

https://dspace.org/

Risk assessment of above ground biomass for fuel use in eucalyptus species cultivated on acid mine drainage in the Witwatersrand Basin gold fields

Mosito, Nosipho Sylvia Vanessa

2016

http://hdl.handle.net/10539/21591

Mosito, Nosipho, Sylvia, Vanessa (2016) Risk assessment of above ground biomass for fuel use in eucalyptus species cultivated on acid mine drainage in the Witwatersrand Basin gold fields, University of Witwatersrand, Johannesburg, <http://wiredspace.wits.ac.za/handle/10539/21591>

Downloaded from a DSpace repository. https://dspace.org/

Risk assessment of above ground biomass for fuel use in *Eucalyptus* species cultivated on acid mine drainage in the Witwatersrand Basin gold fields

Nosipho Sylvia Vanessa Mosito
(Student number- 520988)
School of Chemistry
University of the Witwatersrand, Johannesburg

A research report submitted to the Faculty of Science in partial fulfilment of the requirements for the degree of Master of Science.

Supervisor- Prof. Luke Chimuka

Co-supervisor –Dr Peter Dye

Advisor – Isabel Weiersbye

Johannesburg, April 2016

DECLARATION

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



(Signature of candidate)

18th day of April 2016 in Johannesburg.

ABSTRACT

South African gold mines are associated with the generation of a lot of mine waste in the form of milled rock (tailings). Tailings contain the mineral pyrite which, when exposed to air and water, cause acid mine drainage (AMD). Due to the large environmental impact (footprint and scale) of the tailings storage facilities (TSFs) on soils and groundwater, there has been much research done in phytoremediation. Some plants, such as *Eucalyptus*, used in this method are able to control seepage by using their extensive roots but this may inadvertently extract some contaminants from the water and accumulate them in the above ground parts of the tree. Upon harvesting of these plants, there is the potential for them to be used as biofuel for the generation of bioenergy, and by industry or the public as timber/construction wood, firewood, charcoal, generation of electricity, etc. In this study, three species of *Eucalyptus* trees grown by the University of the Witwatersrand in three site-species trials on AMD were evaluated for their concentrations of elements in leaves, bark, branches/twigs and stem wood, in order to determine the safety of the biomass for fuel, and the potential for environmental pollution (dissemination of metals) that could be caused by combustion. The study focused on *Eucalyptus camaldulensis, E. grandis x camaldulensis* hybrid and *E. dunnii* trees grown for eight years in three different trial sites, with one trial ("Mispah") situated at AngloGold Ashanti's Vaal River Mining Operations (VR, near Orkney – Klerksdorp) and two trials ("Madala", "Red Soil") situated at the West Wits Mining Operations (WW, near Carletonville). The sites were typical of soils on the mine properties (WW Madala: Clovelly, WW Red Soil: Hutton, VR Mispah: Hutton and Mispah), and impacted by seepage from adjacent TSFs. Three entire above-ground trees were harvested per species (three trees per site, nine in total), weighed fresh and after drying. Samples of leaves, bark, twig/small branches, and main stem wood were analysed for their elemental contents; alongside a Certified Reference Material (CRM) (Orchard Leaves no. 1571); using Leco CNS analyser, Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) to determine the concentrations of major and trace elements such as Aluminium (Al), Barium (Ba), Calcium (Ca), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Nickel (Ni), Phosphorus (P), Sulphur (S), Strontium (Sr), Titanium (Ti), Zinc (Zn), Vanadium (V), Chromium (Cr), Cobalt (Co), Copper (Cu), Arsenic (As), Gold (Au), Mercury (Hg), Lead (Pb), Uranium (U), carbon (C) and ash content. The CRM was used to validate the two analytical methods.

There was variation in the concentrations of nutrients measured. There were no significant differences noted in the metal/loids concentrations between all the *Eucalyptus*

species studied ($p>0.05$). Variation between sites could not be determined as there were no replicates available to perform the comparison. The World Health Organisation (WHO) maximum permissible level (MPL) in plants for arsenic (As) is 1 mg/kg. The MPL was excedded in all tissues of all three *Eucalyptus* species studied. Arsenic concentrations of 5.09, 4.36 and 5.48 mg/kg were found in the wood of *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. A risk assessment performed found that there was no evidence that there will be adverse effects caused by supplying fuelwood from these contaminated *Eucalyptus* trees. Even though high arsenic concentrations were recorded in this study, if the wood is used as fertilizer in a vegetable bed, the transfer of the arsenic to the common vegetables is below the daily oral reference dose. The general trend in the concentration of metals and metalloids in different plant tissues was in the order of leaves > bark > branches/twigs > wood. The results of the biomass exposure assessment showed that the exposure through use of the ash as fertiliser was lower than the oral reference dose for Mn, Fe, Ba and As. The biomass risk assessment showed that the best-performing tree, in terms of wood production on AMD, was the *E. camaldulensis*. The risk of other metal/loids was not evaluated as there was no good agreement between the results recorded with those certified of the CRM. It is suspected that the CRM used was old.

ACKNOWLEDGEMENTS

This study was supported by NRF THRIP funding (project TP13080525596 "Re-valuation of gold and uranium mine land and hazardous waste") to Ed Witkowski and Isabel Weiersbye; and AngloGold Ashanti (AGA) Ltd South Africa funding to Isabel Weiersbye for the Ecological Engineering and Phytotechnology Programme (EEPP): Mine Woodlands Project at the School of Animal, Plant and Environmental Sciences (AP&ES) at the University of the Witwatersrand. We are grateful to the Environmental Management and Land Management Departments of AGA for facilitating the Mine Woodlands Project, and supporting the research and risk assessments in order to optimise phytotechnologies for pollution control and land re-valuation. I would like to extend my heartfelt appreciation to the EEPP staff and Nelson Mandela Metropolitan University (NMMU) students that assisted me during sample collection; Desmond Misselhorn, Leon Hoenderdos, Lorato Letsholo, Jacob Mahlangu (Oom J), Mthokozisi Mbazana and Linda Mkhumbeni.

Thank you to Innocent Rabohale who assisted with sample preparation, milling and arranging for sample analysis and to Dr Julien Lusilao and Siyethemba Mabaso of the School of Chemistry who undertook the ICP-OES and ICP-MS analyses.

To Prof Luke Chimuka, thank you for your support, patience and kindness through all my struggles over the years. To Isabel Weiersbye, thank you for giving me the opportunity to complete the Masters Degree course and for making this all possible. To Dr Peter Dye, thank you for all the support since sample collection to date and for taking my numerous phone calls (some at inconvenient times).

To all my colleagues at South32 SA Limited especially Lindie Moore and Clinton Lee for their support, giving me hope and allowing me time off in the past few months, knowing that things were stressful at work. Lastly, to my family and friends, especially my husband Orapeleng, who have been my pillars of strength through a very difficult time in our lives and for giving me hope. THANK YOU. God bless you all.

TABLE OF CONTENTS

DECLARATION II

ABSTRACT III

ACKNOWLEDGEMENTS V

LIST OF FIGURES VIII

LIST OF TABLES X

LIST OF ACRONYMS XII

LIST OF ELEMENTS XV

CHAPTER 1: INTRODUCTION 1

1.1 General Overview 1

1.2 Problem statement 2

1.3 Motivation 3

CHAPTER 2: LITERATURE REVIEW 4

2.1 Origin and characteristics of *Eucalyptus* species 4

2.2 Bio-energy plantations 5

2.3 Phytoremediation 6

2.3.1 Trees in phytoremediation 7

2.3.2 Nutrient uptake by plants and their effects on human health 11

2.3.3 Metal and metalloid uptake by plants and their effects on human health 14

2.3.4 The Mine Woodlands Project (MWP) 19

2.3.5 Economic opportunities for Eucalyptus trees under the MWP 23

2.4 Risk assessment of *Eucalyptus* trees grown on acid mine drainage for use as fuelwood 24

CHAPTER 3: MAIN AIMS AND SPECIFIC OBJECTIVES 27

3.1 Main Aims 27

3.2 Specific Objectives 27

3.3 Research Questions 27

CHAPTER 4: MATERIALS AND METHODS 28

4.1 Site Description 28

4.1.1 Rainfall *28*
4.1.2 Soils *35*
4.1.3 Groundwater *38*
4.2 STUDY SPECIES 40
4.3 EXPERIMENTAL DESIGN 41
4.4 SAMPLING PROCEDURE 41
4.5 SAMPLE PREPARATION FOR CHEMICAL ANALYSIS 42
4.6 RISK ASSESSMENT METHODOLOGY 43
4.7 CALCULATIONS 43
4.8 DATA ANALYSIS/ STATISTICS 45
CHAPTER 5: RESULTS AND DISCUSSION **47**
5.1 RESULTS 47
5.1.1 Nutrients *49*
5.1.2 Metals and Metalloids *51*
5.1.3 Carbon and Ash content *54*
5.1.4 Amount of nutrients, metals and metalloids per tree *54*
5.2 DISCUSSION 56
5.2.1 Nutrients *56*
5.2.2 Metals and Metalloids *56*
5.2.3 Amount of nutrients, metals and metalloids per tree *58*
5.2.4 Comparison to maximum permissible levels *58*
5.2.5 Risk Assessment *59*
CONCLUSION AND RECOMMENDATIONS 63
REFERENCES **65**
APPENDIX A **91**
APPENDIX B **95**
APPENDIX C **101**
APPENDIX D **108**
APPENDIX E **113**
APPENDIX F **119**
APPENDIX G **121**

LIST OF FIGURES

Figure 1: Location of the Mine Woodlands Project trials (Map from Weiersbye, *et al.*, 2006) 20
Figure 2: Conceptual model of woodlands plantation comprising plants like *Eucalyptus* to control the seepage and groundwater contamination by acid mine drainage in the Witwatersrand Basin (AngloGold Ashanti Report to Society, 2004). 23
Figure 3: The 4-step Risk Assessment Process (USEPA, 1991) 26
Figure 4: Map of South Africa showing the AGA's Vaal River operation and West Wits operation. 29
Figure 5: Location of the WW's Madala and Red Soil sites 30
Figure 6: Location of woodlands trials at the VR Mispah site 30
Figure 7: Long-term mean monthly rainfall for AGA's VR operation near Klerksdorp (Herbert, 2008). 31
Figure 8: Mean monthly minimum and maximum temperature for AGA's VR operation near Klerksdorp (Herbert, 2008). 32
Figure 9: Long-term mean monthly rainfall for AGA's WW operation near Carletonville (Herbert, 2008). 33
Figure 10: Mean monthly minimum and maximum temperature for AGA's WW operation near Carletonville (Zuurbekom station between Soweto and Carletonville) (Herbert, 2008). 34
Figure 11: Soil Map for Vaal River Mine (WSP, 2009a) 36
Figure 12: Soil Map for WW's Red Soil site (McLeroth, 2015) 37
Figure 13: Soil Map for WW's Madala site (McLeroth, 2015) 37
Figure 14: Vaal River South Mass Model from 2007 (TDS in mg/L) (WSP, 2009a) 38
Figure 15: Interpolated groundwater elevation and flow direction (WSP, 2009b) 39
Figure 16: Interpolated groundwater TDS concentrations (WSP, 2009b) 39
Figure 17: Typical examples of three *Eucalyptus* species; *E. camaldulensis, E. dunnii, E. grandis x camaldulensis hybrid.* 41
Figure 18: Comparison of nutrients Ca (left) and S (right) concentration in wood averaged across all three *Eucalyptus* species. 50
Figure 19: Comparison of nutrients Ca (left) and S (right) concentration in all tissues averaged across all three *Eucalyptus* species. 50
Figure 20: Comparison of metal/loids Mn (top left), Fe (top right), Ba (bottom left) and As (bottom right) concentration in wood across *Eucalyptus* species. 52

Figure 21: Comparison of metal/loids Mg (top left), Fe (top right), Ba (bottom left) and As (bottom right) concentration in all tissues averaged across all three *Eucalyptus* species.....53
Figure 22: Comparison of metal/loids to the WHO maximum permissible levels.....59

LIST OF TABLES

Table 1: Example of *Salix* species with potential for phytoextraction of various metals 7
Table 2: Examples of plants with potential for phytoextraction of various metals 9
Table 3: Essential nutrients for plant growth, their principal forms for uptake and typical concentrations 12
Table 4: Normal range of metal and metalloid concentrations in plants 17
Table 5: WHO Maximum Permissible Limits (MPL) of metal/loids in plants 18
Table 6: Critical toxicity levels and thresholds for hyperaccumulators 19
Table 7: Annual volume increment in *Eucalyptus* plantations in different parts of the world (Adapted from FAO, 2001) 21
Table 8: Coefficients for the estimation of standing tree volume using the Schumacher and Hall model (Bredenkamp, 2000) 44
Table 9: Standard industry conversion factors for roundwood 45
Table 10: CRM agreements to measured values 47
Table 11: Statistical comparison of concentrations of metals in three *Eucalyptus* species Tukey HSD, The hypothesis where H_0: $v_1=v_2=v_3$and H_1: $v_1\neq v_2$/ $v_2\neq v_3$/ $v_1\neq v_3$. 48
Table 12: Calculated wood volume, wood mass and content of nutrient, metal or metalloid per tree 55
Table 13: Average content per site (species combined) 55
Table 14: Average content of nutrients, metals and metalloids in *Eucalyptus* species consumed and available in ash per household per day (assuming no volatilisation during combustion) 58
Table 15: Transfer Factor from soil to vegetables (Jolly *et al.*, 2013) 60
Table 16: Average concentration of metal and metalloids in typical vegetables found in gardens 60
Table 17: Daily intake of metal/metalloids per *Eucalyptus* species 61
Table 18: Risk Assessment 62
Table 19: Mean concentrations of phosphorus, potassium and % nitrogen for wood from three *Eucalyptus* species ± standard deviation 114
Table 20: Mean concentrations of phosphorus, potassium and % nitrogen for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 114
Table 21: Mean concentrations of calcium, sulphur and magnesium for wood from three *Eucalyptus* species ± standard deviation 115

Table 22: Mean concentrations of calcium, magnesium and sulphur for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 115
Table 23: Mean concentrations in mg/kg of manganese, iron, zinc, copper and nickel for wood from three *Eucalyptus* species ± standard deviation 116
Table 24: Mean concentrations of manganese, iron, zinc, copper and nickel for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 116
Table 25: Mean concentrations of chromium, lead and arsenic for wood from three *Eucalyptus* species ± standard deviation 117
Table 26: Mean concentrations of chromium, lead and arsenic for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 117
Table 27: Mean % dry mass of carbon and ash content for wood from three *Eucalyptus* species ± standard deviation 118
Table 28: Mean % dry mass of carbon and ash content for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 118

LIST OF ACRONYMS

Acronym	Description
AGA	AngloGold Ashanti
AMD	Acid Mine Drainage
ANOVA	Analysis of Variance
AP&ES	Animal, Plant and Environmental Sciences
DME	Department of Minerals and Energy
EEPP	Ecological Engineering and Phytotechnology Programme
FAO	Food and Agriculture Organisation of the United Nations
HSD	Honest Significant Difference
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ITRC	Interstate Technology & Regulatory Council
MAI	Mean Annual Increment
MWP	Mine Woodlands Project
MPL	Maximum Permissible Level
NMMU	Nelson Mandela Metropolitan University
RSD	Relative Standard Deviation
SE	Standard Error
TDS	Total Dissolved Solids
TSF	Tailings Storage Facility
VR	Vaal River

Acronym	Description
AGA	AngloGold Ashanti
WHO	World Health Organisation
Wits	University of the Witwatersrand
WW	West Wits

LIST OF ELEMENTS

Element	**Name**
Al	Aluminium
As	Arsenic
B	Boron
Ba	Barium
C	Carbon
Ca	Calcium
Cl	Chlorine
Co	Cobalt
Cr	Chromium
Cu	Copper
Fe	Iron
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
Ni	Nickel
P	Phosphorus

Element	Name
Pb	Lead
S	Sulphur
Si	Silicon
Zn	Zinc

CHAPTER 1: INTRODUCTION

1.1 General Overview

According to the South African Department of Tourism, Economic and Environmental Affairs (2002) and the Chamber of Mines of South Africa (2004), South Africa's mining industry generates a lot of mine waste, often stored in tailings storage facilities (TSFs), with gold mines in the Witwatersrand Basin accounting for 30 percent of this waste per annum. The gold and uranium mines in the Witwatersrand Basin area have waste dumps accommodating approximately 6 billion tonnes of gold tailings (Robb and Robb, 1998a; b; Wymer, 2001) and contain an estimated 30 million tonnes of sulphur (Witkowski and Weiersbye, 1998). The sulphide material produced after gold and uranium mining is often associated with pyrite material, which when exposed to air and water causes acid mine drainage (AMD) (Akcil and Koldas, 2006).

Releases of AMD are associated with elevated levels of sulphates, chlorides and inorganic contaminants such as metals (iron, aluminium and manganese), and are characterized by low pH and high electrical conductivity (Winde *et al.*, 2004 a; b; c; Akcil and Koldas, 2006). Acid mine drainage has the potential to contaminate surface- and groundwater, and soils, which could result in the disruption of the growth and reproduction of both aquatic and terrestrial flora and fauna and potentially impact human health (Akcil and Koldas, 2006; Nengovhela *et al.*, 2006). In order to ensure that the mining companies around the Witwatersrand Basin Goldfields obtain their closure certificates, they need to ensure that the area is rehabilitated (including ensuring that the AMD is controlled). It is costly to install pumps to extract contaminated groundwater and then treat the water to reduce contaminants to acceptable levels (Vivier, 2005). A less costly and more sustainable solution lies in introducing technologies such as phytoremediation to control the spread of the AMD and immobilize or extract contaminants. The use of trees for this purpose is particularly attractive, owing to their deep root systems, potentially high water use and high biomass (Dye *et al.*, 2008a).

Gold TSFs provide suitable environments for some plant species. There were 286 indigenous and 90 alien species that have been identified to survive and grow on and around gold TSFs in the Witwatersrand Basin (Weiersbye *et al.*, 2006). Several research studies have been conducted on tolerant indigenous trees (Weiersbye, 2007; Weiersbye and Witkowski, 2007) and also on the growth of exotic plants used for phytoremediation on and around these TSFs (Weiersbye *et al.*, 2006a; b; Dye and Weiersbye, 2010). Trees can be particularly useful for phytoremediation because they have deep roots, high biomass and mostly stay evergreen

throughout the seasons (Dye, 2010). Plant biomass can be an important product in some areas and is used to generate energy for households or industry (Jain, 1994; van den Broek *et al.*, 1996; Sims *et al.*, 1999; Abbasi and Abbasi, 2010). *Eucalyptus* species are particularly useful because growth is fast and the wood burns well (Smit and Meincken, 2012). There are, however, some risks to using wood as a source of fuel (Lyons *et al.*, 1985; van Horen *et al.* 1996; Marufu *et al.*, 1997; Estrellan and Iino, 2010). Some of these risks are air pollution, health risks and environmental degradation. During wood combustion, carbon dioxide and water are released as by products. However, combustion is not always complete in practice, as small amounts of hydrocarbons, carbon monoxide and other gases may be released (Lyons *et al.*, 1985) which lead to air pollution. In this study, the elemental uptake by wood, leaves, bark and branches/twigs of *Eucalyptus* trees grown in site-species trials on tailings affected by AMD for eight years was determined, and a risk assessment conducted to determine the safeness of using this contaminated wood as a fuel source.

1.2 Problem statement

When trees have reached physiological maturity, they may be cut and used as fuelwood by the community or industry. These trees will have accumulated metal/loids, such as chromium, arsenic, copper, lead and other chemicals that are a danger to soil, water, air and people when the wood is burnt. In developing a risk management strategy, it is important that we investigate the amount of metal/loids that accumulate in each of the tree species used in the study. After the wood is used for fire, it has become common for ash to be used by religious churches for medicinal purposes. Peltzer (1999) indicated that for medicinal purposes, the African traditional Pentecostal churches in South Africa use ash from firewood for healing. This indicates the importance of ash from firewood among African traditional believers; however, ash from firewood containing metal/loids such as chromium, arsenic and lead can be dangerous depending on the quantity applied. Ash is also used as organic fertilizer in agriculture and sometimes on the soil where fruits and vegetables that are normally eaten without being cooked first are grown i.e. tomatoes (Pradhan *et al.*, 2009). So when ash is used as fertilizer, it is returned to the soil. The problem starts when chemicals and metal/loids in firewood from trees used for remediation of contaminated gold fields are not accounted for. To ensure the health and safety of the end users of the firewood from trees that were previously used in phytoremediation, and have accumulated a certain quantity of environmentally dangerous chemicals, it is important to measure the amount of metal/loids that have accumulated in the trees.

1.3 Motivation

It is important to note that contamination of agricultural land and groundwater by metal/loids is essentially linked to human activities. A major problem with metal/loids is that they cannot be biodegraded, and therefore reside in the environment for long periods of time if they are not removed. Thus, depending on the kind of depth of contamination, different remediation techniques are normally developed; as in this study where *Eucalyptus* tree species are planted as part of the remediation strategy. One of these methods which was used in this study is phytoremediation. It utilizes the ability of hyperaccumulating plants to extract high amounts of metal/loids and also may bring about hydraulic control, to prevent the spread of contaminated water. Accumulation of metal/loids in the environment is a serious concern for animal and human health. When the trees have matured, the stems might be cut down and used as a fuel source by the community or industry. Therefore, for the safety of people and the environment, it is important to investigate the risk of having these trees used, by assessing the amount of metal/loids, including arsenic, nickel, chromium, lead, sulphur, and other metals that accumulated in the stem wood, leaves, barks, twigs and branches. The risk assessment will also assist in choosing, from among the trees used for this study, the one that is the most benign wood for combustion.

CHAPTER 2: LITERATURE REVIEW

2.1 Origin and characteristics of *Eucalyptus* species

Eucalyptus trees are native to Australia but are grown in many parts of the world with a similar climate (Prosser, and Williams, 1998). *Eucalyptus* falls within the *Myrtaceae* family with 800 species recorded in the genus (Meskimen and Francis, 2012).

The *Eucalyptus* tree species are fast growing trees, such that when they are planted on suitable sites and managed properly, they have high potential for producing commercial products such as pulpwood, mulch, fuelwood and even for remediation of some environmental problems (Djavanchir and Mossadegh, 1973; Abo-Hassan, *et al.*, 1988). In addition, some *Eucalyptus* tree species are resistant to salinity and can tolerate a wide range of soil pH and soil types (Fine *et al.*, 2014). This study assessed the above ground biomass of *Eucalyptus* species cultivated on AMD for fuelwood use. It is important to understand the tolerance of *Eucalyptus* species to high concentrations of metal/loids, and their effects on wood and other aboveground biomass (Assareh *et al.*, 1997).

Another advantage of using trees is that, trees often have deep root systems to help them access groundwater during dry seasons. The best and most feasible combination for acid mine drainage and heavy metal control around the TSFs in the Witwatersrand Basin could be the use of hyperaccumulators such as *Tamarix* to uptake contaminants and the use of high water use trees such as *Eucalypts* for hydraulic control (Bouwer, 2000; Medhurst *et al.*, 2002). This, according to Pulford and Dickinson (2006), is because;

i. Trees have a high root and stem biomass which creates a large sink for phytoextraction and contaminant sequestration;
ii. Some trees like *Eucalyptus* spp. are fast growing;
iii. Silviculture, which is the application of the principles of forest ecology to a stand of trees to help meet specified objectives, is an already established practice and can easily be integrated into phytoremediation programs;
iv. Wood and slash – leaves and twigs from trees can be used as a bio-energy source, and,
v. Trees have high root biomass which can assist in binding soils, reducing erosion.

2.2 Bio-energy plantations

The use of fast growing trees as a source of fuelwood in some areas in South Africa could be beneficial for communities that sustain their livelihoods through this option (Dovie et al, 2005; Shackleton *et al*, 2007). The use of *Eucalyptus* trees could be of particular advantage since they grow fast and have denser wood than willow (when grown under short rotation coppices), which aids in slow burning (Leslie *et al.*, 2012). *Eucalyptus* trees could produce high biomass whilst creating a large water and nutrient demand, characteristics which can be utilized in the phytoremediation of AMD and heavy metal polluted soils (Rockwood *et al.*, 2001). The woody biomass, in the form of wood chips can then be used with coal for energy production (Rockwood *et al.*, 2001). South Africa is one country that has a high biomass resource and promotes renewable energy through the use of biofuels as an alternative energy source, resulting in approximately 13.6% of South Africa's energy supply being met by biomass (DME, 2002). However, the global contribution of South Africa in biofuel production is less than 0.01% (US EIA, 2013).

The use of wood as an alternative energy source has a relatively lower cost than fossil fuels, especially when carbon tax is included (Bennett, 2003). More than 70% of people living in rural villages in sub Saharan Africa still depend on wood as their primary fuel source (Matsika *et al.*, 2013). In South Africa, over 80% of households in rural villages use fuelwood to meet their energy needs, even with the availability of electricity (Banks *et al.*, 1996; Williams and Shackleton, 2002; Madubansi and Shackleton, 2006; Shackleton *et al.*, 2007; Vasicek and Gaugris, 2014). However, the overall use of wood has decreased in South Africa from 2002 to 2013 from 19.3% to 10.5% (Stats SA, 2013).

Woody biomass is also a carbon neutral fuel as the quantity of carbon released to the atmosphere during combustion is equal to the quantity removed from the atmosphere during plant growth stage (Rockwood *et al.*, 2001). All fuels used in industry regardless of their fuel type should comply with clean air standards and regulations. During combustion, carbon dioxide and water are released; however, oxidation sometimes is incomplete, which leads to releases of NOx, SO_2, CO and other hydrocarbons (Lyons *et al.*, 1985).

In some countries, *Eucalyptus* trees have the potential to be grown successfully as bioenergy plantations on polluted ground-water using methods to optimize biomass productivity (Madgwick *et al.*, 1990). Studies in South Africa on tree trials on polluted groundwater over the last decade demonstrated that at least seven *Eucalyptus* species or hybrid clones are tolerant to AMD conditions and produce adequate biomass for poles or bioenergy (CSIR, 2010). The conditions presented by AMD polluted groundwater around the base of

TSFs may influence the feasibility and safety of wood fuel production, and this has generated several research questions that need to be answered, which some are answered by this study.

2.3 Phytoremediation

Worldwide, fuelwood demands, soil and groundwater contamination, and agriculture's impact on nature are growing concerns (Hazell *et al.*, 2008). Human activities, such as mining, have continuously increased the level of heavy metal ions circulating in the environment (Ma and Rao, 1997). Fast growing trees in short rotation woody crop systems may increasingly meet societal needs ranging from renewable energy to environmental mitigation and remediation (Rockwood *et al.*, 2004). Most importantly, the use of short rotation woody crop system was reported to have potential to remediate contaminated soil and groundwater (Mulligan *et al.*, 2001; Susarla *et al.*, 2002). Such woody crops include some *Eucalyptus* species, which have relatively low contaminant concentrations (Mulligan *et al.*, 2001). Other tree species that have been found to be successful in phytoremediation because of their fast growing characteristics are hybrids of willow (*Salix*) and poplar (*Populus*) (ITRC, 2009). Some of the studies that have been conducted using willow for phytoremediation have been listed under **Table 1**.

Several engineering methods were developed with the intention to contain and treat contaminated soils (Smith *et al.*, 1995), but these methods were proven to be costly (Rockwood *et al.*, 2001). For example, non-biological technologies e.g. excavation and land-filling, used to decontaminate soils range between US$100 000 to US$3 million per ha (Weiersbye, 2007). The total cost for rehabilitating abandoned mines using engineering measures in South Africa was estimated to be approximately US$ 14 billion (DME, 2007). Phytoremediation is the use of plants for environmental clean-up. Phytoremediation offers a slow but relatively inexpensive and sustainable way for stabilizing, containing or eradicating AMD (Weiersbye, 2007). This is done through hydraulic control of groundwater by short rotation woody crop systems (*Eucalyptus* species) and by the different plant defence mechanisms to defend against toxic conditions associated with AMD and other toxins (Larcher, 2003). When a plant is confronted with toxic conditions it can accumulate them in the root, leaf or stem (Harborne, 1993). Important to the control of AMD, specifically in the Witwatersrand mining region, are three broad phytoremediation approaches. These include **phytoextraction** of contaminants from soils, waste or water. This approach relies on plants that accumulate contaminants or metals in their biomass, and this can be controlled by cropping the plants (Lasat, 2002). **Phytostabilization** of contaminants within soil relies on the plant root's ability to hydraulically

control and also sequester contaminants. **Phytodegradation** relies on the ability of plants to convert ions like phosphate to substances which are less toxic like proteins in leaves (Weiersbye, 2007).

Table 1: Example of *Salix* species with potential for phytoextraction of various metals

Metal	Plant species	Conc. mg/kg	Substrate	Reference
Cu	*Salix caprea* - foliage and woody branches	681	3-year old trees on highly contaminated soil	Dickinson, 2000
Zn	*Salix* (4 spp)- stems	95-156	Heavy sludge applications	Riddell-Black, 1994
Pb	*Salix*- wood	157.4	Metal contaminated soil	Dickinson, 1997
Ni	*Salix* (2 spp) - leaves	<20	Sludge application	Labrecque *et al.*, 1995
Hg	*Salix* (2 spp) - leaves	<20	Sludge application	Labrecque *et al.*, 1995

2.3.1 Trees in phytoremediation

Use of trees in phytoremediation started since the early 1990's because trees can clean up both organic and inorganic contaminants and produce woodlands at the same time (Gansauer, 2012; Peuke and Rennenberg, 2005). The potential use of trees as a suitable vegetation cover for heavy metal-contaminated land has received increasing attention over the last 15 years (Aronsson and Perttu, 1994; Glimmerveen, 1996). Trees were suggested as a low-cost, sustainable and ecologically sound solution to the remediation of heavy metal-contaminated land (Dickinson, 2000), especially when it was uneconomic to use other treatments or when there was a time pressure on the reuse of the land (Riddell-Black, 1994).

Exploitation of metal uptake into plant biomass as a method of soil decontamination is limited by plant productivity and the concentrations of metals achieved (Baker *et al.*, 1994). Some tree species have an advantage when used for phytoremediation as they grow fast and thus have a higher biomass which means that they can sequester much more contaminants than shrubs, grasses and forbs (Rockwood *et al.*, 2004). Many plants, including trees, shrubs, herbs, forbs and grasses, can be potentially useful in phytoremediation when site conditions are not too harsh, but hyperaccumulators and high biomass plants like trees are the most favoured (Rockwood *et al.*, 2001). Some of the herbaceous plants that have been found to have the potential to extract some metals are indicated in **Table 2** below.

Hyperaccumulators are plants that are able to take up and tolerate very high concentrations of metal/loids and other contaminants e.g. sulphates (Pulford and Dickinson, 2006). These include trees such as the *Tamarix* species (Dennis, 2008). Some tree species also use much water, remain green and develop frost tolerant canopies (Leslie *et al.*, 2012).

Table 2: Examples of plants with potential for phytoextraction of various metals

Metal	Plant species	Reference
Cr (IV)	*Brassica juncea* (L.) Czern	Kumar *et al.*, 1995a; Huang *et al.*, 1997a
Cu	*Brassica juncea*	Ebbs and Kochian, 1997
Ni	*Brassica juncea*	Ebbs and Kochian, 1997
Pb	*B. campetris* L.; *B. carinata* A. Br.; *B. juncea*; *B. napus* L.; *B. nigra* (L.) Koch.; *Helianthus annuus* L.; *Pisum sativum* L.; *Zea mays* L	Begonia *et al.*, 1998; Blaylock *et al.*, 1997; Ebbs and Kochian, 1998
Zn	*Avena sativa*; *B. juncea*; *B. napus* L. *Hordeum vulgare*, *B. rapa*	Ebbs *et al.*, 1997; Ebbs and Kochian, 1998
Cd	*Brassica juncea*	Kumar *et al.*, 1995a; Huang *et al.*, 1997a; Ebbs *et al.*, 1997; Salt *et al.*, 1995b

Trees are faced with harsh and challenging environmental conditions when grown on AMD and heavy metal polluted substrates on TSFs (Sheoran and Sheoran, 2006). Many trees were found to survive in highly saline conditions and therefore decontaminate the polluted groundwater (van der Moezel *et al.*, 1988; 1989; 1991; Marcar, 1993; Sun and Dickinson, 1995; Mahmood *et al.*, 2001; Duggan, 2005; Feikema and Baker, 2011). Although some plants are able to tolerate and survive on and around gold TSFs in the Witwatersrand Basin (Weiersbye *et al.*, 2006), site conditions are complex due to variability of contaminant composition, soil depths, soil water contents, and depth to water table. The different soil

substrates can influence the pH, redox potential, organic matter content, and the forms and concentrations of metal/loids which in turn can impact on trees (Pulford and Dickinson, 2006).

While in this study we used high water use plants to control the spread of metal/loids from the environment, it should be noted that some metal/loids can exist in more than one oxidation state. Not all oxidation states of metals are available for plant uptake (Sparks, 2003; Brady and Weil, 2008). Forms of metal/loids available for plant uptake are those forms that are water soluble and mobile in the plant environment. Dube *et al.* (2001), Cao *et al.* (2009) and Meers *et al.* (2009) have also emphasized that the mobility of metal/loids in the soil is the driving force for heavy metal uptake by plants whereas the mobility of metal/loids is dependent upon their solubility in the soil solution. As a result, non-soluble metal/loids are unlikely to be available for plant uptake.

Nevertheless, the availability and subsequently the toxicity of metal/loids to plants can also be affected by several factors such as adsorption and desorption potential of metal/loids to and from the soil mineral surface, precipitation potential of metal/loids and the dissolution potential of minerals containing and/or adsorbing metal/loids (Menzies *et al.*, 2007).

In this study, the amount of metal/loids and minerals absorbed from the soil/environment by the plant is not all that was actually in the soil. That is because some of these metal/loids and minerals are in a form that cannot be taken up by plants. According to Brown *et al.* (1999) the type of a complex that a particular element forms with the mineral surface plays an important role in the mobility and therefore availability of metal/loids to plants in the soil. There are some heavy metal ions that form inner-sphere complexes with some particular mineral surfaces. Such metal/loids are difficult to be desorbed under normal conditions and as a result are relatively immobile. On the other hand, if the heavy metal ion forms an outer-sphere complex with mineral surfaces, it can be easily desorbed and it can be available to plants. The type of the resulting complex is actually the function of both the form with which a heavy metal exist and the type of mineral adsorbing that particular heavy metal.

Studies conducted by Hunter (2001) in southern India showed that even though *Eucalyptus* trees generally accumulate low concentrations of nutrients like nitrogen, phosphorus, potassium, calcium and magnesium compared to indigenous trees like *Dalbergia sissoo*, they can potentially contain high overall amounts of these elements due to their high biomass.

2.3.2 Nutrient uptake by plants and their effects on human health

Plants need nutrients to survive and grow. A criterion by Arnon and Stout (1939) specifies which elements are identified as essential. These criteria are:

- A deficiency of an essential nutrient makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle;
- Such deficiency is specific to the element in question and can be prevented or corrected only by supplying this element; and:
- The element is involved directly in the nutrition of the plant quite apart from its possible effects in correcting some unfavourable microbiological or chemical condition of the soil or other culture medium.

Thirteen of these nutrients are essential inorganic constituents which are N, P, K, Ca, Mg, S, Fe, Cl, Zn, Mn, Cu, B, and Mo (Mengel *et al.*, 2001; ITRC, 2009). These are taken up from the soil through the roots. Nutrients are further classified by Mengel *et al.*, (2001) as primary macronutrients (N, P and K), secondary macronutrients (Ca, Mg and S) and micronutrients/trace elements (Zn, Fe, Cl, Mn, Cu, B and Mo). Sometimes Co and Ni are considered essential to some plants (Mengel *et al.*, 2001).

The principle forms of uptake in plants (Mengel and Kirkby, 1987) and their typical concentrations (Epstein, 1965; Brown *et al.*, 1978b; Epstein and Bloom, 2005) are indicated in the **Table 3** below for all the above mentioned essential nutrients.

Table 3: Essential nutrients for plant growth, their principal forms for uptake and typical concentrations

Nutrient	Principal forms of uptake	Typical concentrations in plants (mg/kg dry weight)
N	NH_4^+, NO^{3-}	15 000
P	H_2PO^-, HPO_4^{2-}	2 000
K	K^+	10 000
Ca	Ca^{2+}	5 000
Mg	Mg^{2+}	2 000
S	SO_4^{2-}, SO_2	1 000
Fe	Fe^{2+}	100
Mn	Mn^{2+}	50
B	$B(OH)_3$	20
Zn	Zn^{2+}	20
Cu	Cu^{2+}	6
Mo	MoO_2^{4-}	0.1
Cl	Cl^-	100
Ni	Ni^{2+}	0.1

Woody plants such as *Eucalyptus nitens* prefer to take up nitrogen in the form of NH_4^+ (Garnett and Smethhurst, 1999; Garnett *et al.*, 2003). N is essential in plants as it forms part of nucleic acids and proteins which are needed for plant growth (OECD, 2014). An excess of nitrogen in plants may cause an overgrowth of leaves and net primary productivity, but it may also cause long-term declines in the latter (Aber *et al.*, 1989; 1995). When combusted, wood

containing high levels of N releases two primary harmful gases which are nitrogen dioxide (NO_2) and nitric oxide (NO) (WHO, 2010). Higher exposure (>0.053ppm/yr for NO_2 and >5ppm/15min for NO) to these gases can cause damage to respiratory airways (WHO, 2010). The nitrogen is not expected to be different in gold TSFs than in normal conditions as there is no fertiliser being added currently in the study plots.

Phosphorus in plants occurs in nucleic acids and in ATP and aids in plant growth (OECD, 2014). According to Marschner (1995) and Hawkins *et al.* (2008), P toxicity symptoms in plants are characterised by stunted growth, accelerated leaf senescence and brown-grey necrosis on young leaves.

Potassium in plants is an essential nutrient involved in enzyme activation, protein synthesis, osmoregulation, stomatal opening and closing, photosynthesis and cell growth (Pallardy, 2008).

Calcium (Ca) occurs in large amounts in plants and is responsible for cell wall elasticity, N metabolism and enzyme activation (Pallardy, 2008). Ca uptake in plants is in the form of insoluble Ca^{2+} through root permeable channels and stored in large vacuoles of cells (Maathuis, 2009).

