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CONTAMINANT FATE IN *SEARSIA LANCEA* WOODLANDS ON ACID MINE DRAINAGE IN THE WITWATERSRAND BASIN GOLDFIELDS

By

Maxine Kelly Joubert

A Dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg in fulfilment of requirements for the degree Master of Science.

13 October 2017, Johannesburg

Contaminant fate in *Searsia lancea* woodlands on acid mine drainage in the Witwatersrand Basin goldfields

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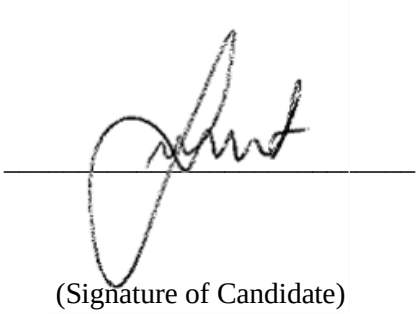
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DECLARATION

I declare this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



(Signature of Candidate)

Signed at Randburg on 13 October 2017.

ABSTRACT

There has been increasing research into plants used for phytoremediation, specifically for phytoextraction and phytostabilisation of heavy metals in soil. There has been little research on trees for their large biomass, especially field studies. There is also a lack of research on trees in South Africa specifically. This study evaluated the fate of contaminants (Na, Mg, Al, S, Cl, Cd, Cr, Mn, Fe, Cu, Zn, and Pb) in *Searsia lancea*, a tree native to South Africa, planted in woodland trials for phytostabilisation and hydrological control on AngloGold Ashanti mining properties, at the base of tailings storage facilities as part of the Mine Woodlands Programme – a collaboration between the University of the Witwatersrand and AngloGold Ashanti.

Trees of average height were harvested from three out of four *S. lancea* plots at four different sites; two sites at the West Wits mining operations (Madala and Redsoils), and two sites at the Vaal River mining operations (West Complex and Mispah). One site at each mining operation had nutrient-poor soil, and one site had nutrient-rich soil for plant growth. Harvesting of above-ground biomass took place first, in which the tree compartments were separated into wood (stems), twigs, and leaves. These were bagged and weighed, and then dried naturally. Sub-samples of wood, twigs, and leaves were taken after weighing the bulk samples. These sub-samples were washed, freeze-dried, and ground using ceramic burr grinders for analysis. Tree roots were excavated using a backacter (TLB), which then proceeded to dig soil pits roughly 2.5 x 3 x 3 m for soil sample collection. Sub-samples of coarse roots and fine roots were taken, but roots were bagged and weighed together. Sub-samples of roots were also washed, freeze-dried, and ground using a ceramic burr grinder for analysis.

Soil samples were taken at certain depths within the pits (0-2, 2-5, 5-10, 10-15, 15-20, 20-30, 40-50, 50-60, 60-70, 70-80, 90-100, 120-130, 145-155, 170-180, 190-210, 240-260 and 280-300 cm). These were bagged and sent for analysis of pH, Electrical Conductivity (EC), and Reduction-Oxidation Potential (Eh). All samples were analysed by X-Ray Fluorescence (XRF). The Mann-Whitney U Test and a non-parametric analysis of variance (Kruskal-Wallis) were used to test for

significant differences in contaminant distribution. Post-hoc pairwise comparisons were performed using Dunn's procedure with a Bonferroni correction for multiple comparisons to test for specific differences between soils (sites), tree compartments and soil using IBM SPSS statistics 24. Bioconcentration Factors (BCF) and Translocation Factors (TF) were calculated to assess the phytostabilisation and phytoextraction abilities of *S. lancea*.

The fate of contaminants was found to be different for different contaminants. Sulphur and Mn were highest in the leaf compartment; Chlorine, Cu, and Zn were highest in the twig compartment; no elements were found to be highest in the wood compartment; Mg and Fe were highest in the coarse roots; and Ca was highest in the fine root compartment. It was also found that *S. lancea* is an accumulator of S, Cl, and Ca with levels of 2 508.92, 2 500.96 and 16 733.46 mg/kg respectively. *Searsia lancea* appears to be a Al, Fe, and Cr stabiliser with TFs < 1 and translocates metals in the sequence Ca > Na > Fe > Mg > Zn > S > Mn > Pb > Cu > Al > Cl > Cr.

BCF results show that *S. lancea* is more of an accumulator than a stabiliser as BCF root: soil pattern was found to be Cl > S > Cu > Cr > Zn > Mn > Mg > Na > Pb > Ca > Al > Fe; while BCF shoot: soil pattern was found to be > 1 in the sequence Cl > S > Ca > Na > Mg > Zn > Cu > Mn > Pb > Cr > Fe > Al, with Al, Cr, Fe, and Pb higher in soil compared to shoot concentrations.

This study demonstrates that certain indigenous tree species are capable of phytoremediation of contaminated sites and that larger biomass species can take up great elemental masses of certain elements.

Key words: phytoremediation, phytostabilisation, phytoextraction, native trees, mine pollution, *Searsia lancea*

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CHAPTER 1

AN INTRODUCTION TO PHYTOREMEDIATION AND THE MINE WOODLANDS PROJECT

1.1 Introduction

The Witwatersrand Basin Goldfields (WBG) is a landscape visibly polluted by tailings storage facilities (TSFs) that are not only unappealing aesthetically, but are polluters of the environment and a health hazard to the public. Reichardt (2013) eloquently unravelled the '*history of mine waste rehabilitation methodology in the South African mining industry from its origins to 1991*', in which he tells of how the industry has not changed its rehabilitation focus for decades – in part due to mismanagement and unguided decision-making, but also due to lack of collaboration and scientific understanding of the environment.

TSFs and their footprints (areas of contaminated soil and residual slimes where tailings have been removed for re-mining) cover about 400 km^2 in the WBG, which works out to about 6 billion tonnes of gold and uranium tailings with an estimated 430 000 tonnes of uranium and 30 million tonnes of sulphur contamination (Weiersbye *et al*., 2006). The volume of waste generated by mining in South Africa was calculated at the rate of 315 million tonnes per annum in 2006 – of which 105 million tonnes was from gold mining alone, at a rate of 200 000 tonnes of waste per ton of gold produced (Weiersbye *et al*., 2006; Weiersbye and Witkowski, 2007). TSFs are also prone to erosion and the production of acid mine drainage (AMD), which have severe impacts on nutrient cycling in polluted soils, on the regeneration of vegetation, and on the biogeochemical cycling of potentially toxic elements (Weiersbye *et al*., 2006). Due to TSFs being so widespread, pollution problems and effective rehabilitation measures are difficult.

Now, under the Minerals and Petroleum Resources and Development Act (MPRDA) Act No. 28 of 2002, it is stated that effective pollution control and rehabilitation measures on TSFs and impacted sites should be demonstrated before a mine closure certificate can be issued (Rossow *et al*., 2009). Most TSFs in the WBG are planted with pasture grass species aimed at rapid attenuation of dust, and as a form of erosion control, but this does not reach long-term site closure objectives and is very costly (Weiersbye *et al.,* 2006; Rossow *et al.,* 2009). Thus, a more ecologically meaningful rehabilitation technique needs to be implemented using indigenous vegetation that minimises site emissions to air,

water, soil, and biota as biodiversity is considered fundamental to ecosystem function and resilience to stress or change, and is thus considered essential to rehabilitation (Cousins and Lindborg, 2004; Rossow *et al.*, 2009).

Today, the field of mine waste rehabilitation is slowly moving away from engineering-based leadership and decision-making, toward a more ecology-based practice, with sound scientific research and collaboration. This is mostly being driven by legislation, but also in part by stakeholders (e.g. local communities) that want mining houses to not only prevent complete destruction of these environments, but to also prevent health and safety hazards.

1.2 Background

1.2.1 Mine Waste and Legislation

Mining has been going on in South Africa since 1886 - soon after gold was discovered – and specifically in the Wonderfonteinspruit area for over 120 years (Naicker et al., 2003; Winde and Stoch, 2010). The Witwatersrand Basin contains the world's largest known gold deposit, and within one year gold mining had extended 30 km's to the west of Johannesburg (Winde and Stoch, 2010). Although the gold mining industry in South Africa began to decline in the 1970s, the country is still one of the top three producers of gold in the world (Winde and Stoch, 2010). Gold mining waste produced in South Africa, compared to other types of waste, was estimated to be around 47% (221 million tons) making it the largest source of mineral waste pollution (Oelofse et al., 2007).

The oxidation of pyrite and other sulphides a few meters into the outermost layer of tailings dams, has occurred because most tailings have been undisturbed for almost a century, leaving them exposed to oxygenated rainwater, and causing the formation of acid mine drainage (AMD) and the pollution of water, soils, and sediments, along with the destruction of ecosystems (Naicker *et al*., 2003; Oelofse *et al*., 2007). The contaminants in the TSFs vary in concentration depending on the age of the fines (the material the TSF is made of), the level of exposure to water, depth of the

contaminants, and the position of the TSF itself (Herbert, 2009). This leads to differential rates of water movement and varying levels of contamination in seepage-affected areas.

Dispersion of trace and metal elements from tailings storage facilities (TSFs) by wind energy also occurs, as mining sites usually have high levels of erosion due to wind and water runoff (Navarro *et al*., 2008). This problem is exacerbated in South Africa as most mining operations are located on, or close to, watersheds (which is also unfavorable with regards to water management) and receive relatively low, erratic, and seasonal rainfall with high evaporation rates and a low ratio of runoff to precipitation (Coetzee, 2004). These factors are directly related to the flow of pollutants, the mechanics of which are as complex as the manner in which they pollute the land. Remediation is thus a challenge for anyone involved in this field. Thankfully South Africa has made huge strides in its environmental legislation.

Before 1991 South Africa had barely any legislation geared towards environmental protection from mining impacts – there were only recommendations and statutes for the structure and abandonment of TSFs (Weiersbye *et al.*, 2006). Mines did not have any obligation to prevent dust pollution – let alone protect ecosystems and their services – until the promulgation of the Atmospheric Pollution Prevention Act 45 of 1965 (amended in 1973). Thereafter, the Chamber of Mines Guidelines from 1979 recommended that wind and water erosion of TSFs be controlled by the most practical means possible using the BATNEEC (Best Available Technology Not Entailing Excessive Cost) concept (Weiersbye *et al*., 2006). Erosion control of the past involved covering the surface of TSFs with waste rock or vegetation (grassing) and grassing is still considered the best form of dust control in the industry – looking at its fast establishment rather than its long-term persistence and erosion control (Weiersbye *et al*., 2006).

Proper legislation only came about with the establishment of the new constitution, specifically the National Environmental Management Act (NEMA) no. 107 of 1998. The Mineral and Petroleum Resources and Development Act (MPRDA) no. 28 of 2002 stems from NEMA and section 24 of the

South African constitution and applies directly to mining, in which it specifies that effective pollution control and rehabilitation measures on TSFs and impacted sites should be demonstrated before a mine closure certificate can be issued. Grassing and waste rock coverage of TSFs for erosion and dust control were, and still are, considered the best control measures in the industry (Weiersbye *et al*., 2006). Grassing is fast to establish and is thus looked at as a quick-fix to these problems, rather than its long-term persistence and erosion control capabilities (Weiersbye, *et al.*, 2006). Halliday (1978) predicted that grassing was only a temporary solution to a long-term problem (the process does abate wind borne erosion in the short-term). This method has been shown to be economically and environmentally unsustainable and Weiersbye (2007) states that only biological technologies and treatments allow for decontamination and rehabilitation simultaneously.

Weiersbye's opinion has been shared with some others in the literature (Pulford and Watson, 2003; Dickinson and Pulford, 2005). These technologies, commonly referred to as phytoremediation, are generally seen as more sustainable and economical compared to past engineering options (pneumatic fracturing, solidification or stabilisation, vitrification, excavation and removal of contaminated soil layers, physical stabilisation with grasses, and washing of contaminated soil with strong acids or heavy metal chelators, among others) (Bhargava *et al.*, 2012). Phytoremediation options will be the focus of this chapter as this study focuses on these applications.

1.2.2 Phytoremediation

Phytoremediation is much more cost effective than chemical and physical treatments and is safe anthropogenically and environmentally. It also offers simultaneous decontamination and ecological rehabilitation of sites (Garbisu and Alkorta, 2001; Regnier *et al*., 2009). In basic terms, phytoremediation is the use of plants to remove, transfer, stabilise, and/or degrade contaminants in different media (Hughes *et al*., 1997; Padmavathiamma *et al*., 2014). Phytoremediation is specifically defined by Garbisu and Alkorta (2001: 273) as "*an emerging green technology that uses plants to remediate soil, sediment, surface water and groundwater environments contaminated with toxic metals, organics, and radionuclides*".

Pulford and Watson (2003) also have a good definition of phytoremediation. They state that phytoremediation is the use of plants to either render contaminants harmless or to remove them from the environment. Phytoremediation is considered an *in-situ* remediation strategy which preserves ecosystems and avoids dramatic landscape disruption (Lasat, 2000). The costs of this type of remediation are variable and dependent on the type of contamination, the condition of the site, and the soil properties. The costs are, however, considerably lower than conventional engineering options – sometimes ranging from 2 to 4 times lower (Cunningham and Ow, 1996; Lasat, 2000; Rockwood *et al*., 2001; Weiersbye, 2007) as they tend to function over a longer period and offer a more long-term solution (Rossow *et al.,* 2009). The earliest attempts at rehabilitation of TSFs (specifically dust control) on the Witwatersrand were made in 1894 with the planting of *Ammophila* species with seeds from Kew Gardens in the United Kingdom, and have been followed by a series of vegetation trials between 1932 and the present day (Thatcher, 1979; Weiersbye and Witkowski, 1998; Weiersbye *et al.*, 2006).

Five main subgroups of phytoremediation have been identified from the various strategies that exist nowadays; phytoextraction (occurs when plants extract metals from soil and store them in compartments of the plant which may then be harvested), phytodegradation (occurs when organic pollutants are degraded by plants and their associated microbes), rhizofiltration (occurs when plant roots absorb metals from waste streams), phytostabilisation (occurs when plants reduce bioavailability and mobility of contaminants by preventing their migration, or by immobilisation), and phytovolatilisation (occurs when pollutants are volatilised into the atmosphere, i.e. evaporation or sublimation) (Pulford and Watson, 2003; Dickinson and Pulford, 2005).

Zipper *et al*. (2011) stated that phytoremediation is considered a passive treatment, meaning that it relies on biological, geochemical, or gravitational processes, as opposed to active treatment methods which rely on the constant addition of alkaline chemicals to neutralize the acidity from AMD. Phytoremediation is passive in its physiochemical processes, such as precipitation of contaminants as solids, but may be considered an active process as plants utilise energy and perform photosynthesis.

Passive treatment systems usually involve minimal cost, low long-term maintenance, and a minimal environmental impact.

There are, however, certain concerns and limitations with phytoremediation. The primary limitations are low plant tolerance, lack of contaminant translocation from roots to shoots, contaminants found beneath the rooting zone of plants, the time taken by the remediation process, lack of plant species suitable for the process, dispersion of plant material to adjacent environments, accumulation of nutrients in topsoil, and food-chain contamination (Hutchings, 2002; Kabata-Pendias, 2004; Dickinson and Pulford, 2005; Van Nevel *et al*., 2007; Favas *et al*., 2014).

The question of low plant tolerance depends on the plant species and the contaminant/s in question, as the upper critical concentration that determines when detrimental effects start to take place, varies from species to species, and between contaminants (Hutchings, 2002). Plants have also developed several biochemical mechanisms that aid them in the adaptation to and tolerance of new, contaminated, or chemically imbalanced soil environments (Kabata-Pendias, 2004). Trees may even exhibit facultative tolerance, through which they redistribute their roots to less contaminated zones (Pulford and Watson, 2003). Thus, plant tolerance and response to heavy metals (HMs) varies, and needs to be investigated for different soil-plant systems – especially when phytoremediation is being investigated (Kabata-Pendias, 2004).

Lack of contaminant translocation from roots to shoots also varies between plant species and contaminants. Translocation is controlled by two processes; root pressure and leaf transpiration (Liphadzi and Kirkham, 2005). Metal uptake into roots is important for phytoextraction, but these must also be translocated into shoots for phytoextraction to occur (Lasat, 2000). In general, the uptake mechanisms of plants are selective, as some plants prefer certain ions over others (Lasat, 2000). For example; Zinc (Zn), Manganese (Mn), Nickel (Ni) and Copper (Cu) are all essential micronutrients for plants and will not exceed the metabolic needs in a non-accumulator plant (Kabata-Pendias, 2004).

As for contaminants found beneath the rooting zone of plants, Dickinson and Pulford (2005) state that root density and the depth of rooting are important in the context of phytoremediation, as contaminants either need to translocate into the biomass of the plant or be bound in the rhizosphere. However, roots in the field may not be in direct contact with the contaminants and thus a low metal uptake is experienced (Van Nevel *et al*., 2007). This may be due to facultative tolerance as mentioned above. Schmidt (2003) showed that only 20% of expected contaminant uptake occurred in field trials compared to pot experiments. This is most likely due to pot experiments being performed under strictly controlled conditions. As such, the following explanations were proposed; 1) in most cases of pot experiments, soils are artificially enriched with heavy metal salts which may increase the probability of the plant accumulating high amounts of heavy metals; 2) the amount of soluble heavy metals is lower in the field compared to pot experiments; 3) if soil amendments are added in pot experiments, the plant roots are always in contact with the amendment, as opposed to field trials where this can be the opposite; 4) pot experiments are generally conducted over short periods due to early harvesting or growth curtailing (Schmidt, 2003). This shows that, although pot experiments are valuable, field trials are imperative to determine how plants and metals will behave in field trials.

The time-frame of phytoremediation processes has also been questioned, as faster, more effective solutions are highly sought after. However, phytoremediation is a slow process and should rather be evaluated on its cost effectiveness as Van Nevel *et al*. (2007) advises. It can also be economically and ecologically sustainable. Duration is, however, an important factor to consider when deciding on the practicality and applicability of the site in question (Terry *et al*., 2003). Time may become less of an issue when the lower cost is noted and even combined with some form of profit making operation such as phytomining or forestry (Van Nevel *et al*., 2007). Alternatively, utilisation of remediation plants for the poorer mining communities (such as medicinal plants, weaving or building materials among others) may be worthwhile if it is safe to do so (Botha and Weiersbye, 2010).

The fate of Heavy Metals (HMs) in the biomass is an important aspect of phytoremediation – especially where phytoextraction is concerned. Some of the metals immobilised in roots that will not

translocate to shoots are Aluminium, Chromium and Mercury, while mobile elements which may be phytotoxic are Cadmium, Copper, Manganese, Nickel, Selenium and Zinc (Dickinson and Pulford, 2005). It is therefore seen as good practice to harvest or coppice the plant biomass (USEPA, 2000) to allow the plants to continually immobilise metals and contaminants.

As for a lack of plant species available as accumulators for phytoextraction or stabilisation; there have been studies conducted, especially over the last 10 years, in which several tolerant species have been identified and mentioned (Boularbah *et al*., 2006; Gbaruko and Friday, 2007; Weiersbye and Witkowski, 2007; Dye *et al*., 2008; Kuzovkina and Volk, 2009; Dye *et al.*, 2014; Conesa *et al*., 2011; Vollenweider *et al*., 2011; Mokgalaka-Matlala *et al*., 2013). More and more species are being found to be suitable for phytoremediation. There is also a growing desire to find and utilise larger biomass species (Rockwood *et al*., 2001; Kuzovkina and Volk, 2009; Dye and Weiersbye, 2010). Thus, there may not be an actual lack of viable species, but a lack of investigation into new, tolerant species.

Dispersion of contaminants to adjacent environments, and accumulation of metals in topsoil are realistic issues regarding phytoextraction. These issues relate to leaf fall and dead biomass, both during harvesting and natural plant growth, which then decompose and subsequently release HMs back into the soil, that were previously contained in the biomass (Van Nevel *et al*., 2007). This will occur if plants are not regularly harvested or coppiced, and depends on whether the plants are seasonal or annual, resulting in the recycling process of HMs to surface soil becoming significant (Dickinson and Pulford, 2005). If HMs are being recycled, it may not be such an issue as they are not necessarily spreading laterally through the surrounding environment, especially if harvesting and coppicing is being practiced.

With regards to food chain exposure, the literature does mention this being a problem (Kabata-Pendias, 2004; Van Nevel *et al*., 2007). However, it has also been noted that plants may accumulate HMs as a natural defence against herbivory (Lasat, 2000). Thus, food chain exposure may

not be an issue at all in certain species. For example, Boyd *et al*., (2002) fed leaves of hyperaccumulator and non-hyperaccumulator species of *Senecio coronatus* (South African populations) to the brown garden snail (*Helix aspersa*). They found that snails preferred non-hyperaccumulator leaves in two out of three trials, and that the mortality rate of snails fed hyperaccumulator leaves was higher than those fed non-hyperaccumulator leaves.

Perez-de-Mora (2006) states that the plants used for phytoremediation on contaminated land should be tolerant to high levels of contaminants, and exhibit low translocation rates from roots to shoots. This is more in line with phytostabilisation, where contaminants are bound in the rooting zone as opposed to being absorbed into the plant itself. It must also be noted that other factors are limiting for plant species to grow on contaminated soils, such as poor soil structure and stability, low nutrient content, and extreme pH levels (Perez-de-Mora, 2006). This is due to erosion and AMD that emanates from TSFs that impact nutrient cycling in polluted soils, the regeneration of vegetation, and the biogeochemical cycling of potentially toxic elements (Weiersbye *et al*., 2006). To overcome this, one can combine the use of plants with soil amendments such as lime, compost, zeolite, fertilisers, and irrigation - among others - to harness natural processes for plant growth and accelerate soil remediation (Perez-de-Mora, 2006; Weiersbye *et al.*, 2006). There are several studies which explain the benefits of using soil amendments (Khan *et al*., 2000; Wong, 2003; Gaur and Adholeya, 2004; and Wang *et al*., 2012).

As can be seen, phytoremediation is conducive to mining environments as it can be adapted to various remediation and environmental needs. There is ultimately no quick-fix for contamination left by mining houses, and a large amount of time and energy does need to go into researching the mine environment to distinguish; what types of contaminants are present and in what quantities, what climate and rainfall the area experiences, what fauna and flora occur naturally, what flora are found on contaminated sites, which of these flora are able to withstand high concentrations of contaminants, and which flora would be best equipped to perform the function of the remediation needed, along with high resilience. The more research that goes into a certain mining environment, the greater the chance

there is of satisfactory long-term rehabilitation. This is something that The Mine Woodlands Programme strives to do.

1.2.3 Waste and Nutrient Uptake by Plants

Soil is the main source of trace elements for plants (Kabata-Pendias, 2004). The partition of metals between the soil solid and solution phases determines the retention of metals by soils, as well as their availability to plants (Hutchings, 2002). In the soil solution, metals can be present in various ways: as free metal ions and soluble metal complexes; adsorbed to inorganic soil constituents at ion exchange sites; bound to soil organic matter; precipitated as oxides, hydroxides and carbonates; and embedded in the structure of silicate minerals (Lasat, 2000).

Contaminants need to be bioavailable (in the correct 'form' to be absorbed by roots) for phytoextraction to occur (Lasat, 2000; Kabata-Pendias, 2004; Dickinson and Pulford, 2005; Nevel *et al*., 2007). Bioavailability depends on metal solubility in the soil solution, and only metals that are in the soil solution as free metal ions and soluble metal complexes and/or adsorbed to inorganic soil constituents at ion exchange sites are bioavailable (Lasat, 2000). Bioavailability of HMs to plants, and thereafter the metal accumulation in plant tissue, can vary hugely according to the actual source of the metal contamination, as well as the site conditions (Hutchings 2002; Pulford and Watson, 2003).

Bioavailability of metals changes with regards to soil properties as they play a crucial role in trace element behaviour, and thus the chemistry of metal interaction with the soil matrix is essential to phytoremediation (Lasat, 2000; Kabata-Pendias, 2004). Acidic and non-acidic soil interact with metals differently. In non-acidic soil (poorly-aerated and reducing) there is generally reduced metal activity (immobilization) due to the sorption of metals onto soil particles (Lasat, 2000) resulting from a greater cation exchange capacity (CEC). With acidic soil (well-aerated and oxidizing) there is generally a lower CEC, and thus desorption of metals from binding sites into the soil solution occurs, due to H^+ competition for binding sites (Lasat, 2000; Kabata-Pendias, 2004). Cation exchange is the release of cations, such as Mg and Ca from soil particles, by protons in acidic soil water - the released cations are

then available for uptake by plants (Kabata-Pendias, 2004). This shows that soil pH affects both metal bioavailability and the metal uptake into roots.

