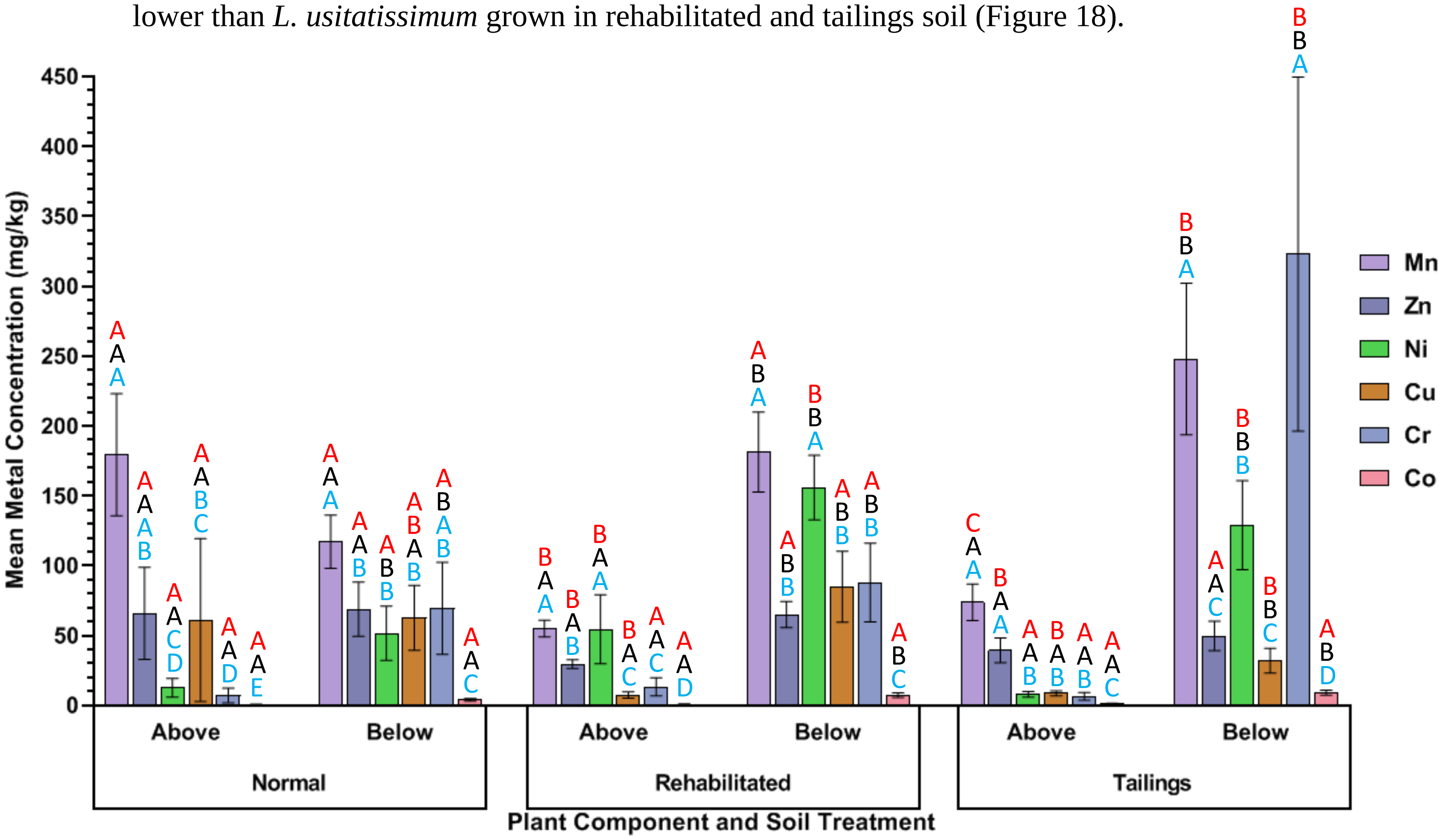


Figure 18: Mean concentration of Mn, Zn, Ni, Cu, Cr and Co in the above and below ground plant component of *L. usitatissimum* after six months of exposure to normal, rehabilitated and tailings soil treatments. Different letters above each bar represent significant differences of the measured variable. Bars with the same letters represent no statistical difference. Red letters compare metal concentrations between soil treatments within the above and below ground plant component. Black letters compare metal concentrations within soil treatments between the above and below ground plant component. Blue letters compare different metal concentrations within soil treatments within the above and below ground plant component. Error bars represent standard deviations of each mean, n = 5.

The mean concentration of Cu in the above ground component of *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in rehabilitated and tailings soil (Figure 18). While the Cu concentration in the below ground component of *L. usitatissimum* grown in tailings soil was significantly ($p < 0.05$) lower than *L. usitatissimum* grown in rehabilitated soil (Figure 18). The mean concentrations of Ni and Cr in the below ground component of *L. usitatissimum* grown in normal soil were significantly ($p < 0.05$) higher than those in the above ground component (Figure 18). The concentrations of all metals in the below ground component of *L. usitatissimum* grown in rehabilitated and tailings soil were significantly ($p < 0.05$) higher than those in the above ground component (Figure 18). The only exception is that the concentration of Zn in *L. usitatissimum* grown in tailings soil did not statistically differ between components (Figure 18). The mean concentration of Co in the above and below ground component of *L. usitatissimum* was significantly ($p < 0.05$) lower than all other metals across soil treatments (Figure 18).

The mean concentration of Mn and Zn in the above ground component of *L. usitatissimum* grown in normal soil did not statistically differ (Figure 18). The Mn concentration was significantly ($p < 0.05$) higher than Ni, Cu and Cr, while Zn was only significantly ($p < 0.05$) higher than Ni and Cr (Figure 18). The mean concentration of Cr was significantly ($p < 0.05$) lower than Cu and did not statistically differ with Ni which did not statistically differ with Cu (Figure 18). The mean concentration of Mn in the below ground section of *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than Zn, Ni and Cu yet did not statistically differ with Cr (Figure 18). Concentrations of Zn, Ni, Cu and Cr did not statistically differ (Figure 18).

The mean concentration of Mn and Ni in the above and below ground component of *L. usitatissimum* grown in rehabilitated soil did not statistically differ and were significantly ($p < 0.05$) higher than Zn, Cu and Cr (Figure 18). The mean concentration of Cu and Cr in the above ground component of *L. usitatissimum* grown in rehabilitated soil did not statistically differ and were significantly ($p < 0.05$) lower than Zn, these three concentrations did not statistically differ in the below ground component (Figure 18). The mean concentration of Mn and Zn in the above ground component of *L. usitatissimum* grown in tailings soil did not statistically differ and were significantly ($p < 0.05$) higher than Ni, Cu and Cr which did not statistically differ (Figure 18). The mean concentration of Zn and Cu in the below ground component of *L. usitatissimum* grown in tailings soil did not statistically differ and were significantly ($p < 0.05$) lower than Mn, Ni and Cr (Figure 18). Concentrations of Mn and Cr did not statistically differ and were significantly ($p < 0.05$) higher than Ni (Figure 18).

Overall, higher concentrations of all metals were present in the below ground component of *L. usitatissimum*. Concentrations of Ni and Cr were higher in the below ground component of *L. usitatissimum* grown in normal soil while only the concentration of Zn showed no difference between components of *L. usitatissimum* grown in rehabilitated and tailings soil. The Ni concentration in the above ground component of *L. usitatissimum* grown in rehabilitated soil was higher than those grown in normal and tailings soil. While the Ni concentration in the below ground component of *L. usitatissimum* grown in normal soil was lower than those grown in rehabilitated and tailings soil. Furthermore, the concentration of Mn and Zn in the above ground component of *L. usitatissimum* grown in normal soil was higher than those grown in rehabilitated and tailings soil. The concentration of Mn and Cr in the below ground component of *L. usitatissimum* grown in tailings soil was higher than *L. usitatissimum* grown in normal and rehabilitated soil. Generally, differences in metal concentration were similar to those of metal amount, higher concentrations were present in the below ground component across soil treatments. Concentrations of Mn and Zn in both components were consistently high across soil treatments. The concentration of Ni in both components was noticeably high in *L. usitatissimum* grown in rehabilitated soil, while the concentration of Mn, Ni and Cr was noticeably high in the below ground component of *L. usitatissimum* grown in tailings soil.

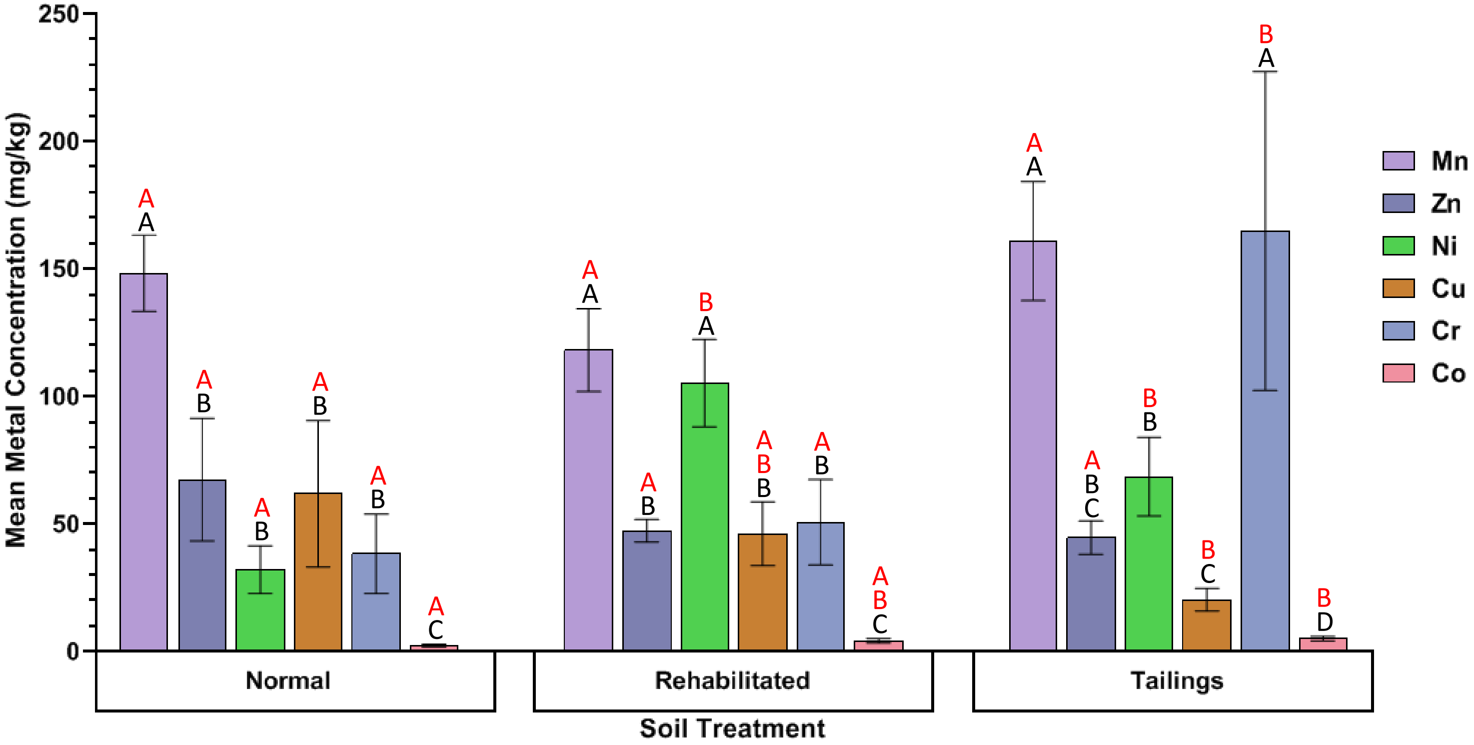
Metal concentration within soil treatment within plant component between species:

The mean concentration of Zn, Ni, Cu and Cr in the above ground component of *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than *H. cannabinus* while the concentration of Mn and Co did not differ (Figure 16&18). The mean concentration of all metals in the below ground component did not statistically differ between *H. cannabinus* and *L. usitatissimum* grown in normal soil (Figure 16&18). The mean concentration of Ni, Cu and Cr in the above and below ground component of *L. usitatissimum* grown in rehabilitated soil was significantly ($p < 0.05$) higher than *H. cannabinus*, concentrations of Zn and Co did not statistically differ (Figure 16&18). The mean concentration of Mn in the above ground component of *H. cannabinus* grown in rehabilitated soil was significantly ($p < 0.05$) higher than *L. usitatissimum* while not statistically differing in the below ground component (Figure 16&18). The mean concentration of Cu and Cr in the above ground component of *L. usitatissimum* grown in tailings soil was significantly ($p < 0.05$) higher than *H. cannabinus*, concentrations of Mn, Zn, Ni and Co did not statistically differ (Figure 16&18). The mean concentration of Mn, Ni and Cr in the below ground component of *L. usitatissimum* grown in tailings was significantly ($p < 0.05$) higher than *H. cannabinus*, concentrations of Zn, Cu and Co did not statistically differ (Figure 16&18).

Overall, metal concentrations, excluding Mn in the below ground component of *H. cannabinus* grown in normal soil and Mn in both components of *H. cannabinus* grown in rehabilitated soil, in the above and below ground component of *L. usitatissimum* were higher than *H. cannabinus* across soil treatments. *Linum usitatissimum* grown in normal soil exhibited noticeably higher concentrations of Zn, Ni and Cu in the above ground component, while *L. usitatissimum* grown in rehabilitated soil exhibited noticeably higher concentrations of Ni, Cu and Cr in both components. *Linum usitatissimum* grown in tailings soil exhibited noticeably higher concentrations of Cu and Cr in the above ground component and noticeably higher concentrations of Mn, Ni and Cr in the below ground component. Generally, higher concentrations of all metals were present in *L. usitatissimum* for each soil treatment, concentrations of Mn and Zn were consistently high in both components of each species across soil treatments, noticeably high Ni concentrations were present in both components of each species grown in rehabilitated soil and noticeably high concentrations of Mn, Ni and Cr were present in the below ground component of *L. usitatissimum* grown in tailings soil.

<u>*L. usitatissimum* - Metal concentration in the total plant component between soil treatments and different metal concentrations within soil treatments:</u>

The mean concentration of Mn and Zn in *L. usitatissimum* did not statistically differ between soil treatments (Figure 19). The mean concentration of Cu in *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in tailings soil while the concentration of Co was significantly ($p < 0.05$) lower (Figure 19). The mean concentration of Ni in *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) lower than *L. usitatissimum* grown in rehabilitated and tailings soil (Figure 19). The mean concentration of Cr in *L. usitatissimum* grown in tailings soil was significantly ($p < 0.05$) higher than *L. usitatissimum* grown in normal and rehabilitated soil (Figure 19).



<u>Figure 19:</u> Mean concentration of Mn, Zn, Ni, Cu, Cr and Co in the total plant component of *L. usitatissimum* after six months of exposure to normal, rehabilitated and tailings soil treatments. Different letters above each bar represent significant differences of the measured variable. Bars with the same letters represent no statistical difference. Red letters compare metal concentrations between soil treatments. Black letters compare different metal concentrations within soil treatments. Error bars represent standard deviations of each mean, n = 5.

The mean concentration of Co in *L. usitatissimum* was significantly ($p < 0.05$) lower than all other metals across soil treatments (Figure 19). The mean concentration of Mn in *L. usitatissimum* grown in normal soil was significantly ($p < 0.05$) higher than Zn, Ni, Cu and Cr which did not statistically differ (Figure 19). The mean concentration of Mn and Ni in *L. usitatissimum* grown in rehabilitated soil did not statistically differ and were significantly ($p < 0.05$) higher than Zn, Cu and Cr which did not statistically differ (Figure 19). The mean concentration of Mn and Cr in *L. usitatissimum* grown in tailings soil did not statistically differ and were significantly ($p < 0.05$) higher than Zn, Ni and Cu (Figure 19). The mean concentration of Zn did not statistically differ with Ni and Cu, while the concentration of Ni was significantly ($p < 0.05$) higher than Cu (Figure 19).

Metal amount in the total plant component within soil treatment between species:

The mean concentration of Mn and Co did not statistically differ between *H. cannabinus* and *L. usitatissimum* grown in normal soil, concentrations of Zn, Ni, Cu and Cr were significantly ($p < 0.05$) higher in *L. usitatissimum* (Figure 17&19). The mean concentration of Zn, Ni, Cu, Cr and Co in *L. usitatissimum* grown in rehabilitated soil were significantly ($p < 0.05$) higher than *H. cannabinus* which had a significantly ($p < 0.05$) higher Mn concentration (Figure 17&19). The mean concentration of Zn and Cu did not statistically differ between *H. cannabinus* and *L. usitatissimum* grown in tailings soil, concentrations of Mn, Ni, Cr and Co were significantly ($p < 0.05$) higher in *L. usitatissimum* (Figure 17&19).

Overall, *L. usitatissimum* exhibited higher concentrations of all metals in the total plant component, excluding Mn in *L. usitatissimum* grown in rehabilitated soil, than *H. cannabinus* across soil treatments. Concentrations of Zn, Cu, Cr and Co in *H. cannabinus* showed no difference between soil treatments, while the total concentration of Mn in *H. cannabinus* grown in tailings soil was lower than *H. cannabinus* grown in normal and tailings soil. Furthermore, *H. cannabinus* grown in rehabilitated soil exhibited a noticeably higher concentration of Ni. Only the concentrations of Mn and Zn in *L. usitatissimum* showed no difference between soil treatments, while the concentration of Ni in *L. usitatissimum* grown in normal soil was lower than *L. usitatissimum* grown in rehabilitated and tailings soil. Furthermore, the Cr concentration in *L. usitatissimum* grown in tailings soil was noticeably higher than *L. usitatissimum* grown in normal and rehabilitated soil. Concentrations of Zn, Ni, Cu and Cr in *L. usitatissimum* grown in normal soil were higher than *H. cannabinus*. Concentrations of all metals, excluding Mn, in *L. usitatissimum* grown in rehabilitated soil were higher than *H. cannabinus*.

Concentrations of all metals, excluding Zn and Cu, in *L. usitatissimum* grown in tailings soil were higher than *H. cannabinus*. The concentration of Co in both species was lower than all other metals across soil treatments. The concentration of Mn in *H. cannabinus* was higher than all other metals, excluding Zn within rehabilitated soil, across soil treatments. The same was true for *L. usitatissimum* grown in normal soil. Concentrations of Mn and Ni in *L. usitatissimum* grown in rehabilitated soil were noticeably higher than other metals. The same was true for the concentrations of Mn, Ni and Cr in *L. usitatissimum* grown in tailings soil. Generally, higher metal concentrations were present in *L. usitatissimum* for each soil treatment, the concentration of Mn was consistently high in both species grown across soil treatments, higher concentrations of Ni were present in both species grown in rehabilitated soil and a high Cr concentration was present in *L. usitatissimum* grown in tailings soil.

4.5 – Remote Sensing and Site Classification

Supervised image classification (SIC) was conducted to determine an area (ha) estimate of different land categories present at Onverwacht TSF (Figure 20) available for the cultivation of *H. cannabinus* and *L.* usitatissimum. This classification approach allowed for calculation of the measured and expected yield (t), economic value (R) and amount (g/ha) of various metals extracted by *H. cannabinus* and *L. usitatissimum* if cultivated onsite using the results of SIC, the greenhouse growth trial and the metals in plant tissue analysis. Atmospherically corrected scenes presenting surface reflectance for Landsat 8 and Sentinel 2B were obtained from United States Geological Survey (USGS) Earth Explorer and Copernicus Open Access Hub respectively.

Congruency between the results obtained from the greenhouse growth trial, metals in plant tissue analysis and supervised image classification (SIC) could be achieved by maximising the likelihood that each land area, if applicable, was classified based on the present soil. Therefore, scenes from both satellites were obtained for the mid-Winter period of 2020 to minimise the effect of vegetative cover over the site and maximise the soil spectral signature. Furthermore, this time period is when the soil samples were collected from Onverwacht TSF for use in the greenhouse growth trial. The creation of composite images from individual bands of Landsat 8 and Sentinel 2B utilised pan sharpening to a 15 m pixel resolution and resampling to a 10 m pixel resolution respectively. The resultant composite images created using Sentinel 2B bands were more defined than those of Landsat 8 because of the smaller pixel size and therefore further image analysis was conducted using the Sentinel 2B scene.