Magnesium (Mg) is an essential part of the chlorophyll molecule in plants (Maathuis, 2009). Mg^{2+} is up-taken from soil and plays a major role as an enzyme cofactor and in energy transfer (Maathuis, 2009). A deficiency in Mg results in chlorosis (Pallardy, 2008)..

Sulphur (S) forms part of most amino acids. It is responsible for protein synthesis in plants (Hänsch and Mendel, 2009). S is mostly present in soils as sulphate (SO_4^{2-}) ion (Maathuis, 2009). This is how it is taken up by the roots of the plant. S toxicity can occur in saline soils with high concentrations of sulphates (Maathuis, 2009).

Zinc (Zn) is essential for enzymes responsible for protein synthesis and energy production (Hänsch and Mendel, 2009). It is mainly taken up in the form of Zn^{2+} ion from the soil (Hänsch and Mendel, 2009). In severe Zn deficiency cases, necrotic areas appear on the leaf surface (Pallardy, 2007).

Copper (Cu) is important in plant growth as it is essential for "photosynthesis and mitochondrial respiration, carbon and nitrogen metabolism, oxidative stress protection and cell wall synthesis" (Hänsch and Mendel, 2009). It normally occurs in two oxidation states Cu^{2+} and Cu^{+}, which allows it to act as both a reducing and oxidative agent that can become toxic to the plant (Hänsch and Mendel, 2009). Copper, when taken up by the plant, therefore binds to proteins to prevent the toxicity elevation in the plant tissues (Hänsch and Mendel, 2009).

Copper deficiency can be identified by wilting new leaves, terminal shoot dieback and total necrosis and collapse of shoot (Snowball and Robson, 1991).

Iron (Fe) has a similar function to copper, but added to its function is also nitrogen assimilation and hormone synthesis (Hänsch and Mendel, 2009). It is very intimately linked to the metabolism of copper. Fe exists in two oxidation states in soil, ferric (Fe^{3+}) and ferrous (Fe^{2+}), which are not equally available to plants (Pallardy, 2008). Plants predominantly prefer to assimilate Fe^{3+} (Pallardy, 2008).

Potassium, calcium, zinc, copper, iron and magnesium all form oxides when combusted which cling to particulate matter which is airborne or remain in the ash (Dare *et al.*, 2001). Some particulate matter (roughly 80%) released from burning wood can be as small as less than one micron (≤1 μm) and is therefore respirable and can cause serious respiratory diseases (Smith, 1987; NARSTO, 2004). Sulphur when combusted forms sulphur dioxide which is a gas that causes several respiratory illnesses (USEPA, 2015).

2.3.3 Metal and metalloid uptake by plants and their effects on human health

Through phytoabstraction to exert hydraulic control on AMD-contaminated groundwater and soils, *Eucalyptus* trees may be able to accumulate metals to potentially toxic levels, and thus pose a threat to end users. Whether these *Eucalyptus* trees do accumulate metals or metalloids, and to what extent, is the subject of this study. This also presents an opportunity (i.e. helping to decontaminate polluted soil and water by phytoremediation, or "biomining" of useful metals) and it also presents a risk to the use of the *Eucalyptus* products that need to be managed, if the concentrations of metals exceed harmful levels. Some of the metals and metalloids of concern are arsenic, lead, uranium and chromium as they are dangerous to human health (Rauf *et al.*, 2011) and are also known to occur at concentrations above the normal surface geochemical background concentrations in gold tailings dams and the surrounding contaminated soils on the Witwatersrand Basin (Witkowski and Weiersbye, 1998).

According to the US Agency for toxic substances and disease registry, arsenic is listed as the Number 1 hazardous substance (US Department of Health and Human Services, 1999). Arsenic can be hazardous for humans and animals when ingested (Hajar *et al.*, 2014). Arsenic uptake in plants is influenced by arsenic concentration in the soil (Marin et al., 1992, 1993b; Xie and Huang, 1998; Rauf *et al.*, 2011). Arsenic species are bioactive and toxic. Long-term exposure to low concentrations of arsenic in drinking water can lead to skin, bladder, lung and prostate cancer (Zhang *et al.*, 2002). The prolonged ingestion of soluble arsenic is said to cause

peripheral neuropathy, gastrointestinal symptoms, diabetes, renal system effects, cardiovascular disease and cancer (WHO, 2010).

Chromium is a non-essential metal in plants (Rauf *et al.*, 2011). Chromium is highly toxic to plants and affects the plant's development (Rauf *et al.*, 2011). Chromium can occur in various oxidation states (-2 to +6) but the +4 [Cr(IV)] and +6 [Cr(VI)] oxidation states are the most toxic and are of the most environmental concern. According to Fendorf (1995), Fendorf and Li (1996) and Bencquer *et al.* (2003) the mobility and phyto-availability of chromium depends on the prevailing oxidation state. The trivalent chromium [Cr (III)] occurs in soils as a cation (i.e. $CrOH^{2+}$) and it is easily adsorbed to most soil mineral surfaces, where it forms inner-sphere complexes (Fendorf, 1995, Fendorf and Li, 1996 and Bencquer *et al.*, 2003). As a result Cr (III) is relatively less mobile and less available to the environment in general. Cr (VI) is more toxic to living organisms owing to its high redox potential and also being able to penetrate biological membranes (Ponce *et al.*, 2015). It can adsorb or accumulate in some plant species. According to Khan *et al.* (2008) prolonged exposure to chromium can lead to liver, kidney and lung damage.

Lead has various oxidation states but the one that is more stable under many environmental conditions is the mobile and toxic Pb^{2+} (Raungsomboon *et al.*, 2008). For example, at room temperature lead(IV) Chloride ($PbCl_4$) decomposes to give lead(II) chloride ($PbCl_2$) and chlorine gas (Cl_2), and lead(IV) oxide (PbO_2) decomposes on heating to give lead(II) oxide (PbO)and oxygen (O_2) (Raungsomboon *et al.*, 2008). Although Pb^{2+} is the dominant form of lead in the soils, Pb^{4+} can be found occasionally in some highly oxidized soils (Raungsomboon *et al.*, 2008). Because lead enters the soil in different forms, its reactions may differ widely from place to place. Once lead is in the soil, it may react with anions such as phosphate (PO_4^{3-}), sulphate (SO_4^{2-}) or carbonate ion (CO_3^{2-}) to form less soluble salts such as lead carbonate [$Pb_3(OH)_2(CO_3)_2$] and chloropyromorphite [$Pb_5(PO_4)Cl$] (Yoon, 2005; Chrysochoou *et al.*, 2007 and Cao *et al.*, 2009). Soil organic matter (humic and fulvic acids) can also form complexes with lead which will then be adsorbed onto soil solids and as a result immobilize lead (Yoon, 2005). Lead has no known biological function in animals or plants (Álvarez-Ayuso *et al.*, 2012). Toxic levels of lead mainly are responsible for hematological, gastrointestinal and neurological dysfunctions in humans (Lockitch, 1993; Mandal and Suzuki, 2002; Álvarez-Ayuso *et al.*, 2012).

Mercury is categorised as one of the priority hazardous substances according to the Agency for Toxic Substances and Disease Registry (ASTDR). Mercury in soil originates from natural processes, anthropogenic activities associated with mining Zn, Pb and V ores and re-

deposition on the earth's surface from the atmosphere (Wang *et al.*, 2012). The most common forms of mercury found at contaminated sites are the mercuric form (Hg^{2+}), mercurous form (Hg_2^{2+}), elemental mercury (Hg^0) and methyl or ethyl mercury (USEPA, 1997). The most toxic of these is methyl mercury (Ulrich *et al.*, 2001). Mercury can be readily taken up by plants and transferred through the food chain to animals and humans (Wang *et al.*, 2012). A study done by Zheng *et al.* (2007b) showed that although mercury was taken up by vegetables grown near a Zinc smelting plant, the transfer factors of mercury were not as detrimental compared to other metal/loids. When ingested, mercury can accumulate in the brain and liver, leading to major toxic effects in the central nervous system that result in cerebral palsy, muteness and mental retardation (Guzzi and La Potta, 2008; Soghoian and Sinert, 2008).

The normal ranges of metals in plants are indicated in **Table 4** below.

Table 4: Normal range of metal and metalloid concentrations in plants

Elements	Range	Reference
Arsenic (As)	0.02 – 5 mg/kg	British Herbal Medicine Association, 1996.
Chromium (Cr)	0.006 – 18 mg/kg	Pawlisz, 1997; Zayed and Terry, 2003
Copper (Cu)	0.4 – 45.8 mg/kg	Kabata-Pendias and Pendias, 1984
Iron (Fe)	640 – 2486 mg/kg	Lavilla *et al.*, 1999
Mercury (Hg)	<1 – 300 ng/kg	Yu *et al.*, 2011
Magnesium (Mg)	0.73 – 1.41 mg/kg	Witkowski and Lamont, 1996
Zinc (Zn)	1 – 160 mg/kg	Kabata-Pendias and Pendias, 1984
Aluminium (Al)	200 ≥1 000 mg/kg	Jansen *et al.*, 2002; Chenery 1948 &1949
Manganese (Mn)	15 – 100 mg/kg	Misra and Mani, 1991
Nickel (Ni)	0.1 – 3.7 mg/kg	Kabata-Pendias and Pendias, 1984

For medicinal purposes, the African traditional Pentecostal churches in South Africa use ash from firewood for healing (Peltzer, 1999). This indicates the importance of ash from firewood among African traditional believers. However, ash from firewood containing chemicals such as copper, arsenic, and chromium can be dangerous depending on the quantity consumed or applied. Bill Hinkley in Curtis (1998) was quoted as saying about the ash, "It can kill you at moderate amounts over a longer period. And it's a carcinogen at low levels".

According to the Florida Centre for Solid and Hazardous Waste management (CCA), chronic exposure to dusts or mists containing chromium salts may cause:

- Ulceration and perforation of the nasal septum;
- Respiratory irritation may occur with symptoms resembling asthma;
- Other symptoms may include conjunctivitis, anorexia, nausea, gastritis, duodenal ulcers and colitis. Liver damage may occur;
- Chronic skin exposures may lead to a skin rash, and entry of chromium salts into open wounds may cause chromium ulcers;
- Acute poisoning from ingestion of chromium and its salts may cause dizziness, intense thirst, abdominal pain, shock and reduction in urinary output and vomiting;
- Prolonged skin exposure might cause ulceration of the skin, and
- Acute exposure to dry chromium salts may cause severe irritation of the eyes, nose throat, bronchial tubes and lungs.

The permissible limits of some of the metal/loids in plants according to the World Health Organisation (WHO) (1996) are indicated in **Table 5** below. The concentrations of metal/loids from this study were evaluated against this standard.

Table 5: WHO Maximum Permissible Limits (MPL) of metal/loids in plants

Elements	**Permissible value of plants (mg/kg)**
Cr (Chromium)	1.30
Cu (Copper)	10
Pb (Lead)	2
Ni (Nickel)	10
As (Arsenic)	1*

* Rauf *et al.*, 2011; Ahmad, 2000 and Manirul *et al.*, 2015.

The critical toxicity levels and thresholds set to define plants as hyperaccumulators are indicated in **Table 6** below.

Table 6: Critical toxicity levels and thresholds for hyperaccumulators

Metal/loids	Critical toxicity level (mg/kg) (Krämer, 2010)	Threshold (mg/kg) (Maestri *et al.*, 2010; Krämer, 2010)
As	<2-80	>1000
Cd	6-10	>100
Co	≥0.4	>1000
Cu	20-30	>1000
Cr	0.2-1	>1000
Pb	0.6-28	>1000
Mn	200-3500	>10000
Hg	0.001-5	>1000
Ni	10-50	>1000
Zn	100-300	>10000

2.3.4 The Mine Woodlands Project (MWP)

Approximately 286 indigenous and 90 alien plant species were identified to survive and grow on and around gold TSFs in the Witwatersrand Basin in South Africa (Weiersbye *et al.*, 2006); which proves that gold TSFs provide suitable environments for some plant species. Several studies were conducted on tolerant indigenous trees in the Witwatersrand Basin (Weiersbye, 2007; Weiersbye and Witkowski, 2007), and also on the growth of exotic plants used for phytoremediation on and around these TSFs (Dye and Weiersbye, 2010; Weiersbye *et al.*, 2006). The use of *Eucalyptus* trees for this purpose is believed to be effective, owing to their deep root systems, potentially high water use and high biomass (Dye *et al.*, 2008). Trees can be particularly useful for phytoremediation because they mostly stay evergreen throughout the seasons (Dye, 2010). Plant biomass can be an important product in some areas and is used

to generate energy for households or industry (Jain, 1994; van den Broek *et al.*, 1996; Sims *et al.*, 1999; Abbasi and Abbasi, 2010).

AngloGold Ashanti (AGA) and the University of the Witwatersrand (Wits) are exploring the potential of phytoremediation as an ecological engineering approach to the sustainable rehabilitation of mine TSFs through the Mine Woodlands Project (MWP) (AGA, 2004; 2008; 2010). This project explores the potential of open woodlands and savannah vegetation on top of the TSFs as an evapotranspiration (ET) cover to control ingress of rainwater and leaching of oxidation products to groundwater, and dust. The project also has closed-canopy woodlands on seepage zones around TSFs to control AMD and groundwater pollution. Several site-species trials were established near Welkom, Klerksdorp and Carletonville (**Figure 1**), with over 40 different shrub and tree species that are being tested for their ability to control dust and seepage.

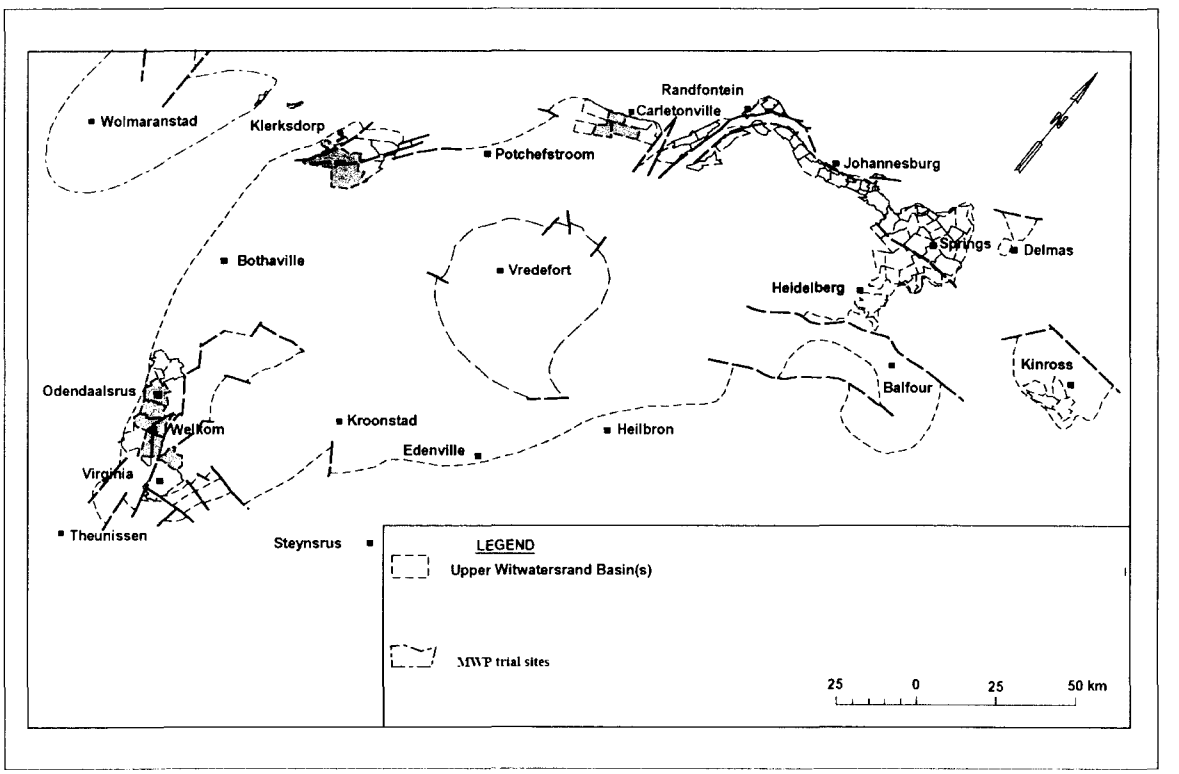

Figure 1: Location of the Mine Woodlands Project trials (Map from Weiersbye, *et al.*, 2006)

Seven species in the *Eucalyptus* genus have been grown on site-species woodlands trials as part of the MWP. These include: *Eucalyptus camaldulensis* (Dehnh.), *Eucalyptus dunnii* (Maiden.), *Eucalyptus grandis x camaldulensis* hybrid, *Eucalyptus macarthurii* (H. Deane and Maiden.), *Eucalyptus melliodora* (A. Cunn. ex Schauer.), *Eucalyptus grandis x nitens* hybrid and *Eucalyptus sideroxylon* (A. Cunn. ex Woolls). **Table 7** shows the mean annual increments expected in these species in different parts of the world.

Table 7: Annual volume increment in *Eucalyptus* plantations in different parts of the world (Adapted from FAO, 2001)

Location	MAI (m^3 ha^{-1} yr^{-1})	Rotation or stand age	Reference
E. camaldulensis			
Argentina	20-25	7-15 years	Lamprecht, 1990
Israel (irrigated plantation)	30	7-15 years	Lamprecht, 1990
Turkey (heartwood growth)	17-20	7-15 years	Lamprecht, 1990
Turkey (1st coppice generation)	25-30	7-15 years	Lamprecht, 1990
Morocco	3-11	7-15 years	Lamprecht, 1990
Portugal	2-10	7-15 years	Lamprecht, 1990
Italy	6-7	7-15 years	Lamprecht, 1990
Colombia	45	3 years	Newman, 1981
Guatemala	12.5-17.6	4.5 years	Otarola and Ugalde, 1989
Nicaragua	2.4-16.8	4.5 years	Otarola and Ugalde, 1989
South Africa	35.4	16 years	Dye *et al.*, 2008a
E. grandis			
Australia - NSW	16	29 years	Hillis and Brown (1984)
Brazil - Aracruz Florestal	55	7 years	Evans (1992)[a]
Costa Rica	1-49	2-4 years	Sánchez (1994)
Costa Rica	49-112	6.5 years	Vásquez and Ugalde (1995)[b]
South Africa	35	N/A	NAS 1980
Swaziland - Shiselwene Forestry	18	9 years	Evans (1992)
Uganda	17-45	N/A	NAS 1980
Zimbabwe	40	N/A	NAS 1980[c]
E. grandis x camaldulensis			
South Africa	29.57	7 years	Little *et al.*, 2002[d]

Location	MAI (m^3 ha^{-1} yr^{-1})	Rotation or stand age	Reference
South Africa: Zululand region	23	7 yrs	Smith *et al.*, 2006[e]

a- Clonal plantations;

b- Experiments with fertilizer and spacing;

c- With irrigation;

d- With manual weed checks;

e- Under optimum planting densities when coupled with fertilization and weeding during establishment.

The expectation is that these *Eucalyptus* plantations are able to control run-off and AMD through hydraulic control (**Figure 2**).

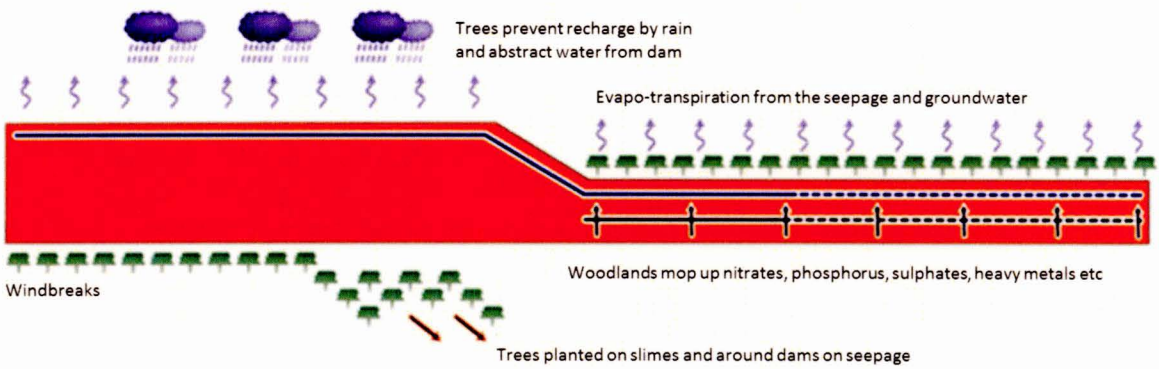

Figure 2: Conceptual model of woodlands plantation comprising plants like *Eucalyptus* to control the seepage and groundwater contamination by acid mine drainage in the Witwatersrand Basin (AngloGold Ashanti Report to Society, 2004).

2.3.5 Economic opportunities for *Eucalyptus* trees under the MWP

Growing *Eucalyptus* trees presents several economic opportunities in the continent of Africa and elsewhere. Timber can be used for pulp and paper production, charcoal production, solid wood products, plywood and veneer, poles for construction, firewood and biomass for energy (Couto *et al.*, 2003). The leaves of some species can be used for the production of essential oils, while the whole plant can be used for land reclamation, wind breaks, stabilization, control of AMD and carbon sequestration (Couto *et al.*, 2003; Mokgalaka *et al.*, 2009). Both international and local studies proved that *Eucalyptus* trees perform better in accumulation of contaminants, productivity, growth and evapotranspiration when grown in

contaminated waste sites compared to other species like *Acacia nilotica, Dalbergia sissoo* and *Phragmites australis* (Singh *et al.,* 2010; Shukla *et al.*, 2011; Morris, 1984; Sun *et al.,* 1994, Zohar and Schiller, 1998; Dye, 2008a). Some communities rely on the forests for their survival. Areas where there is poor infrastructural development and government services are normally prone to have communities that rely on trees in these forests (Shackleton *et al.*, 2007). The use of these forests for bioenergy can also be beneficial for poverty alleviation in these communities (Shackleton *et al.*, 2007).

E. camaldulensis is also used in a number of pharmaceutical applications for the treatment of skin ailments (Mabona and Van Vuuren, 2013). Its bark is used as a pharmaceutical product to wash pimples (Hutchings, 1996; Babayi *et al.*, 2004; Ayepola and Adeniyi, 2008 and Musa *et al.*, 2011). Eucalypts are also a good source of essential oils which are obtained from their leaves (Boland *et al.* 1982; Weston, 1984). The leaves when crushed emit a peppermint aroma which can be used to synthesise peppermint flavours (Weston, 1984). Some foliage from trees such as poplar is also used for manufacturing of animal feed products (Balatinecz and Kretschmann, 2001). The focus of this project is the use of *Eucalyptus* trees for fuelwood to local communities for domestic heating, cooking and recreational purposes e.g. braai.

2.4 Risk assessment of *Eucalyptus* trees grown on acid mine drainage for use as fuelwood

Risk assessment can be viewed as the natural human intelligence process that a person consciously or subconsciously uses every day to protect himself/herself from harm (Lee, 1999). Risk assessment is a valuable tool that attempts to improve environmental planning and business outcomes (Lee, 1999). From the current scenario, *Eucalyptus* trees have the potential to provide biofuels when grown around TSFs as part of MWP to control AMD, surface and groundwater pollution. During AMD control, nutrients and toxic metals could be accumulated by the plants. It has been proven that planting trees at contaminated sites such as landfills and tanneries has the potential to decrease the environmental impacts associated with leachate (Duggan, 2005; Shukla *et al.*, 2011). The accumulation of metal/loids and radioactive elements in the tree biomass might also be of concern. Thus there might be a risk of metal/loids accumulated by *Eucalyptus* being released into the atmosphere during combustion of the woody biomass or contained in the left-over ash.

While the *Eucalyptus* tree species are used in this study for hydraulic control on AMD through consumption of seepage and evapotranspiration, it must also be noted that there are parts of the trees that are consumed by human beings without government regulations. This involves the use of *Eucalyptus* leaves in medicines that can be bought over the counter. *Eucalyptus* leaves are a traditional herbal remedy (Chevallier, 1996). The essential oil found in the leaves, which is also a common ingredient in many over-the-counter cold remedies, is a powerful antiseptic and is used all over the world for relieving coughs and colds, sore throats and other infections (Chevallier, 1996). The most risk to human health is that these can also be obtained from the tree by making incisions in the trunk (Grieve, 1971; Lassak and McCarthy, 1983).

Some of the other environmental impacts that can be experienced by the use of *Eucalyptus* trees for fuelwood use can vary according to the following:

i. Water quantity impacts caused by the use of exotic species that have a high evapotranspiration rate;
ii. Soil quality positive impacts caused by the improvement of soil organic matter if trees are planted on degraded land;
iii. Air quality impacts experienced when harvesting trees for fuelwood can be as a result of increased dust emissions and also release of some contaminants (depending on the land used for planting these trees) into the environment via combustion. The other air quality impacts could be the release of greenhouse gas emissions from logging equipment used during harvesting.

A human health risk assessment follows four basic steps (USEPA, 1991) which are illustrated in **Figure 3** below. The same method was used in this study to identify the risk associated with the harmful effects of domestic use of contaminated trees:

i. Hazard identification was calculated directly from the concentration or content of metals and metalloids found in the *Eucalyptus* trees; and
ii. Exposure assessment, dose-response assessment and risk characterization was assessed through various literature sources.

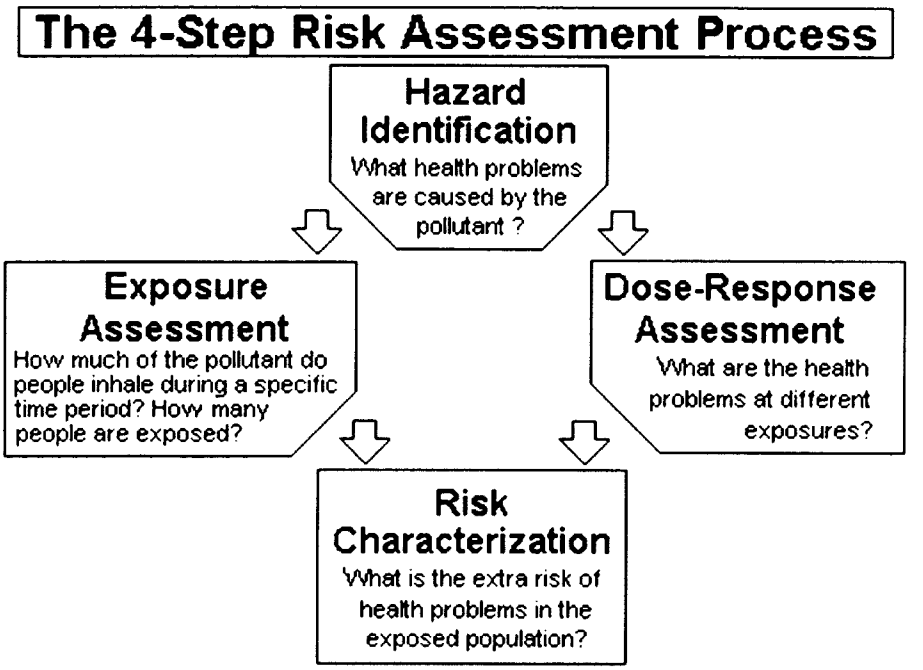

Figure 3: The 4-step Risk Assessment Process (USEPA, 1991)

The equation Risk = Hazard x Exposure was then used following the above highlighted process.

CHAPTER 3: MAIN AIMS AND SPECIFIC OBJECTIVES

3.1 Main Aims

- The aim of this study was to determine concentration and content levels of metals and metalloids in selected *Eucalyptus* trees grown on acid mine drainage in the Witwatersrand Basin and to determine which species produces the most benign wood for combustion and also which species produces the riskiest wood.

3.2 Specific Objectives

The objectives for this study were,

a) To determine and compare metal/loids concentrations and content among three *Eucalyptus* tree species;
b) To compare the metal concentration between tissue types (wood, bark, leaves, branches and twigs) for each species;
c) To determine whether metal/loids concentrations are within the WHO MPLs for plants;
d) To determine which species produces the most benign wood for combustion.

3.3 Research Questions

a) What is the concentration and content of metals in the wood of three *Eucalyptus* tree species/hybrid clones cultivated on acid mine drainage?
b) Do elemental allocations vary with tissue types and with species?
c) Which species will be less harmful when used as fuelwood?

CHAPTER 4: MATERIALS AND METHODS

4.1 Site Description

The study was conducted on site-species trials that were established at AGA's West Wits mine operation near Carletonville (26°26'12"S 27°21'12"E) and AGA's Vaal River mine operation near Klerksdorp (26°59'57"S 26°46'28"E) as part of the broader Mine Woodlands Project. The study locations, Vaal River (VR) and West Wits (WW) mine operations are 112 kilometres away from each other (**Figure 4, Figure 5 and Figure 6**). At Carletonville (WW mine), there are two sites named Red soil and Madala. At Klerksdorp (VR mine), there are two sites named Mispah and West Complex.

4.1.1 Rainfall

The area where VR operation is located normally receives about 657 mm of rain per year, with most rainfall occurring mainly during mid-summer (Herbert, 2008). **Figure 7** below shows the average rainfall values for the study area per month. It receives the lowest rainfall (4 mm) in July and the highest (120 mm) in January. The monthly distribution of average maximum temperatures (**Figure 8**) for the location ranges from 18.5°C in June to 29.1°C in January. The region is coldest during July where the mercury drops to 3.6°C on average.

The WW area normally receives about 703 mm of rain per year, with most rainfall occurring mainly during mid-summer. The chart below (**Figure 9**) shows the average rainfall values for the location per month. It receives the lowest rainfall (2 mm) in July and the highest (129 mm) in January. The monthly distribution of average maximum temperatures (**Figure 10**) for this area ranges from 17.5°C in June to 26.9°C in January. The area is the coldest during July when the mercury drops to -1.4°C on average.

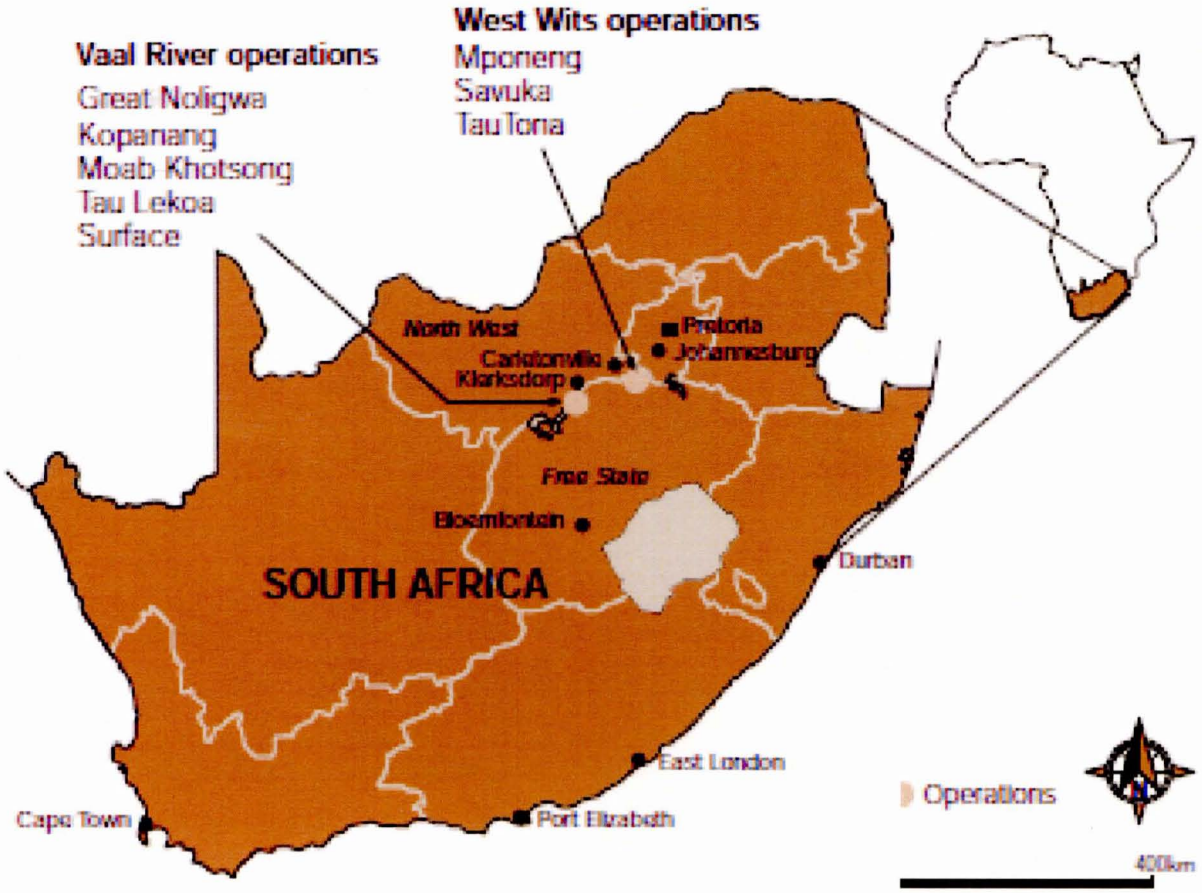

Figure 4: Map of South Africa showing the AGA's Vaal River operation and West Wits operation.



Figure 5: Location of the WW's Madala and Red Soil sites



Figure 6: Location of woodlands trials at the VR Mispah site

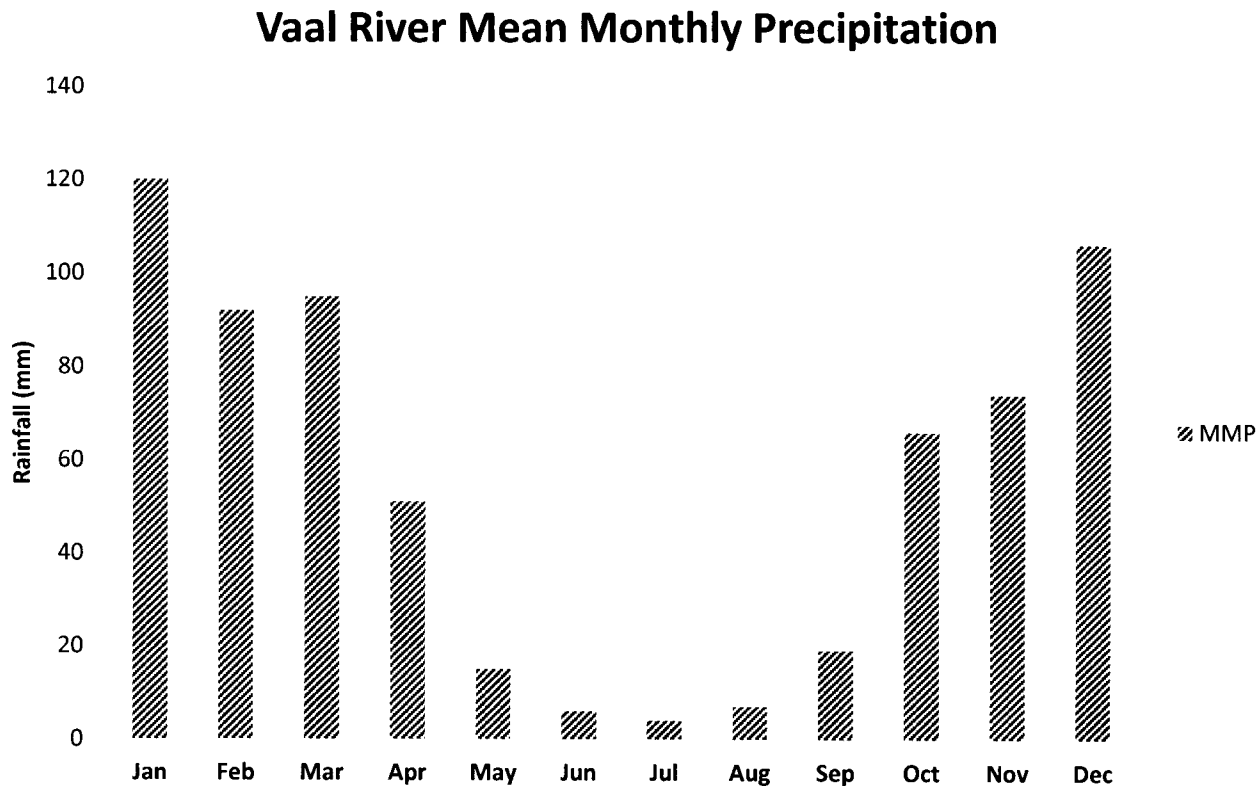

Figure 7: Long-term mean monthly rainfall for AGA's VR operation near Klerksdorp (Herbert, 2008).

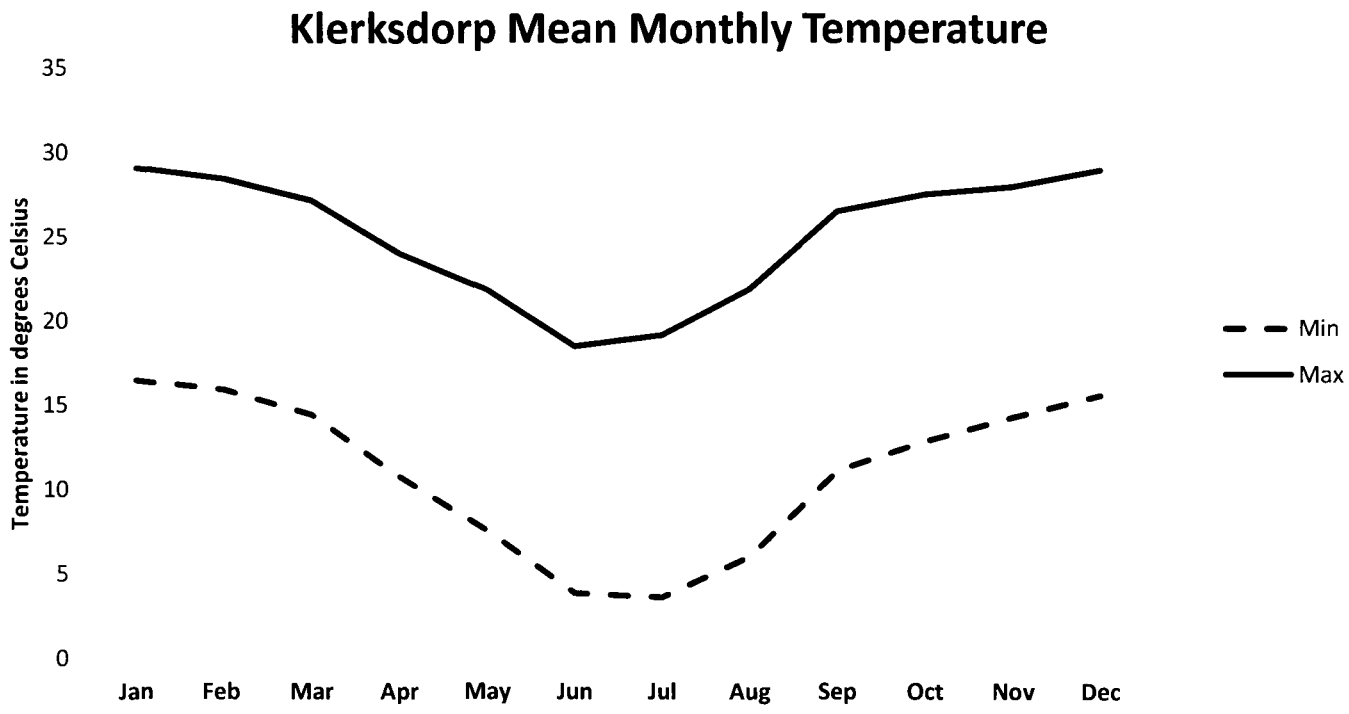

Figure 8: Mean monthly minimum and maximum temperature for AGA's VR operation near Klerksdorp (Herbert, 2008).