The reason plants take up various toxic metals is that they need certain macronutrients (Nitrogen, Phosphorous, Potassium, Sulphur, Calcium, Magnesium) and micronutrients (Iron, Zinc, Manganese, Copper, Molybdenum) to complete their life-cycles (Lasat, 2000). Plants have evolved certain mechanisms to take up, translocate, and store these nutrients whereby the movement of metals across membranes is controlled by proteins with transport functions (Lasat, 2000).

In general, some plants prefer certain ions over others and thus certain mechanisms in certain plants are selective (Lasat, 2000; Alkorta *et al.*, 2010). This selectivity depends exclusively on the structure and properties of the membrane transporters, and certain characteristics allow transporters to recognise, bind, and mediate the transport of specific ions across membranes in the plant (Lasat, 2000). As mentioned, ion transport is mediated by membrane proteins with transport functions (Lasat, 2000). These transmembrane transporters have an extracellular binding site on which ions can attach just before transport (Lasat, 2000). It is important to note that the binding site is only receptive to certain ions (known as transporter specificity) (Lasat, 2000).

Of the total number of ions associated with the roots, only a certain number are absorbed into cells (Lasat, 2000). A significant ion fraction is physically adsorbed at the extracellular, negatively charged, sites of the root cell walls, and cannot be translocated to the shoot, and thus cannot be removed by harvesting (phytoextraction) (Lasat, 2000). This is not the only mechanism responsible for immobilization of metals by the roots. Complexation and sequestration in cellular structures, such as the vacuole, also prevent ions from being transported (Lasat, 2000). Uptake of metals into root cells is important for phytoextraction. These metals must however, also be translocated (metal-containing sap must move from the roots into the shoots for phytoextraction) (Lasat, 2000).

1.2.4 Tolerance of Plants to Heavy Metals (HMs)

High intracellular micronutrient levels (Zn, Mn, and Ni) can ultimately be toxic to plants even though they are needed (Lasat, 2000). Liphadzi and Kirkham (2005) present normal and toxic metal element concentrations in soil and plants for Fe, Cu, Mn, Zn, Ni, Cd and Pb. What is interesting is that non-accumulator species developed mechanisms to control homeostasis of intracellular ions by regulation of ion influx (where there is stimulation at low concentrations and inhibition at high concentrations), and extrusion of intracellular ions back into external solution (Lasat, 2000). Hyperaccumulators (mentioned below) possess additional mechanisms for detoxifying metals, such as complexation, sequestration, precipitation, and binding (Lasat, 2000) that allow these types of plants to survive on polluted media.

There are several mechanisms for tolerance of metal-rich soils, namely; exclusion, accumulation, and indication. Through exclusion, a species will prevent toxic-metal uptake into the roots, resulting in species with little potential for phytoextraction (Lasat, 2000). Accumulators detoxify increased metal levels in soil through bioaccumulation, whilst indicators have poor control over metal uptake and transport processes, resulting in the extent of metal accumulation reflecting metal concentration in the rhizospheric soil (Lasat, 2000). Indicator species are typically used in mine prospecting to find new ore bodies (Lasat, 2000).

For successful plant-based decontamination of land, plants are required to concentrate metals above 1-2%. These levels are extremely high, but are achievable using hyperaccumulators (Lasat, 2000). However, the extent of metal removal is limited by the plants ability to extract and tolerate only a finite amount of contaminants (Lasat, 2000).

Plants do have certain limitations however, and phytoextraction is severely limited by biomass production – especially with regards to hyperaccumulators as they are generally slow growing and produce little biomass (Dickinson and Pulford, 2003). With highly productive species, the potential for biomass production is about 100 tons of fresh weight per hectare, but values of these parameters are

limited to an annual removal potential of a maximum of 400 kg of metal per hectare per annum (Lasat, 2000). The use of trees in phytoextraction would therefore be much more beneficial, as there would be a greater amount of metals per hectare per annum removed due to the large biomass that trees produce.

1.2.5 Hyperaccumulators

A hyperaccumulator species is conventionally defined as a species which is capable of accumulating metals at levels 100 times greater than in non-accumulator species (Lasat, 2000; Pulford and Watson, 2003). Hyperaccumulator plants have highly expressed metal sequestration systems, and may even have a higher requirement for metals compared to non-accumulators (Lasat, 2000; Pulford and Watson, 2003). They may also accumulate nonessential metals. Lasat (2000) explains; Zn, Mn, Ni and Cu are all essential micronutrients and will not exceed the metabolic needs of the plant in a non-accumulator plant, with concentrations always <10 parts per million (ppm) - whereas in hyperaccumulator plants, micronutrients can be concentrated to thousands of ppm. These plants are also typically endemic to areas of naturally high metal content, as well as mining areas (Pulford and Watson, 2003).

For a plant species to be classified as a hyperaccumulator, the concentration criterion depends on the type of metal it is accumulating (Boularbah *et al*., 2006). Thus, a hyperaccumulator will concentrate more than 10 ppm Hg, 100 ppm Cd, 1000 ppm Co, Cr, Cu and Pb, or 10 000 ppm Ni and Zn (Lasat, 2000). About 450 plant species have been identified as hyperaccumulators (Boularbah *et al*., 2006), while new species are constantly being discovered (Pulford and Watson, 2005). It has been found that generally, the metal concentration in the shoots of hyperaccumulators is greater than that found in the roots, and it has even been suggested that metal hyperaccumulation plays a role in protecting plants against fungal and insect attacks (Pulford and Watson, 2003). However, hyperaccumulators usually have a slow growth rate and low biomass (Terry *et al*., 2003).

1.2.6 The fate of contaminants in trees

A number of factors contribute to the fate of contaminants - namely metals, heavy metals and metalloids – within a plant. These can be seen as temporal variations in tissue concentrations of metals, variability of tissue concentrations within a tree, the bioavailable contaminants in the soil solution, and the toxicity of the trace elements to the plant among others. Therefore, different tree species have different tolerance levels to HM pollution (Baycu *et al*., 2006).

Temporal variation in tissue concentrations of metals means that after several years there is a noticeable decrease in HM concentrations in the plant tissues (Dickinson and Pulford, 2005). There is natural dilution of HM concentrations due to tree growth, with relatively rapid rates of metal uptake during the first few years of plant growth in trees, specifically while the roots are becoming established and trees are growing (Dickinson and Pulford, 2005). This is expected, generally, as the plant takes up surrounding bioavailable metals during its growth. The only instance where this may differ is when contaminants are continually added to the substrate and bioavailable metals are continually available for uptake. However, during a plant's lifecycle it will naturally take up less and less of the metals as it grows older and reaches an equilibrium.

Different tissues will contain different concentrations of heavy metals. In general, leaves of a tree will have high concentrations of metals as a survival trait but during leaf fall toxic metals are lost and added to the system again (Dickinson and Pulford, 2005). It has also been found that at the end of the growing season, metals from the leaves and bark of trees translocate to the wood (Dickinson and Pulford, 2005).

It is also widely known that there is variation in metal concentration between different tree tissues or organs (Dickinson and Pulford, 2005; Liu *et al*., 2013). In general, it has been found that there are higher concentrations of metals in roots > leaves > bark > wood (Dickinson and Pulford, 2005). However, different elements show different patterns of distribution; for example, lead, mercury, arsenic, cobalt, tin, and chromium have been found primarily in root tissue, while cadmium,

copper, zinc, cobalt, nickel, molybdenum, and boron have been found in the aboveground tissues (Hutchings, 2002; Dickinson and Pulford, 2005). However, metals tend to remain absorbed in the root system rather than being translocated and mobility and translocation ultimately depend on the metal type and plant species (Dickinson and Pulford, 2005). However, the knowledge of HM uptake in trees is limited, and thus further research needs to be conducted in this area.

1.2.7 The use of Woodlands on Contaminated Land

Woody vegetation can be an efficient sink for nutrients and HMs (Pulford and Watson, 2003; Weiersbye and Witkowski, 2007). It was initially thought that the colonisation of trees on contaminated land was rare but a survey in 1998 conducted by Forest Research showed that this was the opposite, as 250 sites covering 3200 hectares from 27 former contaminating land-uses were identified as supporting a range of 20 tree species (Hutchings, 2002). Hutchings (2002) goes on to say that this study was not very comprehensive or systematic and so probably underestimated the occurrence of woody vegetation on contaminated land. The England Forestry Strategy has also made the establishment of woody biomass a strategic priority on derelict and contaminated land, among others (Hutchings, 2002).

Woodlands can satisfy all criteria that remediation requires; by removing or treating pollutants, breaking or removing the pollutant pathway and protecting or removing the receptor of contaminants (Hutchings, 2002). This is because woody vegetation has a number of advantages that herbs and shrubs do not. Trees have a large biomass, genetic variability, established management practices, possible secondary economic value, and offer site stability (Dickinson and Pulford, 2005; Liu *et al*., 2013). Thus, trees can offer remediation and reclamation at the same time.

Trees are seen as being less tolerant to HM stress than herbs, but it has been found that some species can grow at sites with moderate to considerable contamination of HMs, and may even be planted specifically for phytoremediation, to take advantage of their growth and large biomass (Vollenweider *et al*., 2011). The reason trees may be able to grow on contaminated land could be due

to acclimation (roots specifically avoiding contaminated soil), or contaminants being concentrated primarily in roots, resulting in limited translocation into shoot tissue (Dickinson and Pulford, 2005). However, Brunner *et al*. (2008) found that tree fine roots accumulated about 10 - 20 times more than controls, and that the HMs accumulated in the fine roots only made up 0.03 – 0.2 % of the total amount in the soils. The partitioning of metals within a stem can also vary within a tree (Pulford and Watson, 2003). Trees can thus grow and survive in contaminated soil but their growth rate may be reduced, in some cases greatly, depending on the species and the site (Pulford and Watson, 2003; Dickinson and Pulford, 2005). This may not be a problem if the trees are providing a long-term ecologically sustainable rehabilitation strategy.

Trees have great genetic variability in general and specific genotypes may be selected based on traits for high resistance to contaminants or for high or low HM uptake as well as genotypes for disease resistance or large biomass (Dickinson and Pulford, 2005). This has been done in a number of cases as well as the Mine Woodlands Programme (Weiersbye and Witkowski, 2007; Dye and Weiersbye, 2010).

The fact that there are established management agronomic practices means that the use of trees or woodlands on contaminated land is a great possibility – all that needs to be done is for forestry practices to be developed or adapted from arable land for use on contaminated land (Dickinson and Pulford, 2005). Trees are generally grown in short-rotation coppice systems (SRC), where tree parts are harvested on a 3-5 year cycle with a coppice lifecycle of about 30 years (Dickinson and Pulford, 2005). Extensive research into a rehabilitation strategy, high biomass species, and the area in question is paramount for site-specific, and species-specific, scenarios.

Trees already have an economic value and the development of certain SRC systems will also be driven by the end users of trees (for example biomass fuel, chipboard, paper, etc.) (Dickinson and Pulford, 2005). Dye *et al*. (2014) do note, however, that the biomass should not be used as timber products due to the contaminants contained in the tissues, but rather used in carbon sequestration for

example. The use of trees should also be increasingly prioritised as there is huge public acceptability of this low-cost restoration technique (Dickinson and Pulford, 2005).

Trees not only have public acceptability and aesthetic value, but they offer other advantages such as; stabilisation of contaminated land, a decrease in wind and water erosion, root stability to soil, and their leaf fall contributes a significant amount of organic matter (OM) into the surface soil (Dickinson and Pulford, 2005).

One issue affecting many people's perceptions about the use of trees on contaminated land is that trees naturally acidify soil and in so doing they may further mobilise HMs and contaminants thereby increasing the risk of their migration into groundwater and surface water (Hutchings, 2002; Dickinson and Pulford, 2005). However, there has been no field evidence of this occurring, and it is thought that the HMs mobilised in this way are most likely to be immobilised in the tree tissues before this can take place (Hutchings, 2002).

Another argument against the use of trees is that litter fall releases HMs back into the soil and that this defeats the purpose of the trees entirely (Hutchings, 2002; Kabata-Pendias, 2004). Conversely, it is thought that if OM and plant cover can be built up to a point where the natural decay of vegetation produces additional OM, then normal cycling (normal geochemical cycling of the HMs when they start to reach more 'natural' concentrations in the soil) will establish, and the risk of toxicity will be reduced along with the possibility of HM leaching (Hutchings, 2002).

It should thus be noted that evidence has been found that woody species have the inherent capacity to modify resource allocation under stress conditions, and that woody vegetation can be an efficient sink for nutrients and HMs (Pulford and Watson, 2003; Weiersbye and Witkowski, 2007). Although Hutchings (2002), amongst others, has noted that woody species, namely trees, will only work to remediate low contaminant scenarios, Dye *et al*. (2008), Weiersbye and Witkowski (2007), and Dye and Weiersbye (2010) have stated that there is not only a need to understand how certain

species respond to AMD specifically, but that the primary benefits of using woody species is that they can be used for carbon sequestration and soil rehabilitation as well as phytostabilisation, hydraulic control, and phytoextraction. Thus, trees provide several positive features over and above rehabilitation.

It can therefore be seen that, although the use of trees or woodlands for phytoremediation efforts was initially thought to be inadequate and problematic, there is opinion and evidence that this is an effective approach – especially concerning phytostabilisation, phytoextraction, and hydraulic control. The negative thoughts concerning this approach will most likely dissipate with further research. Domínguez *et al*. (2009) also stated that afforestation of contaminated land by trees is considered a feasible strategy for the extensive stabilisation of contaminants.

1.2.8 The Mine Woodlands Programme

The Mine Woodlands Programme (MWP) is a joint project run by AngloGold Ashanti (AGA) in conjunction with The University of the Witwatersrand (Wits) which is supported by the South African National Research Foundation (NRF) and the South African Department of Trade and Industry programme (THRIP). This project illustrates the need for research, in combination with a remediation program, to obtain the best results for the best remediation effort possible. The MWP stems from the Ecological Engineering and Phytotechnology Programme that AGA has been running with Wits since 1995 to "*quantify the effectiveness of strategically planted woody, semi-woody and herbaceous indigenous species on and around TSFs to limit seepage, extract or immobilize pollutants, reduce wind pollution, rehabilitate tailings and soil and protect groundwater and rivers from contamination*" (AGA, 2004; 2009).

The MWP began in 2001 and combines phytoremediation with ecological engineering to reduce environmental impact and liability for AGA. The programme demonstrates the effectiveness of woodlands in reducing or preventing the spread of AMD from TSFs as well as exhibiting a solution that is not only long-term, but economically and ecologically sustainable as well (Dye and Weiersbye,

2010). Ecological engineering may be defined as the design of ecosystems for the benefit of both humans and nature (Mitsch, 1996), in which natural energy sources are utilised to manipulate and control environmental systems. The MWP focuses on the use of strategically placed trees down-slope of TSFs to limit the spread of contaminants through immobilisation or sequestration, and increased evapotranspiration (ET) (Dye and Weiersbye, 2010). This is achieved with the use of deep-rooted trees to consume mine water (Dye *et al*., 2014).

Trials have been established in order to identify suitable tree species (indigenous and exotic) and silvicultural practices, and to determine the long-term sustainability of the trees (Dye and Weiersbye, 2010). There are also several benefits that the woodlands produce, such as immobilisation of contaminants (in their biomass or bound with organic contaminants in the rooting zone or surface soil horizons) and reduced water flow, and thus contaminant flow, through shallow soil horizons and shallow aquifers into adjacent areas and other surface water channels (Dye and Weiersbye, 2010; Dye *et al*., 2014). This has been demonstrated and reviewed in previous literature such as Dickinson (2000) and Pulford and Watson (2003). As of 2009 the MWP had planted approximately 500 000 trees in trials on AMD and tailings – most of them being indigenous species (Regnier *et al.*, 2009).

Species selection for the MWP was based on a tree's likely suitability to the harsh mine and climatic conditions, as the area is too arid in general to promote forest development (Dye *et al*., 2014). The mean annual precipitation (MAP) is low at about 500 – 800 mm and varies from year to year, and is confined to the summer growing season (Dye *et al*., 2014). The potential evaporation rates range from 2200 – 2400 mm which is very high, exceeding the MAP (Dye *et al.*, 2014).

Species used in the trials include both exotic species (e.g. *Casurina cunninghamiana, Eucalyptus camaldulensis, Pinus halepensis)* and indigenous species *(e.g. Tamerix usneoides, Celtis africana, Olea africana).* There has been much research into the use of our native *Tamerix usneoides,* as well as the exotic *Eucalyptus* species for use in acid mine drainage phytoremediation for the Mine

Woodlands Programme (Weiersbye *et al*., 2006; Weiersbye and Witkowski, 2007; Dye *et al*., 2008; Grindley, 2014; and Wilson *et al*., 2017).

It has been found that the implementation of phytoremediation in arid regions requires the consideration of salinity and the mobility of contaminants (Padmavathiamma *et al.*, 2014). The plant selection needs to be unique and preferably indigenous so as to benefit from the plant adaptations and survival mechanisms for the region in question, as well as a varied plant community that includes drought-, metal-, and salt-tolerance in the plants which can degrade, accumulate, and/or stabilize the environment (Padmavathiamma *et al.,* 2014). Padmavathiamma *et al.* (2014) also noted that many trials in arid environments have not taken advantage of indigenous plant diversity, resulting in poor colonisation. The MWP is an example of a trial that has taken advantage of indigenous plant diversity as well as the other factors mentioned above.

1.3 Problem Statement

Due to several hydrological factors, surface and subsurface seepage waters with a high level of total dissolved solids (TDS), sulphates, chlorites and metalloids, radio nuclides, and certain metals drain from TSFs into lower landscape areas and groundwater aquifers, and enter drainage lines over variable distances from the source (Herbert, 2009). Treatment of seepage at its source is an important consideration especially when the spread of seepage from TSFs leads to larger areas needing rehabilitation and remediation measures. Trees have been looked at for their large biomass and water use potential to help with this issue, as well as for their potential to immobilise contaminants either within their biomass or bound in the rhizosphere (Dickinson, 2000; Pulford and Watson, 2003). The MWP offers a solution to this problem and is investigating the efficiency of certain tree species, and the engineered woodlands they are planted in, as a rehabilitation technique.

Research, however, has shown that while high densities of trees can increase ET and lower water tables, this may lead to an accumulation of salts in the rooting-zone which ultimately raises osmotic potential in the rhizosphere and reduces availability of water to the trees – compromising the

viability of the trees potential for hydrological control (Thorburn, 1999). There is therefore a need to track the fate of contaminants and the uptake and immobilisation potential of the tree species, as well as to continually analyse the soil within the plots to look for contaminant build-up in the rooting-zone (Dye and Weiersbye, 2010).

This study will thus determine the fate of certain contaminants, and the uptake and immobilization of contaminants in *S. lancea* being used in the MWP Vaal River (VR) and West Wits (WW) trials planted in 2003-2004, as well as investigate potential contaminant build-up in the rooting-zone of these trees. It will also provide a documented account of an indigenous South African tree species planted in varied, engineered woodlands in a semi-arid environment, for phytoremediation purposes.

1.4 Aim

The aim of this study was to determine the fate of certain contaminants on four AGA woodland sites which form part of the MWP. The study focused specifically on the biomass production and fate of certain contaminants within the indigenous tree species *Searsia lancea*.

1.5 Objectives

- Research Question 1: What is the fate of contaminants within *S. lancea* trees and how do these compartments compare between sites?

  The first objective was: 1a) to determine the concentration of sodium (Na), Mg, aluminium (Al), chlorine (Cl), Ca, chromium (Cr), manganese (Mn), Fe, copper (Cu), Zn, arsenic (As), Hg, lead (Pb), and uranium (U) in *S. lancea* within the tree compartments (leaves, twigs, wood, bark, coarse roots and fine roots) by harvesting the trees; and, 1b) to compare the tree compartments between each mining site. Trees were harvested and subsamples were taken and analysed via X-Ray Fluorescence for total elemental concentration. Tree compartment concentration was compared between mining sites with a Kruskal-Wallis statistical test.

Hypothesis 1: Tree compartment elemental concentrations will differ between sites.

- Research question 2: How does the average biomass of *S. lancea* trees compare between the four mining sites, and does this biomass correlate to total elemental mass within the trees?

The second objective was: 1) to find the biomass for *S. lancea* on each mining site; 2) to find the total elemental mass for the trees on each mining site; and 3), to test whether biomass and total elemental mass correlates with biomass. Biomass was assessed by harvesting trees and measuring the wet and dry biomass, and then used to determine the total elemental mass from the measured elemental concentrations. The correlation was tested using Pearson's Correlation Coefficient.

Hypothesis 2: Biomass will differ between each mining site due to soil type, and biomass will correlate strongly with total elemental mass.

- Research Question 3: Do *S. lancea* trees at VR and WW perform better as phytoextractors or phytostabilisers in terms of soil elemental concentration and tree elemental concentrations?

The third objective was: 1) to find the total concentrations of contaminants (as above) in the soil of the rooting zone of each tree by digging soil pits; 2) to calculate the root and shoot elemental concentrations for *S. lancea* at each mining site; 3) to calculate translocation factors (TF) for each site for *S. lancea*; and 4) to calculate the bioconcentration factors (BF) for each site for *S. lancea*, to determine whether it performs better as a phytostabiliser or phytoextractor.

Hypothesis 3: *S. lancea* will perform better as a phytostabiliser for HM rehabilitation on these sites.

CHAPTER 2

STUDY AREAS
&
STUDY SPECIES

2.1 Introduction

This study was conducted on two of AngloGold Ashanti's (AGA) mining properties, the first being their West Wits (WW) property about 75 km West of Johannesburg and about 8 km South of Carletonville, South Africa. The second mining property, Vaal River (VR), is located in the North West and Free State Provinces, South Africa – about 180 km from Johannesburg (Figure 2.1).

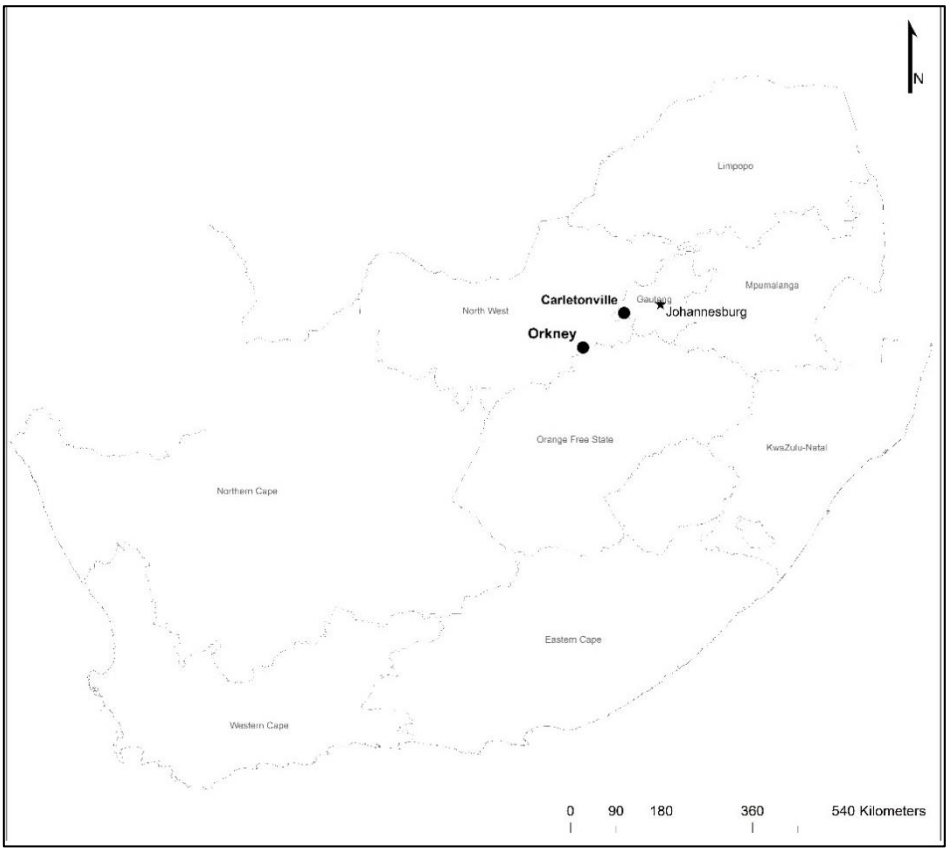


Figure 2.1. Location of West Wits and Vaal River Mining Sites.

The site conditions in these regions are generally considered to be harsh or marginal due to a number of limiting factors. The topography from Klerksdorp to Carletonville is undulating and broken with convex ridges and inter-leading slopes. The mean annual precipitation (MAP) for the Welkom area is about 500 mm and for the Carletonville area it is 700 mm with a coefficient of variation of 25 – 30% (Herbert, 2009). This means that in Welkom and surrounds MAP may be expected to vary from 350 – 650 mm, and in the Carletonville area and surrounds it may vary from 490 – 910 mm in any

year. Most rainfall is experienced as harsh thundershowers between November and March, with only about 10% experienced between April and August, leading to winter drought (Herbert, 2009).