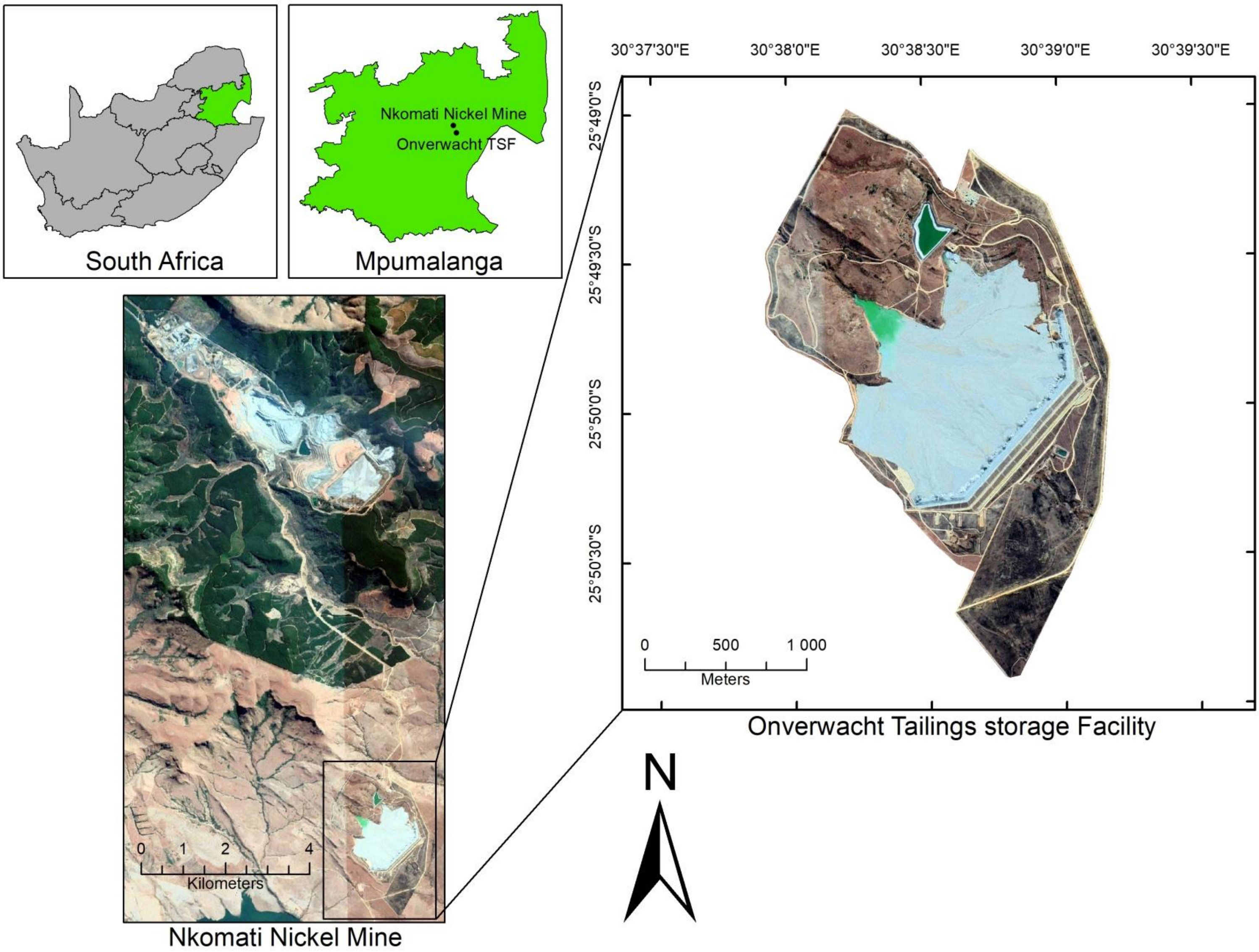


Figure 20: Site map of the locality of Nkomati Nickel Mine and the relative location of the Onverwacht tailings storage facility, Mpumalanga, South Africa. Satellite images of July 2020 were obtained from Google Earth, imported to and geo-referenced in Arcmap (10.4). The edges of the Onverwacht TSF were defined using the fence border present at the site.

Multiple composite images were created using various bands, which measure different wavelength (nm) regions of the electromagnetic spectrum and combinations from the pool of bands available for the Sentinel 2B scene (Table 9&10). Image classification of each composite image was conducted using the supervised image classification tool in ArcGIS (10.4). Initially, training samples were created using the natural colour composite image as a reference for deciding where training samples would be drawn. Based on the natural colour composite image, onsite observations and recommendations from the TSF resident technician, seven land categories were defined: water, tailings sludge, tailings dust, rehabilitated soil, grassland, burnt grassland and dirt road.

Table 9: Parameters of bands used in the creation of composite images using the scene obtained from Sentinel 2B during the mid-Winter period of 2020.

Band Number	Band Name	Pixel Resolution (m)	Wavelength (nm)
2	Blue	10	492.3
3	Green	10	558.9
4	Red	10	664.9
8	Visible Near Infrared	10	832.9
8A	Visible Near Infrared	20	864.0
11	Shortwave Infrared	20	1610.4
12	Shortwave Infrared	20	2185.7

Table 10: Composite image name and band combination order used in the creation of composite images using the scene obtained from Sentinel 2B for the mid-Winter period of 2020.

Composite Image Name	Band Combination Order
Natural Colour	4-3-2
Colour Infrared	8-4-3
Short Wave Infrared	12-8A-4
Agriculture	11-8-2
Geology	12-11-2

Training samples were drawn in each of the defined land categories. Training samples were evaluated to determine whether they were representative of each of the defined land categories by examining the spectral profile of each land categories' relative training samples (Figure 21).

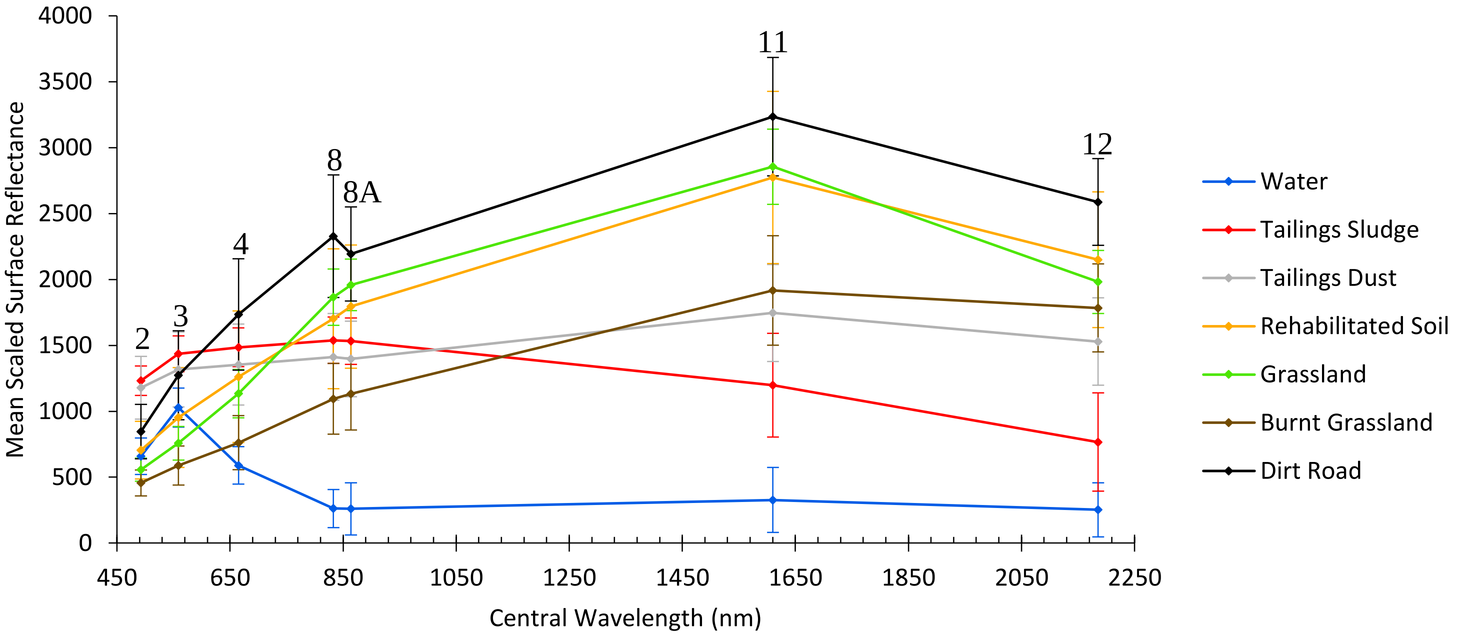


Figure 21: Mean scaled surface reflectance of training samples drawn in each land category at the central wavelength of each band used in composite images created using the Sentinel 2B scene. Specific band numbers are presented above each group of points.

The greatest degree of spectral separation between land categories was observed in the shortwave infrared (SWIR), band 11 and 12, region of the electromagnetic spectrum (Figure 21). Spectral separation between land categories was lower in the visible, band 2, 3 and 4, and visible near infrared (VNIR), band 8 and 8A, regions of the electromagnetic spectrum (Figure 21). The high spectral separation between land categories in band 11 and 12 led to the selection of the geology composite image, of band order and combination 12-11-2, as the image used in the classification process (Figure 22B).

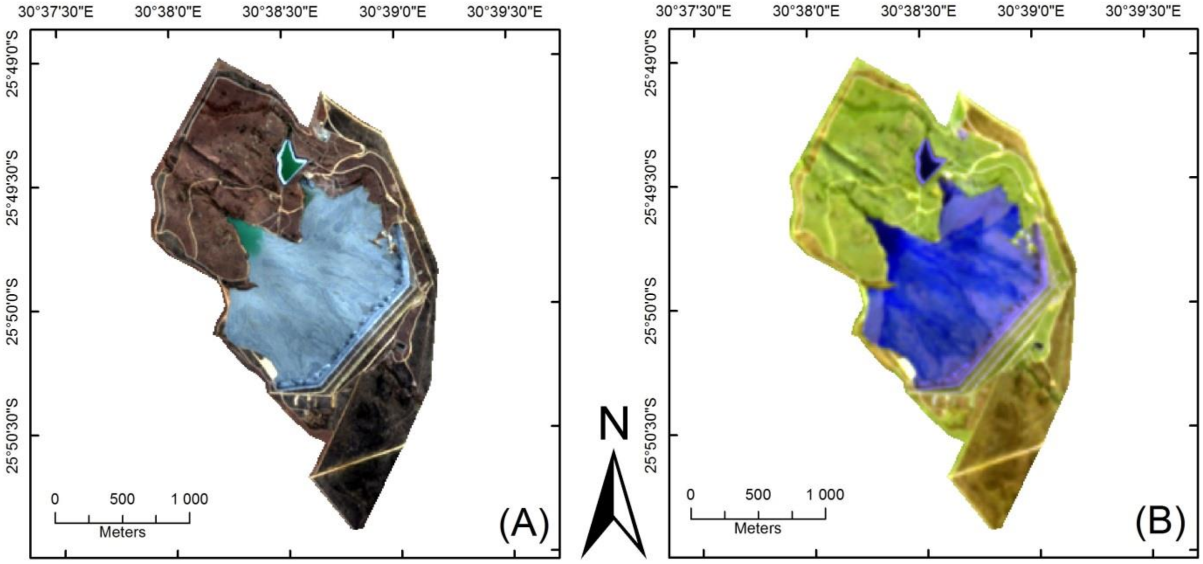


Figure 22: Comparison of 10 m pixel resolution composite images created using the Sentinel 2B scene. Natural colour image (A) constructed using band order: red (4), green (3) and blue (2). Geology image (B) constructed using band order: SWIR (12), SWIR (11) and blue (2).