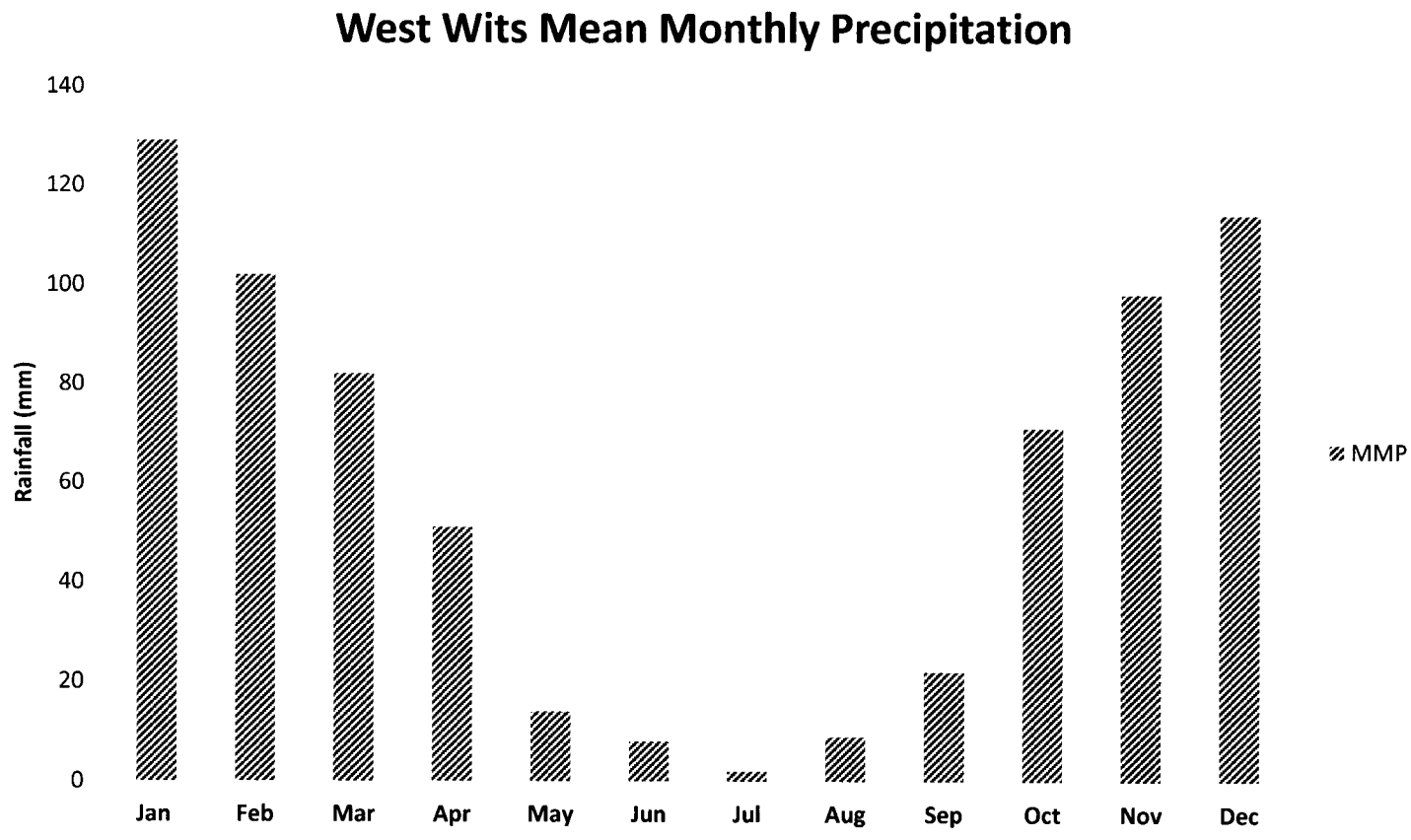

Figure 9: Long-term mean monthly rainfall for AGA's WW operation near Carletonville (Herbert, 2008).

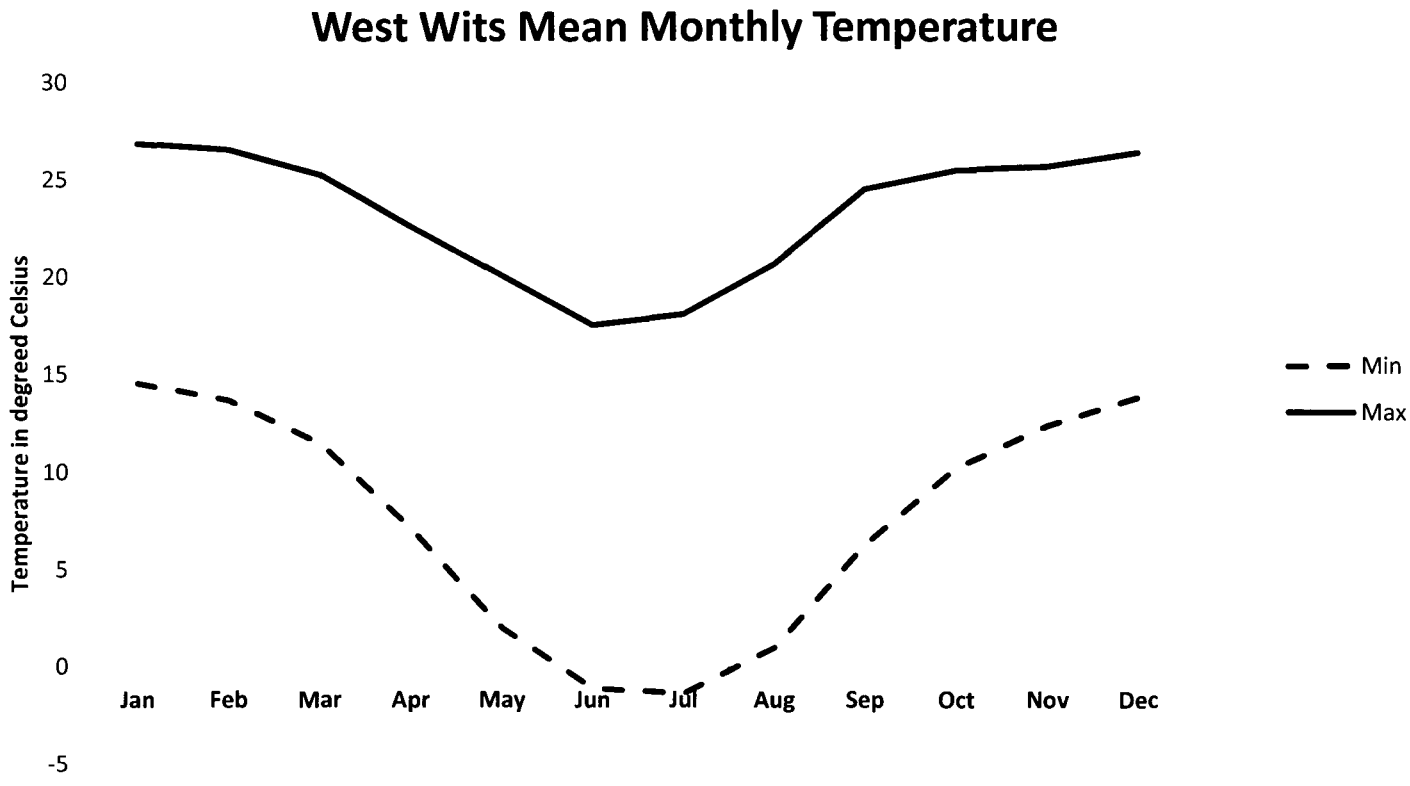

Figure 10: Mean monthly minimum and maximum temperature for AGA's WW operation near Carletonville (Zuurbekom station between Soweto and Carletonville) (Herbert, 2008).

4.1.2 Soils

The soils at VR's Mispah site consist of well drained deep red Hutton and Mispah soils (**Figure 11**) ideal for growing *Eucalyptus* trees (Herbert, 2008). These are shallow, red, sandy loam soils with flat to gentle slopes and 1-10% exposed dolomitic surface rock (WSP, 2009a).

At WW's Red Soil site there are deep reddish brown soils (**Figure 12**) (greater than 200 cm depth) well drained clay loam to clay textured Hutton soils originating from weathered diabase (Herbert, 2008; McLeroth, 2015). The soils' parent material is diabase (dominant) and shale and has an orthic A-horizon with 20-35% clay and a red apedal B-horizon with 50-30% clay (McLeroth, 2015).

At WW's Madala site, the soil profile was classified as yellow brown apedal soils with a shale (or occasionally ferricrete) parent material with a midslope and a very gently concave slope (McLeroth, 2015). The area is dominated by Clovelly soil forms with an orthic A-horizon with 20-35% clay and apedal B-horizon with 20-35% clay (**Figure 13**) (McLeroth, 2015).

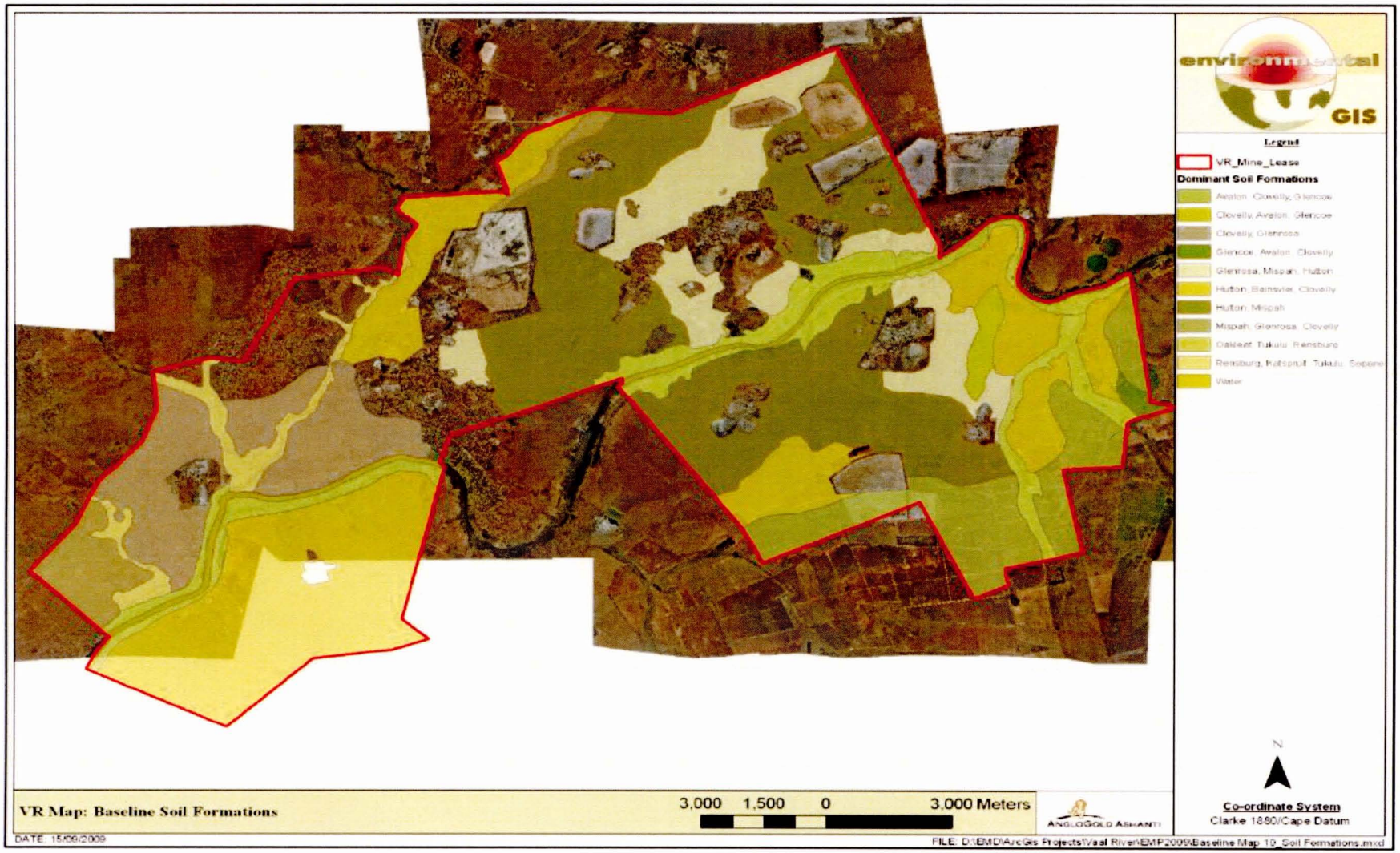

Figure 11: Soil Map for Vaal River Mine (WSP, 2009a)

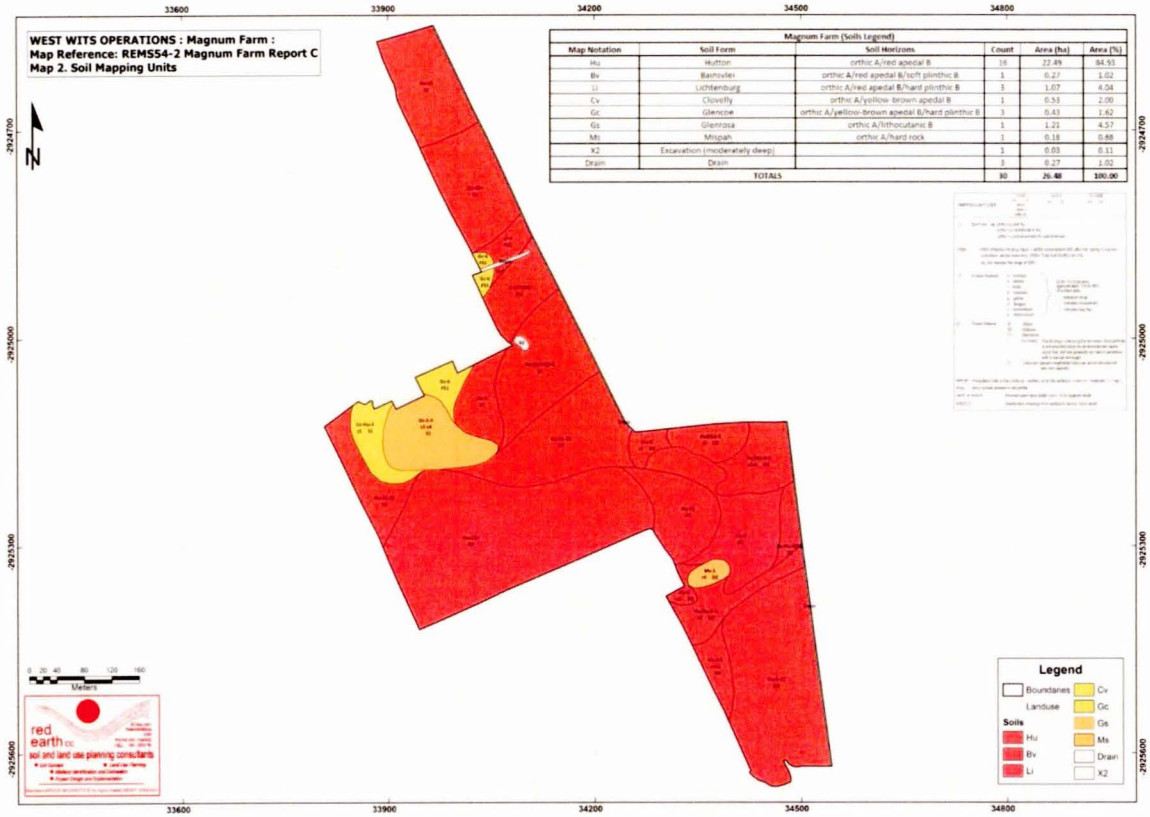

Figure 12: Soil Map for WW's Red Soil site (McLeroth, 2015)

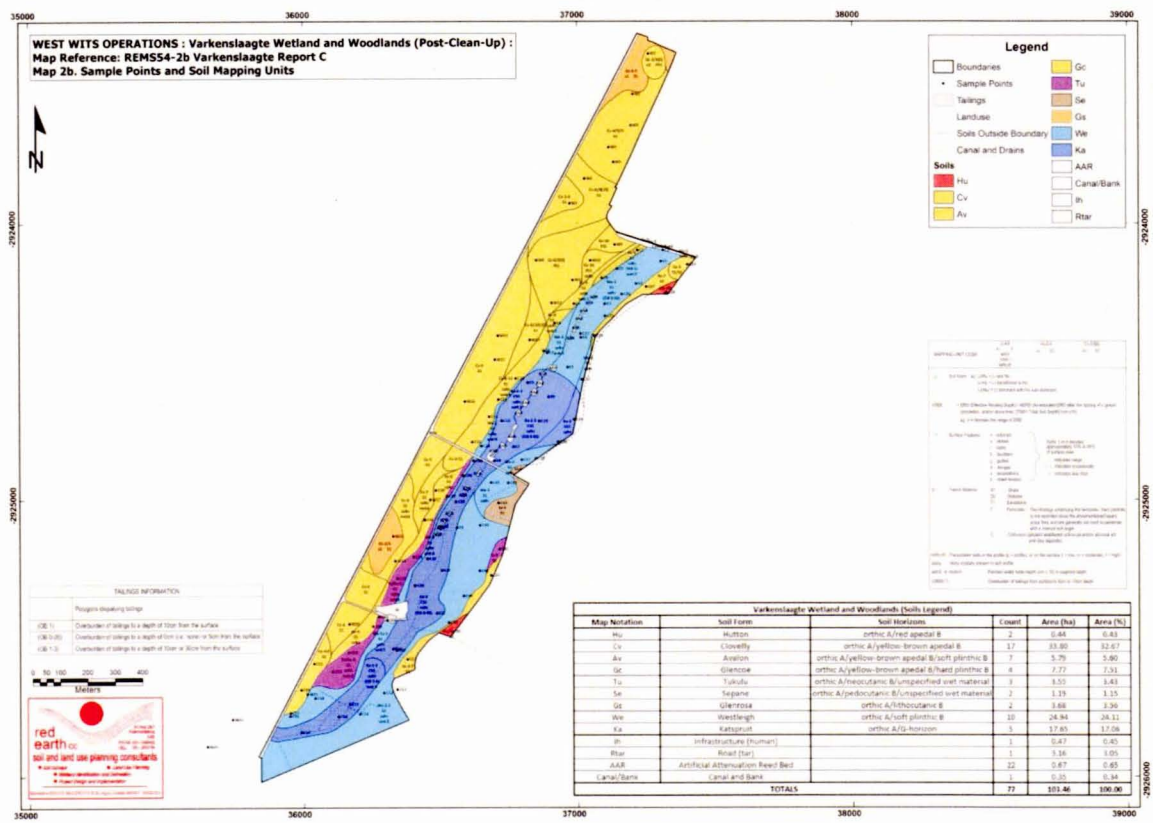

Figure 13: Soil Map for WW's Madala site (McLeroth, 2015)

4.1.3 Groundwater

The VR's Mispah site's groundwater was modelled (**Figure 14**) and showed that polluted groundwater (total dissolved solids (TDS) >1000mg/L) originated from the Mispah and Great Noligwa areas (WSP, 2009a).

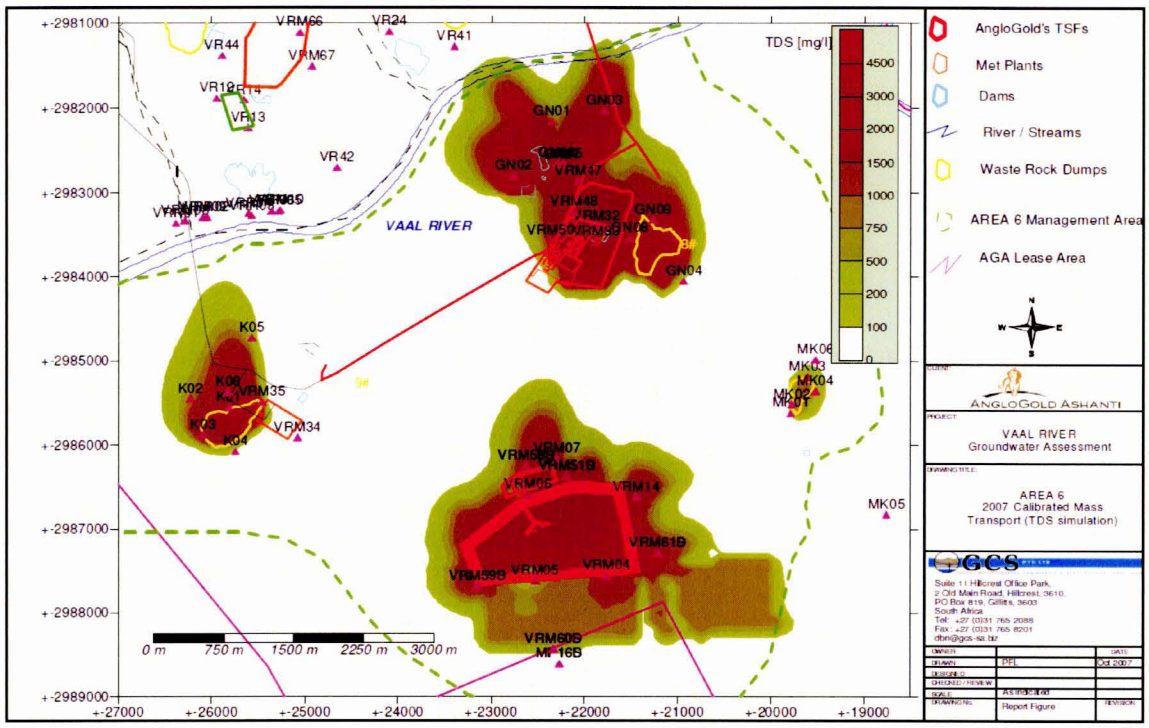

Figure 14: Vaal River South Mass Model from 2007 (TDS in mg/L) (WSP, 2009a)

At WW's Madala and Red Soil sites, the groundwater flow and elevation is depicted in **Figure 15** below [Water Levels (WL) in blue]. The aquifers present in the areas are defined as a 15 to 25 m semi-confined to confined weathered and fractured rock aquifer system (WSP, 2009b). The weathered aquifer system is situated above an inter-bedded impervious shale bedrock aquifer system that extends down to approximately 75 to 250 metres above mean sea level (WSP, 2009b). The TDS is illustrated in **Figure 16** which shows polluted water (above 3200 mg/L TDS) at point MBH44 and MBH18/MBH37.

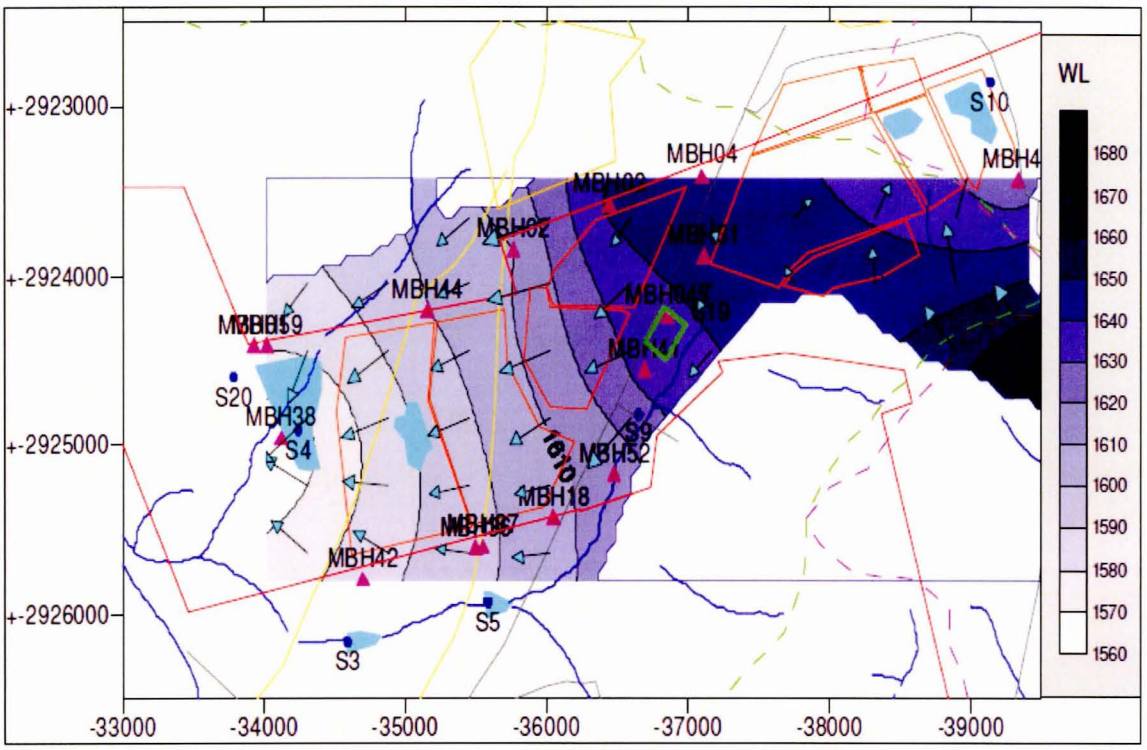

Figure 15: Interpolated groundwater elevation and flow direction (WSP, 2009b)

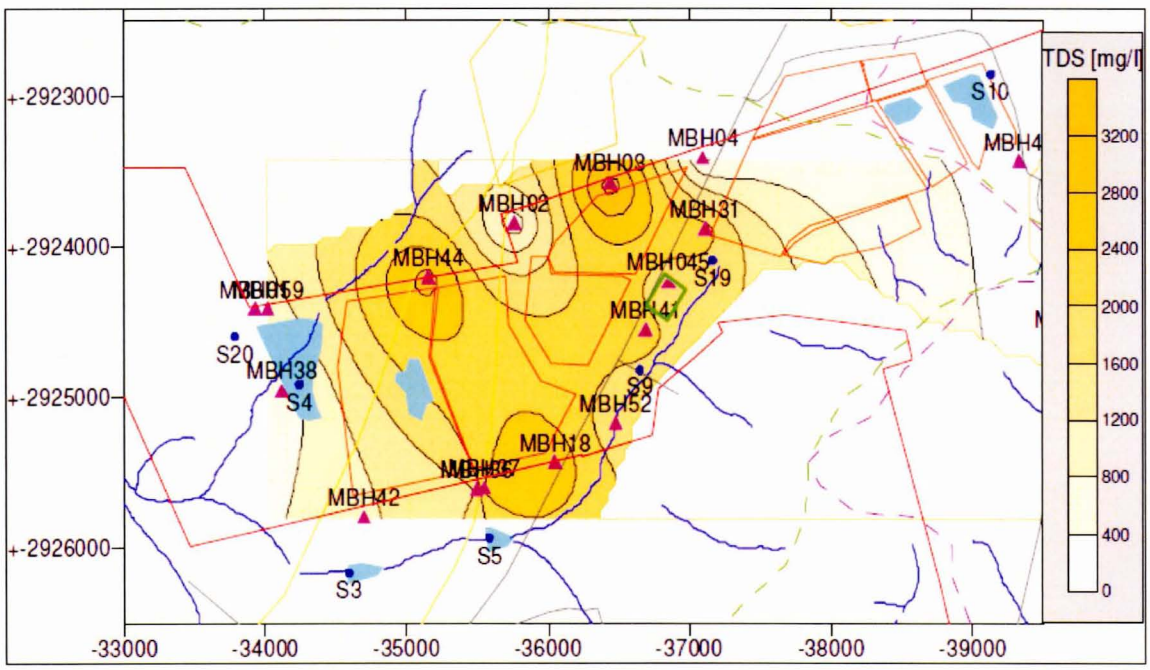

Figure 16: Interpolated groundwater TDS concentrations (WSP, 2009b)

4.2 Study Species

There were at least seven species of *Eucalyptus* grown in the woodlands trials as part of the Mine Woodlands Project (MWP). The exotic species trials were established in 2004. This study focused on three of these species; *E. camaldulensis, E. dunnii* and *E. grandis x camaldulensis hybrid* clone.

E. camaldulensis is also called Red River gum and is a tall-growing perennial, single stemmed tree with an average mature height of 30 m (Bren and Gibbs, 1986), although some authors (e.g. Boland, 1984; Brooker *et al.*, 2002) record trees to 45 m. They often thrive in areas with mean annual rainfall of 1000 mm. According to Hunter (2001), the species cannot adjust easily to prolonged drought. However, it can tolerate reduced periods of low rainfall, inundation and salinity (Van der Moezel *et al.*, 1989). *E. camaldulensis* is a hardy, fast growing gum that is tolerant of salinity, waterlogging, drought and frost, with a range of amenity and wood uses. It commonly grows on riverine sites, whether of permanent or seasonal water (Brooker *et al.*, 2002). This eucalyptus species is the most extensive on grey heavy clay soils along river banks and on floodplains subject to frequent or periodic flooding, preferring deep moist subsoils with clay content (Costermans, 1989). *E. camaldulensis* is a free producer of seeds (Cunningham *et al.*, 1981). According to Jacobs (1955) river red gum could reach ages of 500 to 1000 years.

E. dunnii is commonly known as Dunn's White Gum, Killarney Ash, or White Gum. The species is tall (~50 m) and fast-growing (especially in fertile soils) with rough brown and flaky barks (Trueman *et al.*, 2013). According to Trueman *et al.* (2013), *E. dunnii* is reasonably frost tolerant and adapted to a range of soil types, but prefers moist, deep, fertile and well-drained soils.

E. grandis x camaldulensis is a hybrid obtained by in-vitro rooting of clones of *E. grandis* (Hill ex. Maiden) and *E. camaldulensis* (Dehnh.). The hybrid was developed to combine good growth with drought tolerance traits that both species share.

Eucalyptus camaldulensis	*Eucalyptus dunnii*
	
Eucalyptus grandis x camaldulensis	
	

Figure 17: Typical examples of three *Eucalyptus* species; *E. camaldulensis*, *E. dunnii*, *E. grandis x camaldulensis hybrid*.

4.3 Experimental Design

The study was conducted in three sites. A fourth trial at the VR site could not be included in the experimental design due to destruction by fire of trees resulting in uneven replication. The plan of study looked at the difference between the species and tissues rather than the variation between sites as there were not enough trees harvested to create replicated species within each site. Therefore, the experimental design factorial for the *Eucalyptus* trees at each site is; 3 *Eucalyptus* species x four tissue types (leaf, stem wood, bark, branches/twigs) = 12 samples.

4.4 Sampling procedure

The experiment included three *Eucalyptus* tree species/ hybrids of the same age. Nine trees were destructively sampled in 2012 at three sites as it was important to minimise damage to the plots (i.e. one tree per species per site). The diameter at breast height (dbh) of each tree on each site was recorded so as to select a representative sample for each species (i.e. each site

saw one tree per species felled; one small from site 1, one medium from site 2, one large from site 3). Samples of leaves, stem wood, bark, and twigs/branches from three tree species at each site were harvested within the same week in late-growing season in June 2012. Tree felling was performed and harvesting of leaves, branch/twig, bark and stem wood occurred immediately after felling. A representative sample of approximately 25 leaves (North, West, East and South aspects of the tree) was collected from the top of the canopy. The stem samples were collected in two or three disks per tree and the bark was removed and stored separately to be air dried. The branches/twigs samples were collected for both a branch sample and twig sample (three each). The branches/twigs samples were washed with distilled water and air dried. After the collection of the leaf samples, they were washed with distilled water and freeze dried. Dry sample mass was recorded. These samples were later prepared for the metal analysis.

4.5 Sample preparation for chemical analysis

The samples were collected, cleaned and prepared for analysis according to Tan (2005). The tree samples of wood, bark, leaves and branches/twig were analysed using ICP-OES to determine the concentration of the following elements: Aluminium (Al), Barium (Ba), Calcium (Ca), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Nickel (Ni), Phosphorus (P), Sulphur (S), Strontium (Sr), Titanium (Ti) and Zinc (Zn). Data were expressed in mg/kg.

The metals and metalloids were analysed using ICP-MS for Vanadium (V), Chromium (Cr), Cobalt (Co), Copper (Cu), Arsenic (As), Barium (Ba), Gold (Au), Mercury (Hg), Lead (Pb) and Uranium (U). Data were expressed in μg/kg.

The dried and ground samples were prepared for elemental analysis using the ICP-OES technique (Tan, 2005). Approximately 0.250 ± 0.010 g (weighed mass in Appendix G) of the ground sample was weighed into clean microwave digestion tubes. The samples were microwave digested using a mixture of concentrated hydrogen peroxide (4 ml) and nitric acid (16 ml) at 800 W for about 30 min. Blanks and certified reference materials were prepared using the same procedures. The samples were cooled to room temperature and transferred to 50 ml polyurethane volumetric flask where they were diluted to the mark using de-ionized water (Millipore, USA). The diluted samples were then kept in a fridge until analysis. The concentrations of elements in the samples were determined on an ICP- OES instrument (Spectro Genesis, Germany).

Elements, which read over their calibration limits, were serially diluted to read within the limits by 10x, 20x, 50x and 100x. The CHNS 932 Leco elemental analyser was used to

analyse the ground samples for the light elements C and N (LECO Instruments, St. Joseph, USA). The instrument was calibrated by analysing a series of blanks prior to analysing the calibrating standards. The calibrating standards used were protein standards. Approximately 0.2g of the ground sample was encapsulated within a tin capsule for the analyses of C, N and ash. The samples were combusted at temperatures between 950° - 1000° C. Data were expressed in % dry mass.

The ash content was determined by using the TAPPI standard T 211 om-85 (Munalula and Meincken, 2009). Wood samples were weighed before they were placed in a furnace at 575^0C for 3 hours. Data were expressed in % dry mass.

4.6 Risk assessment methodology

The risk assessment was designed using the USEPA (1991) methodology:

- Hazard was measured from metals and metalloids in the tree biomass;
- Exposure was determined from literature.

The exposure was calculated by using transfer factors from literature to estimate the amount of metals or metalloids that could be accumulated by various vegetables planted in soil fertilised with contaminated ash. The calculation was:

Concentration of metal/loids in vegetables = transfer factor x concentration found in *Eucalyptus* plants (this is assuming the concentration of metal/loids was still the same for ash as that found in *Eucalyptus* plants)

Then daily intake of metal/loids through consumption of vegetables = daily vegetable consumption x concentration of mean vegetable metal concentration (Jolly *et al.*, 2013).

4.7 Calculations

The concentration of metals from the original dry tissue (leaf, branch/twig, stem wood, bark) was calculated as follows,

Dilution factor (DF) = Total volume/Total mass digested and analysed

Metal concentration = DF x Metal reading from ICP-OES or ICP-MS x further dilutions = mg/kg (Dennis, 2008).

To convert µg/kg to mg/kg, data was divided by 1000.

The final data was expressed in mg/kg (ppm) or µg/kg (ppb) dry mass of tissue. For graphs, all data was presented in mg/kg.

To calculate whole plot biomass, the Schumacher and Hall equation, published in the South African Forestry Handbook, 2000 by Bredenkamp, was used to estimate the stem volume (Equation 1).

$$lnV = b_0 + b_1 \ln(dbh + f) + b_2 \ln H \qquad \textit{(Equation 1)}$$

where: *ln* = natural logarithm to the base e

V = stem volume (m^3 under bark), usually to 75mm tip diameter

dbh = breast height diameter (cm – over bark)

f = correction factor

H = tree height (m)

b_0, b_1, b_2 = coefficients (**Table 8**)

The volume was calculated by using the coefficients as indicated in **Table 8**.

Table 8: Coefficients for the estimation of standing tree volume using the Schumacher and Hall model (Bredenkamp, 2000).

Species	b_0	b_1	***F***	b_2	**Author of original equation**
E. grandis x camaldulensis	-10.6435	1.9185	0	1.1494	Du Plessis, 1996

The volume for *E. camaldulensis* and *E. dunni* was estimated by using the *E. grandis* generic equation 2 by Schumacher and Hall (1933).

$$\log volume = -4.584 + \log dbh + \log ht \qquad \textit{(Equation 2)}$$

To calculate the dry mass of trees harvested, the standard industry conversion factors for volume to dry mass (Other *Eucalyptus* species) as detailed by Bredenkamp (2000) were used and are presented in **Table 9** below.

Table 9: Standard industry conversion factors for roundwood

Species	tonne/m^3	m^3/tonne
Eucalyptus grandis	0.68	1.47
Other *Eucalyptus* species	0.83	1.25

4.8 Data Analysis/ Statistics

In this chapter the statistical methods used in the data analysis are described.

The mean, standard deviation, number of samples (n), standard error, and 95% confidence interval formed part of the descriptive statistics which were calculated using Microsoft® Excel Data Analysis. Data was checked for normality using MiniTab® 17.1.0 software. Where normal distribution of all data was observed, wood, bark, branch/twig and leaf, and chemical data of the three *Eucalyptus* plants growing in three different sites was analysed using the Tukey's Honest Significant Difference (HSD) to statistically conduct the mean separation test. It was used in conjunction with a one way ANOVA to find means that are significantly different from each other.