The mean daily maximum temperature is 28 to 30^{o} C which peaks in January, while the mean daily minimum temperature of -2 to 1^{o} C is usually experienced in July (Herbert, 2009). This leads to very high annual evaporation potential of 2200 – 2400 mm with a mean monthly value of 100 – 110 mm in June and 260 – 300 mm in December, which generally leads to very high annual water deficits (Herbert, 2009).

The soil and vegetation descriptions will be made hereunder for each separate site as the soil type and underlying strata does vary. While the general climate may be similar within VR and WW, each trial is subject to different conditions such as soil types, drainage, and plume depth and flow, which in turn may have an impact on the trees and thus affect their growth patterns and uptake potentials. This will not be discussed in detail here, but has been considered in Grindley (2014). It also reminds us that site conditions are an important consideration in determining remediation options for a particular area.

The MWP has several trial plots currently planted on both mining properties. Four of these were chosen for this study due to three different soil types, with one site at WW and VR with better soil conditions for plant growth and one site with poor soil conditions for plant growth. The two West Wits sites and two Vaal River sites will be discussed hereunder.

2.2. West Wits Mining Operations

The two WW trial plots chosen for this study were the Redsoils Trial and the Madala Trial (Figure 2.). The natural vegetation on the WW mining operation is Gauteng Shale Mountain Bushveld (SVcb10) which is a savanna biome vegetation subunit found in the Gauteng and North West provinces (Mucina and Rutherford, 2006). The vegetation is characterised by short, semi-open thicket, dominated by a variety of woody species, and an understory dominated by different types of grasses

(Mucina and Rutherford, 2006). The study area of WW falls into the Varkenslaagte surface water sub-catchment (AGA, 2011a). The Varkenslaagte drains to the north-west and is a tributary to the Wonderfonteinspruit, that in turn drains into the Mooi River, and ultimately into the Vaal River (AGA, 2011a).



Figure 2.2. Map of the West Wits mine lease area showing TSFs and sampling areas.

2.2.1. WW Redsoils Trial

The Redsoils trial is located on an old farming property also known as Magnum Farm (Figure 2.). The soil type is red apedal of Inanda and Hutton forms with a sandy clay loam to clay loam to sandy loam texture (McLeroth, 2015). These soils are deep and well-draining, but they are also moderately leached due to high infiltration rates (McLeroth, 2015).



Figure 2.3. Redsoils Woodlands Trial showing tree locations (S4-S6) and Redsoils control pits (C1 – C3). Aerial photo: CD:NGI 2012 50 cm.

2.2.2. WW Madala Trial

The Madala trial (Figure 2.) is located above what was natural drainage line and is now known as the Varkenslaagte canal, part of which has been converted into a line of remediation reedbeds for the retention and filtering of the water. The soil type found here is yellow-brown apedal of Clovelly, Glencoe, Avalon and Magwa forms (McLeroth, 2015). These soils are shale derived and moderate to deep, while being reasonably well draining (McLeroth, 2015). The effective rooting depth is

considerably reduced due to the gravelly nature of the soil, which includes iron concretions and hard plinthite stones with occasional solid indurated underlying bands that have developed due to a perched fluctuating water table over a long period (McLeroth, 2015).



Figure 2.4. **Madala Woodlands Trial showing tree locations (S1-S3) and Madala control pits (C1 – C3). Aerial photo: CD:NGI 2012 50 cm.**

2.3. Vaal River Operations

The Mispah and West Complex Trials were chosen for this study from those planted on the VR mining property (Figure 2.). The VR mining operation covers approximately 12 000 hectares in the North West and Free State Provinces of South Africa. The area is a warm temperate summer rainfall region with high temperatures, and frequent frosts and veld fires in the winter (Schultze, 1997; Mucina and Rutherford, 2006). The main geological units consist of the Ventersdorp Supergroup, the

Black Reef Quartzite Formation, and the overlying Malmani Subgroup which consists of chert-rich and chert-poor dolomites (AGA, 2005).



Figure 2.5. Map showing the Vaal River mine lease area with relevant TSFs and sampling sites.

2.3.1. VR Mispah Trial

The Mispah trial is adjacent to and down-slope of the Mispah TSF (Figure 2.). The soils at Mispah are deep, well-drained clay loam to clay textured Hutton soils derived from highly weathered dolomite (McLeroth, 2015). The effective rooting zone is 3.5 – 4.0 m, and the polluted groundwater depth is 5 – 6 m. The natural vegetation type is Vaal Reefs Dolomite Sinkhole Woodland (Gh12),

which is a grassland biome vegetation subunit found in North-West and Free State provinces. However, most vegetation growing on the VR mining operation is degraded grassland due to mining transformation. The natural vegetation type supports a grassland-woodland complex and is characterised by clumps of woodland that form on dolomite sinkholes. Dominant tree taxa include *Searsia lancea*, *Acacia erioloba*, and *Celtis africana* (Mucina and Rutherford, 2006).



Figure 2.6. **Vaal River Mispah Woodlands Trial showing tree locations (S10-S12) and control pits (C1 – C4). Aerial photo: CD:NGI 2010 50 cm.**

2.3.2. VR West Complex East Trial

The West Complex east trial is located adjacent to the West Complex TSF (Figure 2.). This TSF has a contamination plume characterised by elevated sulphate, chloride, nitrate and metal levels (Dye et al., 2008; AGA 2011a; Dye and Weiersbye, 2010; Grindley, 2014) with some radionuclides (Rosner and van Schalkwyk, 2000). The main geological units found in the area, as mentioned below, act as a sort of hydraulic control due to varying degrees of porosity and permeability and thus dictate the levels and the movement of water in the area (Grindley, 2014).



Figure 2.7. **Vaal River West Complex Woodlands Trial showing tree locations (S7-S9) and control pits (C1 – C3). Aerial photo: CD:NGI 2010 50 cm.**

The geological profile of the area consists of soil to depths of 2 m with weathered dolomite from depths of 2 m – 8 m, and a zone of cavities filled with hydrated manganese oxides and iron oxides, occasionally barium or cobalt, forming a soft black earth mass (AGA, 2003; Grindley, 2014). The soils in the region are clayey, silty sand with residual dolomite, lavas or Ventersdorp lavas that are area dependent (AGA, 2005; Grindley, 2014). Borehole data in the area show that water tables range from 0.2 – 11 m below the surface (Grindley, 2014). The West Complex TSF was first deposited in the 1950s and is currently about 30 m high from ground level (AGA, 2011b; Grindley, 2014).

2.4. The Woodlands Trials

Each trial area is 5 ha and consists of 2 ha of exotic species next to 3 ha of indigenous species with a total of 20 tree species. For each species, there are four randomly located replicate plots containing 63 trees planted in a 7 x 9 tree grid, with an inter-row spacing of 3 m and an in-row spacing of 2.5 m. There are therefore a total of 252 trees per species per trial originally planted. Not all trees reach maturity, due to death by fire for example.

Trees planted in the exotic plots;

Casurina cunninghamiana, Eucalyptus camaldulensis, Eucalyptus dunnii, Eucalyptus grandis x camaldulensis hybrid, Eucalyptus grandis x nitens hybrid, Eucalyptus macarthurii, Eucalyptus melliodora, Pinus halepensis, Eucalyptus sideroxylon.

Trees planted in the indigenous plots;

Celtis africana, Combretum erythrophyllum (QuaQua), Combretum erythrophyllum (Uppington), Dombeya rotundifolia, Dovyalis caffra, Olea africana, Rhus lancea (Botubela), Rhus lancea (Uppington), Rhus pendulina, Schinus molle, Tamerix usneoides, Ziziphus murcronata.

The species planted in the trials have been found to be tolerant, or are on record as being tolerant, to the conditions at both mining environments. Some of the exotics chosen to be part of the

trials are being used because of their potential for use after coppicing as either timber or for pole production, or for aromatic oils (Weiersbye, *Pers. Comm.*).

Indigenous tree species are more difficult to use after coppicing as they are generally multi-stemmed. Most indigenous species are suited to a particular environment, and don't take contaminants into their leaves, but still have a generally high level of contaminant uptake (Weiersbye, *Pers. Comm.*). They are not pruned however, as maximum leaves are needed for extraction of water and contaminants (evapotranspiration – movement of water to increase uptake) (Weiersbye, *Pers. Comm.*).

2.5. Site Selection

Sites were selected due to minimal disturbance by fire, and because the sites on each mining property, WW and VR are comparable, as one site in each area has well-draining, nutrient rich soils suitable for tree growth and the other site has poorly-draining, nutrient-poor soils for tree growth.

2.6. Tree and Plot Selection

One tree of average height was selected from each plot, giving a total of three trees per site. The ease of access for harvesting was also taken into consideration as a backacter (TLB) was needed for root excavation and to dig the soil pits. Trees were chosen at least one tree in from the outermost tree to negate edge effects if possible. Certain plots had some trees missing due to death and in these cases, edge effects could not be avoided.

2.7. Study Species

Searsia lancea (S. lancea), formerly known as *Rhus lancea*, is known commonly as the Karee tree (English), Rooikaree (Afrikaans), Mushakaladza (venda) and umHlakotshane (Xhosa) (Wanenge, 2009). *S. lancea* is a multi-stemmed species of bush and tree form with a dense, evergreen canopy, and can grow up to 8 m tall and may be single or multi-stemmed (Spuy, 1971). It is one of the few indigenous tree species to maintain a high leaf-area throughout the year (Dye *et al.*, 2008). The leaves are characterised by dark green dorsal surfaces and pale green ventral surfaces, while the roots are

phreatophytic (in drier areas, these trees can reach water via a large tap root) (Weiersbye and Witkowski, 2007). This tree is Republic of South Africa (RSA) National Tree Number 386 (Wanenge, 2009). *S. lancea* belongs to the Anacardiaceae family, is widespread in the vicinity of the Highveld goldmines, and appears to tolerate a wide variety of mining sites (Weiersbye *et al*., 2006; Weiersbye and Witkowski, 2007; Gundiza *et al*., 2008).

The species has been found to tolerate AMD polluted groundwater, the harsh conditions on Highveld mines (fires, frost, wind, drought and AMD polluted soils), and competition from neighbouring trees - as a result *S. lancea* has been planted adjacent to TSFs for phytostabilisation and hydrological control (Dye *et al*., 2008; Dye and Weiersbye, 2010). These factors ensure that *S. lancea* can grow readily in closed canopy stands, and that there is suitably high extraction of AMD throughout the year (Dye *et al*., 2008). Sap flow data reported by Dye *et al*. (2009) showed that *S. lancea* has high transpiration rates throughout the year, which indicated that it is a useful species for hydraulic control of AMD. The tree can also accumulate up to 3 % sulphur in its leaves which also makes it a good candidate for hydraulic control of seepage from TSFs (Dye *et al*., 2009) and in turn phytoextraction. *S. lancea* seedlings were transplanted onto the site-species trials in January 2003. The *S. lancea* used were propagated from cuttings originally from Uppington, Northern Cape, South Africa (Herbert, 2009).

CHAPTER 3

METHODS AND MATERIALS

3.1 Introduction

The aim of the sampling was to collect soil samples and harvest whole trees at two sites at AngloGold Ashanti's Vaal River and West Wits mining operations in order to obtain representative samples of different tree compartments for elemental comparisons, as well as soil – tree comparisons. This was to determine the fate of contaminants in *S. lancea* trees planted for hydrologic control and phytostabilisation purposes, and to determine if *S. lancea* is an extractor or stabiliser of certain contaminants.

Due to this project being funded by THRIP and the NRF, not all sample preparation and analysis was conducted by me. However, where preparation and analysis was conducted externally, I was able to gain experience in the techniques used for soil pH and X-Ray Fluorescence (XRF) analysis.

All harvesting was done by myself, an EEPP supervisor and a team provided by EEPP sub-contractors. Sub-sampling was done by me for all trees except for trees at West Complex due to logistical limitations. B. McLeroth conducted the sub-sampling there.

All plant sample preparation was conducted by me while all soil samples were prepared and analysed at the Vaal River lab (Sieving, weighing and pH) and at the Wits lab (XRF) by EEPP personnel. As various people were involved with this rigorous process not all are named here. Please see the acknowledgement section for all persons concerned.

3.2 Tree Selection

Three *Searsia lancea* plots were chosen randomly at all four sites (Madala and Redsoils trials at West Wits and Mispah and West Complex trials at Vaal River). One tree of average height, per plot, was also randomly chosen, taking into consideration that a TLB (backacter) would need to access the area in order to harvest the roots. Trees were not chosen from the outermost rows in order to negate

edge effects; however, in plots where trees were performing poorly and only a few trees were available – edge effects could not be avoided.

3.3 Harvesting

Harvesting of above-ground biomass took place from 16 February to the 19 March 2015. The harvesting of the roots and the soil pit sampling took place thereafter from the 20 March to 21 May 2015.

S. lancea trees were stripped of their trifoliate leaves first. Leaves were removed by hand, one by one, being careful to remove the petiole as well (Figure 3..). Next, twigs were removed from branches and stems. Twigs were defined as being less than or equal to 1 cm in diameter and kept separate from branches (Figure 3..). The branches were then sawed from the stump using a bow-saw and cut into smaller, more manageable sized pieces. When removing the stump; the grass or debris was removed from the base of the tree and then soil was scraped away with a spade until the tops of roots became evident. This was usually no more than a few centimetres. The stumps were then removed by hand with a bow-saw, or if very wide with a chainsaw. Harvesting was found to be easiest when removing one stem or branch at a time; removing the leaves and then separating the twigs from the stems.



Figure 3.1. **Stripping leaves of *S. lancea* at West Complex.**



Figure 3.2. **Cutting of *S. lancea* twigs after stripping at Vaal River Mispah site.**

A section from the top and bottom of the main stem or branch of each tree was collected for density measurements. If present, dead biomass was separated from live biomass.

Root harvesting and soil pit sampling was conducted using a TLB (backacter). The TLB dug all pits roughly 2.5 m by 3 m with depths ranging from 1 – 3.5 m at the sites of the harvested trees (Figure 3.. and Figure 3.4.). Not all sites were conducive to a 3 m deep pit as the underlying rock strata were either too shallow or there were boulders present. Thus, pit depth ranged from site to site. However, pits at each respective site were dug to the same depth in order for soil samples and data to be comparable.

Once the root ball was removed from the soil pit, it was stripped of its roots (coarse roots and fine roots) and all respective root parts were weighed. Coarse roots were defined as being greater-than 1 cm in diameter, while fine roots were defined as being less-than or equal to 1 cm in diameter. Fine roots and coarse roots were bagged and weighed together.

The pits were dug so that their edges were halfway between the harvested tree and the trees around it. The spacing in general for the planting of the trees was 2.5 m in-tree spacing and 3 m between tree rows, hence the soil pits were dug about 1.5 m from the surrounding trees (Figure 3.5.).

The first metre of soil was dug up by the TLB and placed in a pile close-by to the pit. Next, the root-ball was loosened and pulled up in order to keep as many roots attached to it as possible (Figure 3.6.). The second metre of soil was then dug up and placed in another pile and the same was done with successive piles of soil if the TLB was able to dig deeper.



Figure 3.3. TLB breaking ground on the first soil pit at Redsoils, West Wits site.



Figure 3.4. **TLB in the process of removing soil from a Redsoils soil pit.**

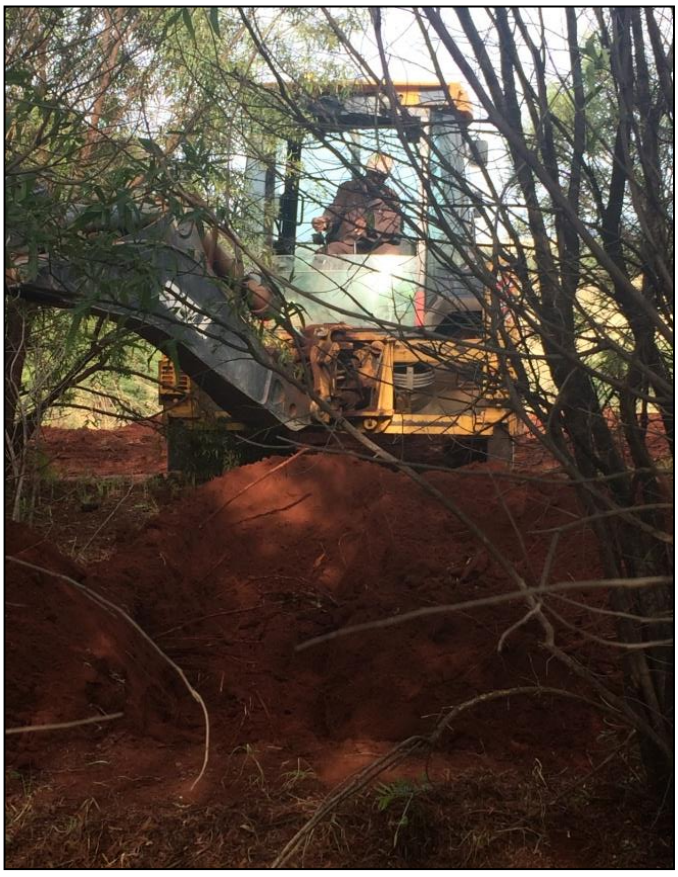


Figure 3.5. **TLB trying to dig a soil pit through two outer trees of a plot; one of the difficulties the TLB encountered whilst digging soil pits.**



Figure 3.6. A Redsoils root ball at West Wits before being cut up.

The pit sizes varied depending on spacing between trees and the TLB access. The pits needed to be large enough to get most, if not all of the root mass per tree as well as large enough and deep enough to obtain soil samples down the soil profile. Each pit size was measured along with the tree spacing in each plot.

3.4 Sub-Sampling

The bags containing the different tree parts were then weighed using a digital hand scale (Travelon Micro Scale, capacity: 50 kg, readability: 0.1 kg). Once weighed, sub-samples were taken randomly of each component using a 2 kg balance, about 100 g leaves, 100 g twigs, two pieces of stem (if available), about 100 g each of fine and coarse root. These sub-samples were bagged into large ziplock bags for transport back to the Wits laboratory for analysis and stored in a – 17-degree Celsius cold-room.

All remaining tree parts were placed into refuse bags for transportation. Thereafter the remainder of the different tree parts were placed into 40 % shade-net bags. The bags were 1.5 x 1.5 m (in general for larger biomass and smaller bags were made for smaller biomass) for drying and/ or

storage (Figure 3.7.). This was to provide ventilation for maximum drying and to prevent moulding. Bags containing leaves were placed onto chicken mesh drying shelves (Figure 3.8.). Bags containing all other components were hung off the floor to prevent moulding and dust accumulation (Figure 3.9.).



Figure 3.7. Example of the 40% shade net bags used to dry the different tree parts.



Figure 3.8. Chicken mesh drying shelves for drying bags of *S. lancea* leaves.



Figure 3.9. Hanging of shade net bags of wood and twigs for drying.

Re-weighing of the dry biomass took place about 6 months after harvesting – initially on 11 November 2015 for a first weighing of a select number of samples and then again on 20 November 2015 to check if there was a change in weight to determine if biomass was dry or not. As biomass was found to be dry, all re-weighing of samples was conducted on 20 November 2015. The samples were weighed in batches again using a piece of shade-net with a known mass. This was then subtracted from the final mass to determine the dry mass of the tree compartments.

3.5 Soil Sampling

Soil samples were taken from faces inside the soil pits in which the roots were harvested. Two opposite faces were sampled for replication at the following depths; Litter, 0-2, 2-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, 50-60, 70-80, 90-100, 120-130, 145-155, 170-180, 190-210, 240-260 and 280-300 cm. Not all pits were sampled up to 300 cm as some soil types did not allow for it.



Figure 3.10. Soil pit sampling in progress using the specially cut PCV pipe.

At each depth about 150 g of sample was taken using a piece of specially cut PVC pipe about 20 cm long with one of the sides cut at an angle of about 45 degrees (Figure 3.10.). The samples were taken from the bottom profile upwards (Figure 3.11.) and were placed in medium sized plastic zip lock bags. The bags were not written on to prevent cross-contamination and were thus double-bagged with

a piece of paper between the bags labelling them. These were then kept cool until transported back to Wits for analysis.



Figure 3.11. An example of a sampled face of a soil pit.

3.6 Sample Preparation and Analysis

3.6.1 Sample Preparation

Plant Samples

Plant samples were washed four times with distilled water (DiH_2O). They were then placed on paper towel to dry (Figure 3.1). Once dry, they were placed in medium plastic zip lock bags. Leaves and fine roots were freeze-dried (Lebanco FreeZone 4.5 l Freeze Drying System), while twigs, stem pieces and coarse roots were oven-dried at room temperature (25^{o} C) until they reached a constant weight. These compartments were oven-dried as they were bulkier and did not dry properly in the freeze-drier. The freeze-drying usually took about a week, while the oven drying took 2-3 weeks, but this was also dependent on the sample. The plant samples were then ground using a ceramic burr

grinder going from the coarsest, to the finest grinder setting. This was done until the samples reached a powder-like consistency (Figure 3.13.).



Figure 3.12. Plant samples set out to dry after washing.



Figure 3.13. Leaf sample ground by a ceramic burr grinder.

Soil Samples

A 10 g sample of soil was taken for each soil sample and mixed with 20 ml of distilled water to measure pH. The remainder of the sample was sieved using a 2 mm plastic sieve to separate soil from gravel and concretions. The sieved soil was then weighed and kept separate for XRF analysis.

3.6.2 Sample Analysis

Soil and plant samples were both analysed by X-ray Fluorescence (XRF) (Spectro, XEPOS 03 STD Gas, 150 VA with Aluminium plates) for total concentrations of Na, Mg, Al, S, Cl, Cd, Cr, Mn, Fe, Cu, Zn, and Pb elements.

3.7 Data Analysis

All data analysis was performed using IBM SPSS Statistics 23. Descriptive statistics (mean, standard deviation, standard error of the mean, etc.) were calculated for all data. Non-parametric tests were used as these data were not normally distributed as assessed by the Shapiro Wilk test for normality. Numerous transformations were done on these data, but they remained non-normally distributed. The Mann-Whitney U test of stochastic equality (Mann and Whitney, 1947) was used for the pairwise comparison between WW and VR sites (for soil elemental data and tree elemental data). The non-parametric analysis of variance (Kruskal-Wallis) (Vargha and Delaney, 1998) was used to test for differences between the soil types, trees and depths.

Post-hoc pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons to find specific differences between soils, trees, compartments and soil depths (Laerd Statistics, 2015).

3.7.1 Elemental mass and elemental concentration calculations

Elemental mass for each tree was calculated for each compartment (leaves, twigs, wood and roots) by taking the mass of the compartment (kg) and multiplying it by the concentration of the

compartment (mg/kg). This gave the elemental mass of the compartment in milligrams. This process was done for each compartment in each tree. The compartment elemental mass for each tree was then added up for a total elemental mass for each tree. This was then averaged for each site. As coarse roots and fine roots were not separated for compartmental mass (kg), the average compartment elemental concentration for coarse roots and fine roots were found and used to calculate the root compartment elemental mass. This was deemed acceptable, as there were no significant differences found between elemental concentration for coarse roots and fine roots. Elemental concentration was determined via XRF for each tree compartment. These values were averaged to find tree elemental concentration. This value was further averaged to find mean tree concentration by site for comparison.

3.7.2 Bioconcentration Factor (BCF)

The BCF, also known as the Enrichment Coefficient for the Roots (ECR) or the root-soil quotient, shows the index for the accumulation of trace elements in plant parts or the transfer of elements from the soil to plant roots. If the result is ≥ 1, the plant is considered a phytostabiliser of the tested metal, and if the result is ≤ 1, the plant is a poor phytostabiliser and more of a phytoextractor of the tested metal. Root and shoot concentrations were averaged per tree. This was compared to the average soil pit elemental concentration at each tree.

$$BCF = \frac{Conc.in\ mg\ kg^{-1} of\ plant\ root/\ shoot}{Conc.in\ mg\ kg^{-1}\ in\ soil} \quad (1)$$

3.7.3 Translocation Factor (TF)

The TF, also known as the shoot-root quotient, if > 1 represents an accumulator species, and if < 1 it represents a stabilizer species. As above, root and shoot concentrations were averaged per tree, but here they were compared to one another.

$$\text{TF} = \frac{Conc.in\ mg\ kg^{-1}\ of\ plant\ shoots}{Conc.in\ mg\ kg^{-1} of\ plant\ roots} \quad (2)$$

CHAPTER 4

RESULTS

4.1 Introduction

Results of this study will be presented in this chapter. The order of results will be as follows; 1) the fate of contaminants within *S. lancea* tree compartments; 2) biomass vs. elemental mass of *S. lancea* trees; and 3) the phytoextraction vs. phytostabilisation potential of *S. lancea*.