Using the geology composite image (Figure 22B) and training samples defined using the natural colour composite image (Figure 22A) a raster image was created using the supervised image classification tool. The raster image, presenting the classification of pixels within the geology composite image into each of the seven defined land categories, was converted into a polygon shape file to extract the total area of each land category (Figure 23).

The classified image indicated varying areas (ha) of each land category (Figure 23). Land categories of tailings sludge, grassland and burnt grassland were the most dominant. While the land categories of water, tailings dust, rehabilitated soil and dirt road each covered a smaller area (Figure 23). The area (ha) covered by each land category indicated that the grassland and tailings sludge land categories covered an area greater than 100 ha (Table 11). The burnt grassland category covered an area of 88 ha, while the remaining land categories covered area’s ranging from 42 ha to 6 ha (Table 11).

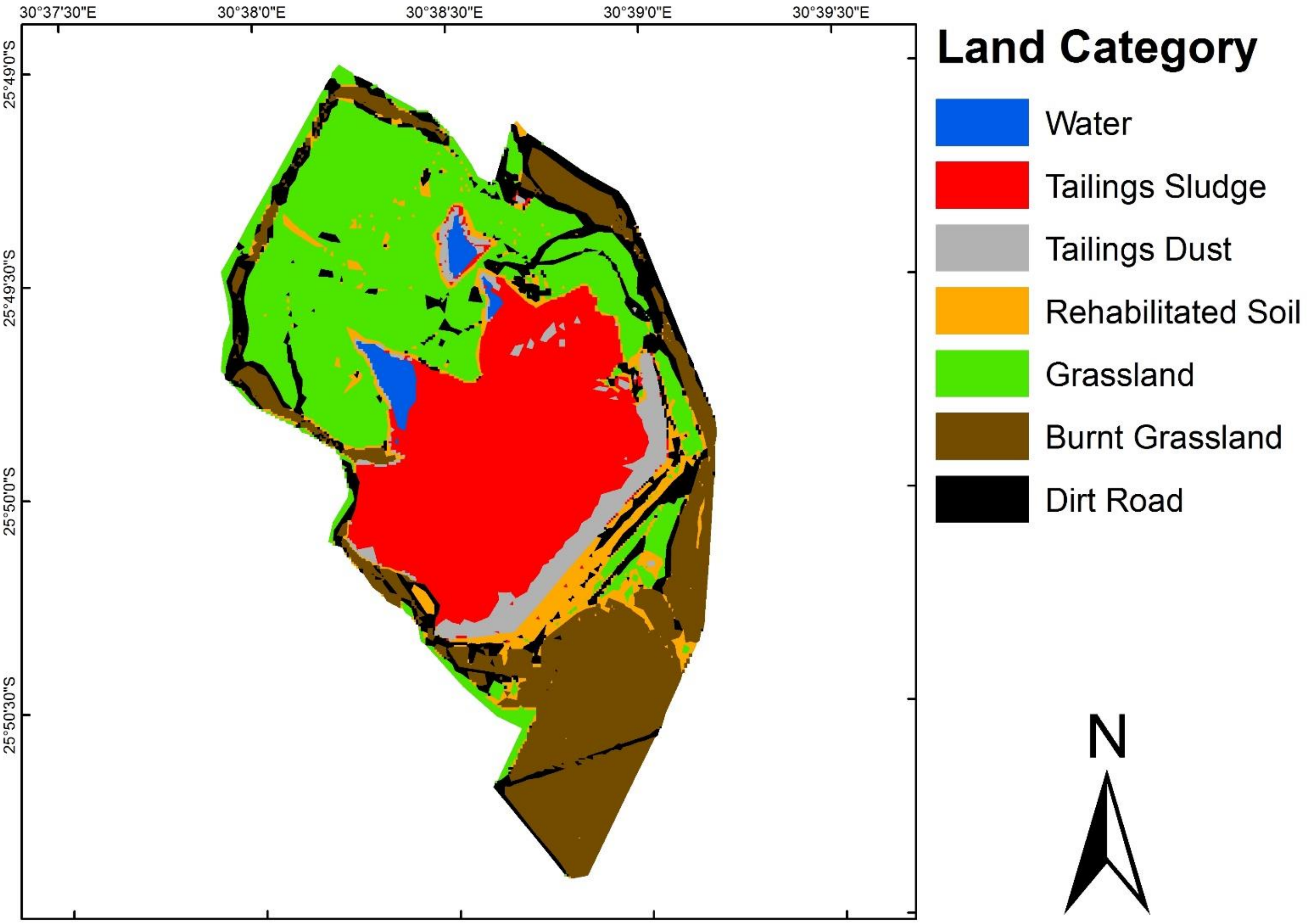


Figure 23: Result of supervised image classification of the geology composite image created using the Sentinel 2B scene into seven land categories.

Table 11: Area (ha) of land categories, in descending order, present at the Onverwacht TSF extracted from the supervised image classification of the Sentinel 2B geology composite image for the mid-Winter period of 2020.

Land Category	**Area (ha)**
Grassland	137
Tailings Sludge	112
Burnt Grassland	88
Dirt Road	42
Rehabilitated Soil	30
Tailings Dust	18
Water	6
Total Site Area	**433**

4.6 – Economic Estimations and Analysis

The results presented are estimations of the measured yield, based on data obtained from the greenhouse growth trial, and the expected yield, based on relevant literature (Arslanoglu *et al*. 2022, Bran *et al*. 2017, Harrison *et al*. 2019, Panoutsou and Alexopoulou 2020), of *H. cannabinus* and *L. usitatissimum* cultivated in available land areas at the Onverwacht tailings storage facility (TSF). The economic value, of measured and expected yield, of each species cultivated on each available land category is presented and compared to the estimated relative cultivation cost (Bran *et al*. 2017, Panoutsou and Alexopoulou 2020). Furthermore, the measured and expected amount of Mn, Zn, Ni, Cu, Cr and Co possibly extracted (g/ha) by *H. cannabinus* and *L. usitatissimum* cultivated in each land category is presented.

Hibiscus cannabinus and *L. usitatissimum* were grown under greenhouse conditions in normal, rehabilitated and tailings soil treatments. Supervised image classifications utilising Sentinel 2B atmospherically corrected imagery were conducted to determine the extent (ha) of different land categories present at the Onverwacht TSF. Five of the seven identified land categories present at the Onverwacht TSF were defined as available for *H. cannabinus* and *L. usitatissimum*, presently or in the future following the site's completion and remediation (Table 11&12). These land categories were grassland, burnt grassland, rehabilitated soil, tailings sludge and tailings dust. The sum of the grassland and burnt grassland land categories areas was used to represent the area of normal soil present onsite (Table 12). The area of the rehabilitated land category was used to represent the area of rehabilitated soil present onsite (Table 12). The sum of the tailings sludge and tailings dust land categories areas was used to represent the area of tailings soil present onsite (Table 12). Therefore, the areas of normal, rehabilitated and tailings soil present at the Onverwacht TSF were determined.

Table 12: Area of normal, rehabilitated and tailings soil treatments present at the Onverwacht TSF. Soil treatment areas were calculated by summing different land category areas, defined through Sentinel 2B supervised image classification, representative of each soil treatment.

Soil Treatment Represented	**Land Category Areas Summed**	**Area (ha)**
Normal	Grassland Burnt Grassland	225
Rehabilitated	Rehabilitated soil	30
Tailings	Tailings Sludge Tailings Dust	130

The measured yield (t/ha) of *H. cannabinus* and *L. usitatissimum* was calculated using the recommended plant density (plants/ha) for each species and the mean above ground mass (kg) of each species after six months of growth in each treatment soil (Table 13). The expected yield of *H. cannabinus* and *L. usitatissimum* cultivated at their recommended plant density are 10.00 and 7.64 t/ha respectively (Harrison *et al*. 2019, Arslanoglu *et al*. 2022). These values were used as the expected yield for the soil treatment in which *H. cannabinus* and *L. usitatissimum* exhibited the highest amount of increase in above ground mass during the greenhouse growth trial. These soil treatments were normal and tailings soil for *H. cannabinus* and *L. usitatissimum* respectively. The expected yield for each species cultivated in the remaining soil treatments was calculated by multiplying the maximum expected yield, 10.00 or 7.64 t/ha, by the relative percent difference observed between the mean above ground mass of *H. cannabinus* and *L. usitatissimum* grown in normal and tailings soil, respectively, and the other soil treatments. These values were then subtracted from the maximum expected yield which would provide an estimation of the expected yield of *H. cannabinus* and *L. usitatissimum* cultivated in the remaining soil treatments (Table 13).

Table 13: Mean above ground mass of 25 *H. cannabinus* and 50 *L. usitatissimum* plants after six months growth in normal, rehabilitated and tailings soil treatments. The measured and expected yield of each species cultivated in each soil treatment are presented.

Species	**Soil Treatment**	**Mean Above Ground Mass (kg)**	**Recommended Plant Density (plants/ha)**	**Measured Yield (t/ha)**	**Expected Yield (t/ha)**
H. cannabinus	Normal	0.011780	300 000	0.141360	10.00
	Rehabilitated	0.004344		0.052128	0.80
	Tailings	0.011616		0.139392	9.86
L. usitatissimum	Normal	0.002980	20 000 000	1.192000	7.26
	Rehabilitated	0.001276		0.510400	1.21
	Tailings	0.003130		1.252000	7.64

As expected, all measured yield values were lower than expected yield values. The lowest difference between these values was observed in *H. cannabinus* and *L. usitatissimum* cultivated in rehabilitated soil (Table 13). Interestingly, although *L. usitatissimum* cultivated in normal and tailings soil had lower mean above ground masses than *H. cannabinus*, the measured yield values of each were higher than those of *H. cannabinus* (Table 13). These values were also the only measured yield values that were greater than one tonne (Table 13).