The hypothesis set up was:

Null hypothesis:	All means are equal (H_0: $v_1=v_2=v_3$)
Alternative hypothesis:	At least one mean is different (H_1: $v_1\neq v_2$/ $v_2\neq v_3$/ $v_1\neq v_3$)
Significance level:	$\alpha = 0.05$

The experimental design used one tree species per site (three different sites) therefore a paired t-test was not possible in the statistical analysis. The metal/loids and nutrients found in highest concentrations in wood were subjected to the Tukey HSD test; i.e. Ba, As measured by ICP-MS and Ca measured by ICP-OES.

The ash content was determined by using the TAPPI standard T 211 om-85. The mean, standard deviation, number of samples (n), standard error, 95% confidence interval formed part of the descriptive statistics which were calculated using Microsoft® Excel Data Analysis.

The mean concentration of potentially harmful metals in wood of the different *Eucalyptus* species was compared to the MPLs in plants. A risk assessment was then conducted using the methodology in section 4.6.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 Results

The measured results were checked against the CRM's known concentrations. For ICP-MS, results with an agreement of 80-120% were accepted (Downer, 2008). For ICP-OES, results with an agreement of 90-110% were accepted (Downer, 2008). Only those measurements that fall within this acceptable range were discussed. The results of elements without a certified reference value for Orchards Leaves (1571) were not discussed. The calculations of CRM agreements are indicated in **Table 10** below.

Table 10: CRM agreements to measured values

Element	Measured value	Certified Value	% agreement
ICP-MS results in µg/kg			
V	432.9±17.2	No reference value	NA
Cr	14969±14.57	2300 ±300	No agreement
Co	190.1±5.25	2000*	10%
Cu	9283±203.8	12000±1000	77%
As	9936±872.3	10000±2000	99%
Ba	41737±2130.9	44000*	95%
Au	10±4.78	No reference value	NA
Hg	109.9±5.6	155±15	71%
Pb	26583±340.9	45000±3000	59%
U	7.25±0.7	29±5	No agreement
ICP-OES results in mg/kg			
Al	309.33±17.66	No reference value	NA
Ba	59.03±4.21	44*	75%
Ca	21980.61±484.97	20900±300	95%
Fe	332.55±9.02	300 ± 20	90%
K	7327.90±128.93	14700±300	50%
Mg	7311.29±215.92	6200±200	85%
Mn	89.00±2.24	91 ± 4	98%
Na	529.54±103.19	82 ± 6	15%
Ni	20.25±0.04	1.3 ± 0.2	6%

Element	Measured value	Certified Value	% agreement
P	7741.47±553.07	2100±100	27%
S	1962.54±415.37	1900*	97%
Sr	32.70±0.35	37 ± 1	88%
Ti	14.58±0.22	No reference value	NA
Zn	49.12±1.96	25 ± 3	51%

*Published non certified values

This section specifically reports the concentration of metal/loids in wood of *Eucalyptus* tree species as wood is the tissue that is primarily used for fuel. The comparison of the concentration of metal/loids in other plant tissues (leaves, branches /twigs, bark) was also done. The elements were grouped and are presented as follows:

- Nutrients: nitrogen (N), phosphorus (P), potassium (K); calcium (Ca), sulphur (S), magnesium (Mg);
- Metals and metalloids: manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), nickel (Ni) cobalt (Co), chromium (Cr), lead (Pb), arsenic (As), barium(Ba), mercury (Hg);
- Carbon and Ash content.

The metals which reported the highest concentrations in wood were subjected to the Tukey HSD test; i.e. As measured by ICP-MS and Ca measured by ICP-OES. The results (**Table 11**) show that there was no significant difference noted between the concentrations of As and Ca in three different *Eucalyptus* species.

Table 11: Statistical comparison of concentrations of metals in three *Eucalyptus* species Tukey HSD, The hypothesis where H_0: $v_1=v_2=v_3$and H_1: $v_1\neq v_2$/ $v_2\neq v_3$/ $v_1\neq v_3$.

Element	F- value	P-value	Tukey HSD Test
As	2.40	0.171	$v_1=v_2=v_3$; no significant difference noted between the concentration of arsenic in 3 *Eucalyptus* species
Ca	2.10	0.204	$v_1=v_2=v_3$; no significant difference noted between the concentration of calcium in 3 *Eucalyptus* species

5.1.1 Nutrients

Only the measured result of Ca and S had an agreement of above 90% when compared to the CRM. All other nutrients were either below or above the 90%-110% acceptable range.

The mean concentration ± SD of Ca and S in wood dry mass of the *Eucalyptus* species are presented graphically in **Figure 18**. Grouping information using the Tukey Method and 95% Confidence Interval showed that the means were not significantly different between the three species for calcium and sulphur but were significantly different for magnesium ((*E. dunnii* ≠ (*E. cam* = *E. gxc*)).

The mean concentration ± SD of Ca and S dry mass in leaves, branches/twigs, bark and wood of the *Eucalyptus* species are presented graphically in **Figure 19** and in Appendix E.

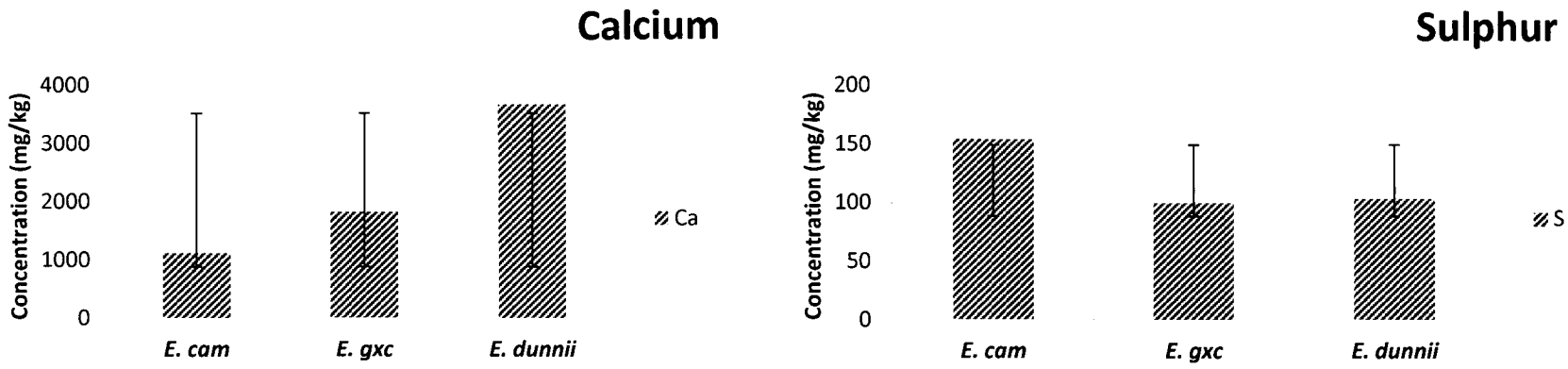

Figure 18: Comparison of nutrients Ca (left) and S (right) concentration in wood averaged across all three *Eucalyptus* species.

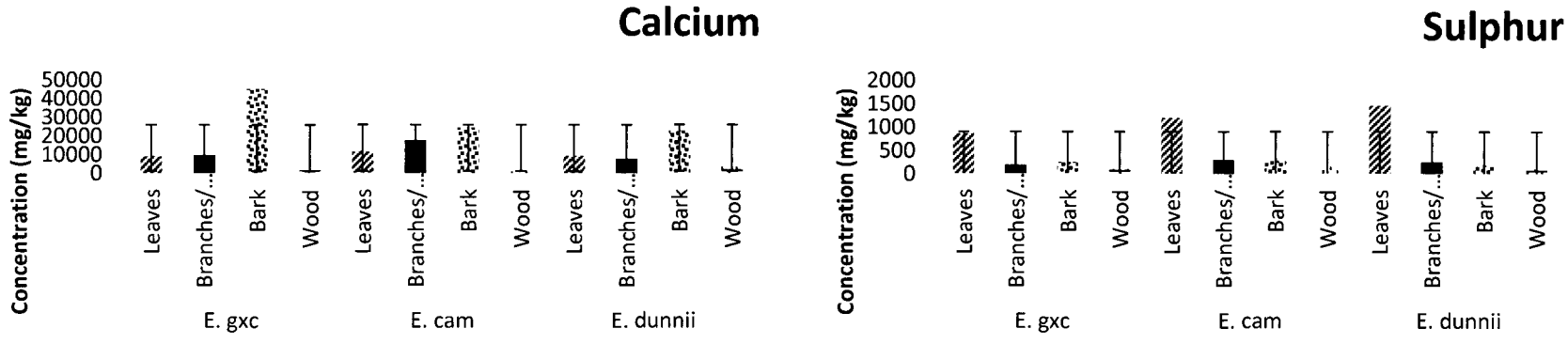

Figure 19: Comparison of nutrients Ca (left) and S (right) concentration in all tissues averaged across all three *Eucalyptus* species.

5.1.2 Metals and Metalloids

Only the results of Mn, Fe, As and Ba had an agreement of above 80% when compared to the CRM. These elements are the only ones discussed. The mean concentration ± standard deviation (SD) of Mn, Fe, As and Ba in wood dry mass of the *Eucalyptus* species are presented graphically in **Figure 20**. Grouping information using the Tukey Method and 95% Confidence Interval showed that the means were not significantly different between the three species for Mn, Fe, As and Ba.

The mean concentration ± SD of Mn, Fe, As and Ba dry mass in leaves, branches/twigs, bark and wood of the *Eucalyptus* species are presented graphically in **Figure 21** and in Appendix E.

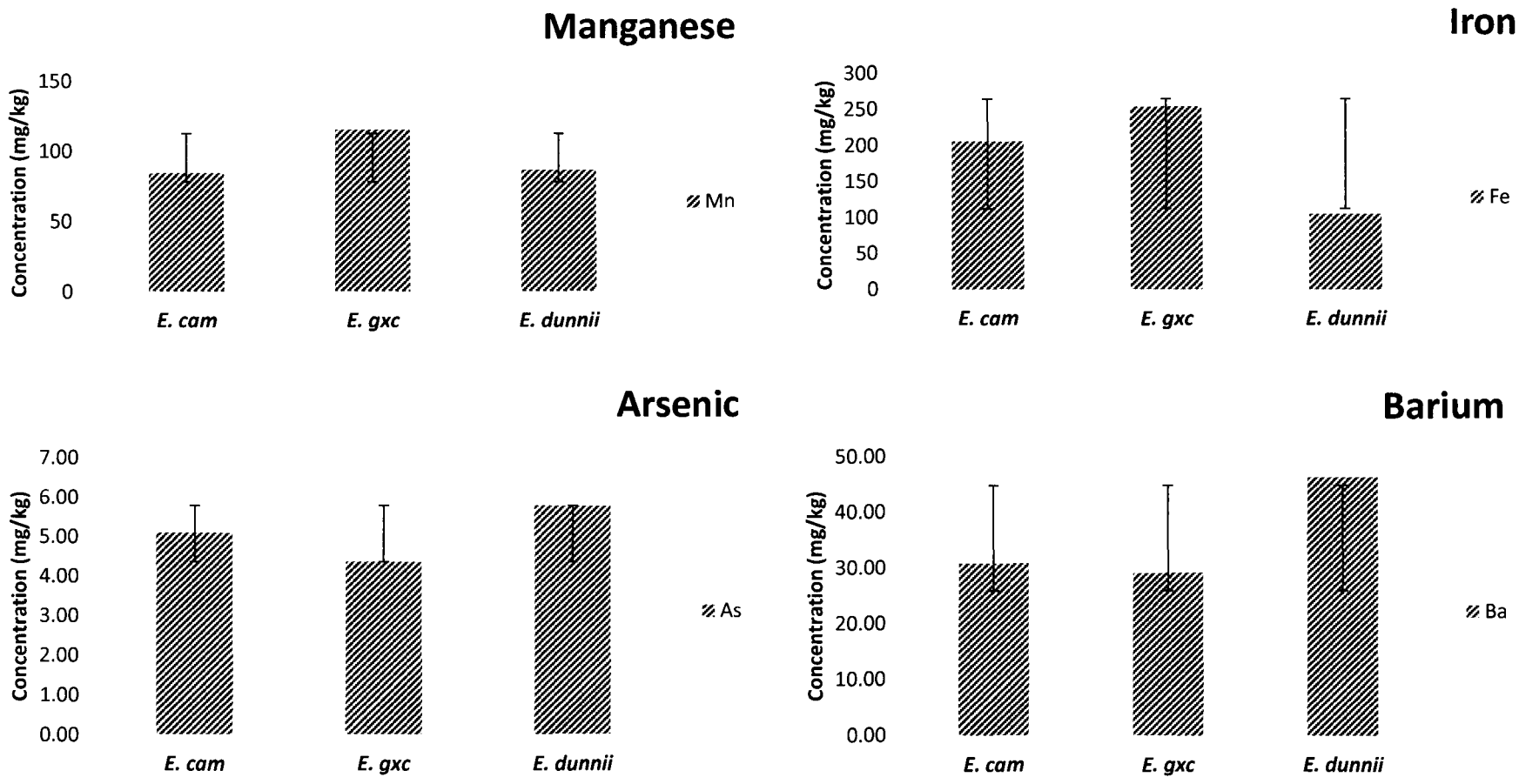

Figure 20: Comparison of metal/loids Mn (top left), Fe (top right), Ba (bottom left) and As (bottom right) concentration in wood across *Eucalyptus* species.

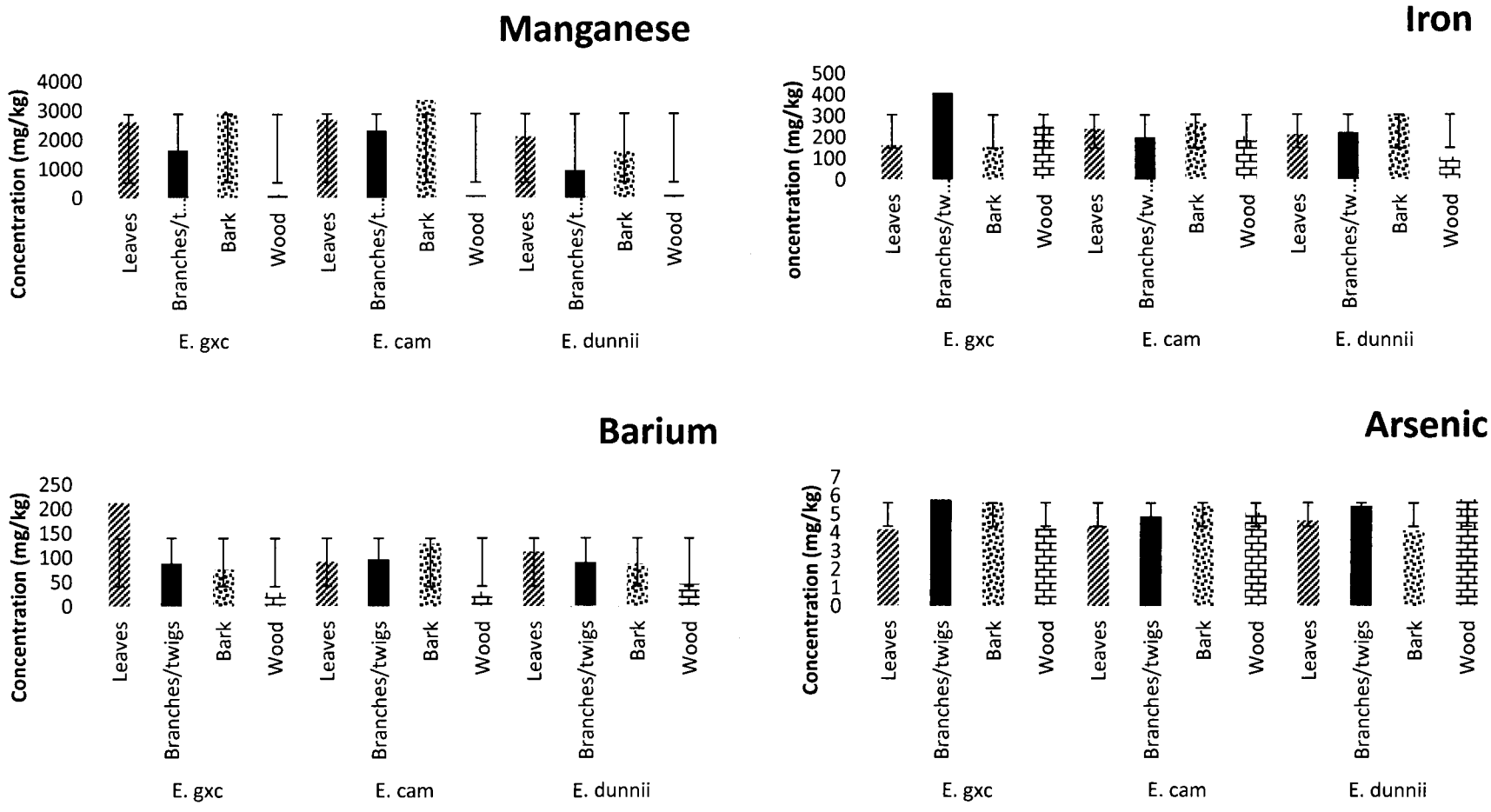

Figure 21: Comparison of metal/loids Mg (top left), Fe (top right), Ba (bottom left) and As (bottom right) concentration in all tissues averaged across all three *Eucalyptus* species.

5.1.3 Carbon and Ash content

There was no CRM value for Carbon and Ash content, therefore this data was not discussed.

5.1.4 Amount of nutrients, metals and metalloids per tree

The concentration of nutrients, metals and metalloids was then scaled up to the whole plot so that the amount of nutrients, metals and metalloids per plot could be estimated. The allometric algorithms as indicated in section 4.7 were used to calculate volumes and mass of wood and content of nutrients, metals and metalloids as indicated in **Table 12** and average content per site indicated in **Table 13** below.

Table 12: Calculated wood volume, wood mass and content of nutrient, metal or metalloid per tree

Tree species	Site	Height (cm)	dbh (cm)	Volume (m3)	Mass (ton)	Mass (kg)	Ca (mg)	S (mg)	Mn (mg)	Fe (mg)	Ba (mg)	As (mg)
E. cam	Mispah	1 013	16.3	0.004	0.004	3.57	4 735.21	697.82	524.43	890.08	125.40	16.63
	Madala	1 790	26.2	0.012	0.010	10.14	13 313.44	1 403.23	7 340.11	1 790.99	348.76	59.32
	Red soil	1 040	9.6	0.003	0.002	2.16	1 543.53	273.73	181.99	410.82	49.71	10.28
E. gxc	Mispah	1 388.2	10.6	0.045	0.038	37.70	74 060.68	3 334.61	5 428.61	3 000.02	699.65	134.57
	Madala	1 500	12.8	0.071	0.059	59.24	52 664.21	5 331.51	4 473.50	6 455.80	2 032.32	291.02
	Red soil	1 830	20.8	0.228	0.189	188.99	493 409.99	22 604.40	36 906.69	108493.59	6 550.70	866.33
E. dunnii	Mispah	2 430	28.6	0.018	0.015	15.03	36 007.58	1 663.85	24 155.08	1 192.75	760.74	97.49
	Madala	1 648	19.9	0.009	0.007	7.09	14 108.58	711.80	11 473.85	650.89	455.26	43.94
	Red soil	1 170	11.1	0.003	0.003	2.81	18 574.79	272.73	3 591.50	403.76	67.70	13.07

Table 13: Average content per site (species combined)

	Ca (mg)	S (mg)	Mn (mg)	Fe (mg)	Ba (mg)	As (mg)
Mispah	38 267.82	1 898.76	10 036.04	1 694.28	528.60	82.90
Madala	26 695.41	2 482.18	7 762.49	2 965.89	945.45	131.43
Red soil	171 176.11	7 716.96	13 560.06	36 436.06	2 222.70	296.56

5.2 Discussion

5.2.1 Nutrients

The calcium concentrations in wood were 1 117.62, 1 821.42 and 3 665.36 mg/kg for *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. The normal concentration range of calcium in plants according to **Table 3** is 1 830.2 to 2 042.5 mg/kg. The concentration of calcium in *E. dunnii* was above this range. The accumulation of calcium in *Eucalyptus* tissues showed the following trend: Bark>branches/twigs>leaves>wood.

In a study conducted on *E. camaldulensis* irrigated with municipal effluent, calcium was recorded as ranging between 11 090-19 950 mg/kg (Singh *et al.*, 2010). This was mainly due to the background Ca soil concentrations in the Singh (2010) study which were on average 12 175 mg/kg. In India, a study of *E. tereticornis* grown in bauxite mining soils showed an increase in Ca uptake (3 480 mg/kg) in the shoot when the soil was inoculated with mycorrhizal bacteria (Khosla and Sudhakara Reddy, 2008). Two of the studied species (*E. cam* and *E. gxc*) were below the findings of these previous studies and one species (*E. dunnii*) was above the normal range of calcium in plants. This study recorded a range of 22 841 to 45 332 mg/kg for stem bark. This might lead to calcium depletion as seen in other *Eucalyptus* stand studies (Spangenberg *et al.*, 1996) in soils as the bark gets removed from site.

Concentrations of sulphur in wood ranged from 99 to 153 mg/kg. The sulphur concentrations were 153.48, 99.35 and 102.70 mg/kg for *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. The accumulation of sulphur in *Eucalyptus* tissues showed the following trend: Leaves>branches/twigs>bark>wood.

The sulphur concentrations recorded for the leaves in this study ranged from 865.15 to 1465.50 mg/kg. In a study conducted in Australia on *E. cladocalyx* seedlings grown on gold tailings, the accumulation of sulphur in leaves was approximately 4 000 mg/kg (Madejón *et al.*, 2012). This is four times more than what was recorded in this study and it could be attributed to the addition of biosolids to the tailings substrate which had background sulphur concentrations of 22 600 mg/kg (Madejón *et al.*, 2012). The sulphur concentrations of the wood can therefore be assumed to be safe for health as they fall below the normal range concentration in plants of 1 000 mg/kg as indicated in **Table 3**.

5.2.2 Metals and Metalloids

Manganese concentration in wood ranged from 84 to 115 mg/kg. The manganese concentrations were 84.35, 115.20 and 86.73 mg/kg for *E. camaldulensis, E. grandis x*

camaldulensis and *E. dunnii* respectively. The accumulation of manganese in *Eucalyptus* tissues showed the following trend: Bark>leaves>branches/twigs>>wood.

The critical concentration levels for manganese in plants ranges from 300 to 500 mg/kg (Kabata-Pendias and Pendias, 1992; Khan *et al.*, 2008). The levels in wood were much lower than this limit although the concentration found in bark, leaves and branches/twigs samples was above this level. In a study conducted on *E. camaldulensis* irrigated with municipal effluent, manganese was recorded as ranging between 134 to 458 mg/kg (Singh *et al.*, 2010). The normal concentration ranges for manganese are 15 to 100 mg/kg. The concentration of manganese that was reported for leaves was however much higher than this and was averaged at 2 475 mg/kg. Excessive levels of manganese can cause damage to the brain, liver, kidneys, the developing foetus etc. (Khan *et al.*, 2008). Care must be taken to ensure these leaves are not supplied to third parties and are disposed of properly.

Iron concentrations in wood were 205.32, 254.21 and 104.94 mg/kg for *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. The accumulation of iron in *Eucalyptus* tissues showed the following trend: Bark>leaves>branches/twigs>>wood.

According to Table 4, the normal concentration ranges for iron in plants are between 640 to 2 486 mg/kg. The wood concentrations are within this range. All the tissue samples also fell within this range. In a study conducted on *E. camaldulensis* irrigated with municipal effluent, iron was recorded as ranging between 570-959 mg/kg (Singh *et al.*, 2010).

In the wood of the *Eucalyptus* trees sampled in this study, the concentration of arsenic was between 4.36 and 5.78 mg/kg. The *Eucalyptus* tree species planted in this study accumulated almost the same amount of arsenic in the wood. Arsenic concentrations of 5.09, 4.36 and 5.78 mg/kg were accumulated in the wood of *E. camaldulensis, E. grandis* x *camaldulensis* and *E. dunnii* respectively. These arsenic levels were considerably higher than the 1 mg/kg maximum permissible limit (Rauf *et al.*, 2011). *E. camaldulensis* and *E. dunnii* were observed to have concentrations above the normal concentration range of arsenic in plants which is 5 mg/kg. The accumulation of arsenic in *Eucalyptus* tissues showed the following trend: Branches/twigs>wood>bark>leaves.

In a case study conducted in Australia on a gold mine tailings facility, it was found that the concentration of arsenic in various types of *Eucalyptus* species was 0.29-5.14 µg/g in mature leaves and 0.11-0.61 µg/g in the stem (King *et al.*, 2008). This was significantly much lower than what was recorded in this study. In another study on plants growing on mine waste in south west England, arsenic ranged from 350 to 2 040 mg/kg (Porter and Peterson, 1975).

Both these studies did not report similar results as were found in this study and this could be due to soil chemistry.

The highest concentration of barium in wood was measured for *E. dunnii* at 46.29 mg/kg. The highest recorded barium concentration in bitter orange tree leaves in a study conducted in Spain was 100 mg/kg (Oliva *et al.*, 2007). Barium concentration in most plants ranges from 4-50 mg/kg (Chaudhry *et al.*, 1977). The concentration measured in this study is lower than both these literature sources.

5.2.3 Amount of nutrients, metals and metalloids per tree

The amount of fuelwood used per household in a study conducted in Bushbuckridge in Mpumalanga was recorded as 3 285 kg/annum (Matsika *et al.*, 2013). This could be estimated at 273.75 kg/household/month (assuming constant usage throughout the year, which might not be the case). This can be further broken down to 9.13 kg/household/day. If contaminated *Eucalyptus* wood is used in this household, the result would be as indicated in **Table 14.**

Table 14: Average content of nutrients, metals and metalloids in *Eucalyptus* species consumed and available in ash per household per day (assuming no volatilisation during combustion)

	Wood Mass (kg)	**Ca (mg)**	**S (mg)**	**Mn (mg)**	**Fe (mg)**	**Ba (mg)**	**As (mg)**
E. camaldulensis	9.13	10 203.83	1 401.30	770.09	1 874.61	281.53	46.45
E. gxc	9.13	16 629.55	1 057.18	347.17	1 158.68	441.07	57.13
E. dunnii	9.13	33 464.77	907.07	1 051.76	2 320.93	266.37	39.76

5.2.4 Comparison to maximum permissible levels

It can be seen from **Figure 22** that arsenic was above four times the WHO permissible levels for plants for all three *Eucalyptus* species. Care must thus be taken when harvesting these trees and selling them as fuelwood.

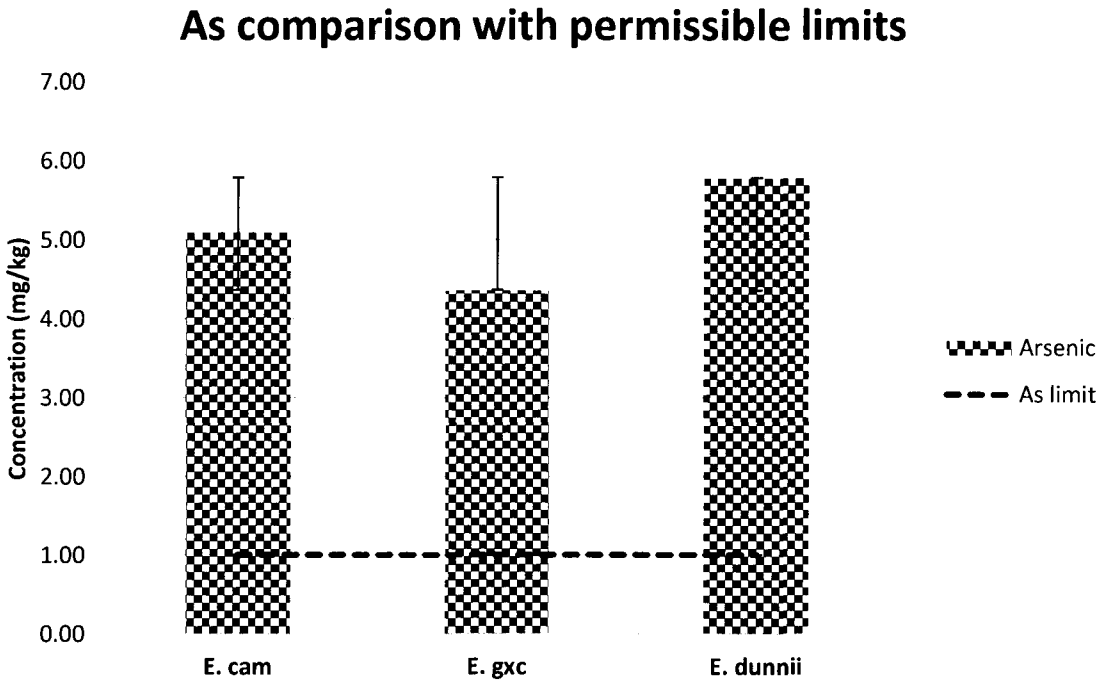

Figure 22: Comparison of metal/loids to the WHO maximum permissible levels

5.2.5 Risk Assessment

The methodology for the risk assessment was highlighted in chapter 4. The hazard as determined in the results chapter is indicated in **Table 15** below. The exposure was determined by assuming that the pathway of these metals and metalloids is through human consumption of vegetables grown in gardens fertilised with ashes from the *Eucalyptus* wood grown in the AMD contaminated sites. The exposure was then calculated and estimated by using literature. According to Madubansi and Shackleton (2005), the average amount of collected wood in 2002 in different settlements in Limpopo was 44.9 kg/person/month (or 1.50 kg/person/day). Assuming the ash content is 2.38% (Munalula and Meincken, 2009), the ash produced from burning this wood would be 1.069 kg/person/month. A transfer factor of metals from soil to plants was adapted from Jolly *et al.* (2013) and is presented in **Table 15** below for the common vegetables. It only covers Mn, Fe and As (there was no reference for Ba found). The estimated averages of metal/loids found in typical garden vegetables are indicated in **Table 16** below.

Table 15: Transfer Factor from soil to vegetables (Jolly *et al.*, 2013)

Elements	Transfer Factor					
	Spinach	Tomato	Radish	Bean	Cauliflower	Carrot
Mn	-	-	-	0.038	-	-
Fe	0.006	0.001	0.001	0.003	0.002	0.002
As	-	0.001	0.001	0.001	0.001	0.001

Table 16: Average concentration of metal and metalloids in typical vegetables found in gardens

Species	Concentration of metal/loids in vegetables						
	Spinach	Tomato	Radish	Bean	Cauliflower	Carrot	Average
Mn							
E. camaldulensis	-	-	-	3.21	-	-	**0.53**
E. gxc	0.69	0.12	0.12	0.35	0.23	0.23	**0.29**
E. dunnii	-	0.09	0.09	0.09	0.09	0.09	**0.07**
Fe							
E. camaldulensis	-	-	-	7.80	-	-	**1.30**
E. gxc	1.53	0.25	0.10	0.76	0.51	0.51	**0.61**
E. dunnii	-	0.10	0.10	0.10	0.10	0.10	**0.09**
As							
E. camaldulensis	-	-	-	0.19	-	-	**0.03**
E. gxc	0.03	0.00	0.00	0.01	0.01	0.01	**0.01**
E. dunnii	-	0.01	0.01	0.01	0.01	0.01	**0.00**

WHO (1998) guidelines specify that the required vegetable daily intake for a human diet is 300 g/person. Assuming an average person of 70kg, then the daily intake is as presented in **Table 17.**

Table 17: Daily intake of metal/metalloids per *Eucalyptus* species

Species	Conc. in wood (mg/kg)	Conc. in vegetables (mg/kg)	Daily intake of metal/loids (mg/d)	Oral reference Dose (mg/d)	Reference for oral reference dose
Mn					
E. camaldulensis	84.35	0.53	0.16	0.5-5.0	WHO, 1993
E. gxc	115.20	0.29	0.09	0.5-5.0	WHO, 1993
E. dunnii	86.73	0.07	0.02	0.5-5.0	WHO, 1993
Fe					
E. camaldulensis	205.32	1.30	0.39	10.0-60.0	WHO, 1993
E. gxc	254.21	0.61	0.18	10.0-60.0	WHO, 1993
E. dunnii	104.94	0.09	0.03	10.0-60.0	WHO, 1993
As					
E. camaldulensis	5.09	0.03	0.01	0.05	WHO, 1966
E. gxc	4.36	0.01	0.00	0.05	WHO, 1966
E. dunnii	5.78	0.00	0.00	0.05	WHO, 1966

To complete the risk assessment, I have rated both the hazard and the exposure as follows:

- For hazards: 1 for least concentration of metal/loid in wood and 3 for most concentration of metal/loid in wood;
- For exposure in Mn: 10 for ≤0.5; 20 for 0.5-2.5; 30 for 2.5-5 and 40 for >5 with reference to the oral reference dose;
- For exposure in Fe: 10 for ≤10; 20 for 10-30; 30 for 30-60 and 40 for >60 with reference to the oral reference dose;
- For exposure in As: 10 for <0.05; 20 for 0.5; 30 for 0.5-1.0 and 40 for >1 with reference to the oral reference dose.

The following equation was used to calculate the risk. The risk assessment is indicated in **Table 18** below.

Risk = Hazard x Exposure (USEPA, 1991).

Table 18: Risk Assessment

Species	Hazard	Exposure	Risk
Mn			
E. camaldulensis	1	10	10
E. gxc	3	10	30
E. dunnii	2	10	20
Fe			
E. camaldulensis	2	10	20
E. gxc	3	10	30
E. dunnii	1	10	10
As			
E. camaldulensis	2	10	20
E. gxc	1	10	10
E. dunnii	3	10	30
Final Risk Assessment Score		***E. camaldulensis***	**50**
		E. gxc	**70**
		E. dunnii	**60**

From the risk assessment done above, there was no evidence that there will be adverse effects caused by supplying fuelwood from these contaminated *Eucalyptus* trees. Even though high arsenic concentrations were recorded in this study, if the wood is used as fertilizer in a vegetable bed, the transfer of the arsenic to the common vegetables is below the daily oral reference dose. All the calculated results for the daily intake of Mn, Fe and As fall below the oral reference dose recommended. This, however, does not rule out harm that might be caused by other metals or metalloids that were not assessed in this section due to the results that were not in agreement with the CRMs. It is suspected that the CRM used was old.

CONCLUSION AND RECOMMENDATIONS

The results of this study highlight the importance of evaluating the environmental impact that can be caused by selling or supplying contaminated wood to the public. It also highlights the different health impacts that can be realised should the ash from the wood be directly or indirectly ingested by the public over a long period of time. While the nutrients accumulated varied, the metal/loids accumulated were almost the same for all the *Eucalyptus* species studied. Concern was raised in regards to the exceeded permissible levels in plants for arsenic for the *Eucalyptus* species studied. Arsenic concentrations of 5.09, 4.36 and 5.48 mg/kg were accumulated in the wood of *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. The concentrations exceeded the WHO MPL of 1 mg/kg for both chromium and arsenic. Arsenic has also been found to volatilise at temperatures below 400°C in hyperaccumulators such as *Pteris vittata* containing high As content (1 170 mg/kg). This causes a threat to human health as As is a Group 1 carcinogen associated with cancers of the lung, bladder, kidney, skin, liver and prostate.

Arsenic has been known to cause a lot of ailments following prolonged exposure. The use of ash resulting from burning this *Eucalyptus* wood for healing purposes or as a fertilizer for a long duration, might result in health impacts to end users. Two species had arsenic levels above the normal concentration range in plants of 0.02 to 5 mg/kg i.e. *E. camaldulensis* and *E. dunnii.* All the species however were above the WHO MPL in plants of 1 mg/kg which seems to suggest that all plant species concentration are unsafe. However, when this arsenic is transferred as ash to vegetables, it was found to be below the daily oral reference dose. Taking into account all the determining properties, it was seen from the risk assessment that the *E. camaldulensis* species was the preferred fuelwood followed by *E. dunnii.* To answer the research objectives;

- The accumulation of metals varies with plant tissues in *Eucalyptus*, with leaves accumulating the majority of metals;
- There was variation in metals accumulated by the different *Eucalyptus* species, with *E. camaldulensis* hybrid accumulating the least amount of harmful metals;
- Arsenic was above the WHO permissible levels in plants for all three *Eucalyptus* species;
- A risk assessment performed found that there was no evidence that there will be adverse effects caused by supplying fuelwood from these contaminated *Eucalyptus* trees. Even though high arsenic concentrations were recorded in this study, if the

wood is used as fertilizer in a vegetable bed, the transfer of the arsenic to the common vegetables is below the recommended daily intake;

- The best option for fuelwood was the *E. camaldulensis* hybrid clone, although there is a concern about other metal/loids that were not evaluated due to non-agreement with CRM.

A considerable amount of metal/loids was accumulated in the leaves. Normally, the stem is the most frequently used part of the tree to fuel fires. A consideration on what will happen to the leaves if they are not combusted will also have to be investigated. The major question raised by this study is how the environmental and health impacts will be managed when selling/supplying this contaminated wood to the public. The other major concern is if arsenic in the wood of these *Eucalyptus* tissues volatilises, is it at all safe to supply this wood to the community. The crucial task will be to ensure that the contamination is reduced before the wood is supplied or to ensure that the ash is treated accordingly. As a future study it would be interesting to also see the relationship between the metal/loids in the soil and those accumulated by the trees. It would also be interesting to check if there is variability between the sites.

REFERENCES

Abbasi, T., and Abbasi, S.A. 2010. Biomass Energy and the Environmental Impacts Associated with its Production and Utilization, Renewable and Sustainable Energy Reviews, 14:919–937.

Aber, J., Nadelhoffer, K., Steudler, P. and Melillo, J. 1989. Nitrogen Saturation in Northern Forest Ecosystems. Bioscience, 39: 378–386.

Aber, J.D., Magill, A., McNulty, S.G., Boone, R.D., Nadelhoffer, K.J., Downs, M., Hallett, R. 1995. Forest Biogeochemistry and Primary Production Altered by Nitrogen Saturation. Water Air and Soil Pollution 85: 1665-1670.