4.2 Fate of contaminants within *S. lancea* tree compartments

Harvested *S. lancea* trees (n = 12) were separated into 5 different compartments (leaves, twigs, wood, coarse roots and fine roots), and subsamples of each compartment were tested for 12 element concentrations (Na, Mg, Al, S, Cl, Cd, Cr, Mn, Fe, Cu, Zn, and Pb) in each tree (N = 60). Despite having significant concentrations of some elements (refer to section 4.1.6 Bark), bark was not considered a separate compartment as it occurs across multiple compartments with relatively low mass, and as such, stripping the bark fell outside the constraints of the project. Although all elements are distributed throughout the trees, some are more evident in certain compartments than others. There were no significant differences found between the five compartments for Al, Pb, Cr, and Na (for $P < 0.05$).

4.2.1 Leaf compartment

Sulphur and Mn had their highest concentrations in the leaf compartment. The mean S concentration was 4367.17 mg/kg in the leaves (95% CI: 3801.02 – 4933.31) and lowest at 1221.49 mg/kg in the wood (95% CI: 952.65 – 1490.34). The concentration pattern observed for S was; leaves > twigs > coarse roots > fine roots > wood. Figure 4.1. shows significant differences between leaves and wood ($p = 0.0005$), leaves and fine roots ($p = 0.0005$), coarse roots and twigs ($p = 0.014$), coarse roots and leaves ($p = 0.0005$); fine roots and twigs ($p = 0.027$), and wood and twigs ($p = 0.0005$).

The mean Mn concentration, as seen in Figure 4.2, was highest at 389.33 mg/kg in the leaves (95% CI: 287.44 – 491.23) and lowest at 72.83 mg/kg in the wood (95% CI: 37.92 – 107.74). The concentration pattern found was; leaves > coarse roots > fine roots > twigs > wood. Significant

differences were found between leaves and wood (p = 0.0005), coarse roots and wood (p = 0.003), and fine roots and wood (p = 0.018).

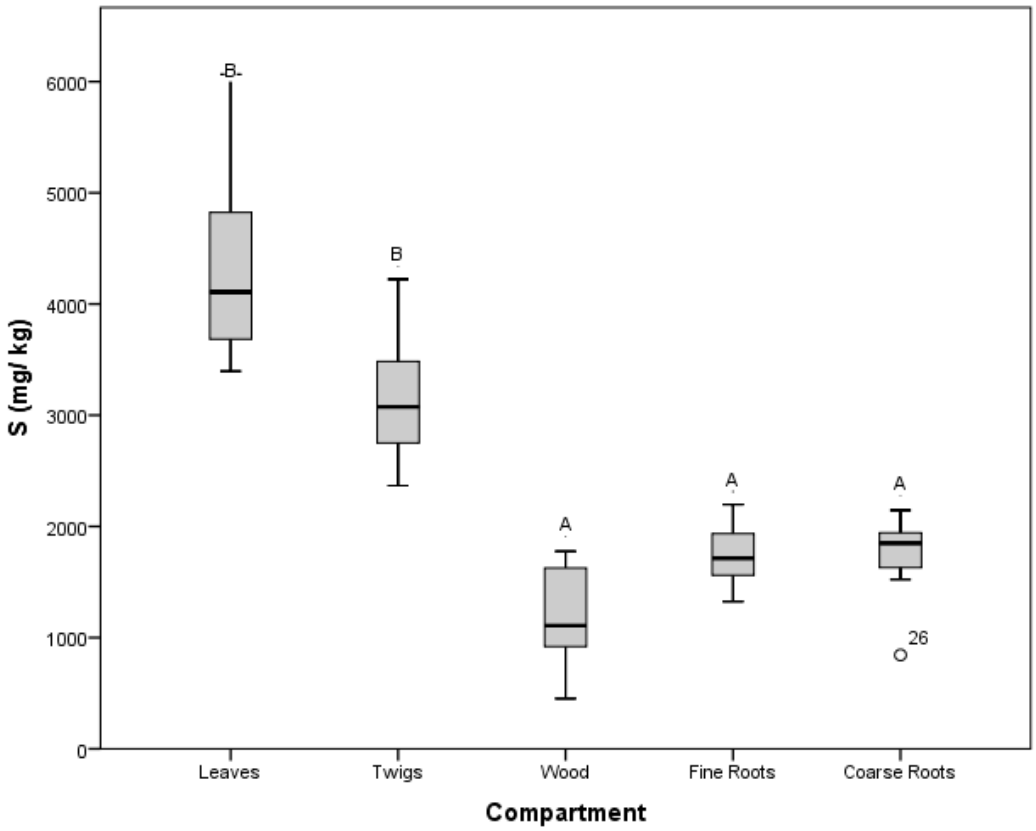


Figure 4.1. **Mean S compartment concentrations. Letters denote significant differences between compartments (N = 60).**

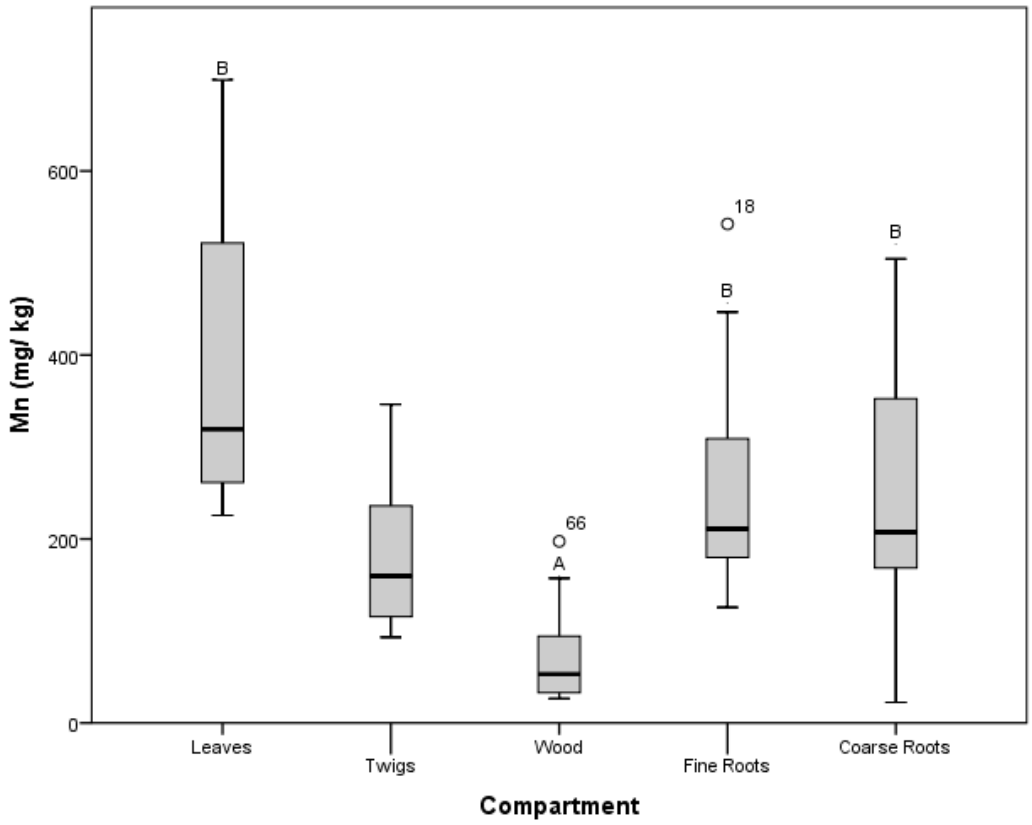


Figure 4.2. **Mean Mn compartment concentrations. Letters denote significant differences between compartments (N = 60).**

Significant differences in the leaf compartments between sites were found for Cl ($p = 0.024$) and Cr ($p = 0.029$). Concentrations of Cl were significantly higher at Madala (5762 mg/kg; 95% CI: 1529.71 – 9994.29 mg/kg) compared to Redsoils (1066.1 mg/kg; 95% CI: 19.66 – 2112.54 mg/kg).

4.2.2 Twig compartment

Chlorine, Cu, and Zn had the highest mean concentrations in the twig compartment. They also shared the same concentration patterns (twigs > leaves > coarse roots > fine roots > wood) with highest mean concentrations found in the twigs at 3814.50 mg/kg (95% CI: 2796.44 – 4832.56 mg/kg), 20.68 mg/kg (95% CI: 18.71 – 22.64 mg/kg), and 46.13 mg/kg (95% CI: 36.57 – 55.70 mg/kg) respectively; and the lowest concentrations found in the wood with means of 1076.06 mg/kg (95% CI: 701.12 – 1450.99 mg/kg), 7.25 mg/kg (95% CI: 5.96 – 8.54), and 6.15 mg/kg (95% CI: 4.89

– 7.41 mg/kg) respectively. As these elements followed the same pattern only Cu is presented here in Figure 4.3.

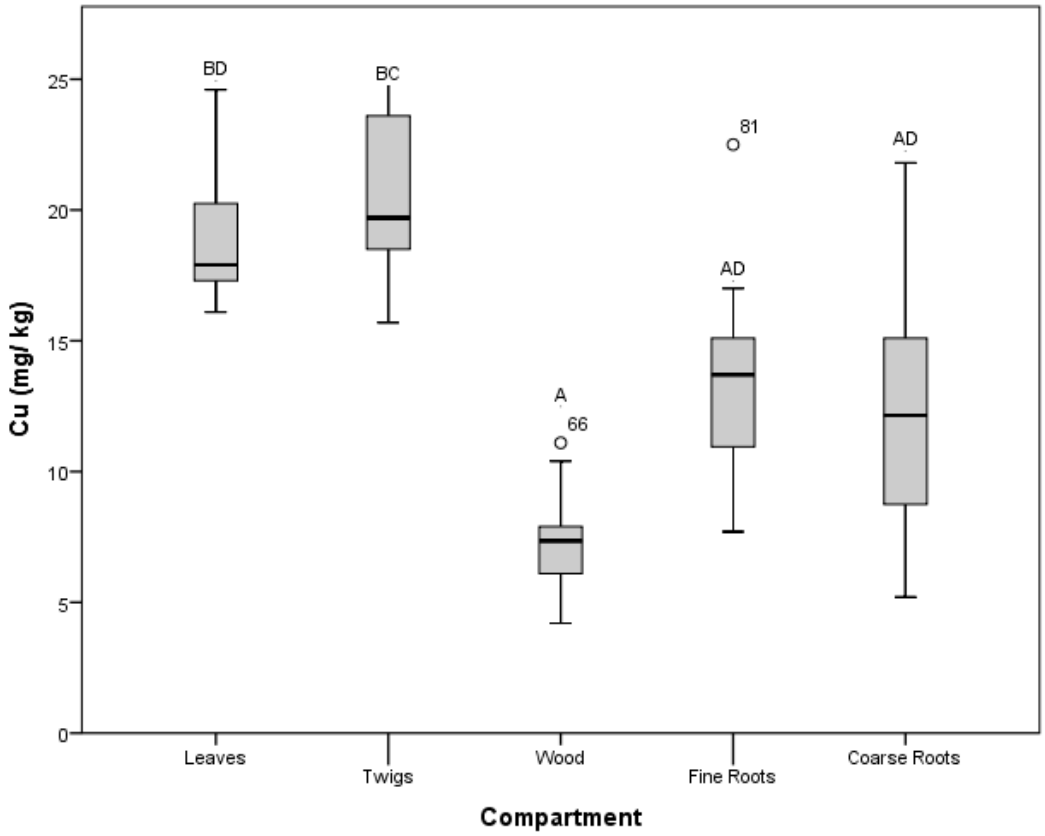


Figure 4.3. Mean Cu compartment concentrations. Letters denote significant differences between compartments (N = 60).

Significant differences at sites were found between twigs for Na ($p = 0.044$), Cl ($p = 0.038$), Cr ($p = 0.038$), Cu ($p = 0.030$), and Zn ($p = 0.034$). Chlorine was significantly higher in West Complex twigs (5553.33 mg/kg; 95% CI: 4790.33 – 6316.34 mg/kg) compared to Redsoils (1754 mg/kg; 95% CI: 1235.68 – 2272.32 mg/kg). Sodium was also significantly higher in West Complex twigs (15480 mg/kg; 95% CI: 13676.59 – 17063.41 mg/kg) compared to twigs at Redsoils (11363.33 mg/kg; 95% CI: 10629.64 – 12097.03 mg/kg).

4.2.3 Wood compartment

No elements were found to have their highest concentrations in the wood compartment, but all elements were found to have their lowest elemental concentrations in the wood compartment.

4.2.4 Coarse root compartment

Iron and Mg were found to be the highest in the coarse root compartment and lowest in the wood compartment, but had different concentration patterns.

The mean Fe concentration was highest at 737.62 mg/kg in the coarse roots (95% CI: 467.19 – 1008.04 mg/kg) and lowest at 117.55 mg/kg in the wood (95% CI: 8.95 – 226.15 mg/kg) (

Figure 4.4.). The concentration pattern found was; coarse roots > fine roots > leaves > twigs > wood. Significant differences were only found between coarse roots and wood ($p = 0.003$).

The highest mean concentration of Mg was 9036.67 mg/kg in the coarse roots (95% CI: 7070.30 – 11003.03 mg/kg) with the lowest concentration of 2218.65 mg/kg in the wood (95% CI: 61.63 – 4498.93 mg/kg). The concentration pattern found was; coarse roots > leaves > twigs > fine roots > wood. Statistically significant differences were found between coarse roots and wood ($p = 0.0005$), leaves and wood ($p = 0.020$), and twigs and wood ($p = 0.029$) (Figure 4.5).

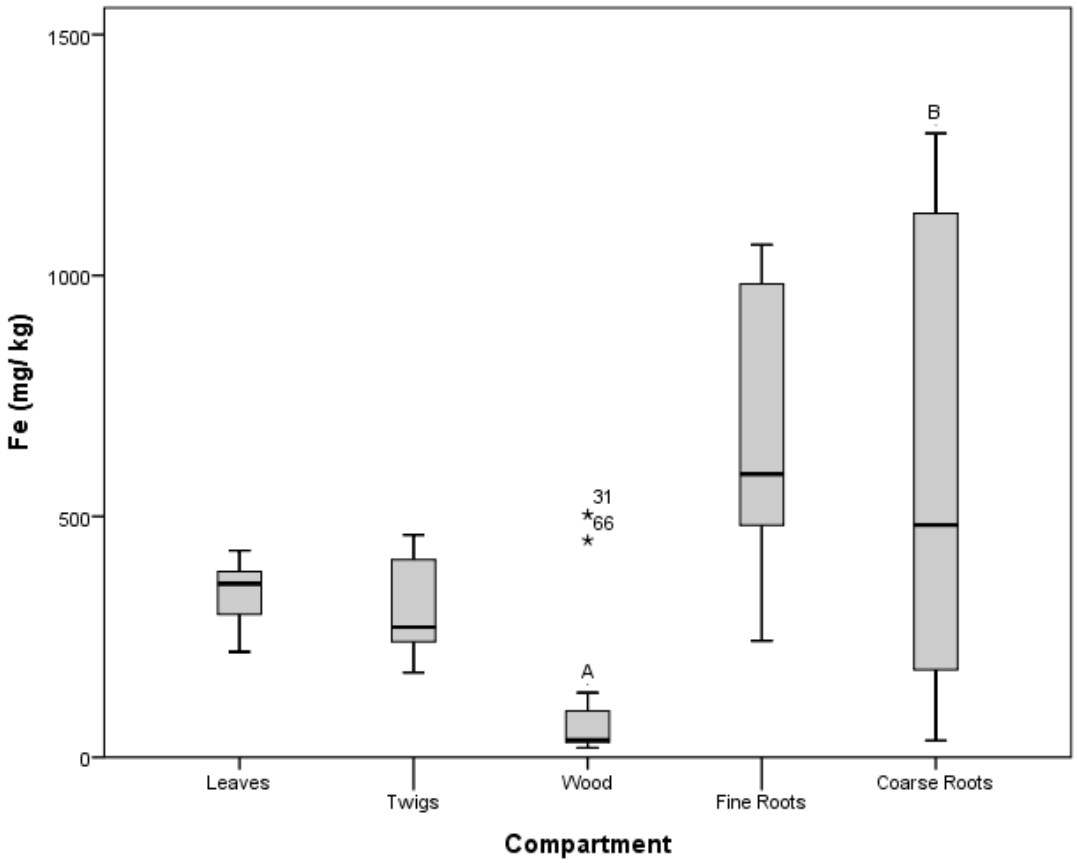


Figure 4.4. **Mean Fe compartment concentrations. Letters denote significant differences between compartments (N = 60).**

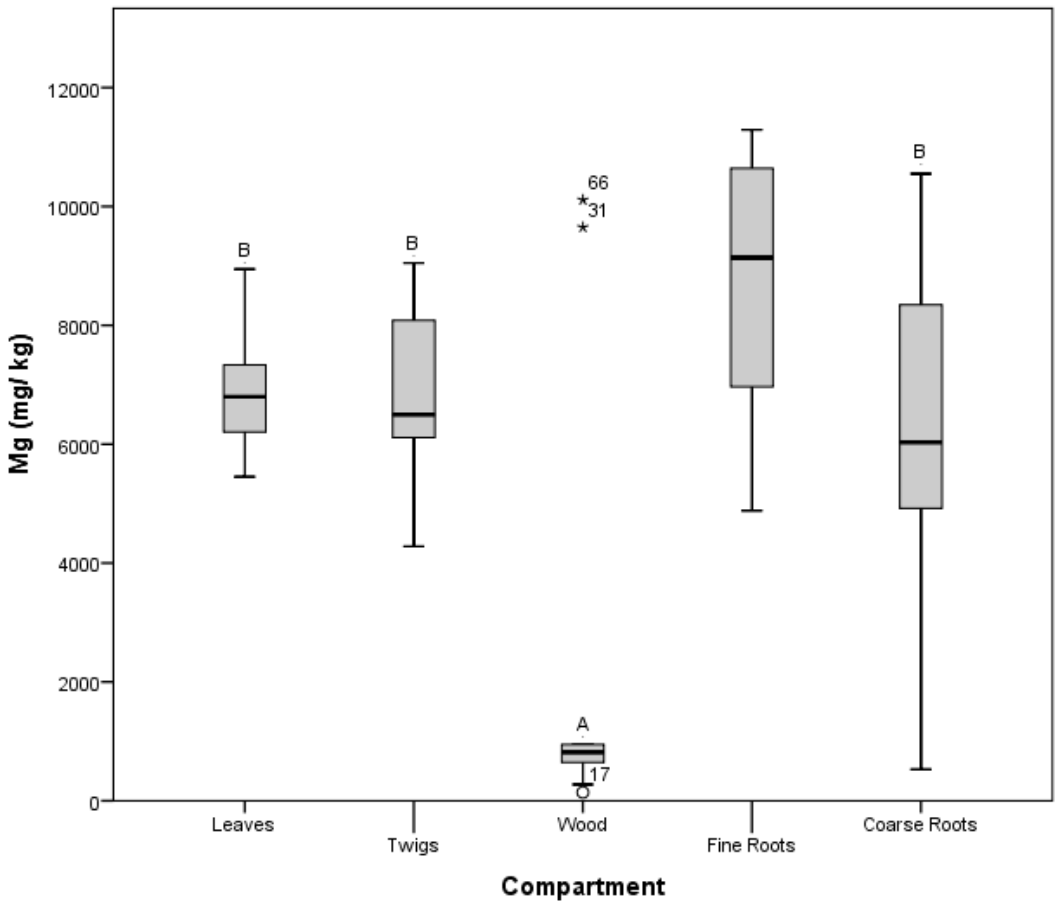


Figure 4.5. **Mean Mg compartment concentrations. Letters denote significant differences between compartments (N = 60).**

Significant differences in coarse roots between sites were found for Al ($p = 0.024$), Cl ($p = 0.026$), and Fe ($p = 0.027$). Aluminium was significantly higher in coarse roots at Redsoils (8225 mg/kg; 95% CI: 445.09 – 16895.09 mg/kg) compared to West Complex (1673.67 mg/kg; 95% CI: 977.79 – 2369.55 mg/kg). Chlorine was significantly higher in coarse roots at West Complex (2572.33 mg/kg; 95% CI: 1237.41 – 3907.86 mg/kg) compared to Redsoils (979.50 mg/kg; 95% CI: 658.15 – 1300.85 mg/kg). Iron was significantly higher in coarse roots at Redsoils (4034.67 mg/kg; 95% CI: 140.46 – 8209.80 mg/kg) compared to West Complex (395.73 mg/kg; 95% CI: 122.17 – 669.30 mg/kg).

4.2.5 Fine root compartment

Calcium concentrations were found to be highest in the fine root compartment. The highest mean Ca concentration was 19 798.17 mg/kg in the fine roots (95% CI: 16.221.79 – 23374.54 mg/kg) and lowest at 10 835.92 mg/kg in wood (95% CI: 7256.67 – 14415.17 mg/kg). The concentration pattern found was; fine roots > twigs > coarse roots > leaves > wood. Statistical differences were found to be significant between fine roots and wood (p = 0.001), twigs and wood (p = 0.003), and coarse roots and wood (p = 0.027) (Figure 4.6).

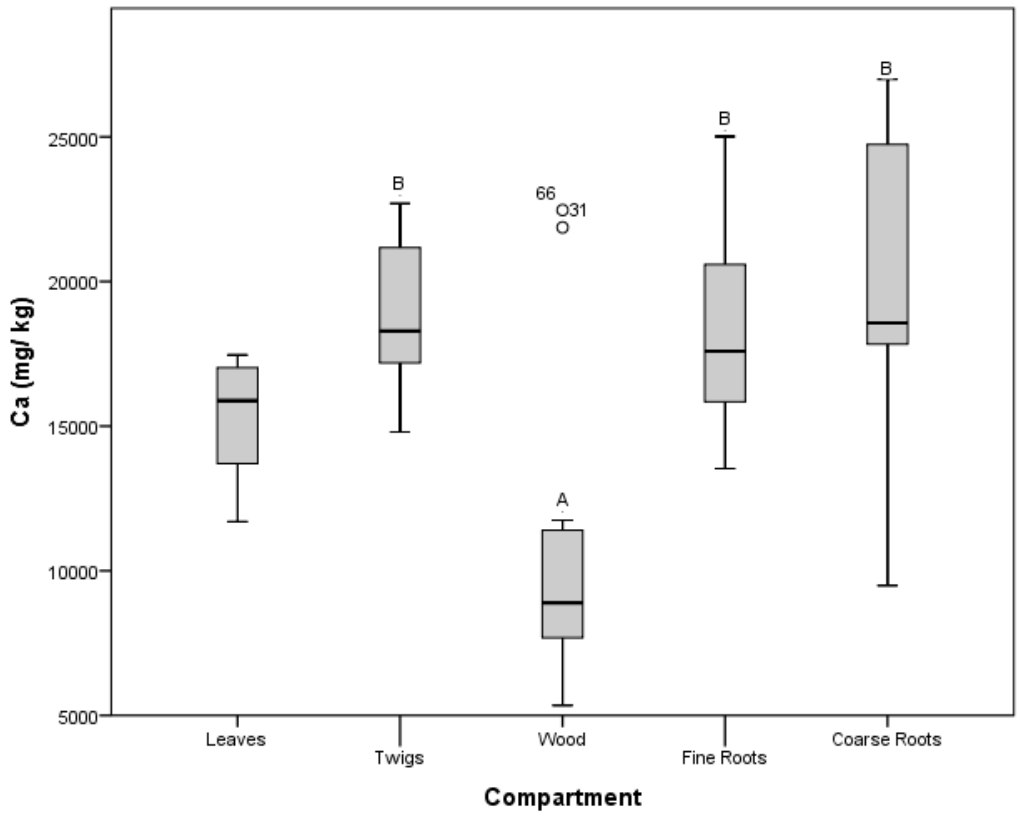


Figure 4.6. Mean Ca compartment concentrations. Letters denote significant differences between compartments (N = 60).

Significant differences were found for fine roots between sites for Mg (p = 0.038), Al (p = 0.023), S (p = 0.023), Cl (p = 0.026), Cr (p = 0.024), and Fe (p = 0.019). Aluminium was significantly higher in fine roots at Redsoils (19276.07 mg/kg; 95% CI: 6287.83 – 32265.51 mg/kg) compared to West Complex (5858 mg/kg; 95% CI: 3137.17 – 8578.83 mg/kg). Conversely, S was found to be significantly higher in fine roots at West Complex (4323.67 mg/kg; 95% CI: 2539.78 –

6107.55 mg/kg) compared to Redsoils (2614.33 mg/kg; 95% CI: 1961.76 – 3266.90 mg/kg). Chlorine was significantly higher in Mispah fine roots (4510.33 mg/kg; 95% CI: 1450.68 – 7569.99 mg/kg) compared to Redsoils (1554.33 mg/kg; 95% CI: 459.41 – 2649.26 mg/kg). Chromium was found to be significantly higher in fine roots at Redsoils (52 mg/kg; 95% CI: 17.93 – 86.07 mg/kg) compared to West Complex (19.3 mg/kg; 95% CI: 15.80 – 22.80 mg/kg). Lastly, Fe was significantly higher in fine roots at Redsoils (7118.67 mg/kg; 95% CI: 2447.41 – 11789.93 mg/kg) compared to Mispah (1412.97 mg/kg; 95% CI: 407.22 – 2418.71 mg/kg).