The measured and expected total yield (t) of *H. cannabinus* and *L. usitatissimum* cultivated in the representative land areas at the Onverwacht TSF was calculated by multiplying the respective yield (t/ha) by the relative available land area (ha) (Table 12&13). The resultant yield values were then multiplied by the value (R/t) of *H. cannabinus* (R 1318.29/t) and *L. usitatissimum* (R 2636.57/t) as stated by Panoutsou and Alexopoulou (2020) and Bran *et al*. (2017). This provided estimations of the measured and expected total yield value of *H. cannabinus* and *L. usitatissimum* cultivated in different available land areas at the Onverwacht TSF (Table 14). Furthermore, the total estimated cultivation cost of *H. cannabinus* and *L. usitatissimum* was calculated by multiplying each land area (ha) by the cultivation cost (R/ha) of each species, R 6872.16 and R 9887.15 respectively at an exchange rate of 1 € = R 16.48 on the 15 June 2022 (Bran *et al*. 2017, Panoutsou and Alexopoulou 2020). Finally, the profit or loss of the measured and estimated total yields were calculated by subtracting the total estimated cultivation cost from the respective measured or expected total yield value (Table 14).

Table 14: Estimated total measured and expected yield, value, profit or loss margin and cultivation cost of *H. cannabinus* and *L. usitatissimum* cultivated in available land areas at Onverwacht TSF.

Species	**Soil Treatment**	**Measured Total Yield (t)**	**Measured Total Yield Value (R)**	**Expected Total Yield (t)**	**Expected Total Yield Value (R)**	**Estimated Total Cultivation Cost (R)**	**Measured Total Yield Profit/Loss (R)**	**Estimated Total Yield Profit/Loss (R)**
H. cannabinus	Normal	31.81	41 929.53	2250.0	2 966 152.50	1 546 236.00	-1 504 306.47	1 419 916.50
	Rehabilitated	1.56	2 061.59	24.0	31 638.96	206 164.80	-204 103.21	-174 526.80
	Tailings	18.12	23 888.68	1281.8	1 689 784.12	893 380.80	-869 492.12	796 403.32
L. usitatissimum	Normal	268.20	707 128.07	1633.5	4 306 837.10	2 224 608.75	-1 517 480.68	2 082 228.35
	Rehabilitated	15.31	40 371.16	36.3	95 707.49	296 614.50	-256 243.34	-200 907.01
	Tailings	162.76	429 128.13	993.2	2 618 641.32	1 285 329.50	-856 201.37	1 333 311.82

As expected, the measured total yield of *H. cannabinus* and *L. usitatissimum* were lower than the expected total yield (Table 14). Therefore, the total yield value of each species was lower than the expected total yield value (Table 14). Both species showed lower measured and expected total yield and yield value estimations when cultivated in rehabilitated soil (Table 14). While value estimations of both species were higher in those cultivated in normal and tailings soil (Table 14). The measured and expected total yield value of *L. usitatissimum* was consistently higher than *H. cannabinus* across soil treatments (Table 14). However, estimations of the measured total yield profit or loss margin of both species indicated that cultivation of each across soil treatments would result in a substantial loss (Table 14). Estimations of these loss margins were higher in *L. usitatissimum* than *H. cannabinus* when cultivated in normal and rehabilitated soil, the opposite is true when cultivated in tailings soil (Table 14).

The estimated total yield profit or loss margin of both species cultivated in normal soil were positive and indicated a profit of ~R 1.4 and ~R 2.1 million for *H. cannabinus* and *L. usitatissimum* cultivation respectively (Table 14). While a profit of ~R 800 000 and ~R 1.3 million was observed for *H. cannabinus* and *L. usitatissimum*, respectively, when cultivated in tailings soil (Table 14). However, both species cultivated in rehabilitated soil showed loss margins of ~R 200 000 (Table 14). Overall, cultivation of both species, based on greenhouse growth trial data, in each soil treatment would result in substantial monetary loss in which the loss incurred by *L. usitatissimum* would be greater than *H. cannabinus* (Table 14). However, cultivation of both species, based on expected yields from relevant literature, would result in monetary gain when cultivated in normal and tailings soil in which the gain produced by *L. usitatissimum* would be greater than *H. cannabinus* (Table 14). While cultivation of both species in rehabilitated soil would result in monetary loss (Table 14).

Estimations of the measured amount (g/ha) of Mn, Zn, Ni, Cu, Cr and Co extracted by *H. cannabinus* and *L. usitatissimum* cultivated in available land areas were calculated. This was done using the recommended plant density (plants/ha) of each species and the amount of each metal (mg) present in the mean above ground mass (g) of 25 *H. cannabinus* and 50 *L. usitatissimum* plants after six months growth in normal, rehabilitated and tailings soil. The same metal amounts and mean above ground mass were used in conjunction with the expected yield (t/ha) of each species to determine the expected amount of metal extracted

The measured amount of Mn extracted by *H. cannabinus* and *L. usitatissimum* was substantially higher than all other metals across soil treatments (Figure 24). The measured amount of Zn extracted by *H. cannabinus* and *L. usitatissimum* grown in normal and tailings soil was higher than the other remaining metals (Figure 24). While *L. usitatissimum* grown in normal soil also exhibited an increased amount of extracted Cu (Figure 24). The measured amount of Ni and Cr extracted by *H. cannabinus* grown in rehabilitated soil was noticeably high compared to other metals excluding Zn (Figure 24). While the measured amount of Ni extracted by *L. usitatissimum* grown in rehabilitated soil was higher than all other metals excluding Mn (Figure 24).

The measured amount of Mn extracted by *L. usitatissimum* was higher than *H. cannabinus* across soil treatments (Figure 24). The same is true for Cu and Zn extracted by *L. usitatissimum* grown in normal and tailings soil respectively (Figure 24). Contrastingly, the measured amount of Zn extracted by *H. cannabinus* was higher than *L. usitatissimum* across soil treatments (Figure 24). The same is true for the amount of Cr extracted by *H. cannabinus* grown in rehabilitated soil (Figure 24).

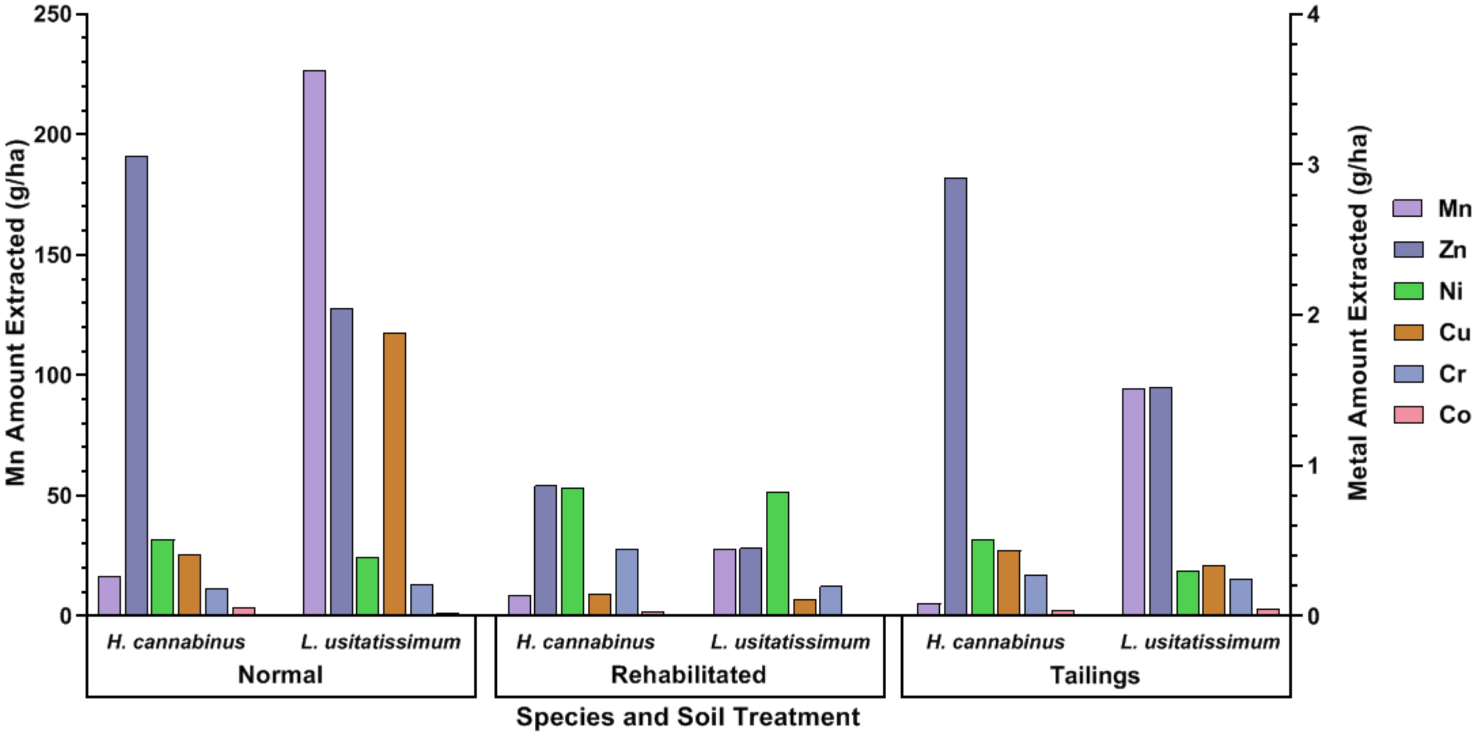


Figure 24: Measured amount of Mn, Zn, Ni, Cu, Cr and Co extracted per hectare by *H. cannabinus* and *L. usitatissimum* cultivated in normal, rehabilitated and tailings soil.

The measured amount Ni, Cu, Cr and Co extracted by *H. cannabinus* and *L. usitatissimum*, excluding the aforementioned comparisons, were similar within each soil treatment (Figure 24). Generally, the measured amount of all metals extracted by *H. cannabinus* and *L. usitatissimum*, excluding Mn, was low across soil treatments. Only the measured amount of Zn and Cu extracted by both species in some cases was higher than 1 g/ha. These results impact the applicability of both species as phytoremediation species in regard to soils contaminated by metals other than Mn.

However, the expected amount of each metal extracted by *H. cannabinus* and *L. usitatissimum* indicated that amounts, greater than 20 g/ha, of metals other than Mn could be extracted across soil treatments (Figure 25). The expected amount of each metal extracted showed the same general differences and similarities between and within *H. cannabinus* and *L. usitatissimum* across soil treatments compared to the measured amount extracted (Figure 25). However, these generalities were exacerbated in which the potential of *H. cannabinus* and *L. usitatissimum* to extract metals, specifically Mn and Zn, was more evident (Figure 25). The same is true regarding the amount of Cu extracted by *L. usitatissimum* grown in normal soil

(Figure 25). Interestingly, the expected amount of all metals extracted by *L. usitatissimum* was higher than *H. cannabinus* across soil treatments (Figure 25). The generally lower amounts of metals extracted, excluding Ni in *L. usitatissimum*, within rehabilitated soil may have been due to the lower rates of growth observed in *H. cannabinus* and *L. usitatissimum* (Figure 25).