Abo-Hassan, A., Kandeel, S. A. and Kherallah, I. E. 1988. New Eucalypt Species Introduction in the Saudi Arabia Central Zone. In Proceedings of the International Forestry Conference, Australian Bicentenary, 5: 1–16.

Ahmad, S.A. 2000. Arsenic: Water Contamination and Health Hazards, 1st edition. Udayan Press, Rajshahi, Bangladesh, p. 48.

Akcil, A., and Koldas, S. 2006. Acid Mine Drainage (AMD): Causes, Treatment and Case Studies, Journal of Cleaner Production, 14: 1139–1145.

Álvarez-Ayuso E., Otones, V., Murciego A., García-Sánchez A. and Santa Regina I., 2012. Antimony, Arsenic and Lead Distribution in Soils and Plants of an Agricultural Area Impacted by Former Mining Activities, Science of the Total Environment 439: 35-43.

AngloGold Ashanti Report to Society. 2004. Environment, South Africa, 7.11. www.anglogoldashanti.com (Access on 2 April 2013).

AngloGold Ashanti Case Study. 2008. Growing Vegetation to Reduce Environmental Damage, South Africa, www.anglogoldashanti.com (Access on 2 April 2013).

AngloGold Ashanti Sustainability Review. 2010. South Africa, www.anglogoldashanti.com (Access on 2 April 2013).

Aronsson, P. and K. Perttu (Eds.). 1994. Willow Vegetation Filters for Municipal Wastewaters and Sludges, A Biological Purification System, Swedish University of Agricultural Sciences, Uppsala, p. 230.

Assareh, M.H., Shariat, A. and Ghamari-Zare, A. 2008. Seedling Response of Three *Eucalyptus* Species to Copper and Zinc Toxic Concentrations, Caspian Journal of Environmental Science, 6 (2): 97–103.

Ayepola, O.O. and Adeniyi, B.A. 2008. The Antibacterial Activity of Leaf Extracts of *Eucalyptus camaldulensis* (Myrtaceae), Journal of Applied Sciences Research 4, 1410-1413.

Babayi, H., Kolo, I., Okogun, J.I. and Ijah, U.J.J. 2004. The Antimicrobial Activities of Methanolic Extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* Against Some Pathogenic Microorganisms, Biokemistri 16, 106-111.

Baker, A.J.M., McGrath, S.P., Sidoli, C.M.D. and Reeves, R.D. 1994. The Possibility of In Situ Heavy Metal Decontamination of Polluted Soils Using Crops of Metal-Accumulating Plants, Resources, Conservation and Recycling, 11(1):41–49.

Balatinecz, J.J. and Kretschmann, D.E. 2001. Properties and Utilization of Poplar Wood, NRC, Research Press National Research, Council of Canada, On KIA OR6, Canada, pp. 277-291.

Becquer, T., Quantin, C., Sicot, M. and Boudot, J.P. 2003. Chromium Availability in Ultramafic Soils from New Caledonia, The Science of the Total Environment 301: 251–261.

Begonia, G.B.; Davis, C.D.; Begonia, M.F.T. and Gray, C.N. 1998. Growth Responses of Indian Mustard [Brassica juncea (L.) Czern.] and its Phytoextraction of Lead from a Contaminated Soil, Bulletin of Environmental Contamination and Toxicology, vol. 61, pp. 38-43.

Bennett, D. 2003. Combustion economics, Short Rotation Crops for Bioenergy: New Zealand, Report, pp. 185- 190.

Benstead. P. 2003. Bioenergy in New Zealand- The way forward, Short Rotation Crops for Bioenergy: New Zealand, Report, pp. 33–38.

Bhati, M. and Singh, G. 2003. Growth and Mineral Accumulation in *Eucalyptus camaldulensis* Seedlings Irrigated with Mixed Industrial Effluents, Bioresources Technology 88, 221-228.

Blaylock, M.J.; Salt, D.E.; Dushenkov, S.; Zakharova, O.; Gussman, C.; Kapulnik, Y.; Ensley, B.D. and Raskin. I. 1997. Enhanced Accumulation of Pb in Indian Mustard by Soil-Applied Chelating Agents. Environmental Science and Technology, vol. 31, no. 3, pp..860- 865.

Boland, D. J, Brophy, J. J., Flynn, T M. and Lassak, E. V. 1982. Phytochemistry 21, 2467

Boland, D.J., Brooker, M.I.H., Chippendale, G.M., Hall, N., Hyland, B.P.M., Johnston, R.D., Kleinig, D.A. and Turner, J.D. 1984. Forest Trees of Australia. Nelson and CSIRO, Melbourne.

Bouwer, H. 2000. Integrated Water Management: Emerging Issues and Challenges, Agricultural Water Management, 45(3), pp. 217-228.

Brady, N.C. and Weil, R.R. 2008. The Nature and Properties of Soils, 14th edition, Pearson Education Inc., New Jersey.

Bredenkamp, B. 2000. Section 4.5, Volume and Mass of logs and standing trees, South African Forestry Handbook., Department of Forest, University of Stellenbosch, Pg 167-174.

Bren, L.J. and Gibbs, N.L. 1986. Relationships between Flood Frequency, Vegetation and Topography in a River Red Gum Forest. Australian Forest Research 16, pp. 357-370.

British Herbal Medicine Association, British Herbal Pharmacopoeia, 1996.

Brooker, M.I.H., Connors, J.R., Slee, A.V. and Duffy, S. 2002. EUCLID: eucalypts of southern Australia, CSIRO Publishing, Collingwood.

Brown, G.E., Foster, A.L. and Ostergren, J.D. 1999. Mineral Surfaces and Bioavailability of Heavy Metals: A Molecular-Scale Perspective. Proceedings of the National Academy of Science. 96: pp. 3388-3395.

Brown, P.H., Welch, R.M. and Cary, E.E. 1978. Nickel: A Micronutrient Essential for Higher Plants. Plant Physiology 85: 801-803.

Cao, X., Wahbi, A., Ma, L., Li, B. and Yang, Y. 2009. Immobilization of Zn, Cu and Pb in contaminated soils using phosphate rock and phosphoric acid. Journal of Hazardous Materials 164: 555-564.

CCA, Bill Hinkley Center for Solid and Hazardous Waste Management, University of Florida, (http://www.ccaresearch.org/) (Accessed 04 January 2015).

Chamber of Mines of South Africa. 2004. Facts and figures –plants, edition 2, part 2, Memoirs of the Botanical Survey of South Africa No. 56.

Chaudhry, F.M., Wallace, A. and Mueller, R.T., 1977. "Barium toxicity in plants," Communications in Soil Science and Plant Analysis, vol. 8, no. 9, pp. 795–797

Chenery EM. 1948. Aluminum in the Plant World. Part I, General Survey in Dicotyledons. Kew Bull, 2: 173–183.

Chenery EM. 1949. Aluminium in the Plant World. Part II. Monocotyledons and Gymnosperms. Kew Bull, 4: 463–466.

Chevallier, A. 1996. The Encyclopedia of Medicinal Plants, London: Dorling Kindersley, pp 141-273.

Chrysochoou, M., Dermatas, D. and Grubb, D.G. 2007. Phosphate application to firing range soils for Pb immobilization: The unclear role of phosphate. Journal of Hazardous Materials 144: 1–14.

Costermans, L.F. 1989. Native Trees and Shrubs of South-Eastern Australia, Weldon, Sydney.

Couto, L., Nicholas, I., Muller, M.D. and Barcellos, D.C. 2003. SRC with *Eucalyptus* in Brazil, Short Rotation Crops for Bioenergy, New Zealand, pp. 279–281.

CSIR, 2010. Council for Scientific and Industrial Research, Division of Natural Resources and the Environment Annual Research Report, NRE, Pretoria, South Africa, pg45.

Cunningham, G.M., Mulham, W.E., Milthorpe, P.E., and Leigh, J.H. 1981. Plants of Western New South Wales, Soil Conservation Service of New South Wales, Australia.

Curtis, R. 1998. "Arsenic and Old Wood", This Old House Magazine, March/April 1998.

Dare, P., Gifford, J., Hooper, R. J., Clemens, A.H., Damiano, L.F., Gong, D. and Matheson, T.W. 2001, Combustion Performance of Biomass Residue and Purpose Grown Species, Biomass and Bioenergy 21: 277–287.

Dennis, A. 2008. Salt Glands and Elemental Concentrations in Leaves of *Tamarix Usneoides E.May ex Bunge* (Tamaricaceae) Grown on Seepage from Highveld Gold Mines, Honours Project, Ecological Engineering and Phytoremediation Programme, School of Animal, Plant and Environmental Sciences (AP&ES). University of the Witwatersrand (unpublished).

Department of Minerals and Energy (DME). 2007. E. Swart, Director of Mine Rehabilitation, Department of Minerals and Energy of South Africa, cited in: The Star Daily Newspaper, Johannesburg, 23rd May.

Department of Tourism, Economic and Environmental Affairs of the Free State Province. 2002. The Waste Lekgotla Conference, Bloemfontein.

Dickinson, N M. 1997. Rehabilitation and Remediation of Metal-Contaminated Soils Using Trees. 4th International Conference on the Biogeochemistry of Trace Elements, Berkeley, California, pp. 437-8.

Dickinson, N.M. 2000. Strategies for Sustainable Woodland on Contaminated Soils. Chemosphere, 41: pp. 259–263.

Djavanchir, K., and Mossadegh, A. 1973. *Eucalyptus*. Tehran University Press. 435p.

Dovie, D.B.K., Witkowski, E.T.F., and Shackleton, C.M. 2005. Monetary Valuation of Livelihoods for Understanding the Composition and Complexity of Rural Households, Agriculture and Human Values 22, pp. 87–103.

Downer, N.R. 2008. Method Development for the Digestion and Analysis of Four Common Sedimentary Lithologies using ICP-OES and ICP-MS, University of Johannesburg, Faculty of Science, Master of Science in Analytical Chemistry Dissertation (unpublished).

Dube, A., Zbytniewski, R., Kowalkowski, T., Cukrowska, E. and Buszewski, B. 2001. Adsorption and Migration of Heavy Metals in Soil. Polish Journal of Environmental Studies 10: 1-10.

Duggan, J., 2005, The Potential for Landfill Leachate Treatment Using Willows in the UK—A Critical Review, Resources, Conservation and Recycling 45, pp. 97–113.

Du Plessis, M. 1996. Tree Volume and Taper Equation for *Eucalyptus grandis* (*Eucalyptus camaldulensis* GXC. Internal Report, HL&H Mining Timber, Tree Improvement Centre, White River.

Dutchinse, 2014. Website article dated 8/05/2014, Very Rare South Africa 5.3M Earthquake – Location: Vaal Reefs Mine – 6.2miles depth http://i2.wp.com/dutchsinse.tatoott1009.com/wp-content/uploads/2014/08/sa_ops.gif (Accessed 5 March 2015)

Dye, P.J., Jarmain, C., Oageng, B., Xaba, J., and Weiersbye, I.M. 2008a. A.B. Fourie, M. Tibbett, I.M. Weiersbye, P.J. Dye (eds). The Potential of Woodlands and Reed-beds for Control of Acid Mine Drainage in the Witwatersrand Gold Fields, South Africa, Mine Closure 2008 –Australian Centre for Geomechanics, Perth, ISBN 978-0-9804185-6-9.

Dye, P.J. and Weiersbye, I.M. 2010. The Mine Woodlands Project in the Witwatersrand Basin Gold Fields of South Africa: Strategy and Progress, Ecological Engineering and Phytotechnology Programme, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg.

Dye, P.J. 2010. Modelling The Effects of Salinity (Chlorides) on Water Uptake by Trees Growing on Sites Affected by Seepage from Gold Tailings Dams, Ecophysiology Research Group Natural Resources and the Environment, CSIR Interim Report (unpublished).

Ebbs, S.D. and Kochian. L.V. 1998. Phytoextraction of Zinc by Oat (*Avena sativa*), Barley (*Hordeum vulgare*), and Indian Mustard (*Brassica juncea*). Environmental Science and Technology, vol. 32, no. 6, pp. 802-806.

Ebbs, S.D. Lasat, M.M.; Brandy, D.J.; Cornish, J.; Gordon, R. and Kochian, L.V. 2010. Heavy Metals in the Environment: Phytoextraction of Cadmium and Zinc from Contaminated Soil. Journal of Environmental Quality, 1997, vol. 26, pp. 1424-1430.

Ellis, R.W. and Eslick, L. 1997. Variation and Range of Mercury Uptake into Plants at a Mercury-Contaminated Abandoned Mine Site, Bull. Environmental Contamination and Toxicology 59: pp. 763-769.

Epstein, E. 1965. Mineral Metabolism. In Plant Biochemistry (J. Bonner and J.E. Verner, eds), Academic Press, London and Orlando, pp. 438-466.

Epstein, E. and Bloom, A. 2005. Mineral Nutrition of Plants: Priciples and Perspectives. 2nd Edition. Sinauer Associates, Inc. Publishers, Sunderland, MA, ISBN 0-87893-172-4

Estrellan, C.R. and Iino, F. 2010. Toxic Emissions from Open Burning, Chemosphere, 80: 193–207.

Evans, J., 1992. Plantation Forestry in the Tropics, Second edition, Oxford Science Publication. USA, p. 403.

FAO. Ugalde L. and Pérez, O. 2001. Mean Annual Volume Increment of Selected Industrial Forest Plantation Species, Forest Plantation Thematic Papers, Working Paper 1. Forest Resources Development Service, Forest Resources Division. FAO, Rome (unpublished).

Feikema, P.M. and Baker, T.G. 2011. Effect of Soil Salinity on Growth of Irrigated Plantation *Eucalyptus* in South-Eastern Australia, Agricultural Water Management, 98:1180–1188.

Fendorf, S.E. 1995. Surface Reactions of Chromium in Soils and Waters. Geoderma. 67: 55-71.

Fendorf, S.E. and Li, G. 1996. Kinetics of Chromate Reduction by Ferrous Iron. Environmental Science and Technology. 30: 1614-1617.

Fine, P., Paresh, R., Beriozkin, A., and Hass, A. 2014. Chelant-enhanced Heavy Metal Uptake by *Eucalyptus* Trees under Controlled Deficit Irrigation. Science of the Total Environment, 493: 995–1005.

Gansauer, G. 2012. Trees in Phytoremediation. Biology Lecture Notes, BZ 572, Colorado State University, Fort Collins.

Garnett, T. and Smethurst, P. 1999. Ammonium and Nitrate Uptake by *Eucalyptus nitens*: Effects of pH and Temperature. Plant and Soil, 214: 133–140.

Garnett, T.P., Shabala, S.N., Smethurst, P.J. and Newman, I.A. 2003. Kinetics of ammonium and nitrate uptake by eucalypt roots and associated proton fluxes measured using ion selective microelectrodes. Functional Plant Biology 30: 1165–1176.

Glimmerveen, I (Ed.). 1996. Heavy Metals and Trees, Institute of Chartered Foresters, Edinburgh, p. 206.

Grieve, M. 1971. A Modern Herbal: The Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folk-Lore of Herbs, Grasses, Fungi, Shrubs, & Trees with All Their Modern Scientific Uses (Vol. 2). Courier Corporation.

Guzzi, G. and La Potta, C.A. 2008. Molecular Mechanisms Triggered by Mercury. Toxicology 244: 1-12.

Hajar, E.W.I., Sulaiman, A.Z.B. and Sakinah, A.M.M. 2014. Assessment of Heavy Metals Tolerance in Leaves, Stems and Flowers of *Stevia rebaudiana* Plant. Procedia Environmental Sciences 20: 386 – 393.

Hänsch, R. and Mendel, R.R. 2009. Physiological Functions of Mineral Micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl), Plant Biology 12: 259–266.

Harborne, J.B. 1993. Introduction to Ecological Biochemistry. Gulf Professional Publishing.

Hawkins, H.J., Hettaschb, H., Mesjasz-Przybylowiczc, J., Przybylowicz, W. and Cramera, M.D. 2008. Phosphorus Toxicity in the *Proteaceae*: A problem in Post-agricultural Lands, Scientia Horticulturae 117: 357–365.

Hazell, P. and Wood, S. 2008. Drivers of Change in Global Agriculture. Philosophical Transactions of the Royal Society B: Bio. Sci., 363 (1491): 495–515.

Herbert, M., 2008, Mine Woodlands Research Project Annual Report: 2007- 08. Restoration and Conservation Biology Research Group, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa (unpublished).

Hillis, W. E. and Brown, A. G., 1984, Eucalypts for Wood Production, CSIRO/Academic Press, London, Pg434.

Huang, J.W., Chen, J. and Cunningham, S.D. 1997. Phytoextraction of Lead Contaminated Soils: In: Phytoremediation of soil and water contaminants. ACS Symposium Series, Kruger, E.L., Anderson T.A. and Coats J.R. (eds), 1997a, p. 283-298.

Huang, J.W., Chen, J.J., Berti, W.R. and Cunningham, S.D. 1997. Phytoremediation of Lead Contaminated Soils: Role of Synthetic Chelates in Lead Phytoextraction. Environmental Science and Technology, 1997b, vol. 31, no. 3, p. 800-805.

Hunter, I., 2001. Above Ground Biomass and Nutrient Uptake of Three Tree Species (*Eucalyptus camaldulensis, Eucalyptus grandis and Dalbergia sissoo*) as affected by Irrigation and Fertiliser, at 3 years of age, in Southern India, Forest Ecology and Management, 144: 189–199.

Hutchings, A. 1996. Zulu Medicinal Plants, University of Natal Press, Pietermaritzburg.

International Agency for Research on Cancer (IARC). 2012. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans, Vol 100 C, Arsenic, Metals, Fibers and Dusts; IARC: Lyon,France.

Jacobs, M.R. 1955. Growth Habits of the Eucalypts. Forestry and Timber Bureau, Canberra, Australia, ISBN 0959459243.

Jain, R.K. 1994. Fuelwood Characteristics of Medium Tree and Shrub Species of India, Bioresource Technology, 47:81–84.

Jansen S, Broadley MR, Robbrecht E. and Smets E. 2002. Aluminum Hyperaccumulation in Angiosperms: A Review of its Phylogenetic Significance. Botanical Review; 68: 235–269.

Jolly, Y. N., Islam, A. and Akbar, S., 2013. Transfer of metals from soil to vegetables and possible health risk assessment, SpringerPlus 2013 2:385.

Kabata-Pendias, A. and Pendias, H. 1984. Trace Elements in Soils and Plants. CRC Press, Boca Raton, Florida.

Kabata-Pendias, A. and Pendias, H. 1992. Trace Elements in Soils and Plants. 2nd edn. CRC Press, London. pp. 227–233.

Kanias, G.D., Tsitsa, E., Loukis, A. and Kilikoglou, V. 1993. Determination and Statistical Analysis of Trace Element and Active Constituent Concentrations in the Medicinal Plant *Eucalyptus camaldulensis* Dehnh (E. Rostratus Schlecht), Journal of Radioanalytical and Nuclear Chemistry, Vol 169, No. 2, p. 483-491.

Khan, S.A., Khan, L., Hussain, I., Shah, H. and Akhtar, N. 2008, Comparative Assessment of Heavy Metals in *Euphorbia helioscopia* L., Pakistan Journal of Weed Science Research 14(1-2): 91-100.

Khosla, B. and Sudhakara Reddy, M. 2008, Response of Ectomycorrhizal Fungi on the Growth and Mineral Nutrition of *Eucalyptus* Seedlings in Bauxite Mined Soil, American-Eurasian Journal of Agriculture and Environmental Science 3:123-126.

King, D.J., Doronila, A.I., Feenstra, C., Baker, A.J.,M. and Woodrow, I.E. 2008. Phytostabilisation of Arsenical Gold Mine Tailings Using Four *Eucalyptus* Species: Growth, Arsenic Uptake and Availability after Five Years, Science of the Total Environment 406: 35-42.

Krämer, U. 2010. Metal hyperaccumulation in plants, Annual Review, Plant Biology 61:28.1–28.18

Kumar, P.B.A.N; Dushenkov, V.; Motto, H. and Raskin, I. 1995. Phytoextraction: The Use of Plants to Remove Heavy Metals from Soils. Environmental Science and Technology, 1995a, vol. 29, no. 5, p. 1232-1238.

Kumar, P.B.A.N.; Motto, H. and Raskin, I. 1995. Rhizofiltration: The Use of Plants to Remove Heavy Metals from Aqueous Streams. Environmental Science and Technology, 1995b, vol. 29, no. 5, p.1239-1245.

Labrecque, M; Teodorescu, T and Daigle, S. 1998. Early Performance and Nutrition of Two Willow Species in Short-Rotation Intensive Culture Fertilized with Wastewater Sludge and Impact on the Soil Characteristics, Canadian Journal of Forestry Research 28. 1621-1635.

Lamprecht, H. 1990. Silviculture in the Tropics: Tropical Forest Ecosystems and their Tree Species-Possibilities and Methods for their Long-Term Utilization, GTZ, Eschborn, Germany, Pg 296.

Larcher, W. 2003. Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups. Springer.

Lasat, M. M. 2002. Phytoextraction of Toxic Metals: A Review of Biological Mechanisms. Journal of Environmental Quality 31, p 109–120.

Lassak, E.V., and McCarthy, T. 1983. Australian Medicinal Plants. Methuen, Australia.

Lavilla I, Filgueiras AV, Bendicho C. 1999 Comparison of Digestion Methods for Determination of Trace and Minor Metals in Plant Samples. Journal of Agriculture and Food Chemistry; 47(12), pp. 5072-5077.

Leco Corporation, 2002. CNS-2000 Elemental Analyzer, CNS-2000 Instruction Manual.

Lee, M. 1999. Risk Assessment Framework for the Management of Sulfidic Mine Wastes, Australian Centre for Mining Environmental Research, Kenmore, 1–20.

Leslie, A.D., Mencuccini, M., and Perks, M. 2012. The Potential for *Eucalyptus* as a Wood Fuel in the UK, Applied Energy, 89:176–182.

Little, K.M., Schumann, A.W., Noble, A.D., 2002, Performance of a *Eucalyptus grandis x E. camaldulensis* Hybrid Clone as Influenced by a Cowpea Cover Crop, Forest Ecology and Management, 168:43-52.

Lockitch, G. 1993. Perspectives on lead toxicity. Clinical Biochemistry 26(5):371-81.

Lyons G.J., Lunny, F., and Pollock, H.P. 1985. A Procedure for Estimating the Value of Forest Fuels. Biomass 8:283–300.

Ma, L.Q., and Rao, G.N. 1997. Chemical fractionation of cadmium, copper, nickel and zinc in contaminated soils. Journal of Environmental Quality 26: 259–264.

Maathius, F. 2009. Plant Physiology of Mineral Macronutrients, Plant Biology 12:250–258.

Mabona, U. and Van Vuuren, S.F. 2013. Southern African Medicinal Plants Used to Treat Skin Diseases, South African Journal of Botany 87:175-193.

Madejón, E., Doronila, A.I., Madejón, P., Baker, A.J.M. and Woodrow, I.E. 2012. Biosolids, Mycorrhizal Fungi and Eucalypts for Phytostabilization of Arsenical Sulphidic Mine Tailing, Agroforestry Systems 84:389–399.

Madubansi, M. and Shackleton, C.M., 2005. Changing Energy Profiles and Consumption Patterns Following Electrification in Five Rural Villages, South Africa, Energy Policy 34 (2006) 4081–4092.

Maestri E, Marmiroli M, Visioli G. and Marmiroli N. 2010. Metal Tolerance and Hyperaccumulation: Costs and Trade-Offs Between Traits and Environment. Environmental Experimental Botany 68:1–13.

Mahmood, K., Morris, J., Collopy, J. and Slavich, P., 2001, Groundwater Uptake and Sustainability of Farm Plantations on Saline Sites in Punjab Province, Pakistan, Agricultural Water Management, 48:1–20.

Mandal, B.K. and Suzuki, K.T. 2002. Arsenic Round the World: A Review. Talanta: 58, 201-235.

Manirul, M.I.; Mayda, U.; Mehraj, H.; Shiam, I.H. and Jamal Uddin, A.F.M. 2015. Evaluation of Arsenic Accumulation by *Colocasia esculenta* (Kochu) from Soil, Journal of Soil Nature 8(2): 1-4.

Marcar, N.E., 1993. Waterlogging Modifies Growth, Water Use and Ion Concentration in Seedlings of Salt-Treated *Eucalyptus camaldulensis, E. tereticornis, E. robusta and E. globulus.* Australian Journal on Plant Physiology. 20:1–13.

Marschner, H. (1995). The Mineral Nutrition of Higher Plants, 2nd edition. Academic Press, London.

Marufu, L., Ludwig, J., Andreae, M.O., Meixner, F.X. and Helas, G. 1997. Domestic Biomass Burning in Rural and Urban Zimbabwe- Part A, Biomass and Energy, 12 (1):53–68.

Matsika, R., Erasmus, B.F.N. and Twine, W.C., 2013, Double Jeopardy: The Dichotomy of Fuel Wood Use in Rural South Africa, Energy Policy 52: 716-725.

McLeroth, B.B., 2015. Soil Survey, Land Capability, Wetland Delineation, Present Land Use, Vegetation Communities, Recommendations (Indigenous Tree Potential, I.E Compartment Divisions, and Land Preparation), and Location of Sites of Cultural and Archaeological Interest, of Tau Tona Waste Rock Dump Footprint (Post-Clean-Up), Varkenslaagte Wetland (Post-Clean-Up) and 'Woodlands', and Magnum Farm, West Wits Mining Operations, prepared for University of the Witwatersrand, Johannesburg and Anglogold Ashanti S.A. Region, Survey Reference: REMS54-Areas A-C Combined - version 1 (unpublished)

McMahon, L., George, B. and Hean, R., 2010b. *Eucalyptus melliodora.* Primefacts- for Profitable, Adaptive and Sustainable Primary Industries, www.dpi.nsw.gov.au/primefacts. (Accessed 20 January 2015).

Medhurst, J.L., Battaglia, M., and Beadle, C.L. 2002. Measured and Predicted Changes in Tree and Stand Water Use Following High-Intensity Thinning Of An 8-Year-Old *Eucalyptus nitens* Plantation. Tree physiology. 22(11): 775-784.

Meers, E., Du Laing, G., Tack, F.M.G. and Verloo, M.G., 2009. Heavy Metal Displacement by Exchangeable Bases (Ca, Mg, K, Na) in Soils and Sediments. Soil Science 174:202-209.

Mengel, K. and Kirkby, E.A. 1987. Principles of Plant Nutrition. 4th Edition. International Potash Institute, IPI, Bern, Switzerland. 685p.

Mengel, K., Kirby, E.A., Kosegarten, H. and Appel, T. 2001. Principles of Plant Nutrition. ISBN-13: 978-1402000089 ISBN-10: 1402000081. 5th Edition

Menzies, N.W., Donn, M.J. and Kopittke, P.M., 2007. Evaluations of Extractants for Estimation of the Phytoavailable Trace Metals in Soils. Journal of Environmental Pollution. 145:121-130.

Meskimen, G. and Francis, J., 2012. *Eucalyptus Grandis* Hill ex Maiden. Eucalipto Rosado, United States Forest Service, http://www.na.fs.fed.us/pubs/silvics_manual/volume_2/eucalyptus/grandis.htm (Accessed on 12 September 2014).

Misra SG, Mani D. 1991. Soil Pollution. Ashish Publishing House, Punjabi Bagh.

Mokgalaka, N.S., Combrinck, S., Lepule, S.P., Regnier, T.C., Weiersbye, I.M. 2009. A.B. Fourie, M. Tibbett (eds). Essential Oil Profiles of *Lippia scaberrima* Sond. from Highveld Gold and Uranium Mines, Mine Closure 2009. Pg 529-537.

Morris, J.D. 1984. Establishment of Trees and Shrubs on a Saline Site Using Drip Irrigation. Australian Forestry, 47:210–217.

Mulligan, C.N., Yong, R.N., and Gibbs, B.F. 2001. Remediation Technologies for Metal-Contaminated Soils and Groundwater: An Evaluation. Engineering Geology, 60(1), 193–207.

Munalula F. and Meincken, M. 2009. An evaluation of South African Fuelwood With Regards to Calorific Value and Environmental Impact, Biomass and Bioenergy, 33:415–420.

Musa, D.A., Nwodo, F.O.C. and Ojogbane, E. 2011. Phytochemical, Antibacterial and Toxicity Studies of the Aqueous Extract of *Eucalyptus camaldulensis* Dehnh, Asian Journal of Plant Science and Research 1, 1-10.

NARSTO, 2004. Particulate Matter Science for Policy Makers: A NARSTO Assesment, edited by P.H. McMurry, M. Shepherd, and J. Vickery, Cambridge University Press, Cambridge, UK, pp 510.

National Academy of Sciences (NAS), 1980, Firewood Crops: Shrub and Tree Species for Energy Production. National Academy Press, Washington, D. C, Pg 237.

Nengovhela, A.C., Yibas, B. and Ogola, J.S. 2006. Characterisation of Gold Tailings Dams of the Witwatersrand Basin with Reference to their Acid Mine Drainage Potential, Johannesburg, South Africa. Water SA. 32 (4): 499–506.

Newman, D. 1981. Third Year Growth of the Species in the *Pulpapel Arboretum*. Research Report, Investigación Forestal, Cartón de Colombia, No. 66. 7p.

Oliva, S.R., Valde´s, B. and Mingorance, M.D., 2008. Evaluation of some pollutant levels in bitter orange trees: Implications for human health, Food and Chemical Toxicology 46: 65–72.

Organisation for Economic Co-operation and Development (OECD). 2014. Consensus Document on the Biology of *Eucalyptus* spp. Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Series on Harmonisation of Regulatory Oversight in Biotechnology No. 58, Pesticides And Biotechnology, www.oecd.org/chemicalsafety/ (Accessed 22 March 2015).

Otarola, A. and Ugalde, L. 1989. Tablas de volumen para *Eucalyptus camaldulensis* en Nicaragua. Proyecto IRENA-CATIE-ROCAP No. 596-0089. Turrialba, C. R. 14 p.

Pallardy, S.G. 2008. Physiology of Woody Plants. 3rd edition. Academic Press publications. ISBN: 978-0-12-088765-1.

Parraga-Aguado, I., Querejeta, J-I., González-Alcaraz, M.N. and Conesa, H.M. 2014, Metal(Loid) Allocation and Nutrient Retranslocation in *Pinus halepensis* Trees Growing on Semiarid Mine Tailings, Science of the Total Environment, 485-486:406-414.

Pawlisz, A. V. 1997. Canadian Water Quality Guidelines for Cr, Environmental Toxicology and Water Quality, 12(2), 123–161.

Peltzer, K. 1999. Faith Healing for Mental and Social Disorders in the Northern Province (South Africa). Journal of Religion in Africa. 387-402.

Peuke, A.D., and Rennenberg, H. 2005. Phytoremediation with Transgenic Trees. Naturforsch C, 60, 199–207.

Ponce, S. C., Prado, C., Pagano, E. and Prado, F.E. 2015. Effect of Solution pH on the Dynamic of Biosorption of Cr (VI) by Living Plants of *Salvinia minima*, Ecological Engineering 74, p. 33-41.

Porter, E. K. and Peterson, P. J. 1975. Arsenic Accumulation by Plants on Mine Waste (United Kingdom). The Science of the Total Environment 4, 365-371

Pradhan, S.K., Holopainen, J.K., and Heinonen-Tanski, H. 2009. Stored Human Urine Supplemented with Wood Ash as Fertilizer in Tomato (*Solanum lycopersicum*) Cultivation and its Impacts on Fruit Yield and Quality. Journal of Agriculture and Food Chemistry. 57(16): 7612-7617.

Prosser, I. P., and Williams, L. 1998. The Effect of Wildfire on Runoff and Erosion in Native *Eucalyptus* Forest. Hydrological Processes, 12(2): 251–265.

Pulford, I.D. and Dickinson, N.W. 2006. In Prasad, m. N. V., Sajwan, K. S. and Naidu, R. (Eds). Phytoremediation Technology Using Trees, Elements in the Environment: Biogeochemistry, Biotechnology and Bioremediation, CRC Press, NW. 375–395.

Pyatt, F.B. 2001. Copper and Lead Bioaccumulation by *Acacia retinoides* and *Eucalyptus torquata* in Sites Contaminated as a Consequence of Extensive Ancient Mining Activities in Cyprus. Ecotoxicology and Environmental Safety 50:60-64.

Rauf, M.A., Hakim, M.A., Hanafi, M.M., Islam, M.M., Rahman, G.K.M.M and Panaullah, G.M. 2011. Bioaccumulation of Arsenic (As) and Phosphorous by Transplanting Aman Rice in Arsenic-Contaminated Clay Soils. Australian Journal of Crop Science 5(12): 1678-1684.

Raungsomboon, S., Chidthaisong, A.; Bunnag, B., Inthorn, D. and Harvey, N.W. 2008. Removal of Lead (Pb2+) by the *Cyanobacterium gloeocapsa* sp. Bioresource Technology 99: 5650–5658.

Riddell-Black, D. 1994. In. P. Aronsson, K. Perttu (Eds.). Heavy Metal Uptake by Fast Growing Willow Species: Willow Vegetation Filters for Municipal Wastewaters and Sludges. A Biological Purification System, Swedish University of Agricultural Sciences, Uppsala, 145–151.

Robb, L.J., and Robb, V.M. 1998a. In: Wilson MGC and Anhaeusser CR (eds.), Environmental Impact of Witwatersrand Gold Mining, The Mineral Resources of South Africa: Handbook, Council for Geoscience. 16: 14–16.

Robb, L.J., and Robb, V.M. 1998b. In: Wilson M.G.C. and Anhaeusser C.R. (eds.). Gold in the Witwatersrand Basin, The Mineral Resources of South Africa: Handbook, Council for Geoscience. 16, Pg 294–349.

Rockwood, D., Alker, G.R., Cardellino, R.W., Ma, L.G., and Tu, C. 2001. Phytoremediation of Contaminated Sites Using Wood Biomass, Florida Center for Solid and Hazardous Waste Management, University of Florida, Gainesville, New York, Report # 01-03, April 2001. 1–96.

Rockwood, D.L., Naidu, C.V., Carter, D.R., Rahmani, M., Spriggs, T.A., Lin, C., and Segrest, S.A. 2004. Short-rotation Woody Crops and Phytoremediation: Opportunities for Agroforestry. In New Vistas in Agroforestry, 51–63.

Sakthivel, V. and Vivekanandan, M. 2009. Reclamation of Tannery Polluted Soil through Phytoremediation, Physiological and Molecular Biology of Plants, 15(2), p. 175-180.

Salt, D.E.; Blaylock, M.; Kumar, N.P.B.A.; Dushenkov, V.; Ensley, D.; Chet, I. and Raskin, I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology, 1995a, vol. 13, p. 468-474.

Salt, D.E.; Prince, R.C.; Pickering, I.J. and Raskin, I. 1995. Mechanisms of Cadmium Mobility and Accumulation in Indian Mustard. Plant Physiology, 1995b, vol. 109 p. 1427-1433.

Sánchez, S. A., 1994, Crecimiento de Eucalyptus deglupta y E. grandis bajo tres sistemas de plantación a nivel de finca en la zona de Turrialba, Costa Rica, Master's Thesis, CATIE, Pg 95.

Schumacher, F.X., Hall, F.S.D., 1933, Logarithmic Expression of Timber Tree Volume, Journal of Agricultural Research, Washington, v.47, pp 719-734.

Shackleton, C.M., Shackleton, S.E., Buiten, E., and Bird, N., 2007, The Importance of Dry Woodlands and Forests in Rural Livelihoods and Poverty Alleviation in South Africa, Forest Policy and Economics, 9:558– 577.

Sheoran, A.S., and Sheoran, V. 2006. Heavy Metal Removal Mechanism of Acid Mine Drainage in Wetlands: A Critical Review. Minerals Engineering, 19(2): 105–116.

Shukla, O.P., Juwarkar, A.A., Singh, S.K., Khan, S., and Rai, U.N. 2011. Growth Responses and Metal Accumulation Capabilities of Woody Plants During the Phytoremediation of Tannery Sludge, Waste Management, 31 (2011): 115–123.

Sims, R.E.H., Senelwa, K., Maiava, T., and Bullock, B.T. 1999. *Eucalyptus* Species for Biomass Energy in New Zealand- Part II: Coppice Performance, Biomass and Bioenergy, 17:333-343.

Singh, G., Bhati, M., Rathod, T. 2010. Use of Tree Seedlings for the Phytoremediation of a Municipal Effluent Used in Dry Areas of North-Western India: Plant Growth and Nutrient Uptake, Ecological Engineering, 36:1299–1306.

Smit, H.C., and Meincken, M. 2012. Time/temperature Combustion Profiles of Various Wood-Based Biofuels, Biomass and Bioenergy, 39:317–323.

Smith, C.W.; Kassier, H.; Morley, T. 2006. The Effect of Initial Stand Density on the Growth and Yield of Selected *Eucalyptus grandis* Clonal Hybrids in Zululand. Pietermaritzburg: Institute for Commercial Forestry Research. (ICFR Bulletin 04/2006).

Smith, K.R. 1987. Biofuels, Air Pollution, and Health (Plenum, N.Y.)

Snowball, K. and A.D. Robson. 1991. Nutrient Deficiencies and Toxicities in Wheat: A Guide for Field Identification, Mexico, D.F.: CIMMYT. ISBN 968-61 27-48-8.

Soghoian, S. and Sinert, R. 2008. Toxicity, heavy metals. http://emedicine.medscape.com/article/814960-overview (Accessed 3 March 2015)

Soil Classification Working Group. 1991. Soil classification: A Taxonomic System for South Africa. Memoirs on the Agricultural National Resources of South Africa, 15:1–57.

Spangenberg, A., Grimm, U, Silva, J. and Fölster, H. 1996. Nutrient Store and Export Rates of *Eucalyptus urograndis* Plantations in Eastern Amazonia (Jari). Forest Ecology and Management, 80: 225-234.

Sparks, D.L, 2003. Environmental soil chemistry, 2nd edition, Elsevier Science, USA.

Sun, D., Dickinson, G.R., Bragg, A. 1994. The establishment of *Eucalyptus camaldulensis* on Tropical Saline Site in North Queensland. Australia Agricultural Ecosystem Environment, 48: 1–8.