4.2.6 Bark

Sub-samples of bark from the top and bottom of the tree stem were analysed. Bark results are not presented above as bark was not stripped from the trees or weighed separately to the other compartments as this fell outside of the time and budget constraints of this study.

Bark contained significantly high mean concentrations of Al (11 580.67 mg/kg; 95% CI: 7761.83 – 15399.50 mg/kg), Fe (3 466.91 mg/kg; 95% CI: 1886.01 – 5047.80 mg/kg), and Pb (1.83 mg/kg; 95% CI: 0.33 – 3.34 mg/kg) compared to the highest compartment concentrations; Al in the coarse roots (2174.49; 95% CI: 1481.73 – 2867.23 mg/kg), Fe in the coarse roots (737.62 mg/kg; 95% CI: 467.19 – 1008.04 mg.kg), and Pb in the wood (0.82 mg/kg; 95% CI: 0.5 – 2.14 mg/kg). Bark also had the highest concentrations of Cr (29.63 mg/kg; 95% CI: 20.05 – 39.2 mg/kg) and Na (13830.00 mg/kg; 95% CI: 11878.89 – 15781.11 mg/kg).

4.3 Total biomass vs. total elemental mass of *S. lancea* trees

4.3.1 Tree Sizes and Densities

Trees were found to be higher in general at the Redsoils and Mispah sites, while the shortest trees were found at the Madala site. Canopy diameter was also found to be wider at the Redsoils and Mispah sites, but did not differ too much between West Complex and Madala sites. There were no major differences in tree densities between the sites.

Table 4.1. ***S. lancea*** **tree height, canopy diameter and density.**

Site	Tree	Height (m)	Canopy Diameter (m)	Canopy Diameter at 90° (m)	Density (g/cm^3)
Madala	S1	3.30	2.90	2.65	0.65
Madala	S2	2.76	3.40	3.16	0.76
Madala	S3	4.33	3.55	3.50	0.83
Redsoils	S4	4.01	4.25	2.47	0.87
Redsoils	S5	4.42	4.50	3.15	0.71
Redsoils	S6	5.05	4.45	4.40	0.41
West Complex	S7	3.30	2.95	2.55	1.58
West Complex	S8	3.45	3.50	3.35	0.66
West Complex	S9	3.06	2.73	2.65	1.98
Mispah	S10	5.25	5.00	4.00	0.61
Mispah	S11	5.50	4.15	3.90	0.93
Mispah	S12	5.25	4.50	4.25	2.10

4.3.2 Biomass vs. site

Mispah had the highest mean tree mass, followed by Redsoils, Madala and West Complex (Figure 4.7. and Table 4.2). Despite apparent variation, there were no significant differences in biomass distribution found between sites (for $p > 0.05$) (Table 4.2.).

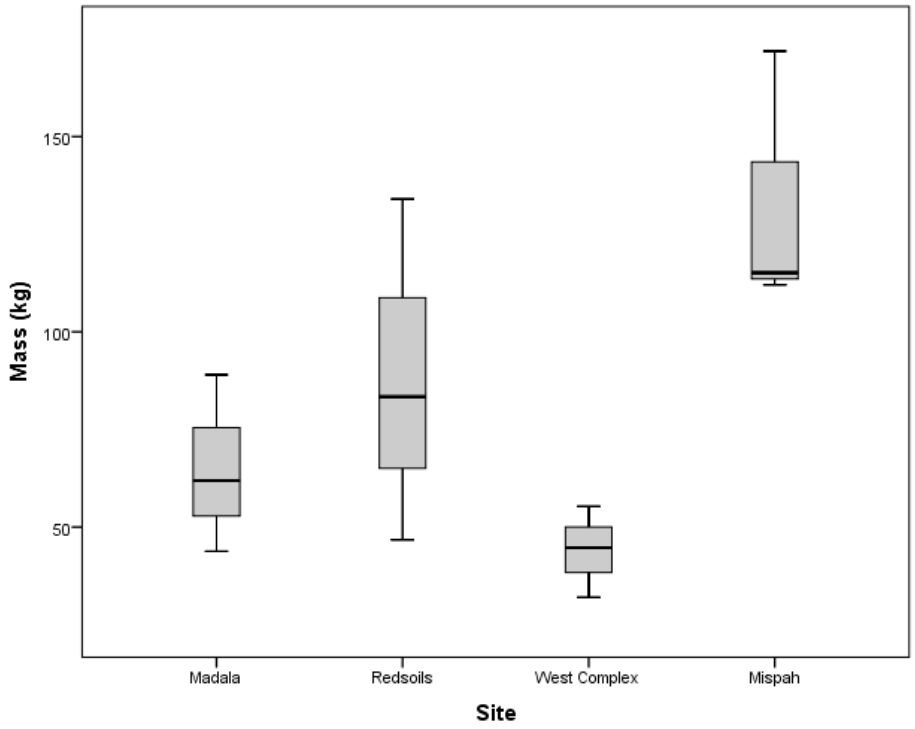


Figure 4.7. **Mean tree mass (kg) for each site (n = 12).**

Table 4.2. **Mass in kilograms of compartments (dry mass) showing total mass of trees.**

Site	Tree	Leaves (kg)	Twigs (kg)	Wood (kg)	Roots (kg)	Total (kg)
Madala	S1	2.6	3.4	7.3	25.7	39.0
Madala	S2	3.6	4.2	22.9	23.9	54.6
Madala	S3	2.6	6.4	44.7	30.1	83.8
Redsoils	S4	1.4	2.1	18.1	20.3	41.9
Redsoils	S5	5.0	5.8	72.2	41.2	124.2
Redsoils	S6	2.3	4.8	39.8	31.3	78.2
West Complex	S7	3.4	4.4	17.3	13.5	38.6
West Complex	S8	3.2	5.9	19.8	19.6	48.5
West Complex	S9	2.6	3.1	9.1	12.4	27.2
Mispah	S10	3.6	13.7	63.6	27.6	108.5
Mispah	S11	5.5	10.9	97.0	48.7	162.1
Mispah	S12	4.3	10.2	67.3	21.1	102.9

4.3.3 Elemental mass in S. lancea trees

Four compartments per tree were averaged for elemental mass for each tree at each site, thus a total of 12 samples were analysed for tree elemental mass (N = 12). It was found that Na, S, Cl, Ca, and Zn showed similar distributions, with the Mispah site showing the highest mean tree elemental mass compared to other sites. The Cl distribution was interesting, as Redsoils mean tree elemental mass was considerably lower than Mispah when it was expected to be similar (Figure 4.8.), but none of these elements showed any significant differences in distribution (for $p > 0.05$).

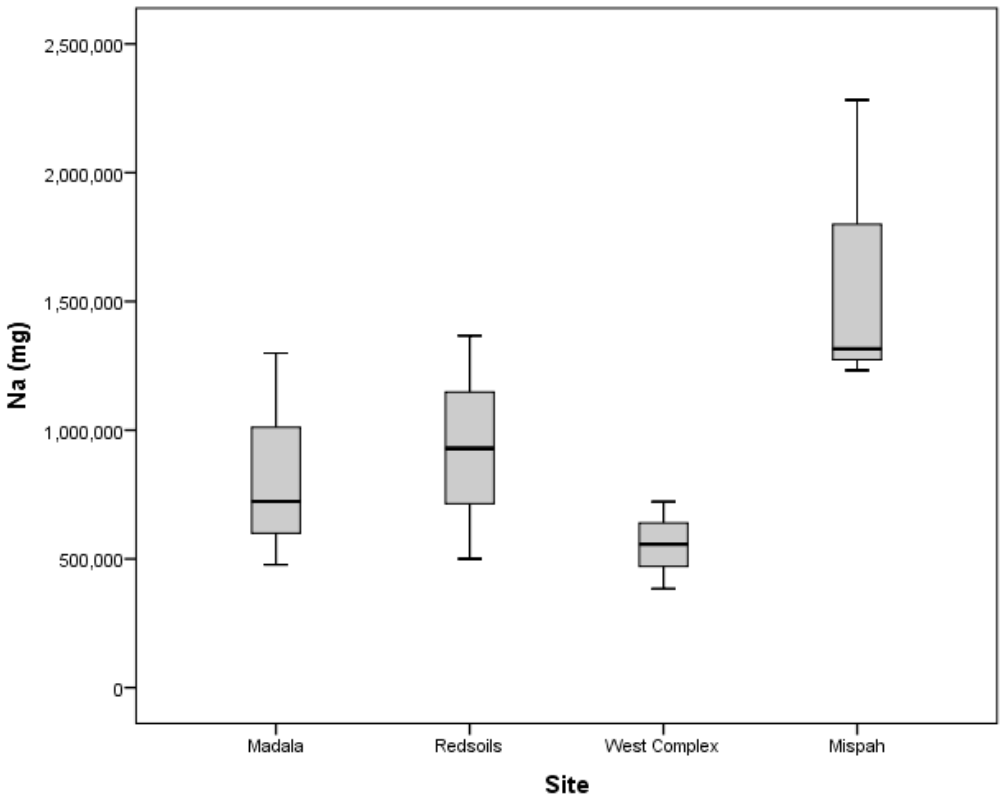


Figure 4.8. Mean elemental mass of Cl for trees at each site (N = 12).

Magnesium, Cr, Mn, and Cu showed similar distributions for mean tree concentration, with the Redsoils and Mispah sites having the highest mean elemental mass. Copper showed the only significant difference in distribution between West complex (425.63 mg; 95% CI: 163.79 mg – 687.47 mg) and Mispah (1357.69 mg; 95% CI: 783.05 mg – 1932.34 mg; $p = 0.039$) (Figure 4.9.). These high tree elemental masses at Redsoils and Mispah may correlate to large biomass.

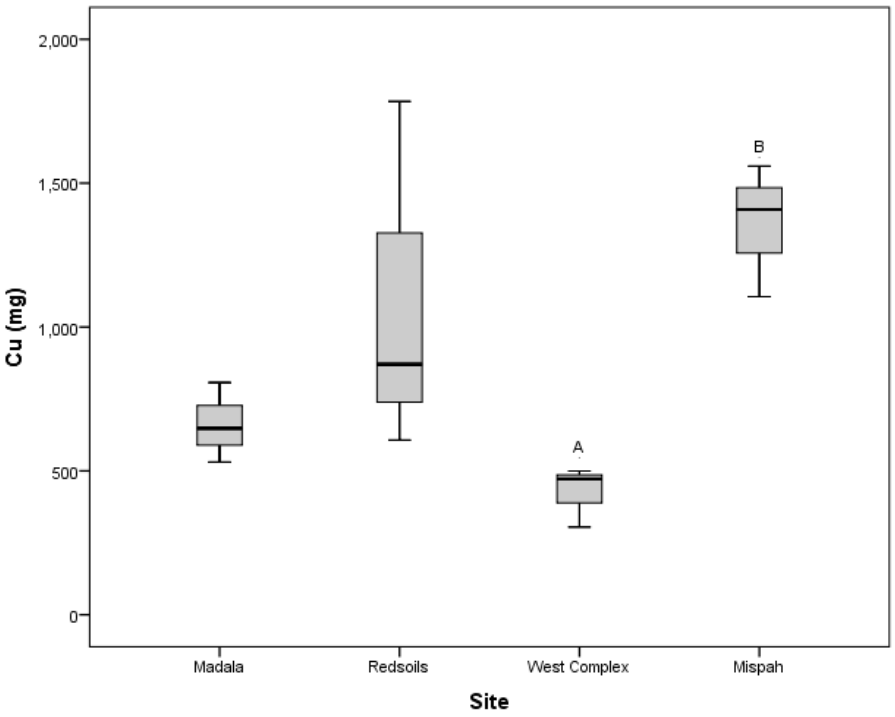


Figure 4.9. Mean elemental mass of Cu for trees at each site with A and B showing significant differences (N = 12).

Interestingly, the mean mass of Al per tree was highest at Redsoils (487755.24 mg; 95% CI: 189137.96 mg – 1164648.434 mg) which also had a significantly higher distribution compared to West complex (62482 mg; 95% CI: 45427.22 mg – 79573.94 mg; p = 0.019) as can be seen in Figure 4.10. Mean Fe was similar to the distribution of Al mean tree mass per site as Redsoils (197039.97 mg; 95% CI: 96907.81 mg – 490987.75 mg) also had a significantly higher distribution compared to West Complex (21881.81 mg; 95% CI: 12855.95 mg – 30907.68 mg; p = 0.013) as seen in Figure 4..

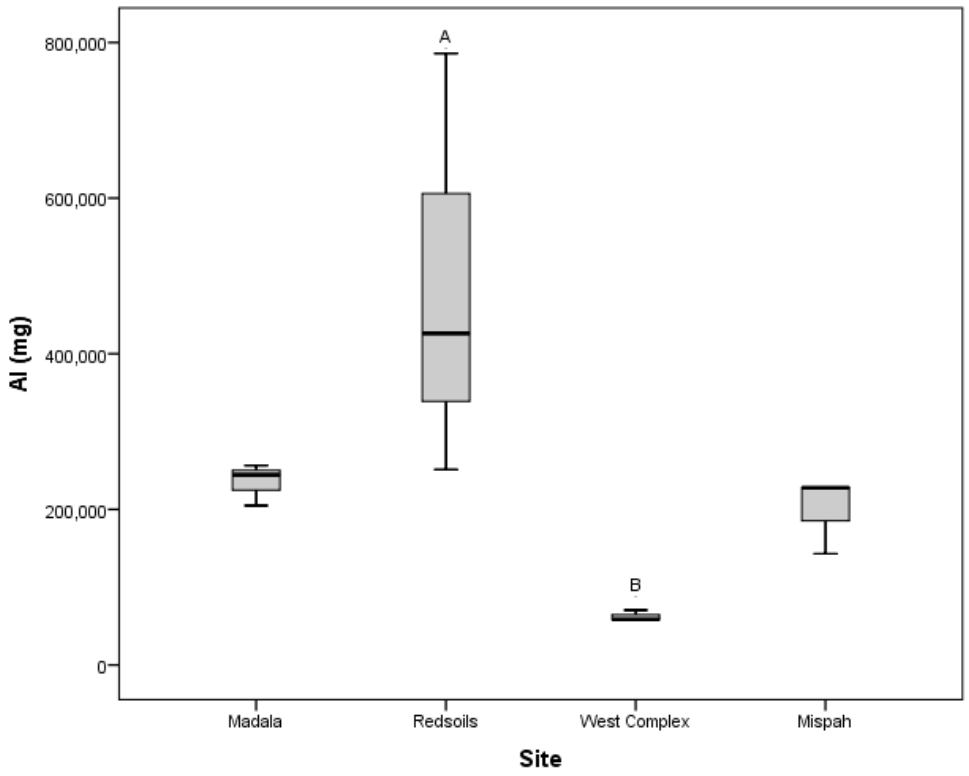


Figure 4.10. **Mean elemental mass of Al for trees at each site with A and B showing significant differences (N = 12).**

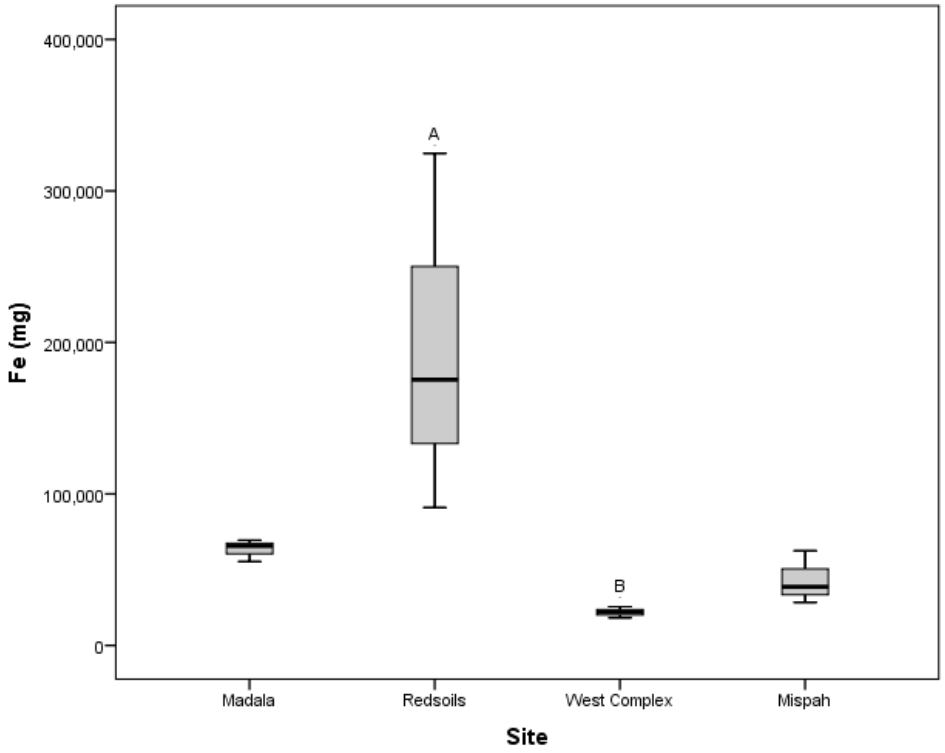


Figure 4.11. **Mean elemental mass of Fe for trees at each site with A and B showing significant differences (N = 12).**

Mean Pb tree mass per site was interesting, as Madala (109.98 mg; 95% CI: 3.41 mg – 223.37 mg) and Redsoils (152.58 mg; 95% CI: 241.2 mg – 546.36 mg) as a pair showed the highest mean tree elemental mass, and not Mispah (57.58 mg; 95% CI: 40.36 mg – 155.52 mg) and Redsoils, even though these were generally the biggest trees, as can be seen in Figure 4.12.

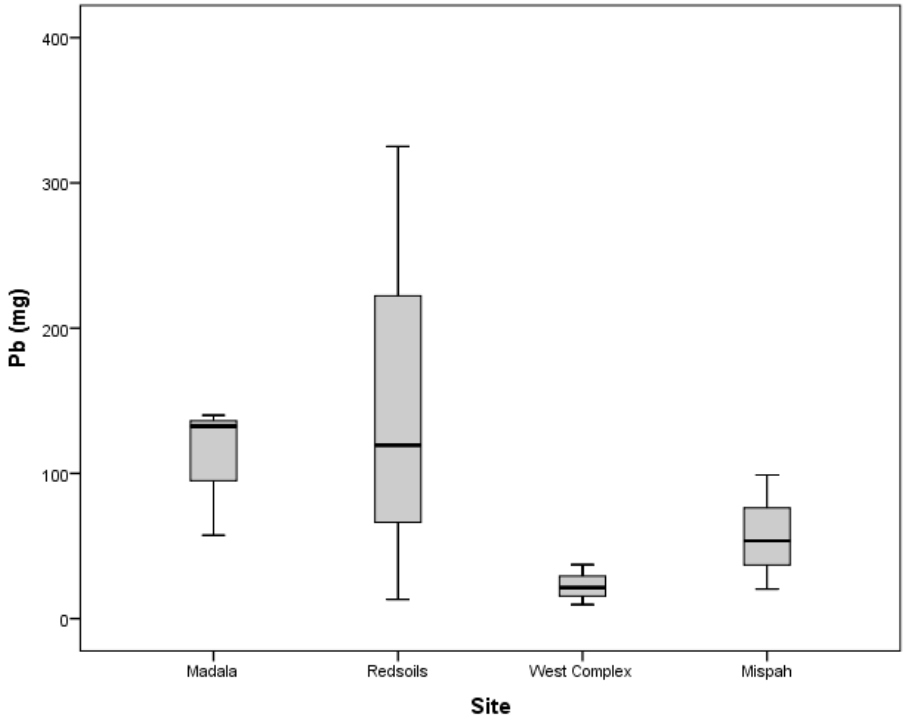


Figure 4.12. Mean elemental mass of Pb for trees at each site (N = 12).

4.3.4 Elemental mass vs. biomass at each site

Mean tree elemental mass was strongly positively correlated to biomass for all elements except Fe, Al, and Pb. Correlations for all other elements were significant ($p < 0.05$) (Table 4.3).

Table 4.3. Pearsons Correlation Coefficient results comparing mean total biomass with mean elemental mass in *S. lancea*.

	Na	Mg	S	Cl	Ca	Cr	Mn	Cu	Zn
Corr. Coeff.	0.970**	0.680*	0.934**	0.843**	0.879**	0.875**	0.639*	0.903**	0.937**
Sig.	0.0005	0.015	0.0005	0.001	0.0005	0.0005	0.025	0.0005	0.0005

* Sig. At the 0.05 level

** Sig. At the 0.01 level

4.4 Phytoextraction vs. phytostabilisation of *S. lancea*

4.4.1 Soil elemental concentrations for each site

Soil pits (n = 13) were dug where *S. lancea* trees were harvested, as well as 3 control pits at each site (four for Mispah) where there were no trees present. Certain depths (refer to Chapter 3: Methods) were sampled (n = 15) from each soil pit. These depths were analysed for the same elements as the tree samples for comparison and averaged for elemental concentration of each pit. Only 11 depths (0-2cm, 2-5cm, 5-10cm, 10-15cm, 15-20cm, 20-30cm, 70-80cm, 90-100cm, 190-210cm, 240-260cm, 280-300cm) were used for pit averages as they were the depths where changes in concentration were observed after examining the initial concentration results. Thus, a total of 224 soil samples were analysed (N = 224). Soil concentrations were found to be significantly different between sites for all elements tested as well as pH (for $p < 0.05$).

pH was found to be highest at West Complex (6.21; 95% CI: 5.93 – 6.51), which was significantly higher than Madala (5.03; 95% CI: 4.84 – 5.22), Redsoils (5.23; 95% CI: 5.04 – 5.42), and Mispah (5.30; 95% CI: 5.09 – 5.51).

4.4.1.a WW Madala

Zinc and Al were found to have the highest mean soil concentration at Madala. Mean soil Zn at Madala (35.18 mg/kg; 95% CI: 31.48 – 38.87 mg/kg) and Redsoils (34.38 mg/kg; 95% CI: 33.26 – 35.51 mg/kg) were found to be significantly higher compared to West Complex (23.46 mg/kg; 95% CI: 19.37 – 27.54 mg/kg; for $p = 0.0005$) and Mispah (20.88 mg/kg; 95% CI: 19.59 – 22.17 mg/kg; $p = 0.0005$) (Figure 4.13.).

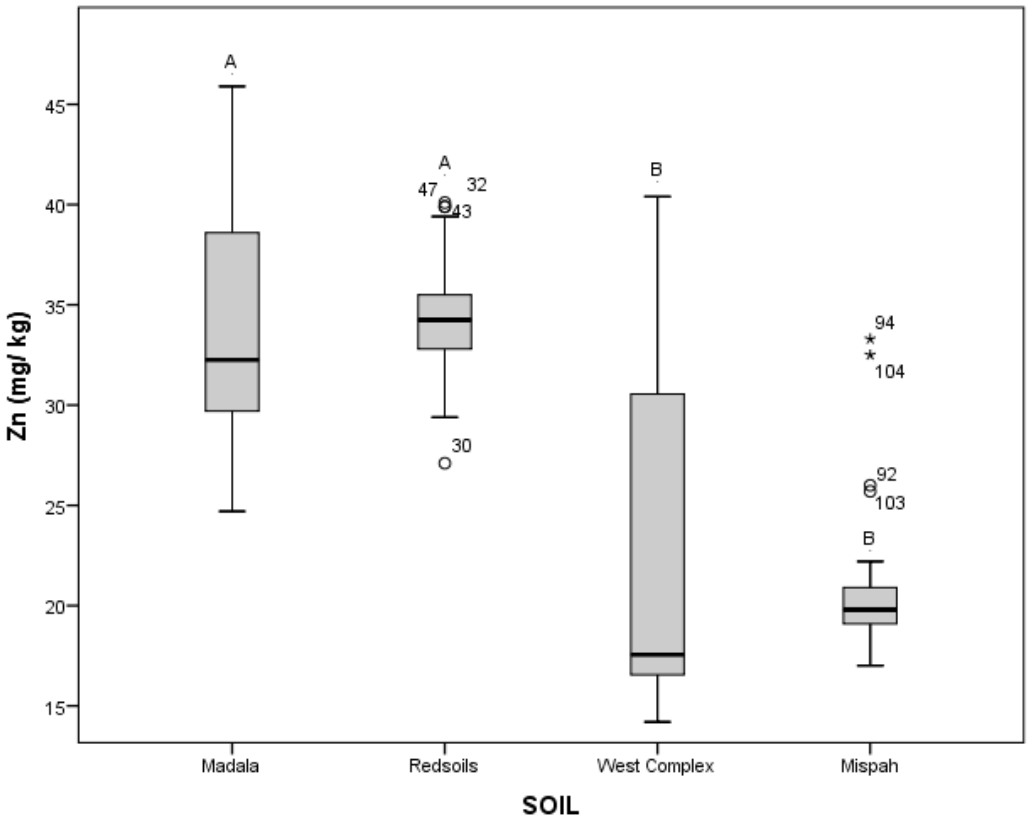


Figure 4.13. **Mean soil Zn concentration at each site. Significant differences are depicted with letters. Circles and stars represent outliers and extreme outliers respectively (N = 224).**

Mean soil Al concentration was found to be significantly lower at West Complex (60898.75 mg/kg; 95% CI: 57838.02 – 63959.48 mg/kg) compared to Redsoils (82971.56 mg/kg; 95% CI: 75406.02 – 90537.1 mg/kg; p = 0.0005), Mispah (85684.24 mg/kg; 95% CI: 81859.21 – 89509.27 mg/kg; p = 0.0005), and Madala (87040.42 mg/kg; 95% CI: 83807.36 – 90273.47 mg/kg; p = 0.006). Mean soil Al concentration at Redsoils was also found to be significantly lower than Mispah (p = 0.020) (Figure 4.14.).