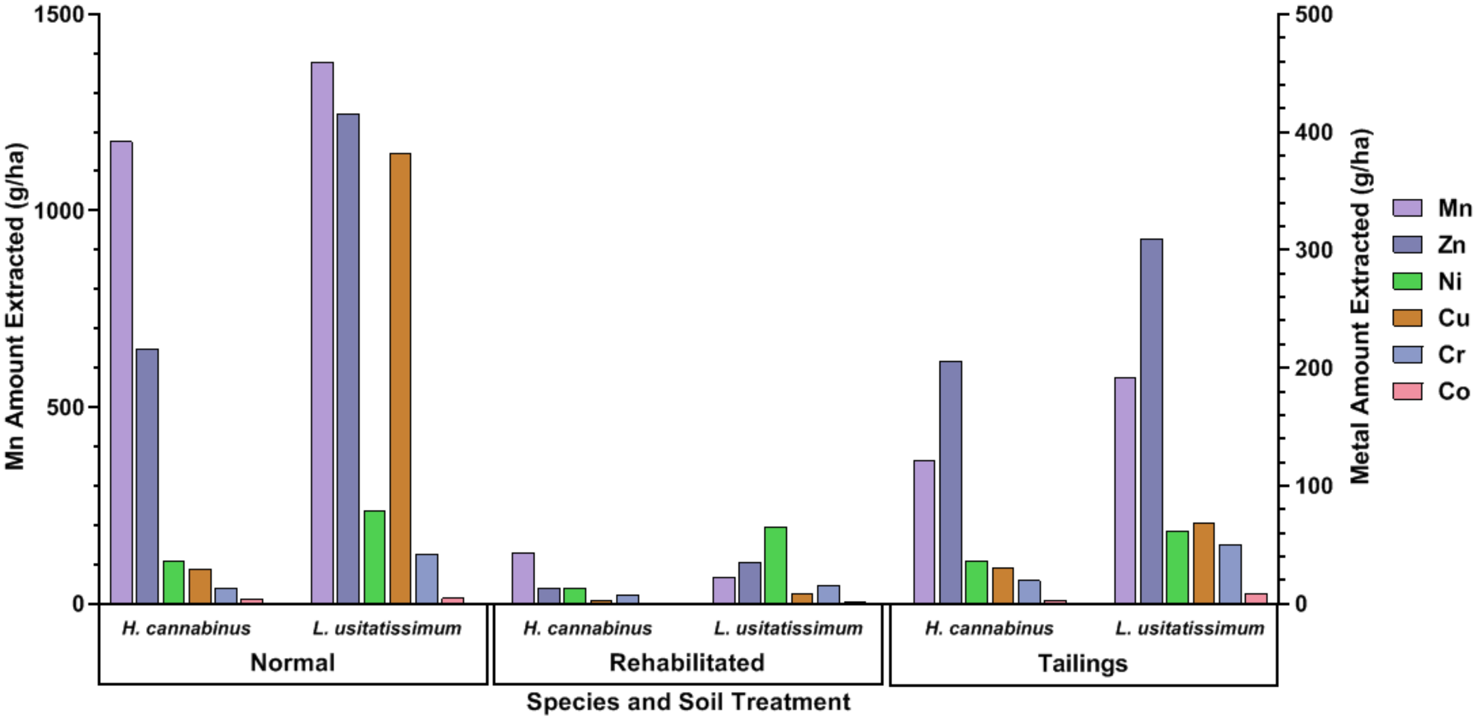


Figure 25: Expected amount of Mn, Zn, Ni, Cu, Cr and Co extracted per hectare by *H. cannabinus* and *L. usitatissimum* cultivated in normal, rehabilitated and tailings soil.

Overall, the measured yield, value and metal extracted by *H. cannabinus* and *L. usitatissimum* were low. Measured yields of *H. cannabinus* were higher than *L. usitatissimum* for each soil treatment while the cultivation of each species in available land areas would result in substantial monetary loss. The only noticeable amount of metal extracted was Mn in which *L. usitatissimum* grown in normal soil would have extracted ~230 g/ha. However, comparisons of the expected yield, value and metal extracted by *H. cannabinus* and *L. usitatissimum* exhibited promising results. The expected total yields of each species cultivated in available land areas were high in which *L. usitatissimum* exhibited higher expected total yields than *H. cannabinus*. However, the expected total yield of both species was substantially low when cultivated in rehabilitated soil. Excluding cultivation in rehabilitated soil both species exhibited the possibility of economic profit when cultivated in available land areas. The estimated total yield profit of *L. usitatissimum* in this case was higher than *H. cannabinus* even though the cost of cultivation was higher than *H. cannabinus*. Furthermore, the expected amount of metal extracted by *L. usitatissimum* was higher than *H. cannabinus* for each soil treatment. *Linum* *usitatissimum* also exhibited a substantially high amount of Cu extracted when cultivated in normal soil. Generally, the cultivation of *L. usitatissimum* in available land areas would result in greater metal extraction, particularly of Mn, Zn and in some cases Cu, and economic benefit compared to *H. cannabinus*. However, based on values calculated using the expected yield, both *H. cannabinus* and *L. usitatissimum* demonstrated the ability to extract various metals at considerable amounts while generating monetary gain when cultivated in either normal or tailings soil treatments.

5 – Discussion

5.1 – General Findings

Current and previous mining activities in South Africa have caused various environmental, human health and societal impacts. This has led to the formation and enforcement of legislation regarding the rehabilitation of current and previous mining landscapes (MPRDA 2002, Swart 2003). Mining landscapes in South Africa present an opportunity in which rehabilitation and economic gain can be achieved through the cultivation of economically valuable fibrous plant species on such landscapes. The purpose of this research was to determine whether *H. cannabinus* and *L. usitatissimum* could be successfully cultivated, producing an economically profitable yield while concurrently extracting various metals, in soils present at an existing mine site. These species were cultivated in normal (non-impacted), rehabilitated (previously impacted), and tailings (impacted) soil treatments, collected from the active Onverwacht tailings storage facility (TSF), over a six month-growth period.

The main findings of this study were that both *H. cannabinus* and *L. usitatissimum* exhibited substantially decreased growth when cultivated in rehabilitated soil. *Hibiscus cannabinus* and *L. usitatissimum* exhibited tolerance to low concentrations of Mn, Zn, Ni, Cu, Cr and Co in normal, rehabilitated and tailings soil treatments. Furthermore, both species exhibited the ability to extract varying amounts of each metal within the total, above and below ground plant mass components. Estimations of the measured yield and expected yield of each species cultivated in each soil treatment indicated that cultivation of *H. cannabinus* and *L. usitatissimum* onsite could possibly result in economic gain while removing metal contaminants from each soil. Finally, this research also demonstrated that the use of remote sensing and image classification could possibly be used to estimate crop yields and metal extraction over large areas using data obtained from greenhouse growth trials. These main findings will be discussed in Sections 5.2 – 5.5.

5.2 – *H. cannabinus* and *L. usitatissimum* Growth Response to Soil Treatments and Soil Physicochemical Variables

Throughout the six-month greenhouse growth trial *H. cannabinus* and *L. usitatissimum* grown in rehabilitated soil exhibited substantial decreases in multiple plant growth parameters (total length, above ground height, below ground root length, total mass, above and below ground mass, basal circumference and leaf number) compared to those grown in normal and tailings soil. However, differences in plant growth parameters of *H. cannabinus* and *L. usitatissimum* grown in normal and tailings soil were minimal. This finding indicates that the presence of moderately higher concentrations of Mn, Zn, Ni, Cu, Cr and Co in tailings soil had a negligible effect on the growth of *H. cannabinus* and *L. usitatissimum*. This in conjunction with the presence of higher concentrations of each metal in rehabilitated soil compared to those in normal soil implies that other soil variables negatively impacted the growth of both species.

The electrical resistance of rehabilitated soil was substantially higher than that of normal and tailings soil. Blizzard and Boyer (1980) and Newman (1969) demonstrated that soil electrical resistance and soil water content have a negative exponential relationship. Furthermore, Pozdnyakov *et al*. (2006) showed that increases in soil electrical resistance were positively correlated with restrictions to water movement through the soil matrix. Decreased soil water content and the impedance of water movement through the soil matrix of rehabilitated soil would impact the process of mass flow in plants grown in rehabilitated soil (Gardner *et al*. 1999, Pozdnyakov *et al*. 2006). This would result in a decrease in nutrient transport from soil, into plant roots, through the xylem and into the above ground plant mass (Gardner *et al*. 1999, Kramer 1944). Furthermore, overall plant transpiration would decrease (Gardner *et al*. 1999, Kramer 1944). The combination of these factors would decrease the growth of plants cultivated in rehabilitated soils. However, values of total N, K and C were consistently lower in rehabilitated soil compared to normal and tailings soil. The same is true for values of available P, organic C and soluble S.

The macronutrients N, P and S are fundamental constituents of proteins and nucleic acids in plant tissue (Marschner 2011). While K is critical to the metabolic functioning of plants, specifically in regard to pH stabilisation, osmoregulation, enzyme activation and membrane transport processes (Marschner 2011). A deficient supply of each to higher plants during cultivation directly impacts growth resulting in reductions in multiple plant growth parameters compared to plants with access to sufficient supplies of each (Marschner 2011, Passioura 2002). The presence of lower concentrations of total N and K, available P and soluble S in rehabilitated soil may further account for the substantial reductions in growth observed in *H. cannabinus* and *L. usitatissimum*. The detrimental effects of deficient supplies of N, P, K and S on *H. cannabinus* growth are confirmed by Hossain *et al*. (2011) and Salih *et al*. (2014) who observed positive linear relationships between *H. cannabinus* growth and increases in N, P, K and S supply. Similar studies which indicated the same general relationship between *L. usitatissimum* growth and N, P, K and S supply further confirm the effects of rehabilitated soil variables on the growth of *L. usitatissimum* (Abd Eldaiem and El-Borhamy 2015, Bakry *et al*.

2015, Taddese and Tenaye 2018, Xie *et al*. 2016). Rehabilitated soil also exhibited lower concentrations of total carbon and organic carbon compared to normal and tailings soil. This could also account for the reduced growth observed in *H. cannabinus* and *L. usitatissimum*.

Soil carbon is related to nutrient cycling, structure and water infiltration and water holding capacity (Kimble *et al*. 2007, Trivedi *et al*. 2018). Furthermore, soil carbon acts as an energy source for soil micro-organisms and is critical for the development and maintenance of mycorrhizal associations (Hoorman 2009). Therefore, soil carbon is fundamental to the maintenance and improvement of soil health, fertility and plant productivity (Kimble *et al*. 2007, Trivedi *et al*. 2018). Furthermore, the lower concentration of carbon within rehabilitated soil could account for the observed decreased nutrient concentrations and substantially higher soil electrical resistance (Gardner *et al*. 1999, Trivedi *et al*. 2018). The combination of these factors could further explain the significantly reduced growth of *H. cannabinus* and *L. usitatissimum* grown in rehabilitated soil.