Sun, D. and Dickinson, G.R. 1995. Salinity Effects on Tree Growth, Root Distribution and Transpiration of *Casuarina cunninghamiana* and *Eucalyptus camaldulensis* Planted on a Saline Site in Tropical North Australia, Forest Ecology and Management 77, Pg 127–138.

Susarla, S., Medina, V.F., and McCutcheon, S.C. 2002. Phytoremediation: An Ecological Solution to Organic Chemical Contamination. Ecological Engineering, 18(5): 647–658.

Tan, K.H. 2005. Soil Sampling, Preparation and Analysis, CRC press.

Trueman, S.J., McMahon, T.V., and Bristow, M. 2013. Production of Cuttings in Response to Stock Plant Temperature in the Subtropical Eucalypts, *Corymbia citriodora* and *Eucalyptus dunnii*. New Forests, 44(2): 265–279.

Ulrich, S.M., Tanton, T.W. and Abdrashitova, S.A. 2001. Mercury in the Aquatic Environment: A Review of Factors Affecting Methylation, Critical Review Environmental Science and Technology 31: 241-293.

United States Environmental Protection Agency (USEPA). 1991. Risk Assessment for Toxic Air Pollutants: A Citizen's Guide. EPA450/3-90-024. Washington, DC.

United States Environmental Protection Agency (USEPA). 1997. Cleaning Up the Nation's Waste Sites: Markets and Technology Trends. EPA/542/R-96/005. Office of Solid Waste and Emergency Response, Washington, DC.

United States Environmental Protection Agency (USEPA). 2015. Sulphur Dioxide. Health. Website (http://www3.epa.gov/airquality/sulfurdioxide/health.html) (Accessed 25 September 2015).

US Department of Health and Human Services. 1999. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Asbestos. Update. Agency for Toxic Substances and Disease Registry.

US EIA, International energy statistics, Energy Information Administration, United States. Available from ⟨http://www.eia.gov/cfapps/ipdbproject/IEDIndex3.cfm⟩; 2013 [accessed 6 September 2013].

Van den Broek, R., Faaij, A., van Wijk, A., 1996. Biomass Combustion for Power Generation, Biomass and Bioenergy, 11(4): 271–281.

Van der Moezel, P.G., Watson, L.E., Pearce-Pinto, G.V.N. and Bell, D.T. 1988. The Response of Six *Eucalyptus* Species and *Casuarina obesa* to the Combined Effect of Salinity and Waterlogging. Australian Journal of Plant Physiology 15, Pg 465–474.

Van der Moezel, P.G., Watson, L.E., Bell, and D.T., 1989. Gas Exchange Responses of Two *Eucalyptus* Species to Salinity and Waterlogging. Tree Physiology 5, Pg 251–257.

Van der Moezel, P.G., Gladys, V.N., Pearce-Pinto, G.V.N., and Bell, D.T. 1991. Screening for Salt and Waterlogging Tolerance in *Eucalyptus* and *Melaleuca* species. Forest Ecology Management 40, Pg 27–37.

Van Horen, C., Nel, R., and Terblanche, P., 1996. Indoor Air Pollution from Coal and Wood Use in South Africa: An Overview, Energy for Sustainable Development, Volume III No. 1: 38–40.

Vasquez, W. and Ugalde, L., 1995, *Tablas de volumen y productos de Eucalyptus grandis en Costa Rica*, CATIE, Turrialba, Costa Rica, Pg 17.

Vivier, J.J.K. 2005. Mine Woodlands Project: Integrated Mass Balance Model, Unpublished report submitted to the University of the Witwatersrand, Johannesburg and Anglogold Ashanti Ltd. AGES (Africa Geo-environmental Services).

Wang, J., Feng, X., Anderson, C.W.N., Xing, Y. and Shang, L. 2012. Remediation of Mercury Contaminated Sites - A Review, Journal of Hazardous Materials 221-222, 1-18.

Weaver, R. W., J. R. Melton, D. S. Wang & R. L. Duble. 1984. Uptake of Arsenic and Mercury from Soil by Bermuda Grass *Cynadon dactylon*. Environmental Pollution 33: 133-142.

Weiersbye, I.M., Cukrowska, E.M., Margalit, N., Feingersh, T., Revivo, G., Braun, O., Stark, R., and Zur, Y., 2006. Acid Mine Drainage (AMD) and Phreatophyte Vegetation: Cycling of Pollutants and Implications for Land-Use Planning, Invited Paper Presented at the Natural Woodlands and Forests Symposium IV, Summerstrand, Port Elizabeth 2006, Department of Water Affairs and Forestry.

Weiersbye, I.M., Margalit, N., Felingersh, T., Revivo, G., Stark, R., Zur, Y., Heller, D., Braun, O. and Cukrowska, E. M. 2006a. Use of airborne hyper-spectral remote sensing (HSRS) to focus remediation and monitor vegetation processes on gold mining landscapes in South Africa. In A.B. Fourie and M. Tibbett (eds), Proceedings of the 1st International Seminar on Mine Closure, Australian Centre for Geomechanics, Perth.

Weiersbye, I.M., Witkowski, E.T.F., and Reichardt, M. 2006b. Floristic Composition of Gold and Uranium Tailings Dams, and Adjacent Polluted Areas, on South Africa's Deep Level Mines. Bothalia. 36 (1): 101–127.

Weiersbye, I. M. 2007. In A, Fourie, M. Tibbett. and J. Wiertz [Eds]. Global review and cost comparison of conventional and phyto-technologies for mine closure. Mine Closure 2007, Australian Centre for Geomechanics, Perth, ISBN 978-0-9804 185-0-7.

Weiersbye, I. M. and Witkowski, E.T.F. 2007. In J. J. Bester., A. H. W. Seydack., T. Vorster., I. J. Van de Merwe. and S. Dzivhani (Eds). Impact of Acid Mine Drainage on the Regeneration Potential of Highveld Phreatophytes. Multiple Use Management of Natural Forest and Woodlands: Policy Refinements and Scientific Progress IV. Department of Water Affairs and Forestry of South Africa, Pg 224–237.

Weston, R.J. 1984. Composition of Essential Oil from Leaves of *Eucalyptus delegatensis*, Phytochemistry, Vol 23, No 9, pp 1943- 1945.

Williams, A., Jones, J.M., Ma, L., and Pourkashanian, M. 2012. Pollutants from the Combustion of Solid Biomass Fuels, Progress in Energy and Combustion Science, 38:113–137.

Winde, F., Wade, P., and Van der Walt, J. 2004a. Gold Tailings as a Source of Waterborne Uranium Contamination of Streams. The Koekemoerspruit (Klerksdorp goldfield, South Africa) as a Case Study Part I of III: Uranium Migration Along the Aqueous Pathway, Water SA Vol. 30 No. 2, pp 219-225.

Winde, F. and Van der Walt, J. 2004b. Gold Tailings as a Source of Waterborne Uranium Contamination of Streams - The Koekemoerspruit (Klerksdorp goldfield, South Africa)

as a Case Study Part II of III: Dynamics of Groundwater-Stream Interactions, Water SA Vol. 30 No. 2, pp 227-232.

Winde, F., Wade, P. and Van der Walt, J., 2004c. Gold Tailings as a Source of Water-Borne Uranium Contamination Of Streams - The Koekemoerspruit (South Africa) as a Case Study Part III of III: Fluctuations of Stream Chemistry and their Impacts on Uranium Mobility, Water SA Vol. 30 No. 2, pp 233-239.

Witkowski, E.T.F. and Weiersbye, I.M. 1998. Establishment of Plants on Gold Slimes Dams: Characterization of the Slimes and Adjacent Soils. Plant, Plant Ecology and Conservation Series No. 6, p3.

Witkowski ETF, Lamont BB.1996. Disproportionate Allocation of Mineral Nutrients and Carbon Between Vegetative and Reproductive Structures in *Banksia hookeriana.* Oecologia 105:38–42.

World Health Organization (WHO). 1966. Specification for the identity and Purity of Food Additives and their Toxicological Evaluations: Some Emulsfiers and Stabilizers and Certain other Substances, Technical Report Series No. 373, Tenth Report of the Joint FAO/WHO Expert Committee on Food Additives (Geneva: 11-18 October 1966), Switzerland.

World Health Organisation (WHO), 1993. Evaluation of Certain Food Additives and Contaminants. In: Forty-First Report of the joint FAO/WHO Expert Committee on Food Additives. WHO, Geneva, Switzerland, WHO Technical Series, 837

World Health Organization (WHO). 1996. Permissible Limits of Heavy Metals in Soil and Plants, (Geneva: World Health Organization), Switzerland.

World Health Organization (WHO). 1998. Health Guideline for the Use of Wastewater in Agriculture and Aquaculture. Report of WHO Science Group, World Health Organization, Geneva, Switzerland, Technical Report Scientific, p778:10.

World Health Organization (WHO). 2010. Exposure to Arsenic: A Major Public Health Concern, (Geneva: World Health Organization), Switzerland.

WSP Environmental (Pty) Ltd, 2009a, Vaal River Operations Environmental Management Programme, AngloGold Ashanti, File reference number 1919ES/ EMP.

WSP Environmental (Pty) Ltd, 2009b, West Wits Operations Environmental Management Programme, AngloGold Ashanti, File reference number 1919ES/ EMP.

Wymer, D. 2001. The Impact of Gold Mining on Radioactivity in Water and Foodstuffs. Proceedings, Chamber of Mines Of South Africa (2001): Conference on Environmentally Responsible Mining in Southern Africa. Muldersdrift/Johannesburg, 25-28, September, 1: 219–230.

Xie Z.M. and Huang C.Y. 1998. Control of Arsenic Toxicity in Rice Plants Grown on an Arsenic-Polluted Paddy Soil. Communications in Soil Science and Plant Analysis 29: 2471–2477.

Xu Q.S., Hu J.Z., Xie, K.B., Yang H.Y., Du K.H., Shi G.X. 2010. Accumulation and Acute Toxicity of Silver in *Potamogeton crispus* L. Journal of Hazardous Materials; 173:86–193.

Yan X.L., Chen T.B., Liao X.Y., Huang Z.C., Pan J.R., Hu T.D., Nie C.J. and Xie H. 2008. Arsenic Transformation and Volatilization during Incineration of the Hyperaccumulator *Pteris vittata* L., Environmental Science Technology 42(5):1479-84.

Yoon, J. 2005. Phosphate-induced Lead Immobilization in Contaminated Soil. Doctoral dissertation, University of Florida, Gainsville, FL.

Zayed A, Terry N. 2003. Chromium in the Environment: Factors Affecting Biological Remediation. Plant Soil 2003; 249:139–56.

Zhang, W., Cai, Y., Tu, C., and Ma, L.Q. 2002. Arsenic Speciation and Distribution in an Arsenic Hyperaccumulating Plant. Science of the Total Environment, 300(1): 167-177.

Zheng, N., Wang, Q. and Zeng, D. 2007. Health Risk of Hg, Pb, Cd, Zn and Cu to the Inhabitants Around Huludao Zinc Plant in China via Consumption of Vegetables. Science of the Total Environment. 2007a, 383: 81-89.

Zheng, N., Wang, Q.C. and Zeng, D.M. 2007. Transfer Characteristics of Mercury, Lead, Cadmium, Zinc and Cuprum from Soil to Vegetable Around Zinc Smelting Plant. Huan Jing Ke Xue. 2007b, 6:1349-1354.

Zohar, Y. and Schiller, G., 1998, Growth and Water Use by Selected Seed Sources of *Eucalyptus* Under High Water Table and Saline Conditions, Agriculture, Ecosystems and Environment 69 (1998), Pg 265-277.

APPENDIX A

SAMPLE CODES

Date	Plot	Tree No.	GPS co-ordinates	Location & species	Sample Type
28/08/2012	3	T17	26°59'21.06"S 26°46'33.55"E	Mispah cam	Branches + Twigs
28/08/2012	3	T17	26°59'21.06"S 26°46'33.55"E	Mispah cam	Wood
28/08/2012	3	T17	26°59'21.06"S 26°46'33.55"E	Mispah cam	Bark
28/08/2012	3	T17	26°59'21.06"S 26°46'33.55"E	Mispah cam	Leaves
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Branches + Twigs
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Leaves
29/08/2012	18	T47	26°25'53.27"S 27°22'6.13"E	Madala gxc	Branches + Twigs
29/08/2012	18	T47	26°25'53.27"S 27°22'6.13"E	Madala gxc	Leaves
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Wood
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Branches + Twigs
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Leaves
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Wood
29/08/2012	18	T47	26°25'53.27"S 27°22'6.13"E	Madala gxc	Wood
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Wood

Date	Plot	Tree No.	GPS co-ordinates	Location & species	Sample Type
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Wood
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Bark
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Wood
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala Cam	Bark
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Bark
29/08/2012	18	T47	26°25'53.27"S 27°22'6.13"E	Madala gxc	Bark
30/08/2012	28	T47	26°26'25.35"S 27°20'40.55"E	Red Soil cam	Branches + Twigs
30/08/2012	28	T47	26°26'25.35"S 27°20'40.55"E	Red Soil cam	Leaves
30/08/2012	17	T48	26°26'25.35"S 27°20'40.55"E	Red soil dunnii	Branches + Twigs
30/08/2012	17	T48	26°26'25.35"S 27°20'40.55"E	Red soil dunnii	Leaves
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Wood
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Leaves
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Branches + Twigs
30/08/2012	17	T48	26°26'25.35"S 27°20'40.55"E	Red soil dunnii	Wood
30/08/2012	28	T47	26°26'25.35"S 27°20'40.55"E	Red Soil cam	Wood

Date	Plot	Tree No.	GPS co-ordinates	Location & species	Sample Type
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Wood
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Wood
30/08/2012	28	T47	26°26'25.35"S 27°20'40.55"E	Red Soil cam	Bark
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Bark
30/08/2012	17	T48	26°26'25.35"S 27°20'40.55"E	Red soil dunnii	Bark
31/08/2012	13	T17	26°59'21.06"S 26°46'33.55"E	Mispah dunnii	Branches + Twigs
31/08/2012	13	T17	26°59'21.06"S 26°46'33.55"E	Mispah dunnii	Leaves
31/08/2012	13	T17	26°59'21.06"S 26°46'33.55"E	Mispah dunnii	Bark
31/08/2012	10	T32	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Branches + Twigs
31/08/2012	10	T32	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Leaves
31/08/2012	13	T17	26°59'21.06"S 26°46'33.55"E	Mispah dunnii	Wood
31/08/2012	10	T32	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Wood
31/08/2012	10	T32	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Bark
31/08/2012	32	TA	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Wood

APPENDIX B

DATA FROM ICP-MS

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
NSPS 1	416.0	3.7	14985.8	1.8	184.8	4.8	9054.1	1.9	8960.1	1.8	39668.2	0.4	15.3	13.2	114.7	4.0	26194.2	1.0	7.4	59.5
NSPS 2	432.5	2.6	14961.9	1.3	190.2	1.5	9443.9	1.3	10210.6	2.4	41619.4	2.7	8.7	20.5	111.1	3.2	26825.9	1.7	6.5	19.8
NSPS 3	450.3	4.6	14959.5	0.9	195.3	5.2	9352.7	1.8	10638.8	3.7	43925.2	1.2	6.1	16.2	103.8	3.0	26731.8	0.7	7.9	26.6
Plot28 T24 Branches & Twigs	104.2	11.3	12800.6	0.2	171.5	5.1	5411.1	2.1	3765.3	2.6	47812.8	2.1	7.3	29.6	85.8	5.0	412.0	2.8	8.5	28.3
Plot28 T47 Branches & Twigs	146.5	7.4	13919.4	1.8	686.3	3.4	6129.6	3.1	6323.0	2.4	106007.2	0.9	9.7	14.8	85.8	3.2	460.1	1.0	8.1	26.5
Plot3 T17 Wood	96.2	3.4	12457.8	1.0	163.7	1.7	2275.2	1.3	4655.2	1.9	35109.2	3.1	54.2	24.2	104.6	3.2	205.5	3.8	0.0	N/A
Plot3 T17 Bark	94.7	3.1	12355.3	3.1	392.5	1.4	2178.3	0.8	6303.8	0.8	163117.1	0.2	39.0	8.4	97.4	1.0	400.7	2.5	7.5	9.0
Plot18 T47 Bark	127.1	11.4	13089.0	0.7	698.3	0.3	2656.9	1.5	5906.6	0.6	52274.0	2.1	40.2	8.9	109.0	3.9	506.7	2.7	67.2	2.1
Plot47 T24 wood	80.2	1.6	12051.6	0.4	155.5	5.0	2106.3	1.9	5938.4	1.6	52678.1	1.2	48.3	11.9	102.7	2.3	2722.5	2.1	3.9	9.5
Plot21 T47 Bark	414.7	2.7	12889.4	2.1	641.1	5.6	3148.4	0.7	4934.1	1.3	68471.1	1.5	71.2	11.2	103.0	2.2	684.9	2.8	125.5	1.6
Plot46 T47 Bark	203.1	2.1	11946.2	1.7	812.4	2.2	3089.1	1.6	5340.7	0.2	53845.0	2.3	47.5	18.9	106.7	2.4	215.1	2.2	17.5	12.7
Plot46 T47 Branches & Twigs	135.8	3.0	13135.5	2.3	665.3	1.9	5955.3	1.8	6696.9	1.8	87977.0	1.1	52.6	14.2	98.2	3.1	973.7	1.1	143.5	6.5

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
Plot21 T47 wood	115.1	2.2	11979.3	2.3	148.7	6.2	2352.1	3.4	4759.5	1.7	23017.3	1.5	27.7	12.1	97.0	1.7	254.0	2.6	0.0	N/A
Plot47 T24 Branches & Twigs	184.0	0.2	11627.4	2.1	303.2	0.7	3292.6	1.5	5058.6	1.6	62908.4	1.3	22.7	13.1	88.3	1.9	861.3	1.4	17.7	29.5
Plot18 T47 wood	65.2	10.2	11927.1	0.6	225.7	6.5	2466.4	2.3	3569.5	2.2	18558.7	2.4	22.2	14.6	99.6	2.6	435.7	2.0	0.0	N/A
Plot17 T48 wood	64.2	26.1	11630.3	2.4	174.5	1.6	1641.4	2.5	6485.3	0.3	50603.9	3.6	18.8	16.3	87.6	2.9	933.0	1.9	0.0	N/A
Plot25 T17 wood	67.8	18.0	12155.3	1.0	129.1	3.7	2001.4	2.4	6576.8	1.9	43942.2	1.6	16.3	12.4	107.7	2.8	226.4	3.7	0.0	N/A
Plot46 T47 wood	86.8	0.8	11584.3	1.7	179.4	1.9	1904.5	3.2	4912.3	3.9	34304.1	2.1	16.8	8.8	93.0	5.3	527.0	1.8	1.0	158.4
Plot17 T48 Branches & Twigs	127.0	11.6	12264.9	3.3	218.6	2.2	4048.6	0.8	7043.9	3.1	12399.4	1.9	14.9	12.6	90.6	2.1	260.3	3.9	12.7	13.4
Plot10 T32 Bark	99.4	4.2	12401.8	1.3	581.2	3.0	2924.9	2.1	5605.9	1.6	12304.6	0.9	16.2	10.3	92.0	2.8	616.0	3.6	11.7	9.4
Plot28 T47 wood	97.2	8.8	11632.3	0.9	354.6	1.8	2695.0	2.6	5847.7	1.0	34379.0	1.3	17.5	9.5	88.1	0.6	277.6	1.0	7.6	30.6
Plot10 T32 Branches & Twigs	119.6	13.4	11748.4	0.2	267.6	3.1	5520.3	2.3	6062.0	1.5	14113.5	1.6	15.1	25.4	87.5	3.5	216.2	1.4	21.1	17.6
Plot27 T29 wood	58.2	19.9	10344.6	1.0	268.6	0.6	1480.2	1.7	6194.3	1.2	64175.9	1.4	15.7	11.7	87.6	4.5	494.5	1.0	0.0	N/A
Plot27 T29 Bark	107.9	9.2	10678.0	0.3	433.3	0.7	2239.5	3.5	4704.2	2.2	63660.4	1.4	11.6	12.4	91.8	1.7	299.3	1.6	18.1	3.6

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
Plot17 T48 Bark	157.9	7.3	11283.5	0.2	1268.0	0.6	2699.8	2.8	4659.1	1.1	88863.3	0.2	8.7	5.7	133.5	1.6	310.2	1.0	11.8	25.2
Plot13 T17 Bark	159.9	3.5	11516.9	1.6	531.5	1.7	2493.4	1.5	2956.2	1.8	10876 4.3	0.6	12.5	5.0	132.4	2.7	247.7	4.6	30.9	1.3
Plot26 T24 Bark	160.2	5.8	11024.1	1.1	193.6	2.0	2851.0	2.8	4549.6	0.6	74162 .8	1.3	9.7	2.4	116.8	1.5	3093 .8	0.5	35.9	3.4
Plot47 T24 Bark	155.9	4.5	10959.2	1.7	466.9	3.4	2380.4	1.1	3883.9	1.1	10951 9.0	0.8	5.1	17.5	95.0	3.4	223.4	2.4	19.9	10.4
Plot13 T17 wood	67.2	7.2	10763.4	1.7	152.1	2.4	1381.5	2.1	4652.7	1.4	24099 .5	0.7	15.3	3.2	87.4	3.7	241.6	2.1	0.0	N/A
Plot10 T32 wood	119.7	2.6	11241.2	3.2	206.8	1.5	2675.1	1.7	4584.1	0.0	34662 .3	1.3	8.1	14.7	88.1	1.7	443.1	1.5	21.3	19.2
Plot3 T17 Branches & Twigs	93.0	3.7	10614.6	1.5	253.3	4.8	7772.6	1.5	3937.9	0.2	15337 0.6	1.5	16.5	8.4	92.6	2.2	546.7	0.4	8.8	12.7
Plot27 T29 Branches & Twigs	114.5	1.8	10750.9	2.3	247.2	2.0	3540.6	0.2	4895.2	1.1	43767 .8	1.9	4.8	7.5	85.9	1.9	283.9	5.5	7.3	27.6
Plot13 T17 Branches & Twigs	128.7	3.6	10955.7	1.6	232.4	0.8	3601.3	0.6	4335.9	1.6	10188 0.4	0.6	12.1	20.7	87.7	0.8	358.4	1.4	27.5	5.7
Plot18 T47 Branches & Twigs	72.2	8.3	10849.3	2.4	233.9	7.5	5374.5	0.6	4610.9	0.6	35973 .8	0.2	82.5	4.4	81.1	3.7	186.2	2.7	4.9	24.5
Plot25 T17 Branches & Twigs	129.2	8.2	10733.6	2.4	197.9	2.8	2355.8	2.3	4633.4	1.9	72816 .0	1.7	33.8	9.0	93.3	1.0	343.6	1.0	32.7	9.2

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
Plot25 T17 Bark	182.8	2.6	10753.4	0.8	254.8	2.4	1792.9	3.0	4251.1	0.6	67613.6	0.9	16.6	13.2	92.2	3.6	916.2	2.3	9.3	4.9
Plot21 T47 Branches & Twigs	152.0	7.7	10124.9	2.4	222.1	1.7	4745.8	1.9	4397.8	3.2	30686.3	1.3	8.3	22.3	88.2	2.6	411.4	1.0	20.8	2.5
Plot28 T47 Bark	183.4	6.7	12313.8	1.3	1807.9	2.0	3626.1	1.4	5023.8	0.4	15580 8.3	2.1	7.2	16.9	89.6	2.3	276.0	0.8	31.4	2.6
Plot26 T24 Leaves	45.2	8.5	10724.3	1.1	616.0	4.1	2051.4	3.2	4027.2	3.3	30547.3	5.1	10.6	4.0	103.8	3.2	351.5	4.1	52.5	4.9
Plot47 T24 Leaves	142.8	4.6	10038.9	1.2	646.0	1.8	4151.3	1.1	4561.6	0.6	56782.1	2.2	22.9	2.2	108.4	2.7	313.0	3.2	65.6	2.9
Plot28 T47 Leaves	261.7	10.9	19585.8	1.0	1172.8	1.0	5352.3	1.9	4435.3	3.0	16259 8.8	1.2	10.4	5.6	103.2	2.4	831.0	1.0	40.2	5.6
Plot13 T17 leaves	326.0	3.3	11077.7	1.1	651.0	3.9	3986.6	3.7	4472.8	2.1	59527.1	2.6	39.3	4.8	108.7	5.5	726.8	5.2	199.3	3.2
Plot27 T29 leaves	159.9	1.1	17578.7	2.3	657.2	4.7	5375.3	3.3	4846.1	3.5	14264 8.8	0.7	9.7	5.0	124.5	3.8	1093.3	3.8	60.7	3.0
Plot3 T17 Leaves	137.4	4.6	1721.8	4.2	550.3	1.7	5056.7	2.3	4126.7	2.3	10047 8	1.2	9.5	3.3	109.8	3.6	702.6	2.4	75.3	5.2
Plot46 T47 Leaves	58.2	1.9	10614.6	1.3	433.3	2.2	2355.8	0.6	4097.8	3.9	48272 5	1.3	8.1	5.0	80.1	3.6	299.3	2.8	32.7	5.1
Plot21 T47 Leaves	107.9	9.2	10849.3	2.4	641.1	1.1	3540.6	0.2	4135.9	1.2	53118	1.6	7.9	9.4	89.0	2.2	310.2	1.1	33.8	4.5
Plot18 T47 Leaves	114.5	1.8	10955.7	0.8	394.5	1.8	3601.3	1.3	4251.1	1.7	46250	1.4	9.7	4.7	88.9	1.7	247.7	2.1	35.6	6.2
Plot25 T17 Leaves	128.7	3.6	10750.9	2.4	589.2	0.6	4740.1	0.9	4344.8	2.9	52287	1.4	9.1	4.9	84.7	1.5	343.6	1.7	47.4	2.8

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
Plot10 T32 Leaves	159.9	3.5	135441	1.1	694.3	1.1	5374.5	1.3	4110.9	1.3	109789	0.2	14.6	3.9	118.6	0.8	616.0	1.5	82.5	2.4
Plot17 T48 Leaves	160.2	5.8	109629	1.9	696.4	1.4	5472.3	3.0	4533.4	2.1	135400	0.6	21.0	3.3	107.7	1.0	861.3	0.6	87.7	1.7

APPENDIX C

DATA FROM ICP-OES

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Blank	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
0.1 ppm	0.106	0.109	0.099	0.098	0.101	0.098	0.098	0.101	0.103	0.107	0.097	0.098	0.095	0.099
0.5 ppm	0.493	0.502	0.502	0.502	0.497	0.505	0.508	0.495	0.503	0.506	0.494	0.507	0.501	0.507
1 ppm	0.995	1.003	1.004	0.998	0.993	1.003	0.995	0.997	0.996	1.002	1.002	1.006	0.96	1.002
NSPS 1	325.651	54.910	22114.228	322.144	7274.549	7234.469	87.675	415.832	19.840	7134.269	1482.966	32.665	14.830	50.301
% RSD	5.624	0.435	1.073	0.455	0.364	0.195	0.924	0.378	1.615	0.698	0.671	1.130	4.236	0.450
NSPS 2	311.751	58.853	21442.846	338.129	7234.213	7144.285	87.730	555.556	20.284	7873.701	2196.243	32.374	14.488	46.863
% RSD	1.274	0.467	0.921	0.230	0.792	0.404	0.252	1.157	1.276	7.403	0.722	0.701	2.418	0.540
NSPS 3	290.581	63.327	22384.770	337.375	7474.950	7555.110	91.583	617.234	20.641	8216.433	2208.417	33.066	14.429	50.200
% RSD	2.479	0.186	0.194	0.471	0.678	0.453	0.568	3.305	1.052	6.850	0.601	1.033	4.616	1.690
Plot 17 T48 Leaves	428.287	134.861	11907.371	204.183	4785.857	4272.908	1792.829	677.291	35.259	7599.602	1304.781	27.590	13.745	18.625
% RSD	1.594	0.225	0.397	0.425	2.873	2.891	0.395	2.338	0.484	8.152	0.495	1.325	1.182	1.722
Plot 10 T32 Leaves	470.000	197.800	10930.000	160.500	2930.000	3065.000	3370.000	510.000	34.900	6945.000	720.000	13.400	9.400	25.600
% RSD	1.242	1.437	0.507	0.289	2.244	0.704	0.684	1.775	1.944	4.449	1.361	0.560	1.069	0.983
Plot 25 T17 Leaves	405.487	52.062	7188.626	279.936	5416.500	2142.571	770.925	460.553	29.836	7644.173	1271.526	5.707	11.213	23.528
% RSD	2.020	0.733	0.014	0.517	2.730	0.221	0.310	3.097	0.552	5.522	1.471	0.545	3.396	1.422
Plot 18 T47 Leaves	389.377	46.126	8391.573	164.736	5710.863	4048.522	2481.030	494.209	25.060	9135.383	1098.243	9.385	9.185	18.870
% RSD	0.286	0.302	4.043	0.498	0.337	0.506	0.247	0.954	1.739	2.097	0.406	0.180	0.630	0.666
Plot 21 T47 Leaves	552.789	52.888	11663.347	358.566	3351.594	3819.721	2544.821	1180.279	25.498	8779.880	1200.199	10.359	16.534	31.474

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
% RSD	1.257	1.204	1.285	1.149	4.479	0.416	0.675	4.330	2.775	6.917	1.375	1.854	4.906	3.597
Plot 46 T47 Leaves	383.619	48.027	8499.402	159.426	4593.464	3143.683	2032.682	562.973	32.084	8260.263	777.202	9.466	9.566	17.238
% RSD	2.102	0.185	1.460	0.288	2.536	0.493	0.225	3.144	0.029	4.710	0.379	1.322	0.512	2.660
Plot 3 T 17 Leaves	409.509	147.623	11616.061	184.778	3995.206	2781.662	2337.195	1747.903	27.068	10037.954	1692.968	12.385	11.087	26.968
% RSD	2.375	0.609	1.113	0.142	4.708	0.574	0.884	3.243	1.097	5.891	0.149	1.563	3.329	2.238
Plot 27 T29 Leaves	463.332	38.362	6755.680	239.139	4424.073	4244.719	2625.548	1220.606	22.220	10123.555	1310.283	7.573	12.953	29.892
% RSD	0.635	0.450	0.045	0.282	0.976	0.455	0.245	1.178	0.833	2.950	0.905	0.254	9.829	0.455
Plot 13 T17 Leaves	925.741	72.658	10018.014	190.152	3763.010	3167.534	1911.529	810.649	25.620	9517.614	1781.425	9.107	8.006	22.318
% RSD	2.380	0.840	0.706	1.048	2.648	0.215	0.190	2.836	0.941	5.181	0.483	1.033	2.326	2.692
Plot 28 T47 Leaves	285.342	185.523	11869.243	173.809	2783.340	2002.403	3183.821	555.667	35.443	6783.140	690.829	34.341	7.609	45.855
% RSD	0.705	1.056	0.754	0.879	1.892	0.328	0.366	2.236	1.401	4.534	0.927	1.572	2.282	1.476
Plot 47 T24 Leaves	460.000	66.500	10645.000	270.000	3555.000	2595.000	695.000	350.000	24.100	7045.000	310.000	16.500	16.600	21.400
% RSD	2.337	0.654	1.022	0.830	3.585	0.858	1.235	3.028	2.408	7.965	1.231	1.543	2.667	2.592
Plot 26 T24 Leaves	220.441	44.589	8211.423	185.972	5746.493	2570.140	1277.555	445.892	27.455	8962.926	921.844	7.615	6.613	16.733
% RSD	1.513	0.811	1.966	0.378	2.452	0.296	0.230	2.869	0.754	4.167	0.324	0.453	6.235	2.954
Plot 28 T47 Bark	148.078	184.121	5756.908	220.264	3349.019	4019.824	3519.223	525.631	29.936	8159.792	165.198	136.964	4.505	22.527
% RSD	3.851	0.582	1.505	0.036	1.783	0.764	0.952	2.901	0.959	4.683	0.953	1.229	3.421	1.749
Plot 21 T47 Branches + Twigs	102.405	33.467	9539.078	221.944	1803.607	3396.794	1663.327	480.962	19.840	7284.569	225.451	15.832	3.407	26.453
% RSD	2.554	0.985	2.591	0.617	2.597	0.284	0.418	2.714	1.387	4.693	1.145	0.455	1.610	1.845

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Plot 25 T17 Bark	213.716	75.558	16836.124	172.648	1744.418	5806.419	1191.188	448.565	20.534	7441.188	203.648	19.936	4.585	15.451
% RSD	1.159	0.672	0.758	0.586	2.543	0.542	0.768	2.509	1.380	4.797	0.853	0.944	2.742	1.546
Plot 25 T17 Branches + Twigs	128.797	84.333	23171.463	238.809	2058.353	4456.435	1658.673	459.632	20.683	7683.853	429.656	31.175	3.897	22.982
% RSD	2.490	0.798	0.874	0.207	2.787	0.430	0.366	2.509	1.056	4.202	0.839	0.763	4.993	1.035
Plot 18 T47 Branches + Twigs	75.010	39.453	7001.598	232.421	2786.656	1363.364	1468.238	484.419	20.176	8235.118	214.243	12.185	2.697	11.586
% RSD	1.357	0.822	0.042	0.435	2.625	0.499	0.065	2.410	2.621	4.314	0.304	0.882	22.538	1.356
Plot 13 T17 Branches + Twigs	177.255	113.327	11372.745	215.431	2439.880	4784.569	1367.735	681.363	20.140	8096.192	335.671	18.236	4.509	21.142
% RSD	1.107	0.671	0.719	0.661	2.760	0.102	0.306	2.891	2.143	5.129	0.563	0.503	4.548	1.729
Plot 27 T29 Branches + Twigs	83.267	37.675	4478.958	235.772	2730.461	1813.627	1252.505	991.984	23.347	2885.772	177.756	12.625	4.208	13.627
% RSD	1.367	0.919	0.615	1.311	1.916	1.130	1.036	2.549	0.472	6.881	4.600	0.760	2.874	2.177
Plot 3 T 17 Branches + Twigs	84.764	127.696	21670.327	205.272	2391.174	2885.383	3549.321	888.578	25.160	4437.899	369.010	38.239	4.193	43.630
% RSD	2.316	1.619	0.454	1.205	2.718	0.830	1.116	2.635	2.180	9.886	2.885	0.780	1.059	2.363
Plot 10 T32 Wood	434.305	31.450	2610.823	574.081	1652.356	195.288	172.025	798.722	23.163	5321.486	119.609	8.087	12.280	16.274
% RSD	2.465	1.004	1.314	1.480	3.623	1.499	1.518	3.219	2.286	7.277	1.765	1.380	1.711	2.620
Plot 13 T17 Wood	67.519	21.974	6612.066	143.728	1977.627	1278.466	27.068	978.825	22.573	6132.641	97.083	8.490	3.596	6.692
% RSD	2.821	0.954	0.751	1.472	3.263	1.270	1.052	3.669	2.645	7.656	6.521	1.229	2.718	2.071
Plot 47 T24 Bark	120.555	99.780	44796.245	304.634	5453.456	5957.851	1563.124	848.981	25.170	5768.078	277.367	115.461	4.694	15.481
% RSD	2.696	1.054	3.536	1.018	2.292	0.748	0.782	2.254	1.809	5.443	1.350	0.864	1.111	2.839

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Plot 26 T24 Bark	265.000	67.500	25100.000	216.500	5705.000	6200.000	2630.000	745.000	25.200	5535.000	293.100	43.100	5.000	37.400
% RSD	1.393	1.886	0.659	1.488	2.925	1.251	1.267	3.125	3.277	9.044	1.827	1.201	1.118	3.495
Plot 13 T17 Bark	244.012	94.411	28108.782	444.112	3413.174	4625.749	1901.198	1501.996	24.651	6222.555	187.226	43.912	5.788	9.880
% RSD	2.463	1.828	0.907	1.593	3.117	1.130	1.611	2.927	3.968	8.894	3.566	1.737	4.478	3.411
Plot 17 T48 Bark	221.477	77.862	23974.179	236.189	4383.507	5069.055	675.540	970.777	27.322	7055.645	188.851	72.958	5.504	10.709
% RSD	1.803	1.321	1.111	1.531	3.011	1.408	1.197	3.469	1.096	6.894	2.303	1.507	1.835	2.307
Plot 27 T29 Bark	118.558	60.028	16440.272	234.319	1568.118	5433.480	2163.404	1739.912	22.873	4784.259	190.372	44.347	4.095	10.088
% RSD	2.071	1.916	0.852	1.459	3.666	0.899	1.323	2.131	3.339	6.583	3.118	1.549	1.817	2.693
Plot 27 T29 Wood	160.144	56.609	1988.818	91.753	708.866	1617.412	191.693	1152.157	21.366	4772.364	100.339	6.989	3.395	7.288
% RSD	1.863	0.873	1.338	0.661	2.771	1.247	1.123	2.570	2.496	8.261	4.461	1.150	0.919	1.520
Plot 10 T32 Branches + Twigs	144.085	116.707	11111.111	243.006	3727.018	1558.753	1938.449	1059.153	27.378	6804.556	220.823	18.485	4.496	19.285
% RSD	2.288	1.190	0.007	1.471	2.261	0.810	1.156	2.343	2.856	6.396	3.225	1.255	1.690	2.336
Plot 28 T47 Wood	117.964	29.142	1312.375	176.547	2270.459	723.553	113.473	998.004	21.357	7100.798	138.323	4.691	4.491	10.679
% RSD	1.468	1.096	0.691	0.783	3.081	1.023	1.018	2.717	2.535	7.020	3.185	0.541	1.691	2.833
Plot 10 T32 Bark	360.577	104.367	43319.311	110.877	4717.548	3916.266	3876.202	1121.795	27.244	15194.311	230.869	74.820	3.506	19.932
% RSD	1.260	1.257	2.944	1.157	2.849	0.887	0.907	3.286	1.297	5.733	1.501	1.337	1.027	2.461
Plot 17 T48 Branches + Twigs	332.335	97.505	7864.271	216.766	4890.220	1935.130	209.980	1526.946	27.944	3652.695	260.080	26.048	4.790	12.076
% RSD	1.395	1.042	0.053	1.584	2.941	1.123	1.012	2.881	2.662	5.973	0.669	1.301	1.683	2.837
Plot 46 T47 Wood	219.237	29.465	888.933	108.969	1473.232	75.509	35.957	1173.592	19.577	4679.385	89.992	4.195	3.196	8.290