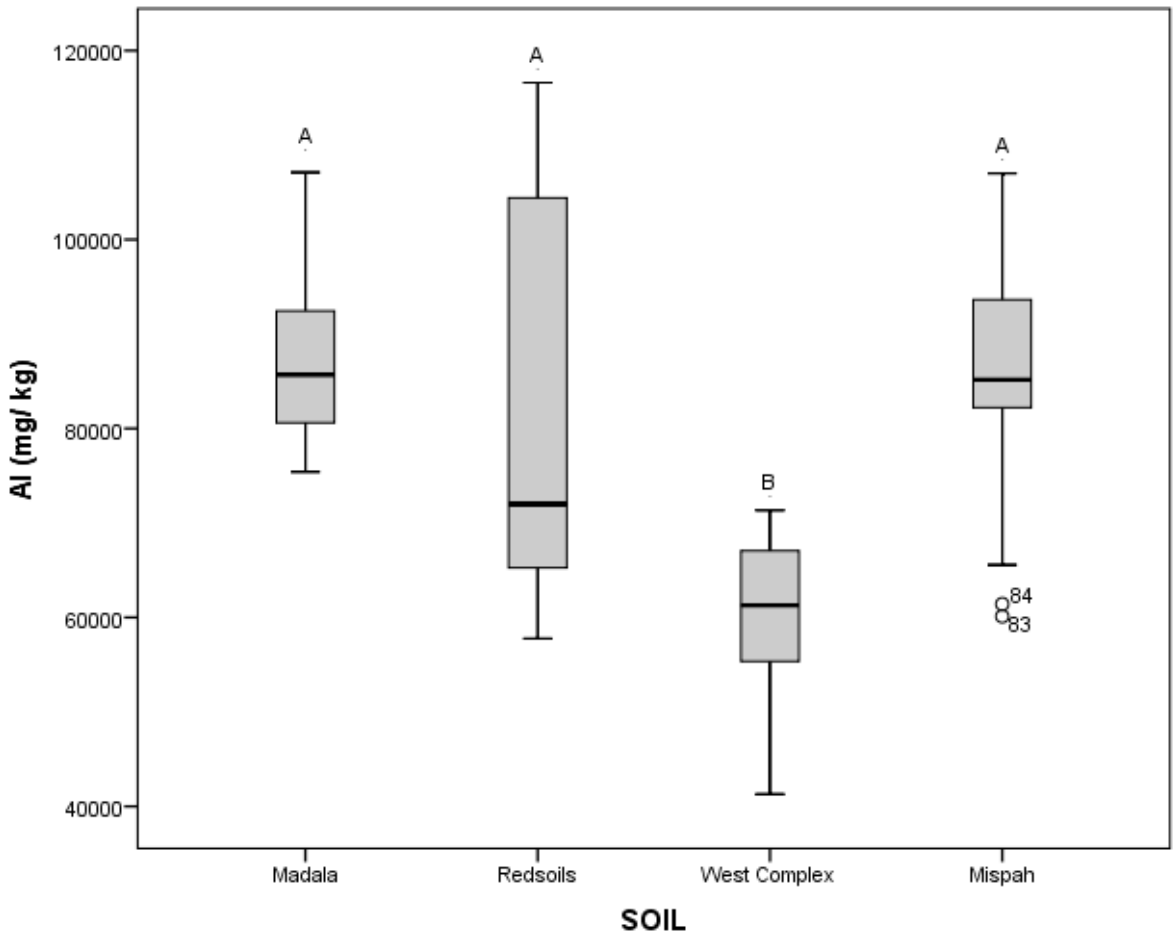


Figure 4.14. Mean soil Al concentration at each site. Significant differences are depicted with letters (N = 224).

4.4.1.b WW Redsoils

Elements found to be highest in mean soil concentration at Redsoils were Pb, Cu, Cr, and Fe. Mean Pb soil concentrations at Redsoils (18.05 mg/kg; 95% CI: 16.57 – 19.54 mg/kg) and Madala (14.99 mg/kg; 95% CI: 13.27 – 16.72 mg/kg) were found to be significantly higher compared to West Complex (10.43 mg/kg; 95% CI: 8.66 – 12.19 mg/kg; $p = 0.0005$ and $p = 0.016$, respectively), and Mispah (8.5 mg/kg; 95% CI: 7.14 – 9.86 mg/kg; for $p = 0.0005$) (Figure 4.15.).

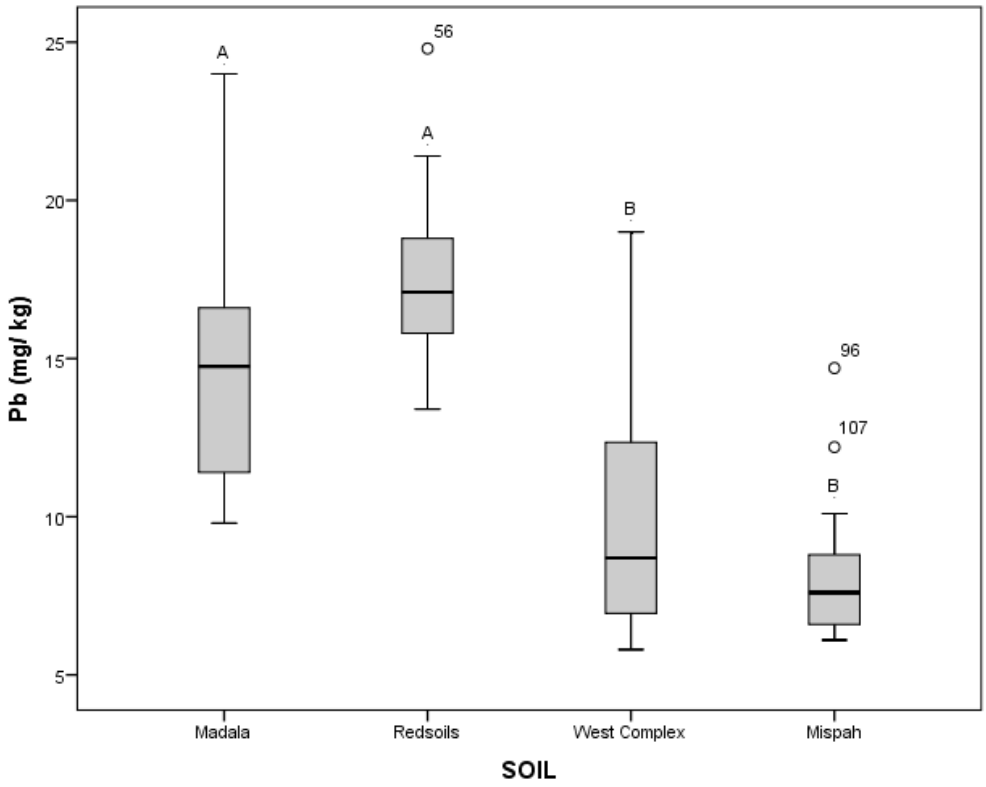


Figure 4.15. Mean soil Pb concentration at each site. Significant differences are depicted with letters. Circles represent outliers (N = 224).

Mean soil Cu concentrations were found to be significantly higher at Redsoils (53.62 mg/kg; 95% CI: 51.79 – 58.97 mg/kg) compared to Madala (35.62 mg/kg; 95% CI: 32.14 – 37.88 mg/kg; p = 0.0005), West Complex (21.35 mg/kg; 95% CI: 19.23 – 23.46 mg/kg; p = 0.0005), and Mispah (19.90 mg/kg; 95% CI: 17.37 – 22.44 mg/kg; p = 0.0005). Madala was significantly higher in mean Cu concentration than West Complex (p = 0.003), while Mispah was also significantly lower than Madala (p = 0.0005) as well (Figure 4.16.).

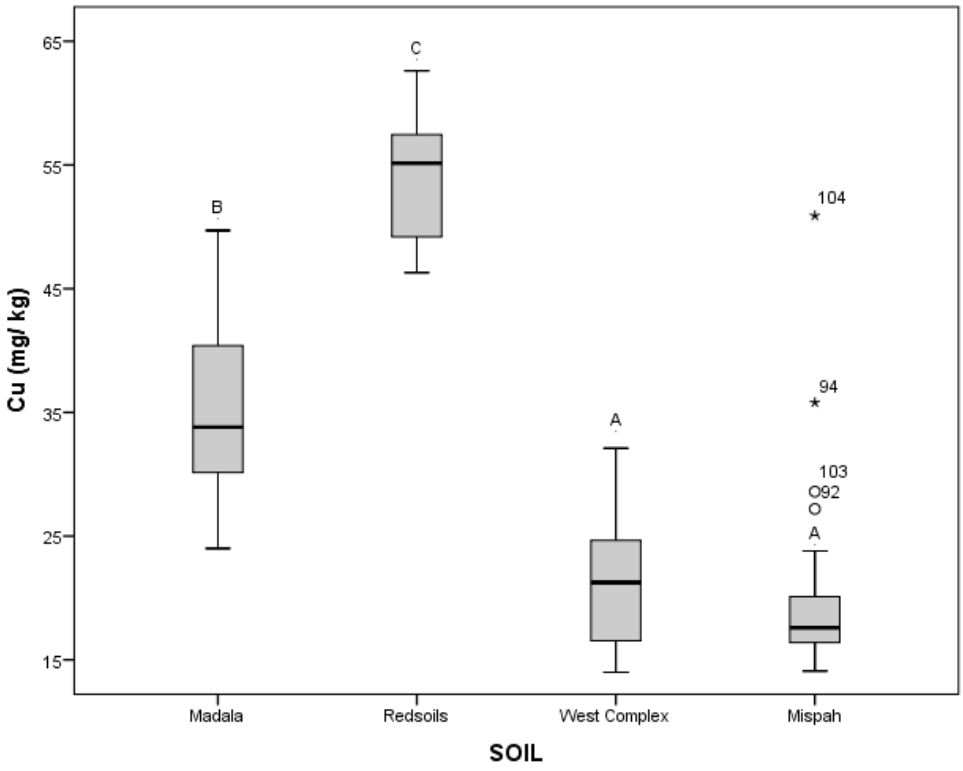


Figure 4.16. **Mean soil Cu concentration at each site. Significant differences are depicted with letters. Circles and stars represent outliers and extreme outliers respectively (N = 224).**

Mean Cr soil concentrations were significantly lower at Mispah (109.91 mg/kg; 95% CI: 100.41 – 119.42 mg/kg) compared to Redsoils (249.13 mg/kg; 95% CI: 236.34 – 261.93 mg/kg; p = 0.0005), and Madala (148.45 mg/kg; 95% CI: 142.26 – 154.65 mg/kg; p = 0.0005); while West Complex (147.47 mg/kg; 95% CI: 108.25 – 186.68 mg/kg; p = 0.0005) and Madala (p = 0.001) were also significantly lower than Redsoils mean Cr concentration (Figure 4.17).

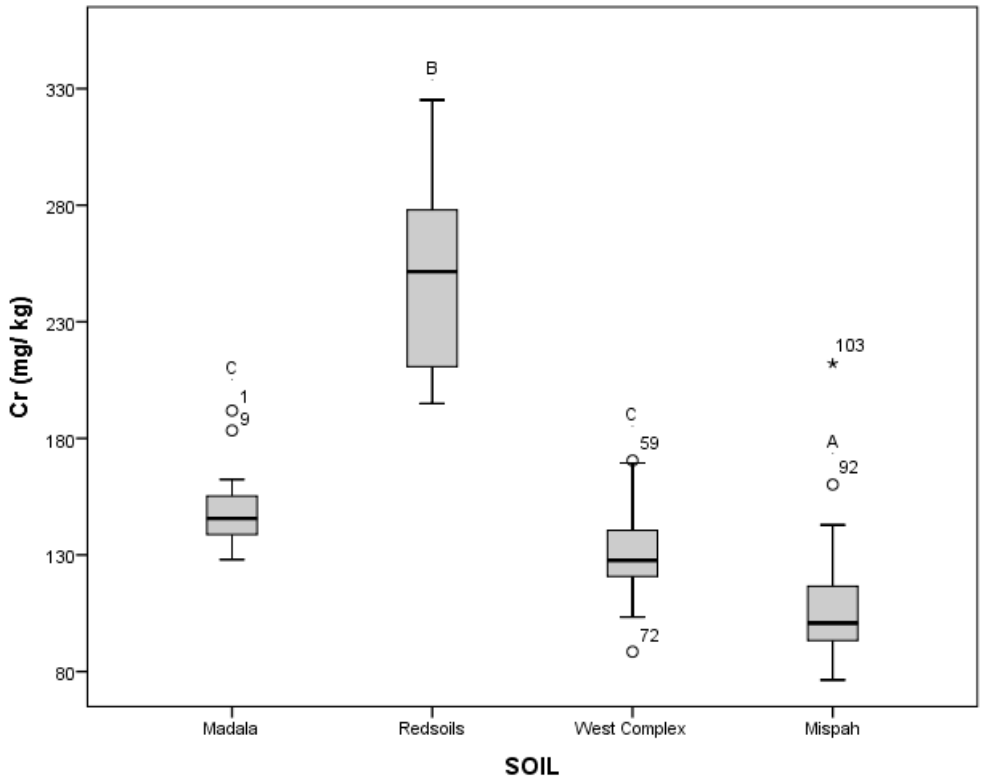


Figure 4.17. **Mean soil Cr concentration at each site. Significant differences are depicted with letters. Circles and stars represent outliers and extreme outliers respectively (N = 224).**

Mean Fe soil concentrations were significantly lower at Mispah (19761.82 mg/kg; 95% CI: 18372.49 – 21151.15 mg/kg) compared to West Complex (23667.08 mg/kg; 95% CI: 22348.80 – 24985.36 mg/kg; p = 0.0005) and Madala (33362.92 mg/kg; 95% CI: 31506.40 – 35219.43 mg/kg; p = 0.0005) (Figure 4.18.). West Complex was also found to be significantly lower in concentration compared to Madala (p = 0.011) and Redsoils (59410.94 mg/kg; 95% CI: 56391.06 – 62430.82 mg/kg; p = 0.0005). Madala was also found to be significantly lower in concentration compared to Redsoils (p = 0.004) and Mispah (p = 0.0005).

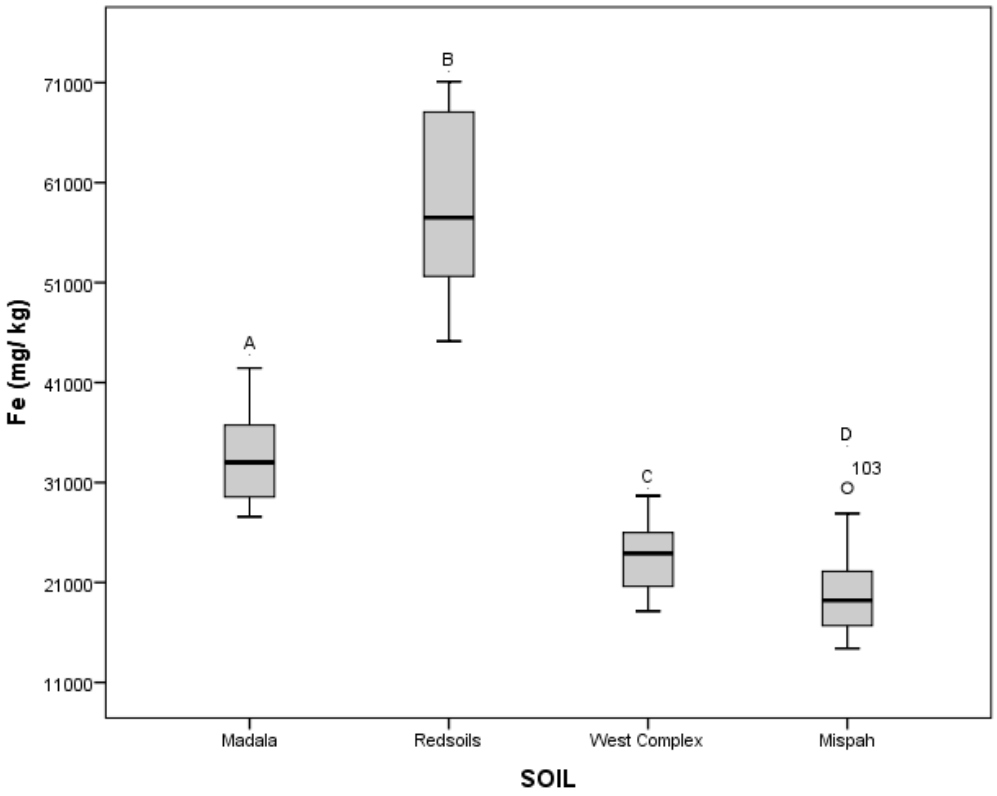


Figure 4.18. **Mean soil Fe concentration at each site. Significant differences are depicted with letters. Circles represent outliers (N = 224).**

4.4.1.c VR West Complex

Mean soil concentrations of Cl, Ca, S, Mn, Mg, and Na were all found to be highest at West Complex. Mean Cl in soil was found to be significantly lower at Redsoils (SPSS did not produce a mean or 95% CI for Cl Redsoils as all values for Cl at Redsoils were below detection limit) compared to Madala (34.39 mg/kg; 95% CI: 0.75 – 68.03 mg/kg; p = 0.0028) and West Complex (276.80 mg/kg; 95% CI: 82.17 – 471.43 mg/kg; p = 0.0005). West Complex was also found to be significantly higher in Cl concentration compared to Mispah (31.98 mg/kg; 95% CI: 20.29 – 8425 mg/kg; p = 0.0005) and Madala (p = 0.0005) (Figure 4.19.).

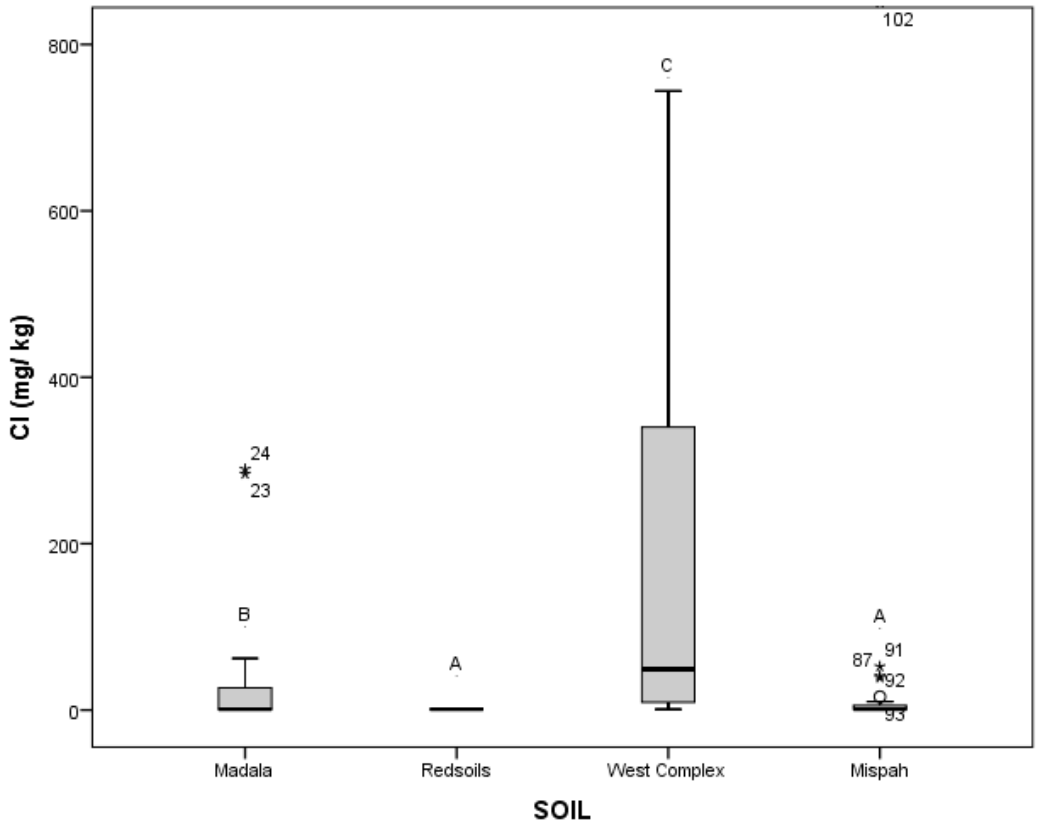


Figure 4.19. Mean soil Cl concentration at each site. Significant differences are depicted with letters. Circles and stars represent outliers and extreme outliers respectively (N = 224).

Mean Ca soil concentration was also significantly lower at Redsoils (190.68 mg/kg; 95% CI: 6.74 – 388.11 mg/kg; p = 0.0005) compared to West Complex (2923.04 mg/kg; 95% CI: 2260.53 – 3585.56 mg/kg; p = 0.0005) (Figure 4.20.). West Complex was also found to be significantly higher than Mispah (406.15 mg/kg; 95% CI: 29.69 – 782.61 mg/kg; p = 0.0005), and Madala (587.4 mg/kg; 95% CI: 102.64 – 1072.16 mg/kg; p = 0.0005).

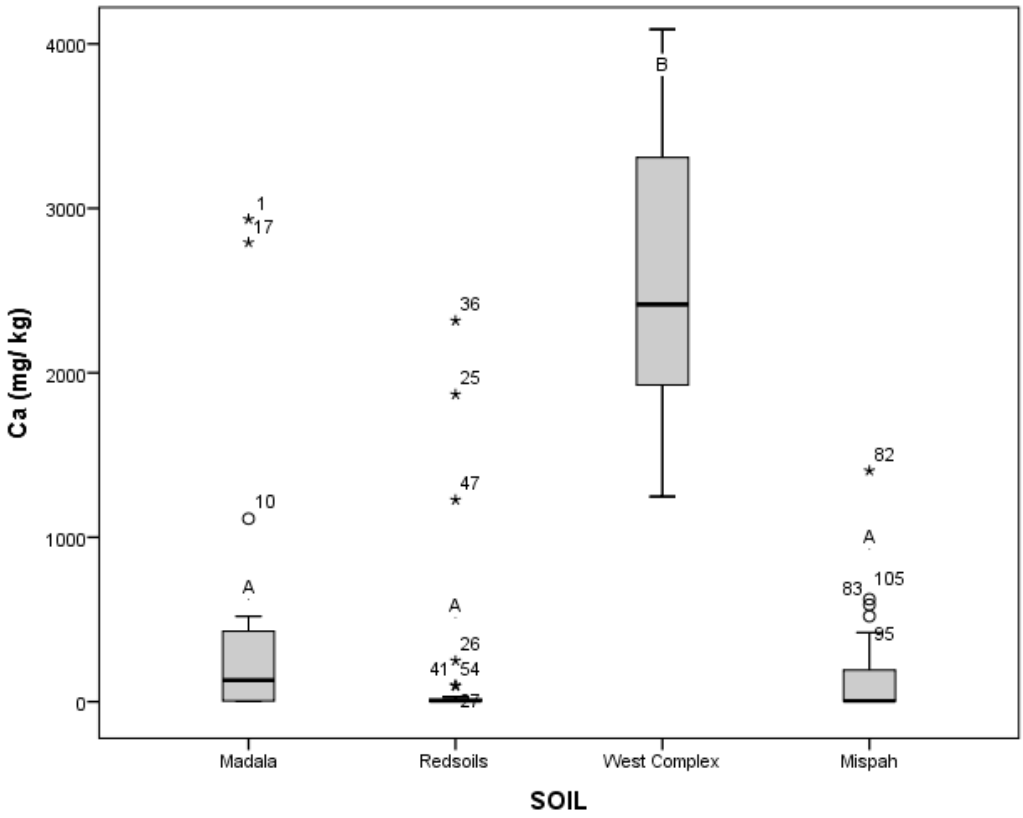


Figure 4.20. **Mean soil Ca concentration at each site. Significant differences are depicted with letters. Circles and stars represent outliers and extreme outliers respectively (N = 224).**

Mean S soil concentrations were found to be significantly lower at Redsoils (218.82 mg/kg; 95% CI: 151.99 – 285.65 mg/kg) compared to Madala (581.07 mg/kg; 95% CI: 362.89 – 799.25 mg/kg; $p = 0.005$) and West Complex (1973.41 mg/kg; 95% CI: 1235.99 – 2710.83 mg/kg; $p = 0.0005$) (Figure 4.21.). West Complex was found to be significantly higher than Mispah (369.18 mg/kg; 95% CI: 249.13 – 489.22 mg/kg; $p = 0.0005$) and Madala ($p = 0.001$).