A lower cation exchange capacity (CEC) was present in rehabilitated soil and could also add to the reasons for the reduced growth of *H. cannabinus* and *L. usitatissimum*. Soil CEC is a measure of the number of base cations (H^+, Ca^{2+}, Mg^{2+}, K^+ and Na^+) that can be adsorbed on soil particle surfaces, this dictates the soil's ability to supply nutrient cations to the soil solution (Chapman 1965, Marschner 2011). Therefore, soil CEC is directly related to soil fertility and plant productivity (Drake *et al*. 1951, Marschner 2011). The CEC of rehabilitated soil was lower than normal and tailings soil, while tailings soil had the highest CEC. Furthermore, the CEC of rehabilitated and normal soil were similar. Furthermore, differences in soil pH and organic carbon may have contributed to the observed differences in the growth of *H. cannabinus* and *L. usitatissimum* between soil treatments. These observations in conjunction with the observed differences in the growth of *H. cannabinus* and *L. usitatissimum* imply that the CEC of each soil may not have been the key soil variable of plant growth in this study. The same may be true of base saturation (BS) of each soil. Soil BS is defined as the percentage of base cations that occupy a soils CEC (Havlin 2020). Gaspar and Laboski (2016) demonstrated that BS has a direct relationship with soil fertility and crop yield, specifically due to the relationship between BS and phosphate availability within soils. However, the observed BS of each soil treatment in this study were similar, all soil treatments had a BS above 90% and were within 2% of one another. This observation indicates that the BS of each soil treatment was not the soil variable directly responsible for the differences observed in *H. cannabinus* and *L. usitatissimum* growth between soil treatments.

Overall, it is plausible that the observed differences in growth parameters of *H. cannabinus* and *L. usitatissimum* between soil treatments was due to varying soil physicochemical variables of each soil treatment. The substantially lower values of multiple plant growth parameters of both species grown in rehabilitated soil, compared to those grown in normal and tailings soil, may specifically be due to the greater soil electrical resistance of rehabilitated soil. However, it is also possible that a combination of this variable with the lower concentrations of total N, K and C, available P, organic C and soluble S led to the significant detrimental impact on *H. cannabinus* and *L. usitatissimum* growth in rehabilitated soil. Furthermore, the observation of minimal and negligible differences in growth parameters between *H. cannabinus* and *L. usitatissimum* grown in normal and tailings soil indicated that the presence of high metal concentrations in tailings soil had less impact on plant growth than other physicochemical soil variables. However, it is worth noting that the growth of each species may have been severely limited due to the small soil volume used as a growth medium in the greenhouse growth trial. This is due to constraints on the proliferation of plant root systems when grown in pots, which results in the phenomenon known as pot bound plants (Gardner *et al*. 1999). This phenomenon was evident in both *H. cannabinus* and *L. usitatissimum* as the roots of both species were curled and layered at the bottom of cultivation pots. Therefore, it is possible that growth trials conducted in field conditions may yield results in which the growth of *H. cannabinus* and *L. usitatissimum* grown in rehabilitated soil would not be as substantially lower than that of each species grown in normal and tailings soil. Furthermore, this limitation may have also impacted the accumulation of metals observed in both species across soil treatments.

5.3 – *H. cannabinus* and *L. usitatissimum* Phytoremediative Capability and Tolerance to Mn, Zn, Ni, Cu, Cr and Co

Determining the phytoremediative capability of a plant species is dependent on the method of phytoremediation in question (Gupta 2013, Khalid *et al*. 2017). The purpose of this research was to determine the phytoextractive capability of *H. cannabinus* and *L. usitatissimum*. This is defined as the ability of a species to extract, from soil or water, translocate and accumulate contaminants in their above ground biomass (Gupta 2013, Khalid *et al*. 2017, Van Nevel *et al*. 2007). Therefore, the accumulation of contaminants in the above ground biomass of plants used in phytoremediation can limit their subsequent use in industrial applications based on contamination thresholds of the relative industry (Gomes 2012, Gupta 2013, Mahar *et al*. 2016). Nevertheless, the extraction and accumulation of various metals was demonstrated by both *H. cannabinus* and *L. usitatissimum* across soil treatments. Accumulation of various metals was present in both the above and below ground mass of each species across soil treatments. However, the amount and concentration of metals were generally higher in the below ground component of each species. This finding indicated the ability of each species to extract metals which could result in soil rehabilitation (Van Nevel *et al*. 2007). However, this finding also indicated that both species may be successfully applied to phytostabalisation strategies for soil rehabilitation (Gupta 2013, Khalid *et al*. 2017). This method of phytoremediation is defined as the reduction in below ground contaminant migration into ecosystems through immobilisation and minimisation of their bioavailability (Gupta 2013, Khalid *et al*. 2017). The increased amounts and concentrations of metals within the below ground component of *H. cannabinus* and *L. usitatissimum* implies that each can be viably utilised as phytostabilisation species.

The results of this research revealed that within a multi-metal (Mn, Zn, Ni, Cu, Cr and Co) contaminated soil both *H. cannabinus* and *L. usitatissimum* consistently accumulated increased amounts and concentrations of Mn and Zn across soil treatments. These two metals are essential micronutrients required for plant growth (Marschner 2011). Manganese is fundamental to processes of photosynthesis, respiration and nitrogen assimilation. While Zn is utilised in enzyme activation, chlorophyll and auxin formation, and starch to sugar conversion (Marschner 2011). The fundamental roles of Mn and Zn in successful plant growth may account for their apparent selection and uptake by *H. cannabinus* and *L. usitatissimum*. Furthermore, *H. cannabinus* grown in rehabilitated soil exhibited a lower amount of Zn in the above ground component, while *L. usitatissimum* exhibited lower amounts of Mn and Zn. This finding may further account for the decreased growth observed in both species grown in rehabilitated soil (Marschner 2011). Furthermore, the low amount of Cu observed in *H. cannabinus* and *L. usitatissimum* grown in rehabilitated soil may also account for the decreased growth in both species. This could be due to the role of Cu in enzyme activation, lignin synthesis, respiration and metabolism (Marschner 2011).

The amount and concentration of Ni in both components of *H. cannabinus* and *L. usitatissimum* grown in rehabilitated soil were generally high compared to other metals. This observation was more evident regarding *L. usitatissimum*. As another essential micronutrient, although required in smaller concentrations, Ni acts as the activation site of the urease enzyme, which is utilised in nitrogen assimilation (Fabiano *et* al. 2015, Marschner 2011, Yusuf *et al*. 2011). Furthermore, Ni is accumulated into plant tissue as a divalent cation and therefore competes with other cations in plant selection for uptake (Hassan *et* al. 2019, Marschner 2011).

It is feasible that the low total N concentration present in rehabilitated soil may have caused both species to increase urease production to utilise sources of nitrogen in the form of urea (Polacco and Holland 1993). This would lead to an increase in each species uptake of Ni, thus possibly restricting the uptake of other metals present in the soil (Polacco and Holland 1993, Yusuf *et al*. 2011). Furthermore, although Ni accumulation in both species grown in rehabilitated soil was noticeably high, the accumulation of Mn was concurrently high. This suggests that both species consistently engaged in Mn accumulation regardless of other soil physicochemical variables across soil treatments. Finally, *H. cannabinus* and *L. usitatissimum* grown in tailings soil exhibited increased accumulations of Ni and Cr. While the accumulation of Cr was more evident regarding *L. usitatissimum*. The increased accumulation of Ni, particularly in the below ground component, observed in both species grown in tailings soil may have been due to the substantially higher Ni concentration present in tailings soil. This finding confirms those of Gupta (2013) and Chandra *et al*. (2017) who observed and demonstrated that the majority of non-hyper-accumulating species retain the majority of Ni within the plant root system. This Ni retention is accomplished through Ni sequestration at cation exchange sites and within root vascular cylinders (Hassan *et al*. 2019, Yusuf *et al*. 2011). Therefore, *H. cannabinus* and *L. usitatissimum* have demonstrated the ability to accumulate Ni, within their root system, and exclude Ni, from their above ground component, when grown in soils with high Ni concentrations. Additionally, increased Cr accumulation was present in the below ground component of each species.

Reale *et al*. (2016) and Oliveira (2012) state that currently Cr has failed to exhibit any fundamental role in plant physiology and metabolism. Therefore, no specific mechanism exists for Cr uptake in plant species (Oliveira 2012). However, Cr in a hexavalent form is soluble in water and can thus be transported into plant tissue through the process of mass flow (Cervantes *et al*. 2001). Yet, Cr^{6+} is converted to its insoluble form Cr^{3+} within plant tissue (Kabata-Pendias and Szteke 2015, Shahid *et al*. 2017). This conversion restricts Cr translocation to the above ground plant component as Cr^{3+} displays the propensity to adhere to plant cellular walls (Kabata-Pendias and Szteke 2015). The accumulation of Cr in plant root tissue causes nutrient imbalances, root injury and leaf chlorosis (Samantaray *et* al. 1998, Shahid *et al*. 2017). Furthermore, high concentrations of Cr can cause enzyme inhibition and oxidative stress (Samantaray *et* al. 1998, Shahid *et al*. 2017). These effects of Cr on plant physiology directly inhibit plant growth (Marschner 2011). However, neither *H. cannabinus* nor *L. usitatissimum* exhibited substantial decreases in growth while containing increased Cr concentrations in their below ground component when grown in tailings soil. This pattern of accumulation is indicative of Cr tolerance, as Mangabeira *et al*. (2011) stated that sequestration of Cr in root cell vacuoles may act as a protective mechanism in plants. Therefore, the minimal impact of Cr on the growth of *H. cannabinus* and *L. usitatissimum*, along with increased Cr accumulation in both species' below ground component suggests that both species have the ability to tolerate and immobilise Cr present in soil.

Generally, the patterns of metal accumulation and selection were similar between *H. cannabinus* and *L. usitatissimum* within each soil treatment. Furthermore, increased amounts and concentrations of each metal were generally present in the below ground component of each species, while lower amounts and concentrations were present in each species' above ground component. This suggests that both species could be utilised as phytoremediation species in both phytoextraction and phytostabilisation strategies of contaminated soil. Overall, the selection and accumulation of metals by each species based on this research is ranked in the descending order of Mn > Zn > Ni > Cu > Cr > Co.