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
% RSD	1.679	1.941	1.639	1.467	2.119	1.190	1.596	2.115	2.215	7.637	2.912	0.731	2.172	1.897
Plot 25 T 17 Wood	111.734	34.441	856.027	97.817	2042.451	173.909	42.151	1141.370	20.224	6512.815	152.683	2.903	3.304	8.510
% RSD	2.668	0.898	1.550	0.716	2.912	1.338	1.447	2.710	1.259	7.772	2.852	1.148	0.377	2.059
Plot 17 T48 Wood	122.255	42.914	2395.210	79.341	1676.647	1606.786	41.417	1127.745	20.659	6007.984	110.679	8.882	3.393	11.078
% RSD	2.691	0.803	0.856	1.874	2.237	0.974	1.501	3.318	3.119	6.368	2.880	1.195	2.791	2.816
Plot 18 T47 Wood	52.653	16.454	1964.499	79.577	2253.690	143.997	137.615	1002.194	21.041	5813.722	88.452	5.684	3.291	7.878
% RSD	1.523	0.851	1.362	1.928	3.519	1.239	1.207	3.093	3.714	7.529	4.647	0.989	7.486	2.738
Plot 47 T24 Branches + Twigs	130.435	53.849	8516.155	198.444	7758.277	2871.959	589.350	996.211	22.138	3340.646	281.213	24.830	5.086	14.659
% RSD	2.277	0.937	0.038	1.251	1.420	1.029	1.078	2.164	2.839	7.136	2.272	0.941	1.646	2.505
Plot 21 T47 Wood	135.446	21.491	714.714	190.224	1839.264	84.266	41.084	1034.586	21.691	5752.699	126.749	3.499	4.398	7.697
% RSD	1.080	1.449	1.557	1.209	2.835	1.043	1.202	2.372	3.478	8.585	2.581	0.722	2.028	1.627
Plot 46 T47 Branches + Twigs	1782.853	79.728	11618.590	749.199	2347.756	1396.234	1500.401	1221.955	28.646	4799.679	155.349	29.147	5.108	15.024
% RSD	1.618	1.253	1.049	1.226	2.502	1.250	0.868	2.499	3.032	7.215	3.137	1.078	1.103	2.716
Plot 46 T47 Bark	136.473	48.397	55781.563	130.561	5611.222	1800.601	2655.311	993.988	23.547	4128.257	294.790	103.607	4.810	8.818
% RSD	2.667	1.816	0.973	1.643	3.620	1.257	1.176	3.662	2.682	7.852	2.862	1.286	4.373	3.131
Plot 21 T47 Bark	334.000	61.900	34190.000	373.000	4240.000	2620.000	3010.000	1420.000	22.500	4110.000	449.000	61.000	9.200	19.400
% RSD	1.096	1.942	1.105	1.228	3.475	1.472	1.117	3.725	1.621	7.234	2.392	1.455	2.583	3.506
Plot 47 T24 Wood	139.200	43.600	2054.000	156.000	944.000	142.700	33.900	962.000	21.800	4770.000	78.900	6.300	3.700	22.000
% RSD	2.256	1.020	0.628	1.769	4.069	0.999	1.028	3.448	5.540	6.762	7.402	1.218	4.247	3.162

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Plot 18 T47 Bark	542.217	49.520	36894.758	220.288	2565.026	2132.853	2364.946	894.358	22.709	7122.849	238.195	73.029	4.402	11.505
% RSD	1.339	1.029	2.137	0.833	3.086	0.971	1.060	2.695	2.698	7.693	1.947	1.395	1.871	3.278
Plot 3 T17 Bark	289.652	151.918	34253.895	217.938	3266.081	2936.476	3595.685	1388.334	25.170	8040.352	229.425	72.014	3.895	23.072
% RSD	1.886	1.762	1.824	1.814	3.322	1.341	1.266	3.491	4.390	8.140	3.076	1.456	1.708	3.307
Plot 3 T17 Wood	80.941	31.898	1325.758	249.203	2651.515	146.830	98.485	912.081	21.232	4844.498	195.375	4.187	3.788	10.666
% RSD	2.679	0.970	1.172	1.111	4.126	1.458	0.967	4.158	3.010	8.691	1.929	0.910	1.159	3.088
Plot 28 T47 Branches + Twigs	239.425	105.547	22146.848	174.681	3571.429	2242.618	1739.824	1156.225	24.441	5476.856	297.287	58.360	4.789	26.038
% RSD	1.703	1.651	0.723	1.832	2.646	0.983	1.167	3.382	2.071	5.397	1.646	1.519	1.602	3.847
Plot 26 T24 Branches + Twigs	103.206	47.495	11012.024	220.040	5410.822	2099.198	1706.413	840.681	25.451	4418.838	439.880	19.439	4.409	15.030
% RSD	3.048	1.117	0.112	1.353	3.286	1.135	0.884	3.174	1.239	8.488	2.387	1.152	2.922	3.280

APPENDIX D

DATA FROM CN & ASH

NORTH WEST UNIVERSITY

ECO ANALYTICA

WITS UNIVERSITY (INNOCENT RABOHALE)

21/8/2014

LECO

Sample no.	Plot no.	Tree no.	Site	Species	Sample type	N %	C %	Ash %
1	26	24	MADALA	E. MELLI	BRANCHES+TWIGS	0.32	44.87	95.16
2	28	47	RED SOIL	E. CAM	BRANCHES+TWIGS	0.23	43.35	94.01
3	3	17	MISPAH	E.CAM	WOOD	0.15	45.99	98.92
4	3	17	MISPAH	E.CAM	BARK	0.13	40.05	90.88
5	18	47	MADALA	E.GXC	BARK	0.11	40.75	90.03
6	47	24	RED SOIL	E.MELLI	WOOD	0	45.82	98.49
7	21	47	MADALA	E.CAM	BARK	0.16	40.74	89.74
8	46	47	RED SOIL	GXC	BARK	0.17	39.52	85.24
9	46	47	RED SOIL	G.GXC	BRANCHES+TWIGS	0	44.01	96.43
10	21	47	MADALA	E.CAM	WOOD	0	46.54	-26.80
11	47	24	RED SOIL	E.MELLI	BRANCHES+TWIGS	0.04	44.21	95.71
12	18	47	MADALA	E.GXC	WOOD	0	45.55	99.13
13	17	48	RED SOIL	G.DUNNII	WOOD	0	44.97	99.09

14	**25**	**17**	**MISPAH**	**E.MELLI**	**WOOD**	0.10	45.56	99.13
15	**46**	**47**	**RED SOIL**	**E.GXC**	**WOOD**	0	45.85	99.69
16	**17**	**48**	**RED SOIL**	**E.DUNNII**	**BRANCHES+TWIGS**	0.34	44.16	97.00
17	**10**	**32**	**MISPAH**	**GXC**	**BARK**	0.11	39.43	89.55
18	**28**	**47**	**RED SOIL**	**E.CAM**	**WOOD**	0.04	45.02	99.27
19	**10**	**32**	**MISPAH**	**E.GXC**	**BRANCHES+TWIGS**	0.12	44.01	96.81
20	**27**	**29**	**MADALA**	**E.DUNNII**	**WOOD**	0	45.03	99.26
21	**27**	**29**	**MADALA**	**E.DUNNII**	**BARK**	0.06	41.79	94.78
22	**17**	**48**	**RED SOIL**	**E.DUNNII**	**BARK**	0.07	40.54	92.69
23	**13**	**17**	**MISPAH**	**E.DUNNII**	**BARK**	0.14	40.67	92.34
24	**26**	**24**	**MADALA**	**E.MELLI**	**BARK**	0.18	41.01	92.36
25	**47**	**24**	**RED SOIL**	**E.MELLI**	**BARK**	0.18	39.33	87.75
26	**13**	**17**	**MISPAH**	**E.DUNNII**	**WOOD**	0	44.72	98.32
27	**10**	**32**	**MISPAH**	**E.GXC**	**WOOD**	0	45.06	98.80
28	**3**	**17**	**MISPAH**	**E.CAM**	**BRANCHES+TWIGS**	0.23	42.54	93.79
29	**27**	**29**	**MADALA**	**E.DUNNII**	**BRANCHES+TWIGS**	0.14	44.76	97.69
30	**13**	**17**	**MISPAH**	**E.DUNNII**	**BRANCHES+TWIGS**	0.22	44.28	96.32
31	**18**	**47**	**MADALA**	**E.GXC**	**BRANCHES+TWIGS**	0.05	43.97	97.47
32	**25**	**17**	**MISPAH**	**E.MELLI**	**BRANCHES+TWIGS**	0.29	43.23	95.00
33	**25**	**17**	**MISPAH**	**E.MELLI**	**BARK**	0.12	42.13	94.91
34	**21**	**47**	**MADALA**	**E.CAM**	**BRANCHES+TWIGS**	0.22	44.15	96.12

35	**28**	**47**	**RED SOIL**	**E.CAM**	**BARK**	0.20	38.79	84.83
36	**26**	**24**	**MADALA**	**E.MELLI**	**LEAVES**	1.27	48.47	95.68
37	**47**	**24**	**MADALA**	**E.MELLI**	**LEAVES**	1.01	48.87	95.09
38	**28**	**47**	**RED SOIL**	**E.CAM**	**LEAVES**	1.02	46.35	93.02
39	**13**	**17**	**MISPAH**	**E.DUNNII**	**LEAVES**	1.63	50.33	95.87
40	**27**	**29**	**MADALA**	**E.DUNNII**	**LEAVES**	1.28	49.87	96.06
41	**3**	**17**	**MISPAH**	**E.CAM**	**LEAVES**	1.21	49.49	95.69
42	**46**	**47**	**RED SOIL**	**E.GXC**	**LEAVES**	0.70	49.24	96.46
43	**21**	**47**	**MADALA**	**E.CAM**	**LEAVES**	1.16	50.33	95.31
44	**18**	**47**	**MADALA**	**E.GXC**	**LEAVES**	0.92	47.73	95.91
45	**25**	**17**	**MISPAH**	**E.MELLI**	**LEAVES**	1.19	49.84	96.58
46	**10**	**32**	**MISPAH**	**E.GXC**	**LEAVES**	0.99	47.71	96.03
47	**17**	**48**	**RED SOIL**	**E.DUNNII**	**LEAVES**	1.25	48.93	94.66
48	**26**	**24**	**MADALA**	**E.MELLI**	**WOOD**	0	44.52	98.96
N5P6 1					**ORCHARDS LEAVES**	2.27	43.73	Not enough sample
N5P6 2					**ORCHARDS LEAVES**	2.26	43.49	Not enough sample
N5P6 3					**ORCHARDS LEAVES**	2.27	43.43	Not enough sample

CRM1					**ORCHARDS LEAVES**	2.25	43.58	Not enough sample
CRM2					**ORCHARDS LEAVES**	2.27	43.76	Not enough sample

This laboratory participates in the following quality control schemes:

1. Agricultural Laboratory Association of Southern

Africa.

2. International Soil-Analytical Exchange (ISE), Wageningen, Nederland.

No responsibility is accepted by North-West University for any losses due to the use of this data

APPENDIX E

DATA TABLES FOR RESULTS

Table 19: Mean concentrations of phosphorus, potassium and % nitrogen for wood from three *Eucalyptus* species ± standard deviation

Concentration (mg/kg)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Phosphorus	5899.33 ± 1135.27	5271.53 ± 568.82	5637.66 ± 751.96
Potassium	2253.75 ± 406.38	1793.09 ± 568.82	1454.38± 662.94
% N	0.05 ±0.09	-0.05±0.04	-0.08±0.04

Table 20: Mean concentrations of phosphorus, potassium and % nitrogen for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	Potassium	Phosphorus	Nitrogen
		mg/kg	mg/kg	%
E. gxc	Leaves	4411.44	8113.55	0.87
	Branches/ twigs	2953.81	6613.12	0.23
	Bark	4297.93	8815.14	0.13
	Wood	1793.09	5271.53	-0.05
E. cam	Leaves	3376.71	8533.66	1.13
	Branches/ twigs	2588.74	5733.11	0.23
	Bark	3618.37	6770.05	0.16
	Wood	2253.75	5899.33	0.05
E. dunnii	Leaves	4324.31	9080.26	1.39
	Branches/ twigs	3353.52	4878.22	0.23
	Bark	3121.60	6020.82	0.09
	Wood	1454.38	5637.66	-0.08

Table 21: Mean concentrations of calcium, sulphur and magnesium for wood from three *Eucalyptus* species ± standard deviation

Concentration (mg/kg)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Calcium	1117.62 ± 348.99	1821.42 ± 869.82	3665.36 ± 2560
Sulphur	153.48 ± 36.74	99.35 ± 17.56	102.70 ± 7.10
Magnesium	318.22 ± 352.42	138.26 ± 60.09	1500.89 ± 192.70

Table 22: Mean concentrations of calcium, magnesium and sulphur for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	Ca (mg/kg)	Mg (mg/kg)	S (mg/kg)
E. gxc	Leaves	9273.66	3419.07	865.15
	Branches/twigs	9910.43	1439.45	196.80
	Bark	45331.88	2616.57	254.62
	Wood	1821.42	138.26	99.35
E. cam	Leaves	11716.22	2867.93	1194.67
	Branches/twigs	17785.42	2841.60	297.25
	Bark	24733.60	3192.10	281.21
	Wood	1117.62	318.22	153.48
E. dunnii	Leaves	9560.35	3895.05	1465.50
	Branches/twigs	7905.32	2844.44	257.84
	Bark	22841.08	5042.76	188.82
	Wood	3665.36	1500.89	102.70

Table 23: Mean concentrations in mg/kg of manganese, iron, zinc, copper and nickel for wood from three *Eucalyptus* species ± standard deviation

Concentration (mg/kg)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Manganese	84.53 ± 38.21	115.20 ± 70.75	86.73 ± 91.19
Iron	205.32 ± 38.61	254.21 ± 277.41	104.94 ± 34.16
Zinc	9.68 ± 1.72	10.81 ± 4.73	8.35 ± 2.38
Copper	2.44±0.22	0.20±0.02	0.20±0.06
Nickel	21.43 ± 0.24	21.26 ± 1.80	21.53 ± 0.97

Table 24: Mean concentrations of manganese, iron, zinc, copper and nickel for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	Mn	Fe	Zn	Cu	Ni
		(mg/kg)				
E. gxc	Leaves	2627.90	161.55	20.57	3.78	30.68
	Branches/twigs	1635.70	408.21	15.30	5.62	25.40
	Bark	2965.49	153.91	13.42	2.89	24.50
	Wood	115.20	254.21	10.81	2.35	21.26
E. cam	Leaves	2688.61	239.05	34.77	4.29	29.34
	Branches/twigs	2317.49	200.63	32.04	6.22	23.15
	Bark	3374.97	270.40	21.67	2.98	25.87
	Wood	84.35	205.32	9.68	2.44	21.43
E. dunnii	Leaves	2109.97	211.16	23.61	4.94	27.70
	Branches/twigs	943.41	222.66	15.62	3.73	23.81
	Bark	1580.05	304.87	10.23	2.48	24.95
	Wood	86.73	104.94	8.35	1.50	21.53

-

Table 25: Mean concentrations of chromium, lead and arsenic for wood from three *Eucalyptus* species ± standard deviation

Concentration (mg/kg)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Chromium	12.02 ± 0.41	11.58 ± 0.34	10.91 ± 0.66
Lead	0.25 ± 0.04	0.47 ± 0.05	0.56 ± 0.35
Arsenic	5.09 ± 0.66	4.36 ± 0.07	5.78 ± 0.98

Table 26: Mean concentrations of chromium, lead and arsenic for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	Cr (mg/kg)	Pb (mg/kg)	As (mg/kg)
E. gxc	Leaves	52.34	0.39	4.15
	Branches/twigs	11.91	0.46	5.79
	Bark	12.48	0.45	5.62
	Wood	11.58	0.47	4.36
E. cam	Leaves	13.84	0.62	4.35
	Branches/twigs	11.55	0.47	4.89
	Bark	12.52	0.45	5.42
	Wood	12.02	0.25	5.09
E. dunnii	Leaves	46.10	0.89	4.62
	Branches/twigs	11.32	0.30	5.43
	Bark	11.16	0.29	4.11
	Wood	10.91	0.56	5.78

Carbon and Ash Content:

Table 27: Mean % dry mass of carbon and ash content for wood from three *Eucalyptus* species ± standard deviation

Content (%)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Carbon	45.85 ± 0.77	45.48 ± 0.40	44.91 ± 0.16
Ash	99.10 ± 0.25	99.21 ± 0.45	98.89 ± 0.50

Table 28: Mean % dry mass of carbon and ash content for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	% Carbon	% Ash
E. gxc	Leaves	48.23	96.14
	Branches/twigs	44.40	97.01
	Bark	39.90	88.28
	Wood	45.48	99.21
E. cam	Leaves	48.72	94.67
	Branches/twigs	43.35	94.64
	Bark	39.86	88.48
	Wood	45.85	99.10
E. dunnii	Leaves	49.71	95.53
	Branches/twigs	44.40	97.01
	Bark	41.00	93.27
	Wood	44.91	98.89

APPENDIX F

SAMPLE TREE HEIGHTS AND DBH

Site	Species of *Eucalyptus*	Plot	Tree	Total height (cm)	Dbh (cm)
Mispah	*gxc*	10	32	1388,2	11
	cam	3	17	1013	16
	dunnii	13	17	2430	29
Madala	*gxc*	18	47	1500	13
	cam	21	47	1790	26
	dunnii	27	29	1648	20
Red Soil	*gxc*	46	47	1830	21
	cam	28	47	1040	9.6
	dunnii	17	48	1170	11

APPENDIX G

SAMPLE WEIGHED DRY MASS

Sample	Mass (g)
Plot 17 T48 Leaves	0.2510
Plot 10 T32 Leaves	0.2500
Plot 25 T17 leaves	0.2497
Plot 18 T47 Leaves	0.2504
Plot21 T47 Leaves	0.2510
Plot46 T47 Leaves	0.2509
Plot3 T17 Leaves	0.2503
Plot27 T29 Leaves	0.2509
Plot13 T17 Leaves	0.2498
Plot28 T47 Leaves	0.2497
Plot47 T24 Leaves	0.2500
Plot 26 T24 Leaves	0.2495
Plot28 T47 Bark	0.2497
Plot21 T47 Branches +twigs	0.2495
Plot 25 T17 bark	0.2508
Plot 25 T17Branches +twigs	0.2502
Plot 18 T47 branches +twigs	0.2503
Plot 13 T17 Branches + twigs	0.2495
Plot27 T 29 Branches +twigs	0.2495
Plot3 T17 branches + twigs	0.2504
Plot 10 T32 wood	0.2504
Plot 13 T17 wood	0.2503
Plot 47 T24 bark	0.2503
Plot 26 T24 Bark	0.2500
Plot 13 T 17 bark	0.2505
Plot 17 T48 Bark	0.2498
Plot 27 T 29 bark	0.2503
Plot 27 T29 wood	0.2504
Plot10 T32 Branches +twigs	0.2502
Plot 28 T47 wood	0.2505

Sample	Mass (g)
Plot 10 T32 Bark	0.2496
Plot 17 T48 branches + twigs	0.2505
Plot46 T 47 wood	0.2503
Plot 25 T17 wood	0.2497
Plot 17 T48 wood	0.2508
Plot18 T47 wood	0.2507
Plot 47 T24 Branches +twigs	0.2507
Plot21 T47 wood	0.2501
Plot 46 T 47 branches + twigs	0.2496
Plot 46 T47 Bark	0.2495
Plot 21 T47 Bark	0.2500
Plot 47 T24 wood	0.2500
Plot 18 T 47 bark	0.2499
Plot 3 T 17 Bark	0.2503
Plot3 T 17 Wood	0.2508
Plot 28 T47 Branches + twigs	0.2506
Plot 28 T24 branches +twigs	0.2495

Risk assessment of above ground biomass for fuel use in *Eucalyptus* species cultivated on acid mine drainage in the Witwatersrand Basin gold fields

Nosipho Sylvia Vanessa Mosito
(Student number- 520988)
School of Chemistry
University of the Witwatersrand, Johannesburg

A research report submitted to the Faculty of Science in partial fulfilment of the requirements for the degree of Master of Science.

Supervisor- Prof. Luke Chimuka

Co-supervisor –Dr Peter Dye

Advisor – Isabel Weiersbye

Johannesburg, April 2016

DECLARATION

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



(Signature of candidate)

18th day of April 2016 in Johannesburg.

ABSTRACT

South African gold mines are associated with the generation of a lot of mine waste in the form of milled rock (tailings). Tailings contain the mineral pyrite which, when exposed to air and water, cause acid mine drainage (AMD). Due to the large environmental impact (footprint and scale) of the tailings storage facilities (TSFs) on soils and groundwater, there has been much research done in phytoremediation. Some plants, such as *Eucalyptus*, used in this method are able to control seepage by using their extensive roots but this may inadvertently extract some contaminants from the water and accumulate them in the above ground parts of the tree. Upon harvesting of these plants, there is the potential for them to be used as biofuel for the generation of bioenergy, and by industry or the public as timber/construction wood, firewood, charcoal, generation of electricity, etc. In this study, three species of *Eucalyptus* trees grown by the University of the Witwatersrand in three site-species trials on AMD were evaluated for their concentrations of elements in leaves, bark, branches/twigs and stem wood, in order to determine the safety of the biomass for fuel, and the potential for environmental pollution (dissemination of metals) that could be caused by combustion. The study focused on *Eucalyptus camaldulensis, E. grandis x camaldulensis* hybrid and *E. dunnii* trees grown for eight years in three different trial sites, with one trial ("Mispah") situated at AngloGold Ashanti's Vaal River Mining Operations (VR, near Orkney – Klerksdorp) and two trials ("Madala", "Red Soil") situated at the West Wits Mining Operations (WW, near Carletonville). The sites were typical of soils on the mine properties (WW Madala: Clovelly, WW Red Soil: Hutton, VR Mispah: Hutton and Mispah), and impacted by seepage from adjacent TSFs. Three entire above-ground trees were harvested per species (three trees per site, nine in total), weighed fresh and after drying. Samples of leaves, bark, twig/small branches, and main stem wood were analysed for their elemental contents; alongside a Certified Reference Material (CRM) (Orchard Leaves no. 1571); using Leco CNS analyser, Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) to determine the concentrations of major and trace elements such as Aluminium (Al), Barium (Ba), Calcium (Ca), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Nickel (Ni), Phosphorus (P), Sulphur (S), Strontium (Sr), Titanium (Ti), Zinc (Zn), Vanadium (V), Chromium (Cr), Cobalt (Co), Copper (Cu), Arsenic (As), Gold (Au), Mercury (Hg), Lead (Pb), Uranium (U), carbon (C) and ash content. The CRM was used to validate the two analytical methods.

There was variation in the concentrations of nutrients measured. There were no significant differences noted in the metal/loids concentrations between all the *Eucalyptus*

species studied ($p>0.05$). Variation between sites could not be determined as there were no replicates available to perform the comparison. The World Health Organisation (WHO) maximum permissible level (MPL) in plants for arsenic (As) is 1 mg/kg. The MPL was excedded in all tissues of all three *Eucalyptus* species studied. Arsenic concentrations of 5.09, 4.36 and 5.48 mg/kg were found in the wood of *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. A risk assessment performed found that there was no evidence that there will be adverse effects caused by supplying fuelwood from these contaminated *Eucalyptus* trees. Even though high arsenic concentrations were recorded in this study, if the wood is used as fertilizer in a vegetable bed, the transfer of the arsenic to the common vegetables is below the daily oral reference dose. The general trend in the concentration of metals and metalloids in different plant tissues was in the order of leaves > bark > branches/twigs > wood. The results of the biomass exposure assessment showed that the exposure through use of the ash as fertiliser was lower than the oral reference dose for Mn, Fe, Ba and As. The biomass risk assessment showed that the best-performing tree, in terms of wood production on AMD, was the *E. camaldulensis*. The risk of other metal/loids was not evaluated as there was no good agreement between the results recorded with those certified of the CRM. It is suspected that the CRM used was old.

ACKNOWLEDGEMENTS

This study was supported by NRF THRIP funding (project TP13080525596 "Re-valuation of gold and uranium mine land and hazardous waste") to Ed Witkowski and Isabel Weiersbye; and AngloGold Ashanti (AGA) Ltd South Africa funding to Isabel Weiersbye for the Ecological Engineering and Phytotechnology Programme (EEPP): Mine Woodlands Project at the School of Animal, Plant and Environmental Sciences (AP&ES) at the University of the Witwatersrand. We are grateful to the Environmental Management and Land Management Departments of AGA for facilitating the Mine Woodlands Project, and supporting the research and risk assessments in order to optimise phytotechnologies for pollution control and land re-valuation. I would like to extend my heartfelt appreciation to the EEPP staff and Nelson Mandela Metropolitan University (NMMU) students that assisted me during sample collection; Desmond Misselhorn, Leon Hoenderdos, Lorato Letsholo, Jacob Mahlangu (Oom J), Mthokozisi Mbazana and Linda Mkhumbeni.

Thank you to Innocent Rabohale who assisted with sample preparation, milling and arranging for sample analysis and to Dr Julien Lusilao and Siyethemba Mabaso of the School of Chemistry who undertook the ICP-OES and ICP-MS analyses.

To Prof Luke Chimuka, thank you for your support, patience and kindness through all my struggles over the years. To Isabel Weiersbye, thank you for giving me the opportunity to complete the Masters Degree course and for making this all possible. To Dr Peter Dye, thank you for all the support since sample collection to date and for taking my numerous phone calls (some at inconvenient times).

To all my colleagues at South32 SA Limited especially Lindie Moore and Clinton Lee for their support, giving me hope and allowing me time off in the past few months, knowing that things were stressful at work. Lastly, to my family and friends, especially my husband Orapeleng, who have been my pillars of strength through a very difficult time in our lives and for giving me hope. THANK YOU. God bless you all.

TABLE OF CONTENTS

DECLARATION II

ABSTRACT III

ACKNOWLEDGEMENTS V

LIST OF FIGURES VIII

LIST OF TABLES X

LIST OF ACRONYMS XII

LIST OF ELEMENTS XV

CHAPTER 1: INTRODUCTION 1

1.1 General Overview 1

1.2 Problem statement 2

1.3 Motivation 3

CHAPTER 2: LITERATURE REVIEW 4

2.1 Origin and characteristics of *Eucalyptus* species 4

2.2 Bio-energy plantations 5

2.3 Phytoremediation 6

2.3.1 Trees in phytoremediation 7

2.3.2 Nutrient uptake by plants and their effects on human health 11

2.3.3 Metal and metalloid uptake by plants and their effects on human health 14

2.3.4 The Mine Woodlands Project (MWP) 19

2.3.5 Economic opportunities for Eucalyptus trees under the MWP 23

2.4 Risk assessment of *Eucalyptus* trees grown on acid mine drainage for use as fuelwood 24

CHAPTER 3: MAIN AIMS AND SPECIFIC OBJECTIVES 27

3.1 Main Aims 27

3.2 Specific Objectives 27

3.3 Research Questions 27

CHAPTER 4: MATERIALS AND METHODS 28

4.1 Site Description 28

4.1.1 Rainfall *28*
4.1.2 Soils *35*
4.1.3 Groundwater *38*
4.2 STUDY SPECIES 40
4.3 EXPERIMENTAL DESIGN 41
4.4 SAMPLING PROCEDURE 41
4.5 SAMPLE PREPARATION FOR CHEMICAL ANALYSIS 42
4.6 RISK ASSESSMENT METHODOLOGY 43
4.7 CALCULATIONS 43
4.8 DATA ANALYSIS/ STATISTICS 45
CHAPTER 5: RESULTS AND DISCUSSION **47**
5.1 RESULTS 47
5.1.1 Nutrients *49*
5.1.2 Metals and Metalloids *51*
5.1.3 Carbon and Ash content *54*
5.1.4 Amount of nutrients, metals and metalloids per tree *54*
5.2 DISCUSSION 56
5.2.1 Nutrients *56*
5.2.2 Metals and Metalloids *56*
5.2.3 Amount of nutrients, metals and metalloids per tree *58*
5.2.4 Comparison to maximum permissible levels *58*
5.2.5 Risk Assessment *59*
CONCLUSION AND RECOMMENDATIONS 63
REFERENCES **65**
APPENDIX A **91**
APPENDIX B **95**
APPENDIX C **101**
APPENDIX D **108**
APPENDIX E **113**
APPENDIX F **119**
APPENDIX G **121**

LIST OF FIGURES

Figure 1: Location of the Mine Woodlands Project trials (Map from Weiersbye, *et al.*, 2006) 20
Figure 2: Conceptual model of woodlands plantation comprising plants like *Eucalyptus* to control the seepage and groundwater contamination by acid mine drainage in the Witwatersrand Basin (AngloGold Ashanti Report to Society, 2004). 23
Figure 3: The 4-step Risk Assessment Process (USEPA, 1991) 26
Figure 4: Map of South Africa showing the AGA's Vaal River operation and West Wits operation. 29
Figure 5: Location of the WW's Madala and Red Soil sites 30
Figure 6: Location of woodlands trials at the VR Mispah site 30
Figure 7: Long-term mean monthly rainfall for AGA's VR operation near Klerksdorp (Herbert, 2008). 31
Figure 8: Mean monthly minimum and maximum temperature for AGA's VR operation near Klerksdorp (Herbert, 2008). 32
Figure 9: Long-term mean monthly rainfall for AGA's WW operation near Carletonville (Herbert, 2008). 33
Figure 10: Mean monthly minimum and maximum temperature for AGA's WW operation near Carletonville (Zuurbekom station between Soweto and Carletonville) (Herbert, 2008). 34
Figure 11: Soil Map for Vaal River Mine (WSP, 2009a) 36
Figure 12: Soil Map for WW's Red Soil site (McLeroth, 2015) 37
Figure 13: Soil Map for WW's Madala site (McLeroth, 2015) 37
Figure 14: Vaal River South Mass Model from 2007 (TDS in mg/L) (WSP, 2009a) 38
Figure 15: Interpolated groundwater elevation and flow direction (WSP, 2009b) 39
Figure 16: Interpolated groundwater TDS concentrations (WSP, 2009b) 39
Figure 17: Typical examples of three *Eucalyptus* species; *E. camaldulensis, E. dunnii, E. grandis x camaldulensis hybrid.* 41
Figure 18: Comparison of nutrients Ca (left) and S (right) concentration in wood averaged across all three *Eucalyptus* species. 50
Figure 19: Comparison of nutrients Ca (left) and S (right) concentration in all tissues averaged across all three *Eucalyptus* species. 50
Figure 20: Comparison of metal/loids Mn (top left), Fe (top right), Ba (bottom left) and As (bottom right) concentration in wood across *Eucalyptus* species. 52

Figure 21: Comparison of metal/loids Mg (top left), Fe (top right), Ba (bottom left) and As (bottom right) concentration in all tissues averaged across all three *Eucalyptus* species.....53
Figure 22: Comparison of metal/loids to the WHO maximum permissible levels.....59

LIST OF TABLES

Table 1: Example of *Salix* species with potential for phytoextraction of various metals 7
Table 2: Examples of plants with potential for phytoextraction of various metals 9
Table 3: Essential nutrients for plant growth, their principal forms for uptake and typical concentrations 12
Table 4: Normal range of metal and metalloid concentrations in plants 17
Table 5: WHO Maximum Permissible Limits (MPL) of metal/loids in plants 18
Table 6: Critical toxicity levels and thresholds for hyperaccumulators 19
Table 7: Annual volume increment in *Eucalyptus* plantations in different parts of the world (Adapted from FAO, 2001) 21
Table 8: Coefficients for the estimation of standing tree volume using the Schumacher and Hall model (Bredenkamp, 2000) 44
Table 9: Standard industry conversion factors for roundwood 45
Table 10: CRM agreements to measured values 47
Table 11: Statistical comparison of concentrations of metals in three *Eucalyptus* species Tukey HSD, The hypothesis where H_0: $v_1=v_2=v_3$and H_1: $v_1\neq v_2$/ $v_2\neq v_3$/ $v_1\neq v_3$. 48
Table 12: Calculated wood volume, wood mass and content of nutrient, metal or metalloid per tree 55
Table 13: Average content per site (species combined) 55
Table 14: Average content of nutrients, metals and metalloids in *Eucalyptus* species consumed and available in ash per household per day (assuming no volatilisation during combustion) 58
Table 15: Transfer Factor from soil to vegetables (Jolly *et al.*, 2013) 60
Table 16: Average concentration of metal and metalloids in typical vegetables found in gardens 60
Table 17: Daily intake of metal/metalloids per *Eucalyptus* species 61
Table 18: Risk Assessment 62
Table 19: Mean concentrations of phosphorus, potassium and % nitrogen for wood from three *Eucalyptus* species ± standard deviation 114
Table 20: Mean concentrations of phosphorus, potassium and % nitrogen for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 114
Table 21: Mean concentrations of calcium, sulphur and magnesium for wood from three *Eucalyptus* species ± standard deviation 115

Table 22: Mean concentrations of calcium, magnesium and sulphur for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 115
Table 23: Mean concentrations in mg/kg of manganese, iron, zinc, copper and nickel for wood from three *Eucalyptus* species ± standard deviation 116
Table 24: Mean concentrations of manganese, iron, zinc, copper and nickel for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 116
Table 25: Mean concentrations of chromium, lead and arsenic for wood from three *Eucalyptus* species ± standard deviation 117
Table 26: Mean concentrations of chromium, lead and arsenic for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 117
Table 27: Mean % dry mass of carbon and ash content for wood from three *Eucalyptus* species ± standard deviation 118
Table 28: Mean % dry mass of carbon and ash content for leaves, branches/twigs, bark and wood from three *Eucalyptus* species 118

LIST OF ACRONYMS

Acronym	Description
AGA	AngloGold Ashanti
AMD	Acid Mine Drainage
ANOVA	Analysis of Variance
AP&ES	Animal, Plant and Environmental Sciences
DME	Department of Minerals and Energy
EEPP	Ecological Engineering and Phytotechnology Programme
FAO	Food and Agriculture Organisation of the United Nations
HSD	Honest Significant Difference
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ITRC	Interstate Technology & Regulatory Council
MAI	Mean Annual Increment
MWP	Mine Woodlands Project
MPL	Maximum Permissible Level
NMMU	Nelson Mandela Metropolitan University
RSD	Relative Standard Deviation
SE	Standard Error
TDS	Total Dissolved Solids
TSF	Tailings Storage Facility
VR	Vaal River

Acronym	Description
AGA	AngloGold Ashanti
WHO	World Health Organisation
Wits	University of the Witwatersrand
WW	West Wits

LIST OF ELEMENTS

Element	**Name**
Al	Aluminium
As	Arsenic
B	Boron
Ba	Barium
C	Carbon
Ca	Calcium
Cl	Chlorine
Co	Cobalt
Cr	Chromium
Cu	Copper
Fe	Iron
K	Potassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
Ni	Nickel
P	Phosphorus

Element	Name
Pb	Lead
S	Sulphur
Si	Silicon
Zn	Zinc

CHAPTER 1: INTRODUCTION

1.1 General Overview

According to the South African Department of Tourism, Economic and Environmental Affairs (2002) and the Chamber of Mines of South Africa (2004), South Africa's mining industry generates a lot of mine waste, often stored in tailings storage facilities (TSFs), with gold mines in the Witwatersrand Basin accounting for 30 percent of this waste per annum. The gold and uranium mines in the Witwatersrand Basin area have waste dumps accommodating approximately 6 billion tonnes of gold tailings (Robb and Robb, 1998a; b; Wymer, 2001) and contain an estimated 30 million tonnes of sulphur (Witkowski and Weiersbye, 1998). The sulphide material produced after gold and uranium mining is often associated with pyrite material, which when exposed to air and water causes acid mine drainage (AMD) (Akcil and Koldas, 2006).

Releases of AMD are associated with elevated levels of sulphates, chlorides and inorganic contaminants such as metals (iron, aluminium and manganese), and are characterized by low pH and high electrical conductivity (Winde *et al.*, 2004 a; b; c; Akcil and Koldas, 2006). Acid mine drainage has the potential to contaminate surface- and groundwater, and soils, which could result in the disruption of the growth and reproduction of both aquatic and terrestrial flora and fauna and potentially impact human health (Akcil and Koldas, 2006; Nengovhela *et al.*, 2006). In order to ensure that the mining companies around the Witwatersrand Basin Goldfields obtain their closure certificates, they need to ensure that the area is rehabilitated (including ensuring that the AMD is controlled). It is costly to install pumps to extract contaminated groundwater and then treat the water to reduce contaminants to acceptable levels (Vivier, 2005). A less costly and more sustainable solution lies in introducing technologies such as phytoremediation to control the spread of the AMD and immobilize or extract contaminants. The use of trees for this purpose is particularly attractive, owing to their deep root systems, potentially high water use and high biomass (Dye *et al.*, 2008a).

Gold TSFs provide suitable environments for some plant species. There were 286 indigenous and 90 alien species that have been identified to survive and grow on and around gold TSFs in the Witwatersrand Basin (Weiersbye *et al.*, 2006). Several research studies have been conducted on tolerant indigenous trees (Weiersbye, 2007; Weiersbye and Witkowski, 2007) and also on the growth of exotic plants used for phytoremediation on and around these TSFs (Weiersbye *et al.*, 2006a; b; Dye and Weiersbye, 2010). Trees can be particularly useful for phytoremediation because they have deep roots, high biomass and mostly stay evergreen

throughout the seasons (Dye, 2010). Plant biomass can be an important product in some areas and is used to generate energy for households or industry (Jain, 1994; van den Broek *et al.*, 1996; Sims *et al.*, 1999; Abbasi and Abbasi, 2010). *Eucalyptus* species are particularly useful because growth is fast and the wood burns well (Smit and Meincken, 2012). There are, however, some risks to using wood as a source of fuel (Lyons *et al.*, 1985; van Horen *et al.* 1996; Marufu *et al.*, 1997; Estrellan and Iino, 2010). Some of these risks are air pollution, health risks and environmental degradation. During wood combustion, carbon dioxide and water are released as by products. However, combustion is not always complete in practice, as small amounts of hydrocarbons, carbon monoxide and other gases may be released (Lyons *et al.*, 1985) which lead to air pollution. In this study, the elemental uptake by wood, leaves, bark and branches/twigs of *Eucalyptus* trees grown in site-species trials on tailings affected by AMD for eight years was determined, and a risk assessment conducted to determine the safeness of using this contaminated wood as a fuel source.