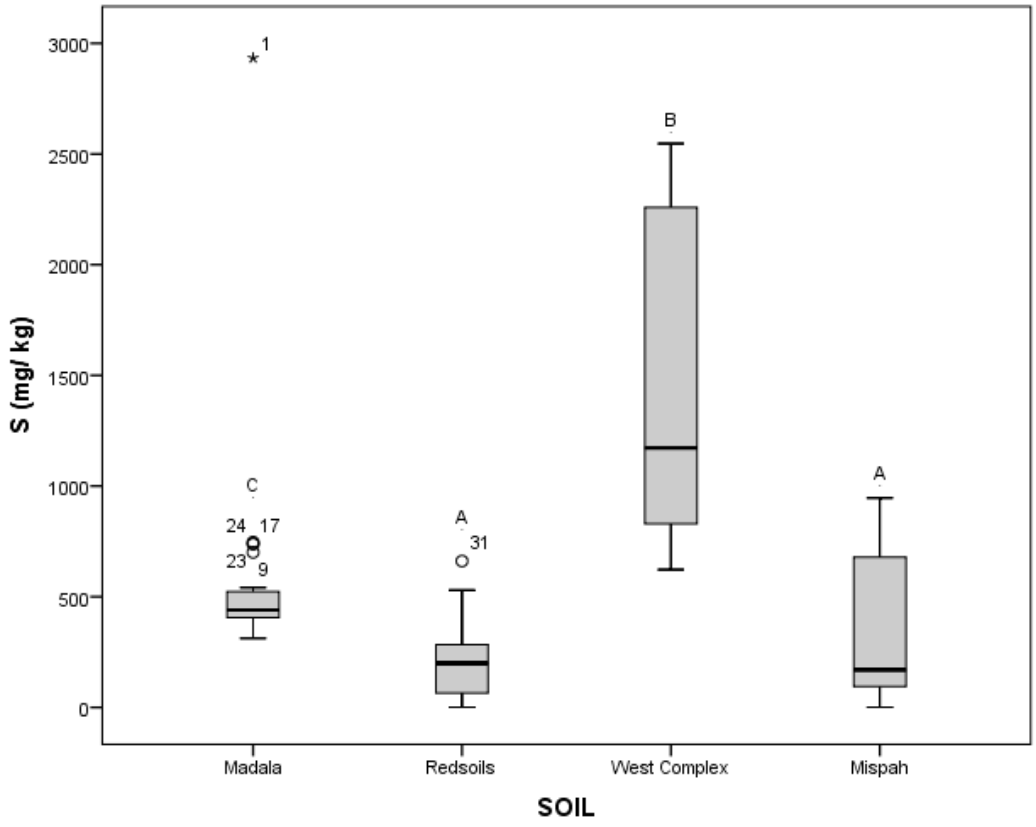


Figure 4.21. **Mean soil S concentration at each site. Significant differences are depicted with letters. Circles and stars represent outliers and extreme outliers respectively (N = 224).**

Mean soil Mn concentrations were significantly lower at Madala (335.51 mg/kg; 95% CI: 259.59 – 411.44 mg/kg) compared to Redsoils (810.22 mg/kg; 95% CI: 766.80 – 853.65 mg/kg; p = 0.0005), West Complex (2895.93 mg/kg; 95% CI: 2066.2 – 3725.17 mg/kg; p = 0.0005), and Mispah (713.94 mg/kg; 95% CI: 631.44 – 796.44 mg/kg; p = 0.0005) (Figure 4.22.). Mispah (p = 0.0005) and Redsoils (p = 0.0005) were also significantly lower in mean Mn concentration than West Complex.

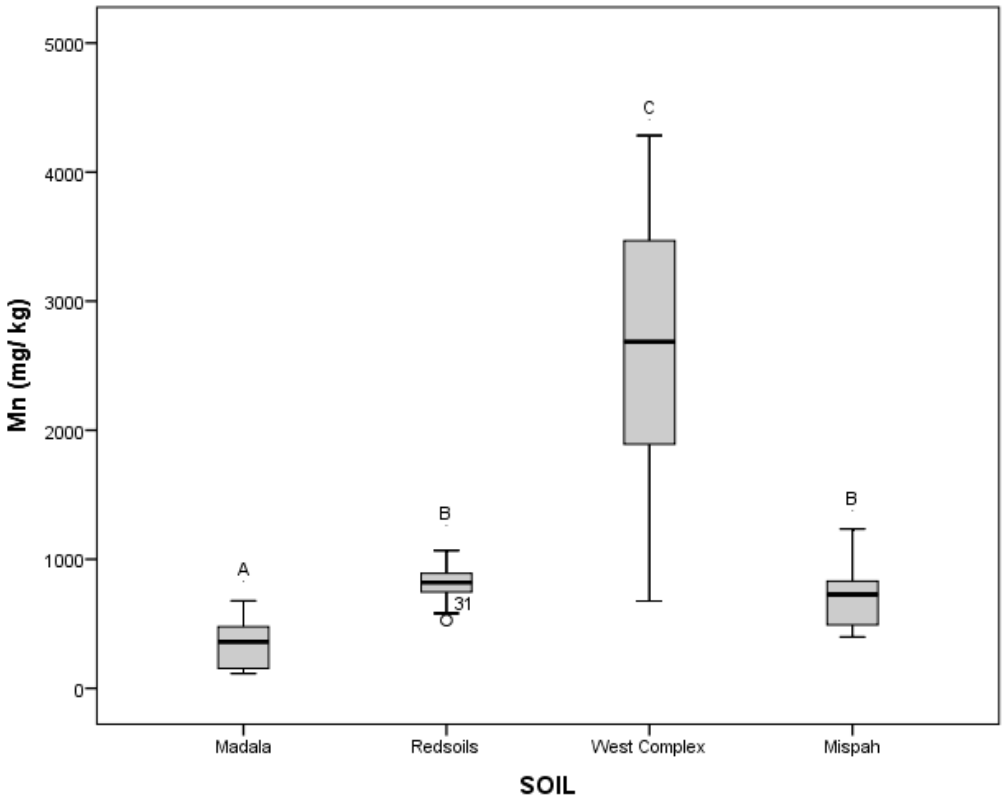


Figure 4.22. **Mean soil Mn concentration at each site. Significant differences are depicted with letters. Circles represent outliers (N = 224).**

The mean soil Mg concentration was significantly lower at Redsoils (1625.31 mg/kg; 95% CI: 1473.09 – 1777.54 mg/kg) compared to Madala (2611.88 mg/kg; 95% CI: 2348.84 – 2874.95 mg/kg; p = 0.0005), Mispah (2566.7 mg/kg; 95% CI: 2429.04 – 2704.35 mg/kg; p = 0.0005), and West Complex (5701.21 mg/kg; 95% CI: 5124.54 – 6277.88 mg/kg; p = 0.0005) (Figure 4.23.). Mispah (p = 0.0005) and Madala (p = 0.0005) were also found to be significantly lower in Mg concentration compared to West Complex.

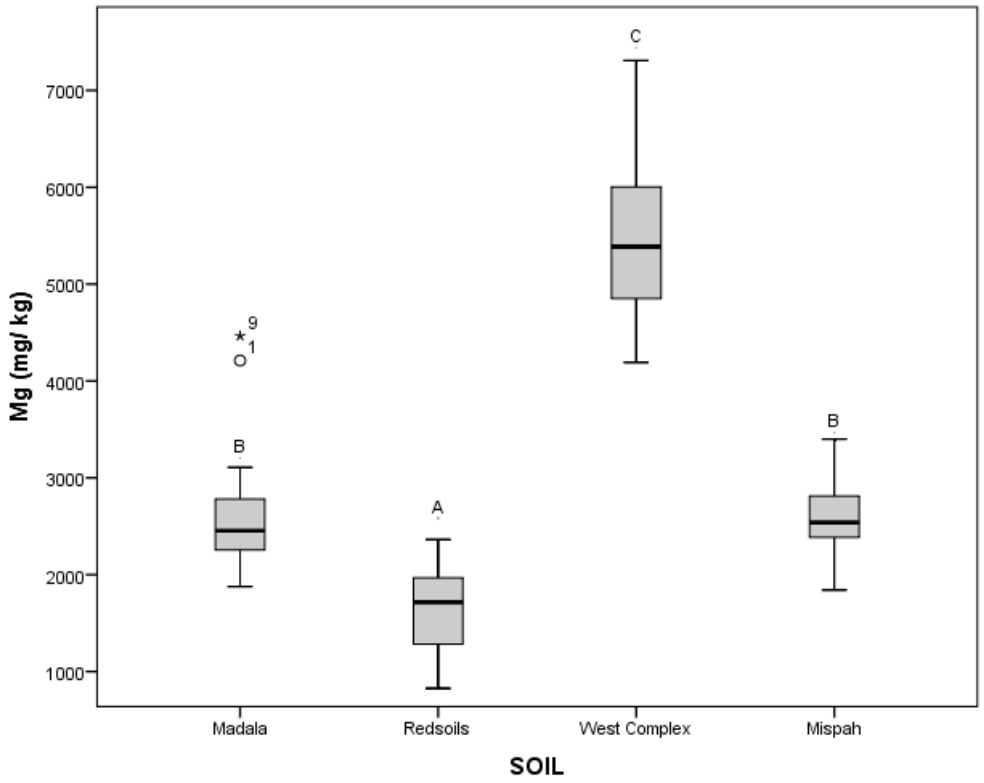


Figure 4.23. **Mean soil Mg concentration at each site. Significant differences are depicted with letters. Circles and stars represent outliers and extreme outliers respectively (N = 224).**

Mean Na soil concentration was significantly lower at Mispah (14701.21 mg/kg; 95% CI: 13398.21 – 16004.39 mg/kg) compared to West Complex (21095.83 mg/kg; 95% CI: 19149.31 – 23042.36 mg/kg; $p = 0.0005$), Madala (19080.83 mg/kg; 95% CI: 18193.16 – 19968.51 mg/kg; $p = 0.001$), and Redsoils (19935.63 mg/kg; 95% CI: 19376.39 – 20494.86 mg/kg; $p = 0.0005$) (Figure 4.24.).

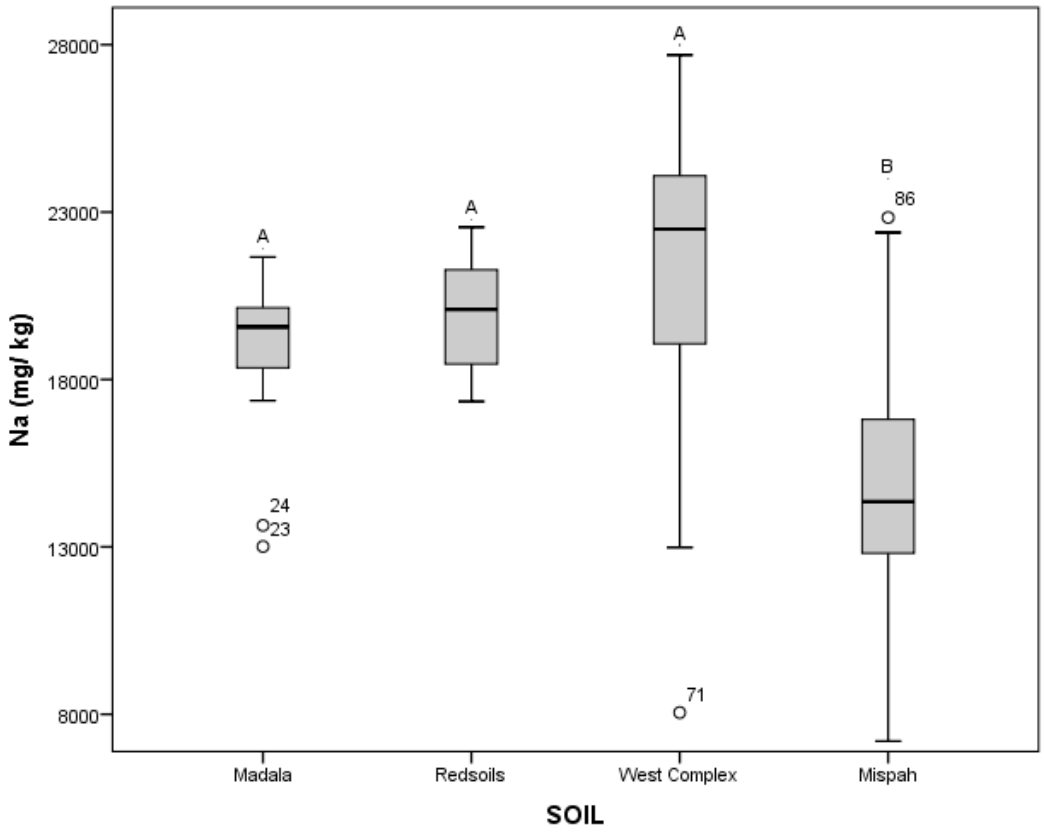


Figure 4.24. **Mean soil Na concentration at each site. Significant differences are depicted with letters. Circles represent outliers (N = 224).**

4.4.1.d VR Mispah

No elements were found to be highest in mean concentration at the Mispah site compared to Redsoils, West Complex and Madala but, as mentioned above, there were many very low concentrations of elements found in the soil at Mispah.

4.4.2 Control Soil Pit Concentrations

Three control pits were sampled at each site, except for Mispah where four soil pits were sampled (n = 13), to compare mean soil element concentrations with trees to mean soil element concentrations without trees. There were significant differences found between sites for pH and S (for $p < 0.05$).

Mispah soil pit S11 (4.69; 95% CI: 4.41 – 4.97) showed a significantly lower mean pH to Control pit 4 (VR-M-C4) (6.1; 95% CI: 5.94 – 6.26; p = 0.0005).

Sulphur was significantly different between control and tree soil pits at both Redsoils and Mispah (both well-draining, nutrient-rich soils). At Redsoils S5 (83.65 mg/kg; 95% CI: 26.92 – 140.37 mg/kg) contained significantly lower S than Control 1 (WW-R-C1) (711.56 mg/kg; 95% CI: 616.01 – 807.11 mg/kg; p = 0.004). At Mispah, S10 (762.47 mg/kg; 95% CI: 703.59 – 821.35 mg/kg) was significantly higher in S than Control 4 (VR-M-C4) (137.22 mg/kg; 95% CI: 60.91 – 213.53 mg/kg; p = 0.001) and S12 (123.34 mg/kg; 95% CI: 3.73 – 250.4 mg/kg) was significantly lower in S than Control 1 (VR-M-C1) (933.83 mg/kg; 95% CI: 780.7 – 1086.97 mg/kg; p = 0.004). No clear patterns were observed here.

4.4.3 Tree elemental concentration for each site

While sub-samples were taken of coarse roots and fine roots for concentration analysis, roots were weighed as a combined total for tree compartment masses due to time and budget constraints. As there were no significant differences found between coarse roots and fine roots ($p > 0.05$), it was deemed acceptable to average the concentrations of fine roots and coarse roots for an overall mean root concentration for each tree. Five sub-samples from each tree were taken (n = 5), from three trees at each site (n = 12), thus a total of 60 samples were analysed for tree elemental concentration (N = 60). Significant differences in tree concentration between sites were found for Na, Mg, Cl, and Cr ($p < 0.05$).

4.4.3.a WW Madala

No mean tree concentrations were significantly higher at Madala compared to the other sites ($p > 0.05$).

4.4.3.b WW Redsoils

Mean tree Cr concentration was significantly lower at Mispah (17.12 mg/kg; 95% CI: 15.66 – 18.58 mg/kg) and West Complex (17.61 mg/kg; 95% CI: 16.19 – 19.03 mg/kg) compared to Madala (23.18 mg/kg; 95% CI: 20.62 – 25.74 mg/kg; p = 0.004 and p = 0.012) and Redsoils (32.63 mg/kg; 95% CI: 26.75 – 38.51 mg/kg; for p = 0.0005) respectively (Figure 4.25.).

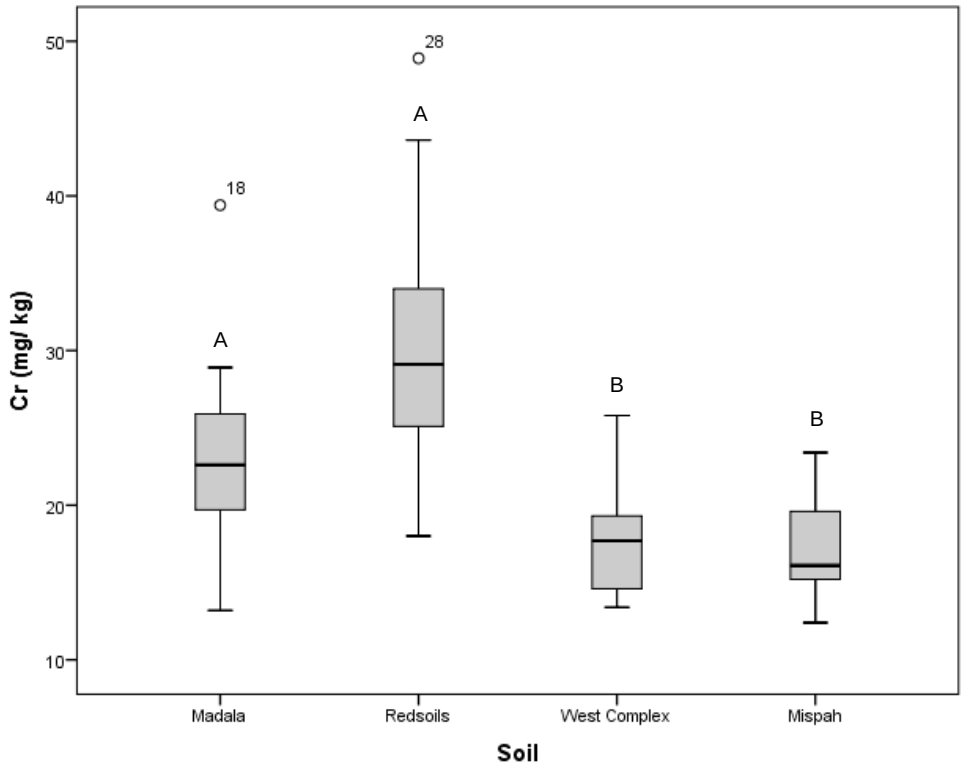


Figure 4.25. Mean tree Cr concentration for each site. Circles denote outliers (N = 60).

4.4.3.c VR West Complex

Mean tree Na concentration was significantly higher at West Complex (14168.1 mg/kg; 95% CI: 13458.04 – 14878.15 mg/kg) compared to Redsoils (12090 mg/kg; 95% CI: 11216.98 – 12963.02 mg/kg; p = 0.0005) and Madala (13583.33 mg/kg; 95% CI: 12433.17 – 14725.5 mg/kg; p = 0.009) (Figure 4.26.).

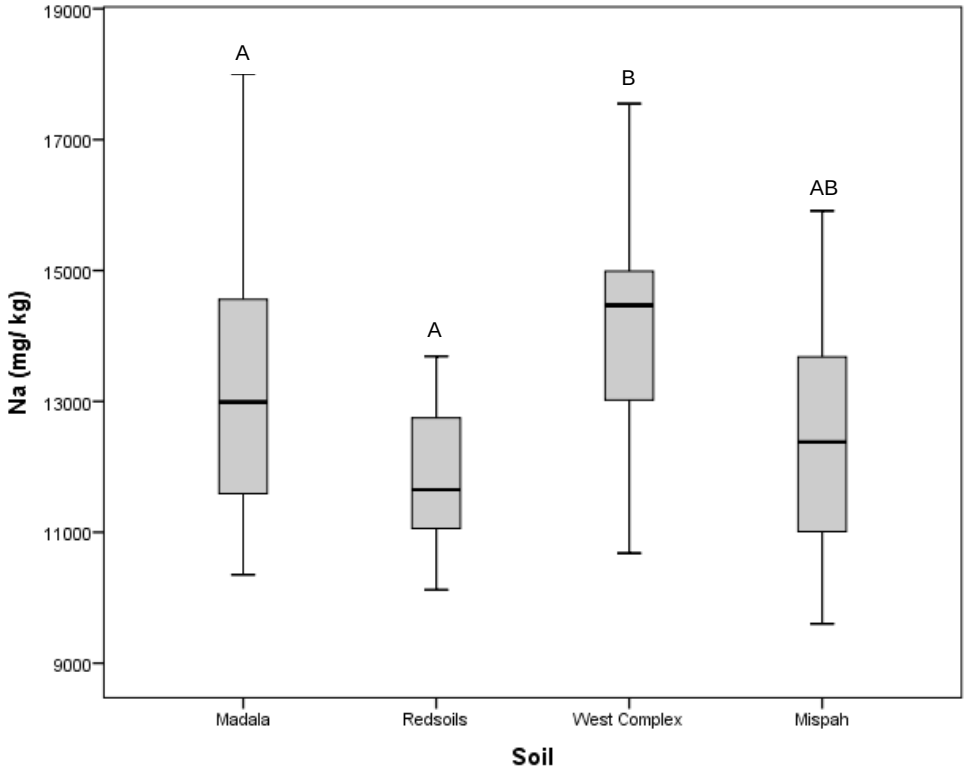


Figure 4.26. Mean tree Na concentration for each site. No outliers are shown here (N = 60).

Similarly, mean tree Mg concentration was higher at West Complex (7280.63 mg/kg; 95% CI: 5557.48 – 9003.38 mg/kg) compared to Madala (4805.42 mg/kg; 95% CI: 3546.74 – 6064.1 mg/kg; p = 0.035) (Figure 4.27.).

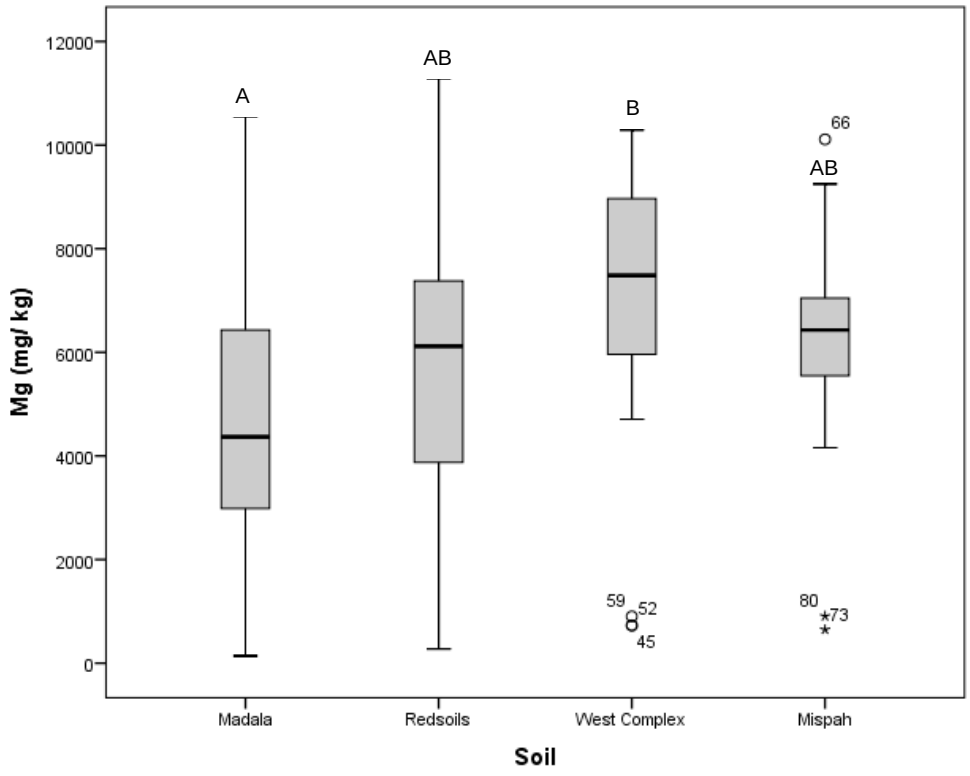


Figure 4.27. Mean tree Mg concentration for each site. Circles and stars denote outliers and extreme outliers respectively (N = 60).