A useful finding was that although *L. usitatissimum* generally exhibited higher accumulated metal concentrations than *H. cannabinus*, the accumulated metal amounts between the two species were generally similar. This was due to the inherently greater biomass of *H. cannabinus* (Kozlowski *et al*. 2005). The total extractive ability of the plant is the product of the mass of the plant tissue and the metal concentration in the tissue. The majority of phytoremediation research and its applications has focused on the use of hyper-accumulating species for specific metals (Gupta 2013, Khalid *et al*. 2017). These species are defined as plants with the ability to tolerate and accumulate metals within their biomass at higher concentrations than those present in their growth medium (Rascio and Navari-Izzo 2011). Multiple studies have demonstrated the phytoremediative potential of hyper-accumulating species, in which selected species have accumulated high concentrations of various metals (Bhargava *et al*. 2012, Mahar *et al*. 2016, Raskin *et al*. 1997, Schwitzguébel 2002). However, the outcome of using hyper-accumulating species, which usually have an inherently low biomass, could be that a low amount of metal is extracted regardless of higher concentrations present in plant biomass (Vamerali *et al*. 2010, Vangronsveld *et al*. 2009). This raises the issue as discussed in Yan *et al*. (2020), whether plant species, of high biomass, extracting relatively lower metal concentrations and perhaps of economic value could be used to achieve the same or similar results as hyper-accumulating species. Overall, both *H. cannabinus* and *L. usitatissimum* exhibited potential for use in phytoremediation. Specifically for soils contaminated with Mn, Zn and/or Cr.

5.4 – *H. cannabinus* and *L. usitatissimum* Economic Value

This research attempted to utilise growth and metal accumulation data of *H. cannabinus* and *L. usitatissimum* grown in greenhouse conditions, in soils collected from an active tailings storage facility, to extrapolate each species growth and metal extraction if cultivated in available land areas onsite. This extrapolation of greenhouse data was then used to determine whether cultivation of each species onsite would produce economic gain while extracting various metals present in onsite soils. Utilisation and extrapolation of cultivation parameters, measured yields, expected yields and economic values of *H. cannabinus* and *L. usitatissimum* to onsite cultivation revealed that both species could possibly produce economic gain if cultivated onsite. Furthermore, both species would possibly extract substantial amounts of various metals, particularly Mn and Zn, when cultivated onsite. This finding indicates that the cultivation of *H. cannabinus* and *L. usitatissimum* concurrently as phytoremediation and industrial plant species would perhaps be possible.

The economic value of *H. cannabinus* and *L. usitatissimum* is due to their use in the manufacture and production of various fibre-based products (Ramesh *et al*. 2017). These products commonly include textiles, polymer composites, paper, cordage, hempcrete, insulation boards and bioenergy (Amaducci and Gusovius 2010, Chen and Liu 2010). Allen *et al*. (2019) identified multiple “fibrous frontier products” through economic complexity analytics for the development and diversification of the South African fibre industry. Fibre products available for the South African industry could include non-woven and woven textiles, motor vehicle parts, paper and pulp products, bioethanol and various wood products (Allen *et al*. 2019). The possibility for development of a South African fibre industry in conjunction with the need and requirement to rehabilitate large areas of degraded active, closed and/or abandoned mine land provides an opportunity to use fibrous plant species as dual-purpose crops (Harrison *et al*. 2019, Mostert *et al*. 2019, MPRDA 2002). This research has demonstrated the phytoremediative ability of *H. cannabinus* and *L. usitatissimum*, further showing that the cultivation of each onsite could produce financial gain while extracting and/or immobilising metals, thus rehabilitating contaminated soils. However, limitations exist within this study and in the application of fibrous plant species as phytoremediative and industrial crops.

The results of this research would benefit from an onsite growth trial, thus allowing for greater accuracy in estimating yield and metal extraction potentials of *H. cannabinus* and *L. usitatissimum* over large areas. Furthermore, the presence of metals in plant biomass may limit their application in fibre product manufacture (Gomes 2012, Gupta 2013, Mahar *et al*. 2016). This is dependent on the amount of metal contained within plant biomass and the desired final product of that plant biomass (Gupta 2013, Mahar *et al*. 2016). Plant biomass produced in metal contaminated soil would need to fall below contamination thresholds relative to the final manufactured product (Gupta 2013, Gomes 2012, Mahar *et al*. 2016). However, based on this research and multiple other studies, the use of fibrous plant species as concurrent phytoremediation and industrial crops is still possible (Griga and Bjelková 2013, Mukhtar *et al*. 2019, Saba *et al*. 2015, Saleem *et al*. 2020). Furthermore, this rehabilitation strategy could possibly fulfil the requirements of mine rehabilitation stated within the Mineral and Petroleum Resources Development Act (MPRDA) of 2002. The requirements stated in the MPRDA (2002) include rehabilitation, skills transfer, job creation and development of post mine land use to active, closed and abandoned mines throughout South Africa. This research has suggested that each of these requirements could possibly be fulfilled through the cultivation of *H. cannabinus* and *L. usitatissimum*, or perhaps other fibrous plant species, on land areas previously or currently impacted by mining activities. This notion has recently begun to gain traction and momentum in multiple sectors of government and scientific research in South Africa (Harrison *et al*. 2019, Mostert *et al*. 2019).

5.5 – The Use of Remote Sensing to Estimate Crop Yield and Metal extraction

This research utilised remote sensing, specifically the process of supervised image classification (SIC), to estimate the extent of available land areas present onsite for the cultivation of *H. cannabinus* and *L. usitatissimum*. The land area extent results obtained from the SIC process were used to extrapolate growth and metal extraction data from the greenhouse growth trial to estimate each variable in a scenario where *H. cannabinus* and *L. usitatissimum* are cultivated onsite. The extrapolation of various greenhouse growth data sets in combination with SIC and/or other remote sensing techniques and processes could prove useful in estimating in field crop growth for different research applications. Furthermore, it is possible that the use of SIC and greenhouse growth data sets could be used to estimate crop yield over larger areas than those addressed in this research. The existence of remote sensing, digital soil mapping and crop growth modelling techniques, that are of greater complexity and accuracy than those used in this research, are available (Hengl and Macmillan 2019, Huang *et al*. 2019, Maas 1988, Scull *et al*. 2003, Spitters 1989). Furthermore, remote sensing techniques which predict and model the presence and concentration of soil metal contamination exist (Shi *et al*. 2018, Wu *et al*. 2011). The combination of these techniques, which would allow for scaling up and out, could aid in decisions regarding the application and commitment to the remediation of metal contaminated soil through the cultivation of various economically valuable field crops.

5.6 – Limitations and Recommendations for Future Studies

The methodology used in this research could be improved to possibly produce additional and/or more accurate results. The soils used in this research had varying physicochemical parameters and metal concentrations. This impacted both plant growth and metal accumulation in each species. Additions of nutrients in the form of fertilizer to each soil may have reduced variations in plant growth observed within each species. This would possibly increase the amount of and perhaps type of metal extracted by each species. Furthermore, the varying metal concentration present in each soil treatment impacted the amount and selection of metals extracted by each species. The addition of an equal amount of each of the tested metals to soil treatments would allow each species to select and extract metals from an equalized pool. This would possibly yield different results in regard to the selection and amount of metal extracted by *H. cannabinus* and *L. usitatissimum*. Additionally, this research may have benefitted from comparing the growth and metal extraction of *H. cannabinus* and *L. usitatissimum* between a control soil, i.e., normal soil, and soil treatments each containing increased amounts of tailings dust, i.e., normal soil mixed with increasing amounts of tailings dust in predetermined ratios. Furthermore, the growth of both species was inherently impacted due to their cultivation in the limited amount of soil used in the greenhouse growth trial. This limitation could be addressed by conducting greenhouse growth trials using larger cultivation pots containing greater soil volumes. However, plant cultivation in field conditions, in which the proliferation of plant root systems is not constrained, would enhance the growth of each species. This would allow for increased accuracy in estimations of each species yield and metal extraction potential. Finally, future studies investigating the use of *H. cannabinus* and *L. usitatissimum*, or other fibrous species, would benefit by comparing plant growth and metal extraction potential to a selected hyper accumulating species cultivated in the same conditions.

6 – Conclusion

The aim of this research was to assess the ability of *H. cannabinus* and *L. usitatissimum* to grow in and extract metals from soil contaminated with nickel and chrome mine tailings and to determine if these species could be utilised concurrently as phytoremediative and economically beneficial plant species. Based on the objectives of this study both *H. cannabinus* and *L. usitatissimum* exhibit potential as both phytoremediative and economically beneficial plant species.

The first objective of this research was to determine whether the growth and subsequent yield of *H. cannabinus* and *L. usitatissimum* was impacted when cultivated in soils associated with mining and soils contaminated with nickel and chrome mine tailings. *Hibiscus cannabinus* and *L. usitatissimum* exhibited substantially reduced growth when cultivated in rehabilitated soil. However, each species exhibited minimal differences in growth parameters between those grown in normal and tailings soil. This suggested that *H. cannabinus* and *L. usitatissimum* were tolerant to varying concentrations of Mn, Zn, Ni, Cu, Cr and Co.

The second objective of this research was to determine whether *H. cannabinus* and *L. usitatissimum* could successfully extract metals present in soils associated with mining and soils contaminated with nickel and chrome mine tailings. Therefore, determining each species' phytoremediative capability. *Hibiscus cannabinus* and *L. usitatissimum* demonstrated the ability to extract varying amounts of Mn, Zn, Ni, Cu, Cr and Co within each soil treatment. Both species were observed to extract higher amounts of Mn and Zn, suggesting that either could be used in phytoextraction strategies of soils contaminated with Mn and/or Zn. However, both species exhibited higher accumulation in the below ground plant component. This suggests that either species could be used in phytostabilisation strategies of soils contaminated with Mn, Zn, Ni, Cu and Cr.

The third objective of this research was to determine whether the cultivation of *H. cannabinus* and *L. usitatissimum* in soils associated with mining and soils contaminated with nickel and chrome mine tailings would result in economic gain. Therefore, determining whether each species can be utilised in or developed into a sustainable fibre industry providing profit to both mine and fibre industry stakeholders. Based on the extrapolation of measured yields to onsite available cultivation areas, cultivation of both species would result in economic loss while removing relatively low amounts of metal. However, based on the extrapolation of estimated yields from relevant literature sources, cultivation of both species would result in economic gain when cultivated in normal or tailings soil present onsite. The same cannot be said for both species when cultivated in rehabilitated soil present onsite.

Overall, *H. cannabinus* and *L. usitatissimum* exhibited phytoremediative and economic potential. Aspects of the current state of mine impacted land in South Africa and the requirements of rehabilitation enforced through South African legislation could possibly be addressed through the application of *H. cannabinus* and *L. usitatissimum,* or other economically valuable fibrous plant species, to mine rehabilitation strategies.

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