Mean tree Cl concentration was significantly lower at Redsoils (1225.7 mg/kg; 95% CI: 1008.27 – 1443.13 mg/kg) compared to Mispah (2579.09 mg/kg; 95% CI: 1943.61 – 3214.56 mg/kg; p = 0.006), Madala (2912 mg/kg; 95% CI: 2110.74 – 3713.27 mg/kg; p = 0.001), and West Complex (3287.2 mg/kg; 95% CI: 2647.91 – 3926.48 m/kg; p = 0.0005) (Figure 4.28.).

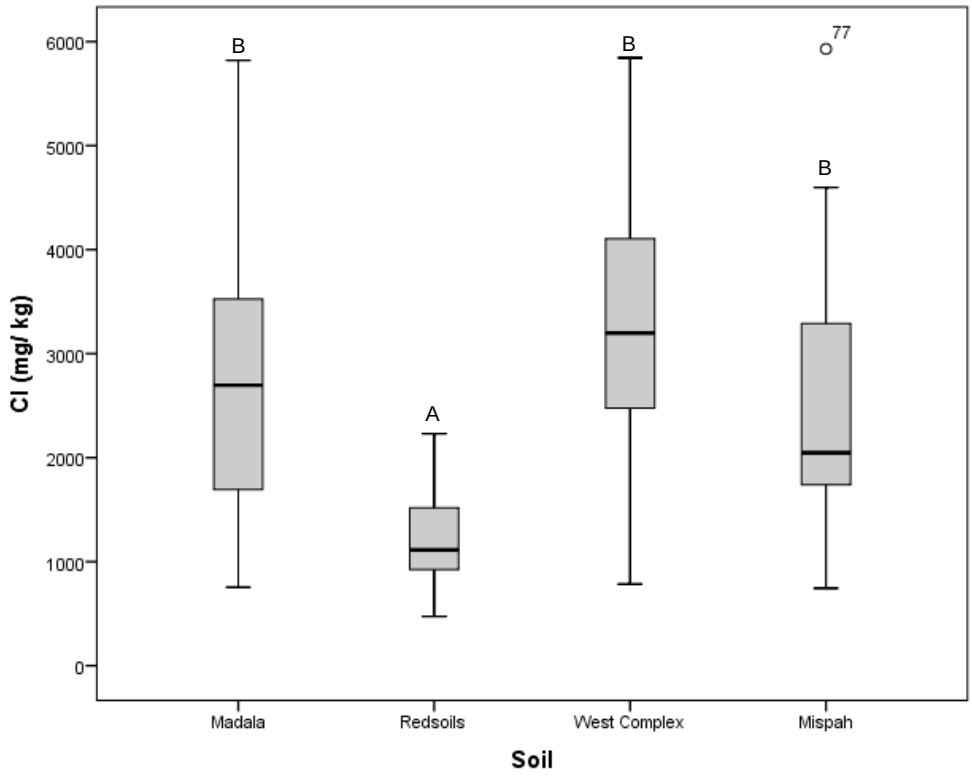


Figure 4.28. Mean tree Cl concentration for each site. Circles denote outliers (N = 60).

4.4.3.d VR Mispah

No mean tree concentrations were significantly higher at Mispah compared to the other sites.

4.4.4 Phytoextraction potential of S. lancea

S. lancea accumulates Na, Mg, Al, S, Ca, Mn, Fe, Cu, Zn, and Pb more in shoots than in roots (Translocation Factor (TF) > 1), but does not accumulate Cl and Cr very well (TF < 1) (Table 4.4.). Soil type did significantly influence shoot translocation factors of *S. lancea* for Al. Aluminium had a mean TF of 1.44 at Redsoils (95% CI: 0.33 – 2.56) which was significantly higher than West Complex with a mean of 0.58 (95% CI: 0.32 – 0.85; p = 0.028). The TFs were found to be in the sequence of Ca > Na > Fe > Mg > Zn > S > Mn > Pb > Cu > Al > Cl > Cr.

Table 4.4. Mean shoot Translocation Factor ± 95% CI for elements in *S. lancea* (n = 12).

Metal	TF	
Na	158.82	± 70.05
Mg	28.67	± 6.08
Al	1.02	± 0.22
S	4.03	± 0.77
Cl	0.60	± 0.19
Ca	2461.19	± 464.01
Cr	0.56	± 0.14
Mn	3.40	± 1.00
Fe	54.70	± 6.57
Cu	1.90	± 0.24
Zn	5.93	± 0.77
Pb	2.42	± 1.51

4.4.5 Phytostabilisation potential of S. lancea

The mean Bioconcentration Factor (BCF) for root and shoot results show that *S. lancea* is better at extracting metals from soil compared to stabilising metals. Aluminium, Fe, and Cr are the only elements not > 1 (Table 4.5.), and are thus stabilised in the rooting zone. These results show that *S. lancea* extraction potential outweighs its phytostabilisation potential.

The root BCFs were found to be in the sequence of Cl > S > Cu > Cr > Zn > Mn > Mg > Na > Pb > Ca > Al > Fe, with only Cl and S concentrations being higher in the root tissue compared to the soil. The shoot BCFs were found to be in the sequence Cl > S > Ca > Na > Mg > Zn > Cu > Mn > Pb > Cr > Fe > Al, with only Al, Cr, Fe, and Pb higher in the soil compared to the shoot tissues.

Table 4.5. Mean root and shoot Bioconcentration Factors ± 95% CI for *S. lancea* (n = 12).

Metal	BCF (shoots)	BCF (roots)
Na	38.29 ± 31.17	0.25 ± 0.06
Mg	6.34 ± 2.01	0.27 ± 0.11
Al	0.03 ± 0.01	0.03 ± 0.01
S	29.33 ± 16.85	7.27 ± 3.91
Cl	1454.16 ± 965.06	4812.68 ± 3805.33
Ca	118.55 ± 55.52	0.05 ± 0.03
Cr	0.40 ± 0.04	0.89 ± 0.33
Mn	1.02 ± 0.49	0.34 ± 0.14
Fe	0.03 ± 0.01	0.00 ± 0.00
Cu	1.69 ± 0.40	0.93 ± 0.26
Zn	2.99 ± 0.62	0.52 ± 0.13
Pb	0.15 ± 0.06	0.11 ± 0.05

CHAPTER 5

DISCUSSION AND CONCLUSION

5.1 Introduction

The aim of this research was to determine the fate of contaminants in *S. lancea* trees planted in plots, as part of the Mine Woodlands Programme, for hydrological control and phytostabilisation. Previous studies conducted by the Ecological Engineering and Phytotechnology Programme looked at the potential for *S. lancea* to be used for hydrological control and phytostabilisation, but did not research where contaminants were stored, or whether the tree would perform better as an extractor or stabiliser specifically. This dissertation aimed to provide a more in-depth look at where *S. lancea* was storing contaminants, and whether the tree performs better as a phytostabiliser or phytoextractor. There is now more information available about *S. lancea's* capabilities in terms of phytoremediation which will allow for improved strategies on the use of the tree in future planting scenarios for the Mine Woodlands Programme, as well as for other phytoremediation plans or programmes. This research has also added to the list of plants that may be used for phytoremediation in South Africa – research which is currently lacking. Some important findings have emerged and these are presented below.

5.2 The fate of contaminants within *S. lancea* tree compartments

The first objective of this study was to determine the fate of contaminants (Na, Mg, Al, S, Cl, Cd, Cr, Mn, Fe, Cu, Zn, and Pb) in the different tree compartments of *S. lancea* using X-Ray Fluorescence (XRF) to quantify elemental concentration in the different tree compartments. Differences in measured concentrations were expected between compartments, as plants usually exhibit concentration patterns with varying concentrations across tree compartments (Pulford and Watson, 2003; Dickinson and Pulford, 2005).

The majority of measured elements were found throughout the entire tree, but were primarily located in certain compartments. This suggests that different elements are translocated more readily than others. These findings may be attributed to the bioavailability of certain contaminants and elements in the soil solution, as trees are only able to absorb what is available to them (Kabata-Pendias, 2004). The concentration pattern found for tested elements in *S. lancea* differed across

compartments, as was expected (Dickinson and Pulford, 2005; Liu *et al*., 2013). Such differences could be attributed, in part, to the physiological traits of plants with regards to environmental contamination.

Very high concentrations of some elements were found to occur in the shoot compartments of the trees, showing that not all trees use the resistance trait of only absorbing metals into the root tissue as stated by Pulford and Watson (2003). Dye *et al*. (2008) found that *S. lancea* accumulates high concentrations of S in the leaves. Other than that, there has been little to no research conducted on *S. lancea* previously in this regard and, as such, no direct comparisons can be made between other compartment concentrations. It is also very difficult to compare compartmentalisation of metals by trees (Pulford and Watson, 2003), as trees and their ability to take up and translocate elements is site and species specific. Further research will have to look at the physiological mechanisms of *S. lancea* to determine which methods the tree species is using to tolerate and accumulate these high concentrations.

As the site affects a tree's ability to take up and translocate elements, certain element concentrations were significantly higher in tree compartments at the nutrient-poor soil sites compared to the nutrient-rich soil sites; specifically, Cl, Na, and S. This suggests that the nutrient-poor, poorly draining soils at West Complex and Madala are more likely to contain higher bioavailable amounts of Cl, Na, and S due to soil properties – or that *S. lancea* is an accumulator of these elements no matter the soil conditions, and that *S. lancea* will take up large amounts of these elements. It may also suggest that trees growing on nutrient-poor sites, although not attaining as much biomass as the nutrient-rich sites, are capable of taking up much the same, if not more, concentrations of these elements compared with the trees growing on nutrient-rich sites due to the accumulator abilities of *S. lancea*.

At the nutrient-rich sites, Cr, Fe, and Al concentrations were found to be significantly higher in trees than those at the nutrient-poor sites, suggesting these elements may be more bioavailable at Redsoils and Mispah. This again suggests that soil properties (pH, CEC, EC, Eh, etc.) are very

important to consider in contamination phytoremediation options and should be considered in more detail in any follow-up studies. It has been mentioned frequently in the literature that locality will impact heavily on the type of phytoremediation chosen (Hutchings, 2002; Dickinson and Pulford, 2005) as heavy metal uptake by trees may also vary due to site-specific conditions (Pulford and Watson, 2003).

Although there is apparent translocation of metals taken up by trees to their bark, it was not included in this study as it fell outside of the project scope. That is not to say that bark is not important in the fate of contaminants in tree species, as was seen by the significantly high concentrations of elements found in the bark sub-samples compared to other elements. The importance of bark is further highlighted by Dickinson and Pulford (2005) who stated that wood and bark tissues are known to contain a significant concentration of heavy metals, which also increases with the age of the trees (Pulford and Watson, 2003; Dickinson and Pulford, 2005). This is due to the process of bark transitioning to wood during growth periods.

Importantly, this pool of HMs in the bark is also considered to be less bioavailable than metals located in the roots and leaves of trees, as they are less likely to be recycled back into the environment. Similarly, although no elements were found to be of highest concentration in the wood compartment, this compartment may represent a much more significant proportion of the total amount of metal(s) or contaminants in a tree when scaled up. This is something that was also highlighted by Pulford and Watson (2003). The small proportion of bark biomass to wood biomass also contributed to bark being excluded as an independent compartment. Although bark had relatively high to extremely high elemental concentrations, its small biomass suggested that its contribution to the overall elemental mass of the trees would be small compared to those of the other compartments.

5.3 Total biomass vs. total elemental mass of *S. lancea* trees

The second objective of this study was; firstly, to determine total dry biomass for *S. lancea* at each mining site by weighing the harvested tree; secondly, to calculate the total elemental mass for the

trees on each mining site by multiplying the compartment mass by the concentration; and thirdly, to test for correlation between total dry biomass and total elemental mass by using the Pearson's Correlation Coefficient. It was expected that biomass would differ between each mining site due to soil type and that total elemental mass increase with greater biomass.

As expected, total biomass was found to be higher at the nutrient-rich sites, Redsoils and Mispah. While there were no significant differences between sites, trees were noticeably larger at Redsoils and Mispah sites compared to Madala and West Complex. It was hypothesised that trees with higher biomass would contain higher elemental mass compared to trees with low biomass. Total elemental mass was generally found to be higher at Redsoils and Mispah. The correlation found between total elemental mass and tree biomass suggests that the greater the biomass of the tree, the greater the elemental mass will be. Wanenge (2009) confirmed that trees on sites with better soil conditions were larger and sequestered more carbon, nitrogen and sulphur.

These findings contrast with the findings above (Section 5.1), where some concentrations were found to be higher in lower biomass trees. This highlights the importance of converting elemental concentration into elemental mass when looking at how much of a contaminant a plant is taken up with regards to phytoextraction (Hutchings, 2002).

5.4 Phytoextraction vs. phytostabilisation of *S. lancea*

The third objective of this study was; firstly, to measure the total concentration of contaminants (as above) in the soil of the rooting zone of each tree by collecting soil samples and analysing them with XRF; secondly, to determine if there was a difference between soil pits with trees and soil pits without trees by using a Kruskal-Wallis Statistical Test; thirdly, to determine what elements *S. lancea* stabilises and which elements *S. lancea* extracts by using Bioconcentration Factors (root/shoot concentration over soil concentration) and Translocation Factors (root concentration over soil concentration). It was expected that *S. lancea* would behave as a phytostabiliser as the trees were

initially planted for hydrological control and phytostabilisation in the Mine Woodlands Project (Dye and Weiersbye, 2010).

5.4.1 Soil concentration at each site

While there were some instances of highest mean concentrations at the nutrient-poor sites, there were also some instances where mean concentrations were found to be highest at the nutrient-rich sites, thus no clear pattern was evident. This again most likely has to do with site-specific conditions of pH, CEC, parent rock material, site heterogeneity and even sampling bias.

Bulked samples may mask the heterogeneity of the contaminant concentration on site (Dickinson and Pulford, 2005). This shows that it is difficult to accurately represent contaminant concentration across a site, and that spatial mapping of contaminants is preferable in order to convey the heterogeneity of any site. This, coupled with bioavailability information, will be a very strong indicator of any phytoremediation effort, as looking at the partitioning of metals allows for a better understanding of metal and contaminant behaviour, compared to only looking at total metal concentration (Favas *et al*., 2011).

Towards this, McLeroth (2015) reported on one *S. lancea* tree soil pit from the Madala site, showing that the pH (4.94 – 5.93) and cation exchange capacity (CEC) (4.1 – 5.7 meq/100g for soil and 18.9 meq/100g for clay) were both low. As low pH and a low CEC usually indicate that bioavailability of heavy metals and contaminants are high, this suggests that metals and contaminants were available for uptake by the trees on this site. Bhargava *et al*. (2012) also stated that the bioavailability of micronutrients will increase with acidification of the rhizosphere.

We can only assume that the metals and contaminants on the other sites were available for uptake as well – especially as these are results from the low-nutrient, poorly draining soil of the Madala site. However, mobility of HMs can also be increased if chloride complexes are formed when high amounts of chloride ions are present. The Cl concentration at Madala and West Complex (poor

soil sites) was found to be relatively high. This further suggests that the mobility of contaminants was high on these sites. This is also something that should be investigated more thoroughly in future.

5.4.2 Soil Pit Elemental Concentrations

Surprisingly, there were no clear patterns that emerged from significance tests in this study, between pits with trees and pits without trees. This may signify that tree uptake does not significantly alter soil concentrations. However, bioavailability testing of the soil would lead to clearer information about what is available for uptake in the pits with and without trees.

In some pits with trees, pH was found to be significantly lower compared to some pits without trees. This suggests that trees may be acidifying the soil. In alkaline soil (high pH), which is poorly-aerated and reducing, there is generally reduced activity of metals as they tend to be adsorbed onto soil particles (high CEC). Conversely, with acidic soil (low pH, which is generally well aerated and oxidising), there is generally a lower CEC, and thus cations are released from soil particles and available for plants to take up (Kabata-Pendias, 2004; Bhargava *et al*., 2012) as was found for the Madala site by McLeroth (2015). This may lead to increased leaching (Mertens *et al*., 2007), but low soil pH may not be considered a negative aspect, as in order for the contaminants to be available for uptake they have to be bioavailable, which acidic soil allows for with a low CEC (Kabata-Pendias, 2004; Dickinson and Pulford, 2005).

There is thus room for research into which is the dominant process (acidification and mobility vs. leaching of mobile contaminants) as there must be a trade-off between trees mobilising contaminants, which increases bioavailability, and leaching potential. The mobilisation of contaminants does raise the importance of spatial aspects of tree planting as there are many trees (of varying species) per plot available to take up and/or stabilise contaminants. It may also be noted that the trees are more likely to take up or stabilise the contaminants before they can move further-afield (Dickinson and Pulford, 2005) – especially in the woodlands.

Interestingly, S was found to be lower in some pits with trees compared to some pits without trees. This suggests that trees are taking up large amounts of S. In his study, Wanenge (2009) found that *S. lancea* takes up large amounts of S and may be considered a sulphur accumulator. As some soil pits with trees were higher in total S concentration compared to pits without trees, it may be suggested that there is spatial heterogeneity of contaminants on site and that the trees may be stabilising as well as taking up contaminants.

5.4.3 Mean tree elemental mass at each site

Mean tree elemental masses were found to be highest in trees at Redsoils and Mispah compared to West Complex and Madala. Mean tree elemental mass was also found to strongly, positively correlate with biomass. This further suggests that biomass does influence the elemental mass of contaminants taken up by tree species as well as that nutrient-rich, well-draining soils are likely to increase nett uptake of contaminants in trees.

5.5 Phytoextraction vs phytostabilisation

Unexpectedly, it was found that *S. lancea* trees have a lower mean elemental concentration compared to mean soil elemental concentration, but according to the Bioconcentration Factors (BCF), *S. lancea* extracts more metals from the soil than it stabilises. *S. lancea* was also found to accumulate more elements in the shoots compared to the roots based on the Translocation Factors calculated. This suggests that *S. lancea* is better at extracting elements than stabilising the elements tested; except for Al, Fe, and Cr which it was found to stabilise. This is most likely due to the *S. lancea* ecotypes being used, which are from the surrounding mining areas – originally Uppington in the Norther Cape. As such, the trees may have developed mechanisms to deal with potential toxicity of the heavy metals and other elements. It is not uncommon for trees to develop coping mechanisms (Lasat, 2000; Pulford and Watson, 2003).

This is something that Lasat (2000), and Pulford and Watson (2003) say will heighten the likelihood of plants being suitable for phytoremediation. Further research will be needed in order to

discover the exact detoxifying mechanisms used by *S. lancea*. Multiple metals and elements, increasing the variability of potential toxins, at one site, are considered a risk in phytoremediation as well (Dickinson and Pulford, 2005; Hermle *et al*., 2006); especially where only one tree species is being utilised. The MWP is most likely addressing this issue by using multiple species, as one species may take up or stabilise certain contaminants that others do not, especially when a tree species is classified as a hyperaccumulator.

Bhargava *et al*. (2012), state that the BCF is traditionally the ratio of metal concentration of the shoot tissue to that of the soil, and is determined by the capacity of the roots to take up metals. Most plants will have a value of < 1, whereas hyperaccumulators generally have a value > 1. If this is the case, then *S. lancea* was shown to be a hyperaccumulator of Na, Mg, S, Cl, Ca, Mn, Cu, and Zn. In fact, the S, Ca, and Cl concentrations in *S. lancea* shoots were above 1000 mg/kg (a published threshold value), and thus *S. lancea* may be classified as a hyperaccumulator of S, Ca, and Cl according to Lasat (2000). These levels surpass those described by Liphdzi and Kirkham (2005) as being toxic to plants.

However, this does not meet two of the three requirements that Lorestani *et al*. (2011) identified, which are that; 1) metal concentrations in roots must be lower than in the shoots, 2) above-ground metal concentrations in the plant must be 100-500 times higher than in the same species planted in non-polluted environments, and 3) shoot concentrations should outweigh soil concentrations of the HM in question. Liphadzi and Kirkham (2005) also state that an important trait of hyperaccumulators is to allocate more of a HM to the shoots compared to the roots, as the root system is the primary target of HM toxicity. This is something that *S. lancea* does seem to be capable of.

Although *S. lancea* does meet one of the criteria (shoot concentrations are greater than root concentrations), is it enough to be called a hyperaccumulator? Favas *et al*., (2014) stated that hyperaccumulators are usually low biomass species, however, phytoextractors with large biomass can generally compensate for slightly lower metal accumulation capacity – as larger amounts of metals can

be removed. As *S. lancea* is a tree, we can say that this statement does apply to this study. Wanenge (2009) showed that *S. lancea* accumulates large masses of S - about 3.5 times greater than other plants. Dye *et al* (2008) also found that *S. lancea* accumulates 3% S in the leaves, which shows that it is a good candidate species for hydrological control and TSF seepage and phytoextraction. This was confirmed by the extremely high concentrations of S found in this study. Thus, *S. lancea* may be considered a S, Cl and Ca accumulator. Further studies should compare these findings with control trees.

As these trees are 14-15 years old, it is unclear as to whether there has been a dilution effect of metals in the plant tissues. The concentrations are certainly high, but they may have been even higher a number of years ago. Liu *et al*. (2013) mentioned that the uptake of heavy metals varied with age and that 2-3 year old trees were more efficient for remediation. However, with plant maturity and a warm climate, transpiration is higher and translocation of elements from roots to shoots may increase as a result. The optimal tree growth years for *S. lancea* should therefore be determined in order to maximise its phytostabilisation and phytoextraction abilities. Harvesting or coppicing may then occur soon after the maximum uptake of contaminants (USEPA, 2000).

However, as these trees were planted primarily for hydrological control adjacent to TSFs, there may be continual bioavailable metals available for uptake due to contaminants continually being flushed from the TSFs when it rains – in which case harvesting may not be necessary as long as the trees are preventing the lateral flow and leaching of contaminants. The semi-arid climate and high temperatures in summer months may explain why the trees are extracting high amounts of contaminants. Dye *et al* (2008) found that *S. lancea* has high transpiration rates throughout the year which means that it is a good candidate for hydrological control of TSF seepage and even phytoextraction. Grindley (2014) also found that mature *S. lancea* plots are effective in reducing water table levels and slowing lateral groundwater movement. As hydrological control is one of the primary reasons for planting of the *S. lancea* woodlands, this would mean that they are fulfilling their purpose

for the MWP. Models have also predicted that the trees will take up increasing amounts of contaminants in future scenarios (Grindley, 2014).

The perception that *S. lancea* stabilises contaminants instead of extracting them, could have arisen from previous studies performed using samples grown in pots (e.g. Wanenge, 2009), and not trees grown in the field. This is not uncommon, as pot trials have yielded different results compared to field trials in the literature (Liu *et al*., 2013). This may be attributed to pot experiments artificially enriching the soil with heavy metals, lower amounts of soluble metals being available to plants in the field, tree roots always being in contact with soil amendments (if added) in pot experiments, and the time periods in which pot trials are conducted, which are short compared to field trials (Schmidt, 2003).

5.6 Limitations of this study

There are a number of limitations of this study, due to time and budget constraints. These should be considered for further research, particularly for *S. lancea*, but also in other phytoremediation studies. Bioavailability of elements in the soil is a critical component to consider in phytoremediation studies, as mentioned above. Trees (and plants in general) cannot take up that which is not available for them to take up. This will also better indicate whether tree or plant species are performing phytoextraction or phytostabilisation, as well as allowing for a better comparison between trees and soil. Control trees (growing on uncontaminated substrate) were not compared to trees in this study. While this was not necessary for this study, it is something that should be considered in future. Data would be able to show how much less, or more than, the concentrations in control trees are when compared to trees on contaminated sites. This will go a long way in proving that trees (specifically indigenous South African trees) are able to decontaminate polluted sites.

5.7 Conclusion

This study has produced field trial data of an indigenous South African tree species (*Searsia lancea*) to add to the, still small, body of phytoremediation knowledge. It has shown that the fate of

selected contaminants (as stated in Chapter 1) in *S. lancea*, while found throughout the trees, differed in concentration between the varying compartments while being primarily located in certain tree compartments. Biomass was found to be strongly positively correlated to total elemental mass. This means that the larger the tree, the greater the amount of a contaminant it is likely to contain. Biomass was also found to be larger on the nutrient-rich sites compared to the nutrient-poor sites, as was expected.

There was no clear relationship found between soil pits with trees and soil pits without trees. This was an unexpected finding, but is most likely due to site heterogeneity. A closer look at bioavailability of contaminants may yield stronger relationships regarding soil concentrations and trees. However, *S. lancea* was found to be more of a phytoextractor than phytostabiliser based on high Bioconcentration Factor and elemental concentration results. This indigenous tree species also translocated most elements from its roots to its shoots based on the Translocation Factors calculated.

This study further confirms that *S. lancea* trees are ideal candidates for phytoremediation and have greater phytoextracting abilities than phytostabilising abilities. This may be in part due to their high evapotranspiration rates and hyperaccumulator abilities. Further work will need to be conducted on the exact detoxifying mechanism used by the tree species and to find the bioavailability of contaminants in soil compared to total concentrations, as the trees will only be able to take up what is available to them.

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