1.2 Problem statement

When trees have reached physiological maturity, they may be cut and used as fuelwood by the community or industry. These trees will have accumulated metal/loids, such as chromium, arsenic, copper, lead and other chemicals that are a danger to soil, water, air and people when the wood is burnt. In developing a risk management strategy, it is important that we investigate the amount of metal/loids that accumulate in each of the tree species used in the study. After the wood is used for fire, it has become common for ash to be used by religious churches for medicinal purposes. Peltzer (1999) indicated that for medicinal purposes, the African traditional Pentecostal churches in South Africa use ash from firewood for healing. This indicates the importance of ash from firewood among African traditional believers; however, ash from firewood containing metal/loids such as chromium, arsenic and lead can be dangerous depending on the quantity applied. Ash is also used as organic fertilizer in agriculture and sometimes on the soil where fruits and vegetables that are normally eaten without being cooked first are grown i.e. tomatoes (Pradhan *et al.*, 2009). So when ash is used as fertilizer, it is returned to the soil. The problem starts when chemicals and metal/loids in firewood from trees used for remediation of contaminated gold fields are not accounted for. To ensure the health and safety of the end users of the firewood from trees that were previously used in phytoremediation, and have accumulated a certain quantity of environmentally dangerous chemicals, it is important to measure the amount of metal/loids that have accumulated in the trees.

1.3 Motivation

It is important to note that contamination of agricultural land and groundwater by metal/loids is essentially linked to human activities. A major problem with metal/loids is that they cannot be biodegraded, and therefore reside in the environment for long periods of time if they are not removed. Thus, depending on the kind of depth of contamination, different remediation techniques are normally developed; as in this study where *Eucalyptus* tree species are planted as part of the remediation strategy. One of these methods which was used in this study is phytoremediation. It utilizes the ability of hyperaccumulating plants to extract high amounts of metal/loids and also may bring about hydraulic control, to prevent the spread of contaminated water. Accumulation of metal/loids in the environment is a serious concern for animal and human health. When the trees have matured, the stems might be cut down and used as a fuel source by the community or industry. Therefore, for the safety of people and the environment, it is important to investigate the risk of having these trees used, by assessing the amount of metal/loids, including arsenic, nickel, chromium, lead, sulphur, and other metals that accumulated in the stem wood, leaves, barks, twigs and branches. The risk assessment will also assist in choosing, from among the trees used for this study, the one that is the most benign wood for combustion.

CHAPTER 2: LITERATURE REVIEW

2.1 Origin and characteristics of *Eucalyptus* species

Eucalyptus trees are native to Australia but are grown in many parts of the world with a similar climate (Prosser, and Williams, 1998). *Eucalyptus* falls within the *Myrtaceae* family with 800 species recorded in the genus (Meskimen and Francis, 2012).

The *Eucalyptus* tree species are fast growing trees, such that when they are planted on suitable sites and managed properly, they have high potential for producing commercial products such as pulpwood, mulch, fuelwood and even for remediation of some environmental problems (Djavanchir and Mossadegh, 1973; Abo-Hassan, *et al.*, 1988). In addition, some *Eucalyptus* tree species are resistant to salinity and can tolerate a wide range of soil pH and soil types (Fine *et al.*, 2014). This study assessed the above ground biomass of *Eucalyptus* species cultivated on AMD for fuelwood use. It is important to understand the tolerance of *Eucalyptus* species to high concentrations of metal/loids, and their effects on wood and other aboveground biomass (Assareh *et al.*, 1997).

Another advantage of using trees is that, trees often have deep root systems to help them access groundwater during dry seasons. The best and most feasible combination for acid mine drainage and heavy metal control around the TSFs in the Witwatersrand Basin could be the use of hyperaccumulators such as *Tamarix* to uptake contaminants and the use of high water use trees such as *Eucalypts* for hydraulic control (Bouwer, 2000; Medhurst *et al.*, 2002). This, according to Pulford and Dickinson (2006), is because;

i. Trees have a high root and stem biomass which creates a large sink for phytoextraction and contaminant sequestration;
ii. Some trees like *Eucalyptus* spp. are fast growing;
iii. Silviculture, which is the application of the principles of forest ecology to a stand of trees to help meet specified objectives, is an already established practice and can easily be integrated into phytoremediation programs;
iv. Wood and slash – leaves and twigs from trees can be used as a bio-energy source, and,
v. Trees have high root biomass which can assist in binding soils, reducing erosion.

2.2 Bio-energy plantations

The use of fast growing trees as a source of fuelwood in some areas in South Africa could be beneficial for communities that sustain their livelihoods through this option (Dovie et al, 2005; Shackleton *et al*, 2007). The use of *Eucalyptus* trees could be of particular advantage since they grow fast and have denser wood than willow (when grown under short rotation coppices), which aids in slow burning (Leslie *et al.*, 2012). *Eucalyptus* trees could produce high biomass whilst creating a large water and nutrient demand, characteristics which can be utilized in the phytoremediation of AMD and heavy metal polluted soils (Rockwood *et al.*, 2001). The woody biomass, in the form of wood chips can then be used with coal for energy production (Rockwood *et al.*, 2001). South Africa is one country that has a high biomass resource and promotes renewable energy through the use of biofuels as an alternative energy source, resulting in approximately 13.6% of South Africa's energy supply being met by biomass (DME, 2002). However, the global contribution of South Africa in biofuel production is less than 0.01% (US EIA, 2013).

The use of wood as an alternative energy source has a relatively lower cost than fossil fuels, especially when carbon tax is included (Bennett, 2003). More than 70% of people living in rural villages in sub Saharan Africa still depend on wood as their primary fuel source (Matsika *et al.*, 2013). In South Africa, over 80% of households in rural villages use fuelwood to meet their energy needs, even with the availability of electricity (Banks *et al.*, 1996; Williams and Shackleton, 2002; Madubansi and Shackleton, 2006; Shackleton *et al.*, 2007; Vasicek and Gaugris, 2014). However, the overall use of wood has decreased in South Africa from 2002 to 2013 from 19.3% to 10.5% (Stats SA, 2013).

Woody biomass is also a carbon neutral fuel as the quantity of carbon released to the atmosphere during combustion is equal to the quantity removed from the atmosphere during plant growth stage (Rockwood *et al.*, 2001). All fuels used in industry regardless of their fuel type should comply with clean air standards and regulations. During combustion, carbon dioxide and water are released; however, oxidation sometimes is incomplete, which leads to releases of NOx, SO_2, CO and other hydrocarbons (Lyons *et al.*, 1985).

In some countries, *Eucalyptus* trees have the potential to be grown successfully as bioenergy plantations on polluted ground-water using methods to optimize biomass productivity (Madgwick *et al.*, 1990). Studies in South Africa on tree trials on polluted groundwater over the last decade demonstrated that at least seven *Eucalyptus* species or hybrid clones are tolerant to AMD conditions and produce adequate biomass for poles or bioenergy (CSIR, 2010). The conditions presented by AMD polluted groundwater around the base of

TSFs may influence the feasibility and safety of wood fuel production, and this has generated several research questions that need to be answered, which some are answered by this study.

2.3 Phytoremediation

Worldwide, fuelwood demands, soil and groundwater contamination, and agriculture's impact on nature are growing concerns (Hazell *et al.*, 2008). Human activities, such as mining, have continuously increased the level of heavy metal ions circulating in the environment (Ma and Rao, 1997). Fast growing trees in short rotation woody crop systems may increasingly meet societal needs ranging from renewable energy to environmental mitigation and remediation (Rockwood *et al.*, 2004). Most importantly, the use of short rotation woody crop system was reported to have potential to remediate contaminated soil and groundwater (Mulligan *et al.*, 2001; Susarla *et al.*, 2002). Such woody crops include some *Eucalyptus* species, which have relatively low contaminant concentrations (Mulligan *et al.*, 2001). Other tree species that have been found to be successful in phytoremediation because of their fast growing characteristics are hybrids of willow (*Salix*) and poplar (*Populus*) (ITRC, 2009). Some of the studies that have been conducted using willow for phytoremediation have been listed under **Table 1**.

Several engineering methods were developed with the intention to contain and treat contaminated soils (Smith *et al.*, 1995), but these methods were proven to be costly (Rockwood *et al.*, 2001). For example, non-biological technologies e.g. excavation and land-filling, used to decontaminate soils range between US$100 000 to US$3 million per ha (Weiersbye, 2007). The total cost for rehabilitating abandoned mines using engineering measures in South Africa was estimated to be approximately US$ 14 billion (DME, 2007). Phytoremediation is the use of plants for environmental clean-up. Phytoremediation offers a slow but relatively inexpensive and sustainable way for stabilizing, containing or eradicating AMD (Weiersbye, 2007). This is done through hydraulic control of groundwater by short rotation woody crop systems (*Eucalyptus* species) and by the different plant defence mechanisms to defend against toxic conditions associated with AMD and other toxins (Larcher, 2003). When a plant is confronted with toxic conditions it can accumulate them in the root, leaf or stem (Harborne, 1993). Important to the control of AMD, specifically in the Witwatersrand mining region, are three broad phytoremediation approaches. These include **phytoextraction** of contaminants from soils, waste or water. This approach relies on plants that accumulate contaminants or metals in their biomass, and this can be controlled by cropping the plants (Lasat, 2002). **Phytostabilization** of contaminants within soil relies on the plant root's ability to hydraulically

control and also sequester contaminants. **Phytodegradation** relies on the ability of plants to convert ions like phosphate to substances which are less toxic like proteins in leaves (Weiersbye, 2007).

Table 1: Example of *Salix* species with potential for phytoextraction of various metals

Metal	Plant species	Conc. mg/kg	Substrate	Reference
Cu	*Salix caprea* - foliage and woody branches	681	3-year old trees on highly contaminated soil	Dickinson, 2000
Zn	*Salix* (4 spp)- stems	95-156	Heavy sludge applications	Riddell-Black, 1994
Pb	*Salix*- wood	157.4	Metal contaminated soil	Dickinson, 1997
Ni	*Salix* (2 spp) - leaves	<20	Sludge application	Labrecque *et al.*, 1995
Hg	*Salix* (2 spp) - leaves	<20	Sludge application	Labrecque *et al.*, 1995

2.3.1 Trees in phytoremediation

Use of trees in phytoremediation started since the early 1990's because trees can clean up both organic and inorganic contaminants and produce woodlands at the same time (Gansauer, 2012; Peuke and Rennenberg, 2005). The potential use of trees as a suitable vegetation cover for heavy metal-contaminated land has received increasing attention over the last 15 years (Aronsson and Perttu, 1994; Glimmerveen, 1996). Trees were suggested as a low-cost, sustainable and ecologically sound solution to the remediation of heavy metal-contaminated land (Dickinson, 2000), especially when it was uneconomic to use other treatments or when there was a time pressure on the reuse of the land (Riddell-Black, 1994).

Exploitation of metal uptake into plant biomass as a method of soil decontamination is limited by plant productivity and the concentrations of metals achieved (Baker *et al.*, 1994). Some tree species have an advantage when used for phytoremediation as they grow fast and thus have a higher biomass which means that they can sequester much more contaminants than shrubs, grasses and forbs (Rockwood *et al.*, 2004). Many plants, including trees, shrubs, herbs, forbs and grasses, can be potentially useful in phytoremediation when site conditions are not too harsh, but hyperaccumulators and high biomass plants like trees are the most favoured (Rockwood *et al.*, 2001). Some of the herbaceous plants that have been found to have the potential to extract some metals are indicated in **Table 2** below.

Hyperaccumulators are plants that are able to take up and tolerate very high concentrations of metal/loids and other contaminants e.g. sulphates (Pulford and Dickinson, 2006). These include trees such as the *Tamarix* species (Dennis, 2008). Some tree species also use much water, remain green and develop frost tolerant canopies (Leslie *et al.*, 2012).

Table 2: Examples of plants with potential for phytoextraction of various metals

Metal	Plant species	Reference
Cr (IV)	*Brassica juncea* (L.) Czern	Kumar *et al.*, 1995a; Huang *et al.*, 1997a
Cu	*Brassica juncea*	Ebbs and Kochian, 1997
Ni	*Brassica juncea*	Ebbs and Kochian, 1997
Pb	*B. campetris* L.; *B. carinata* A. Br.; *B. juncea*; *B. napus* L.; *B. nigra* (L.) Koch.; *Helianthus annuus* L.; *Pisum sativum* L.; *Zea mays* L	Begonia *et al.*, 1998; Blaylock *et al.*, 1997; Ebbs and Kochian, 1998
Zn	*Avena sativa*; *B. juncea*; *B. napus* L. *Hordeum vulgare*, *B. rapa*	Ebbs *et al.*, 1997; Ebbs and Kochian, 1998
Cd	*Brassica juncea*	Kumar *et al.*, 1995a; Huang *et al.*, 1997a; Ebbs *et al.*, 1997; Salt *et al.*, 1995b

Trees are faced with harsh and challenging environmental conditions when grown on AMD and heavy metal polluted substrates on TSFs (Sheoran and Sheoran, 2006). Many trees were found to survive in highly saline conditions and therefore decontaminate the polluted groundwater (van der Moezel *et al.*, 1988; 1989; 1991; Marcar, 1993; Sun and Dickinson, 1995; Mahmood *et al.*, 2001; Duggan, 2005; Feikema and Baker, 2011). Although some plants are able to tolerate and survive on and around gold TSFs in the Witwatersrand Basin (Weiersbye *et al.*, 2006), site conditions are complex due to variability of contaminant composition, soil depths, soil water contents, and depth to water table. The different soil

substrates can influence the pH, redox potential, organic matter content, and the forms and concentrations of metal/loids which in turn can impact on trees (Pulford and Dickinson, 2006).

While in this study we used high water use plants to control the spread of metal/loids from the environment, it should be noted that some metal/loids can exist in more than one oxidation state. Not all oxidation states of metals are available for plant uptake (Sparks, 2003; Brady and Weil, 2008). Forms of metal/loids available for plant uptake are those forms that are water soluble and mobile in the plant environment. Dube *et al.* (2001), Cao *et al.* (2009) and Meers *et al.* (2009) have also emphasized that the mobility of metal/loids in the soil is the driving force for heavy metal uptake by plants whereas the mobility of metal/loids is dependent upon their solubility in the soil solution. As a result, non-soluble metal/loids are unlikely to be available for plant uptake.

Nevertheless, the availability and subsequently the toxicity of metal/loids to plants can also be affected by several factors such as adsorption and desorption potential of metal/loids to and from the soil mineral surface, precipitation potential of metal/loids and the dissolution potential of minerals containing and/or adsorbing metal/loids (Menzies *et al.*, 2007).

In this study, the amount of metal/loids and minerals absorbed from the soil/environment by the plant is not all that was actually in the soil. That is because some of these metal/loids and minerals are in a form that cannot be taken up by plants. According to Brown *et al.* (1999) the type of a complex that a particular element forms with the mineral surface plays an important role in the mobility and therefore availability of metal/loids to plants in the soil. There are some heavy metal ions that form inner-sphere complexes with some particular mineral surfaces. Such metal/loids are difficult to be desorbed under normal conditions and as a result are relatively immobile. On the other hand, if the heavy metal ion forms an outer-sphere complex with mineral surfaces, it can be easily desorbed and it can be available to plants. The type of the resulting complex is actually the function of both the form with which a heavy metal exist and the type of mineral adsorbing that particular heavy metal.

Studies conducted by Hunter (2001) in southern India showed that even though *Eucalyptus* trees generally accumulate low concentrations of nutrients like nitrogen, phosphorus, potassium, calcium and magnesium compared to indigenous trees like *Dalbergia sissoo*, they can potentially contain high overall amounts of these elements due to their high biomass.

2.3.2 Nutrient uptake by plants and their effects on human health

Plants need nutrients to survive and grow. A criterion by Arnon and Stout (1939) specifies which elements are identified as essential. These criteria are:

- A deficiency of an essential nutrient makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle;
- Such deficiency is specific to the element in question and can be prevented or corrected only by supplying this element; and:
- The element is involved directly in the nutrition of the plant quite apart from its possible effects in correcting some unfavourable microbiological or chemical condition of the soil or other culture medium.

Thirteen of these nutrients are essential inorganic constituents which are N, P, K, Ca, Mg, S, Fe, Cl, Zn, Mn, Cu, B, and Mo (Mengel *et al.*, 2001; ITRC, 2009). These are taken up from the soil through the roots. Nutrients are further classified by Mengel *et al.*, (2001) as primary macronutrients (N, P and K), secondary macronutrients (Ca, Mg and S) and micronutrients/trace elements (Zn, Fe, Cl, Mn, Cu, B and Mo). Sometimes Co and Ni are considered essential to some plants (Mengel *et al.*, 2001).

The principle forms of uptake in plants (Mengel and Kirkby, 1987) and their typical concentrations (Epstein, 1965; Brown *et al.*, 1978b; Epstein and Bloom, 2005) are indicated in the **Table 3** below for all the above mentioned essential nutrients.

Table 3: Essential nutrients for plant growth, their principal forms for uptake and typical concentrations

Nutrient	Principal forms of uptake	Typical concentrations in plants (mg/kg dry weight)
N	NH_4^+, NO^{3-}	15 000
P	H_2PO^-, HPO_4^{2-}	2 000
K	K^+	10 000
Ca	Ca^{2+}	5 000
Mg	Mg^{2+}	2 000
S	SO_4^{2-}, SO_2	1 000
Fe	Fe^{2+}	100
Mn	Mn^{2+}	50
B	$B(OH)_3$	20
Zn	Zn^{2+}	20
Cu	Cu^{2+}	6
Mo	MoO_2^{4-}	0.1
Cl	Cl^-	100
Ni	Ni^{2+}	0.1

Woody plants such as *Eucalyptus nitens* prefer to take up nitrogen in the form of NH_4^+ (Garnett and Smethhurst, 1999; Garnett *et al.*, 2003). N is essential in plants as it forms part of nucleic acids and proteins which are needed for plant growth (OECD, 2014). An excess of nitrogen in plants may cause an overgrowth of leaves and net primary productivity, but it may also cause long-term declines in the latter (Aber *et al.*, 1989; 1995). When combusted, wood

containing high levels of N releases two primary harmful gases which are nitrogen dioxide (NO_2) and nitric oxide (NO) (WHO, 2010). Higher exposure (>0.053ppm/yr for NO_2 and >5ppm/15min for NO) to these gases can cause damage to respiratory airways (WHO, 2010). The nitrogen is not expected to be different in gold TSFs than in normal conditions as there is no fertiliser being added currently in the study plots.

Phosphorus in plants occurs in nucleic acids and in ATP and aids in plant growth (OECD, 2014). According to Marschner (1995) and Hawkins *et al.* (2008), P toxicity symptoms in plants are characterised by stunted growth, accelerated leaf senescence and brown-grey necrosis on young leaves.

Potassium in plants is an essential nutrient involved in enzyme activation, protein synthesis, osmoregulation, stomatal opening and closing, photosynthesis and cell growth (Pallardy, 2008).

Calcium (Ca) occurs in large amounts in plants and is responsible for cell wall elasticity, N metabolism and enzyme activation (Pallardy, 2008). Ca uptake in plants is in the form of insoluble Ca^{2+} through root permeable channels and stored in large vacuoles of cells (Maathuis, 2009).

Magnesium (Mg) is an essential part of the chlorophyll molecule in plants (Maathuis, 2009). Mg^{2+} is up-taken from soil and plays a major role as an enzyme cofactor and in energy transfer (Maathuis, 2009). A deficiency in Mg results in chlorosis (Pallardy, 2008)..

Sulphur (S) forms part of most amino acids. It is responsible for protein synthesis in plants (Hänsch and Mendel, 2009). S is mostly present in soils as sulphate (SO_4^{2-}) ion (Maathuis, 2009). This is how it is taken up by the roots of the plant. S toxicity can occur in saline soils with high concentrations of sulphates (Maathuis, 2009).

Zinc (Zn) is essential for enzymes responsible for protein synthesis and energy production (Hänsch and Mendel, 2009). It is mainly taken up in the form of Zn^{2+} ion from the soil (Hänsch and Mendel, 2009). In severe Zn deficiency cases, necrotic areas appear on the leaf surface (Pallardy, 2007).

Copper (Cu) is important in plant growth as it is essential for "photosynthesis and mitochondrial respiration, carbon and nitrogen metabolism, oxidative stress protection and cell wall synthesis" (Hänsch and Mendel, 2009). It normally occurs in two oxidation states Cu^{2+} and Cu^{+}, which allows it to act as both a reducing and oxidative agent that can become toxic to the plant (Hänsch and Mendel, 2009). Copper, when taken up by the plant, therefore binds to proteins to prevent the toxicity elevation in the plant tissues (Hänsch and Mendel, 2009).

Copper deficiency can be identified by wilting new leaves, terminal shoot dieback and total necrosis and collapse of shoot (Snowball and Robson, 1991).

Iron (Fe) has a similar function to copper, but added to its function is also nitrogen assimilation and hormone synthesis (Hänsch and Mendel, 2009). It is very intimately linked to the metabolism of copper. Fe exists in two oxidation states in soil, ferric (Fe^{3+}) and ferrous (Fe^{2+}), which are not equally available to plants (Pallardy, 2008). Plants predominantly prefer to assimilate Fe^{3+} (Pallardy, 2008).

Potassium, calcium, zinc, copper, iron and magnesium all form oxides when combusted which cling to particulate matter which is airborne or remain in the ash (Dare *et al.*, 2001). Some particulate matter (roughly 80%) released from burning wood can be as small as less than one micron (≤1 μm) and is therefore respirable and can cause serious respiratory diseases (Smith, 1987; NARSTO, 2004). Sulphur when combusted forms sulphur dioxide which is a gas that causes several respiratory illnesses (USEPA, 2015).

2.3.3 Metal and metalloid uptake by plants and their effects on human health

Through phytoabstraction to exert hydraulic control on AMD-contaminated groundwater and soils, *Eucalyptus* trees may be able to accumulate metals to potentially toxic levels, and thus pose a threat to end users. Whether these *Eucalyptus* trees do accumulate metals or metalloids, and to what extent, is the subject of this study. This also presents an opportunity (i.e. helping to decontaminate polluted soil and water by phytoremediation, or "biomining" of useful metals) and it also presents a risk to the use of the *Eucalyptus* products that need to be managed, if the concentrations of metals exceed harmful levels. Some of the metals and metalloids of concern are arsenic, lead, uranium and chromium as they are dangerous to human health (Rauf *et al.*, 2011) and are also known to occur at concentrations above the normal surface geochemical background concentrations in gold tailings dams and the surrounding contaminated soils on the Witwatersrand Basin (Witkowski and Weiersbye, 1998).

According to the US Agency for toxic substances and disease registry, arsenic is listed as the Number 1 hazardous substance (US Department of Health and Human Services, 1999). Arsenic can be hazardous for humans and animals when ingested (Hajar *et al.*, 2014). Arsenic uptake in plants is influenced by arsenic concentration in the soil (Marin et al., 1992, 1993b; Xie and Huang, 1998; Rauf *et al.*, 2011). Arsenic species are bioactive and toxic. Long-term exposure to low concentrations of arsenic in drinking water can lead to skin, bladder, lung and prostate cancer (Zhang *et al.*, 2002). The prolonged ingestion of soluble arsenic is said to cause

peripheral neuropathy, gastrointestinal symptoms, diabetes, renal system effects, cardiovascular disease and cancer (WHO, 2010).

Chromium is a non-essential metal in plants (Rauf *et al.*, 2011). Chromium is highly toxic to plants and affects the plant's development (Rauf *et al.*, 2011). Chromium can occur in various oxidation states (-2 to +6) but the +4 [Cr(IV)] and +6 [Cr(VI)] oxidation states are the most toxic and are of the most environmental concern. According to Fendorf (1995), Fendorf and Li (1996) and Bencquer *et al.* (2003) the mobility and phyto-availability of chromium depends on the prevailing oxidation state. The trivalent chromium [Cr (III)] occurs in soils as a cation (i.e. $CrOH^{2+}$) and it is easily adsorbed to most soil mineral surfaces, where it forms inner-sphere complexes (Fendorf, 1995, Fendorf and Li, 1996 and Bencquer *et al.*, 2003). As a result Cr (III) is relatively less mobile and less available to the environment in general. Cr (VI) is more toxic to living organisms owing to its high redox potential and also being able to penetrate biological membranes (Ponce *et al.*, 2015). It can adsorb or accumulate in some plant species. According to Khan *et al.* (2008) prolonged exposure to chromium can lead to liver, kidney and lung damage.

Lead has various oxidation states but the one that is more stable under many environmental conditions is the mobile and toxic Pb^{2+} (Raungsomboon *et al.*, 2008). For example, at room temperature lead(IV) Chloride ($PbCl_4$) decomposes to give lead(II) chloride ($PbCl_2$) and chlorine gas (Cl_2), and lead(IV) oxide (PbO_2) decomposes on heating to give lead(II) oxide (PbO)and oxygen (O_2) (Raungsomboon *et al.*, 2008). Although Pb^{2+} is the dominant form of lead in the soils, Pb^{4+} can be found occasionally in some highly oxidized soils (Raungsomboon *et al.*, 2008). Because lead enters the soil in different forms, its reactions may differ widely from place to place. Once lead is in the soil, it may react with anions such as phosphate (PO_4^{3-}), sulphate (SO_4^{2-}) or carbonate ion (CO_3^{2-}) to form less soluble salts such as lead carbonate [$Pb_3(OH)_2(CO_3)_2$] and chloropyromorphite [$Pb_5(PO_4)Cl$] (Yoon, 2005; Chrysochoou *et al.*, 2007 and Cao *et al.*, 2009). Soil organic matter (humic and fulvic acids) can also form complexes with lead which will then be adsorbed onto soil solids and as a result immobilize lead (Yoon, 2005). Lead has no known biological function in animals or plants (Álvarez-Ayuso *et al.*, 2012). Toxic levels of lead mainly are responsible for hematological, gastrointestinal and neurological dysfunctions in humans (Lockitch, 1993; Mandal and Suzuki, 2002; Álvarez-Ayuso *et al.*, 2012).

Mercury is categorised as one of the priority hazardous substances according to the Agency for Toxic Substances and Disease Registry (ASTDR). Mercury in soil originates from natural processes, anthropogenic activities associated with mining Zn, Pb and V ores and re-

deposition on the earth's surface from the atmosphere (Wang *et al.*, 2012). The most common forms of mercury found at contaminated sites are the mercuric form (Hg^{2+}), mercurous form (Hg_2^{2+}), elemental mercury (Hg^0) and methyl or ethyl mercury (USEPA, 1997). The most toxic of these is methyl mercury (Ulrich *et al.*, 2001). Mercury can be readily taken up by plants and transferred through the food chain to animals and humans (Wang *et al.*, 2012). A study done by Zheng *et al.* (2007b) showed that although mercury was taken up by vegetables grown near a Zinc smelting plant, the transfer factors of mercury were not as detrimental compared to other metal/loids. When ingested, mercury can accumulate in the brain and liver, leading to major toxic effects in the central nervous system that result in cerebral palsy, muteness and mental retardation (Guzzi and La Potta, 2008; Soghoian and Sinert, 2008).

The normal ranges of metals in plants are indicated in **Table 4** below.

Table 4: Normal range of metal and metalloid concentrations in plants

Elements	Range	Reference
Arsenic (As)	0.02 – 5 mg/kg	British Herbal Medicine Association, 1996.
Chromium (Cr)	0.006 – 18 mg/kg	Pawlisz, 1997; Zayed and Terry, 2003
Copper (Cu)	0.4 – 45.8 mg/kg	Kabata-Pendias and Pendias, 1984
Iron (Fe)	640 – 2486 mg/kg	Lavilla *et al.*, 1999
Mercury (Hg)	<1 – 300 ng/kg	Yu *et al.*, 2011
Magnesium (Mg)	0.73 – 1.41 mg/kg	Witkowski and Lamont, 1996
Zinc (Zn)	1 – 160 mg/kg	Kabata-Pendias and Pendias, 1984
Aluminium (Al)	200 ≥1 000 mg/kg	Jansen *et al.*, 2002; Chenery 1948 &1949
Manganese (Mn)	15 – 100 mg/kg	Misra and Mani, 1991
Nickel (Ni)	0.1 – 3.7 mg/kg	Kabata-Pendias and Pendias, 1984

For medicinal purposes, the African traditional Pentecostal churches in South Africa use ash from firewood for healing (Peltzer, 1999). This indicates the importance of ash from firewood among African traditional believers. However, ash from firewood containing chemicals such as copper, arsenic, and chromium can be dangerous depending on the quantity consumed or applied. Bill Hinkley in Curtis (1998) was quoted as saying about the ash, "It can kill you at moderate amounts over a longer period. And it's a carcinogen at low levels".

According to the Florida Centre for Solid and Hazardous Waste management (CCA), chronic exposure to dusts or mists containing chromium salts may cause:

- Ulceration and perforation of the nasal septum;
- Respiratory irritation may occur with symptoms resembling asthma;
- Other symptoms may include conjunctivitis, anorexia, nausea, gastritis, duodenal ulcers and colitis. Liver damage may occur;
- Chronic skin exposures may lead to a skin rash, and entry of chromium salts into open wounds may cause chromium ulcers;
- Acute poisoning from ingestion of chromium and its salts may cause dizziness, intense thirst, abdominal pain, shock and reduction in urinary output and vomiting;
- Prolonged skin exposure might cause ulceration of the skin, and
- Acute exposure to dry chromium salts may cause severe irritation of the eyes, nose throat, bronchial tubes and lungs.

The permissible limits of some of the metal/loids in plants according to the World Health Organisation (WHO) (1996) are indicated in **Table 5** below. The concentrations of metal/loids from this study were evaluated against this standard.

Table 5: WHO Maximum Permissible Limits (MPL) of metal/loids in plants

Elements	**Permissible value of plants (mg/kg)**
Cr (Chromium)	1.30
Cu (Copper)	10
Pb (Lead)	2
Ni (Nickel)	10
As (Arsenic)	1*

* Rauf *et al.*, 2011; Ahmad, 2000 and Manirul *et al.*, 2015.

The critical toxicity levels and thresholds set to define plants as hyperaccumulators are indicated in **Table 6** below.

Table 6: Critical toxicity levels and thresholds for hyperaccumulators

Metal/loids	Critical toxicity level (mg/kg) (Krämer, 2010)	Threshold (mg/kg) (Maestri *et al.*, 2010; Krämer, 2010)
As	<2-80	>1000
Cd	6-10	>100
Co	≥0.4	>1000
Cu	20-30	>1000
Cr	0.2-1	>1000
Pb	0.6-28	>1000
Mn	200-3500	>10000
Hg	0.001-5	>1000
Ni	10-50	>1000
Zn	100-300	>10000

2.3.4 The Mine Woodlands Project (MWP)

Approximately 286 indigenous and 90 alien plant species were identified to survive and grow on and around gold TSFs in the Witwatersrand Basin in South Africa (Weiersbye *et al.*, 2006); which proves that gold TSFs provide suitable environments for some plant species. Several studies were conducted on tolerant indigenous trees in the Witwatersrand Basin (Weiersbye, 2007; Weiersbye and Witkowski, 2007), and also on the growth of exotic plants used for phytoremediation on and around these TSFs (Dye and Weiersbye, 2010; Weiersbye *et al.*, 2006). The use of *Eucalyptus* trees for this purpose is believed to be effective, owing to their deep root systems, potentially high water use and high biomass (Dye *et al.*, 2008). Trees can be particularly useful for phytoremediation because they mostly stay evergreen throughout the seasons (Dye, 2010). Plant biomass can be an important product in some areas and is used

to generate energy for households or industry (Jain, 1994; van den Broek *et al.*, 1996; Sims *et al.*, 1999; Abbasi and Abbasi, 2010).

AngloGold Ashanti (AGA) and the University of the Witwatersrand (Wits) are exploring the potential of phytoremediation as an ecological engineering approach to the sustainable rehabilitation of mine TSFs through the Mine Woodlands Project (MWP) (AGA, 2004; 2008; 2010). This project explores the potential of open woodlands and savannah vegetation on top of the TSFs as an evapotranspiration (ET) cover to control ingress of rainwater and leaching of oxidation products to groundwater, and dust. The project also has closed-canopy woodlands on seepage zones around TSFs to control AMD and groundwater pollution. Several site-species trials were established near Welkom, Klerksdorp and Carletonville (**Figure 1**), with over 40 different shrub and tree species that are being tested for their ability to control dust and seepage.

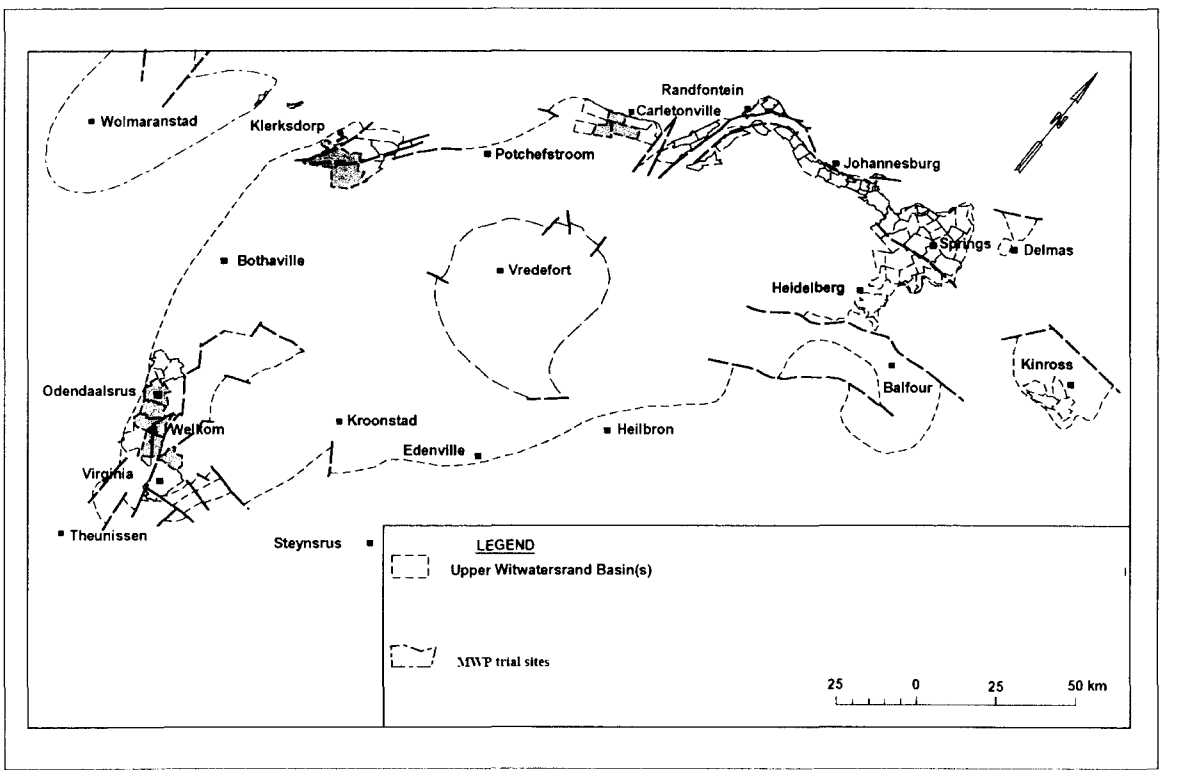

Figure 1: Location of the Mine Woodlands Project trials (Map from Weiersbye, *et al.*, 2006)

Seven species in the *Eucalyptus* genus have been grown on site-species woodlands trials as part of the MWP. These include: *Eucalyptus camaldulensis* (Dehnh.), *Eucalyptus dunnii* (Maiden.), *Eucalyptus grandis x camaldulensis* hybrid, *Eucalyptus macarthurii* (H. Deane and Maiden.), *Eucalyptus melliodora* (A. Cunn. ex Schauer.), *Eucalyptus grandis x nitens* hybrid and *Eucalyptus sideroxylon* (A. Cunn. ex Woolls). **Table 7** shows the mean annual increments expected in these species in different parts of the world.

Table 7: Annual volume increment in *Eucalyptus* plantations in different parts of the world (Adapted from FAO, 2001)

Location	MAI (m^3 ha^{-1} yr^{-1})	Rotation or stand age	Reference
E. camaldulensis			
Argentina	20-25	7-15 years	Lamprecht, 1990
Israel (irrigated plantation)	30	7-15 years	Lamprecht, 1990
Turkey (heartwood growth)	17-20	7-15 years	Lamprecht, 1990
Turkey (1st coppice generation)	25-30	7-15 years	Lamprecht, 1990
Morocco	3-11	7-15 years	Lamprecht, 1990
Portugal	2-10	7-15 years	Lamprecht, 1990
Italy	6-7	7-15 years	Lamprecht, 1990
Colombia	45	3 years	Newman, 1981
Guatemala	12.5-17.6	4.5 years	Otarola and Ugalde, 1989
Nicaragua	2.4-16.8	4.5 years	Otarola and Ugalde, 1989
South Africa	35.4	16 years	Dye *et al.*, 2008a
E. grandis			
Australia - NSW	16	29 years	Hillis and Brown (1984)
Brazil - Aracruz Florestal	55	7 years	Evans (1992)[a]
Costa Rica	1-49	2-4 years	Sánchez (1994)
Costa Rica	49-112	6.5 years	Vásquez and Ugalde (1995)[b]
South Africa	35	N/A	NAS 1980
Swaziland - Shiselwene Forestry	18	9 years	Evans (1992)
Uganda	17-45	N/A	NAS 1980
Zimbabwe	40	N/A	NAS 1980[c]
E. grandis x camaldulensis			
South Africa	29.57	7 years	Little *et al.*, 2002[d]

Location	MAI (m^3 ha^{-1} yr^{-1})	Rotation or stand age	Reference
South Africa: Zululand region	23	7 yrs	Smith *et al.*, 2006[e]

a- Clonal plantations;

b- Experiments with fertilizer and spacing;

c- With irrigation;

d- With manual weed checks;

e- Under optimum planting densities when coupled with fertilization and weeding during establishment.

The expectation is that these *Eucalyptus* plantations are able to control run-off and AMD through hydraulic control (**Figure 2**).

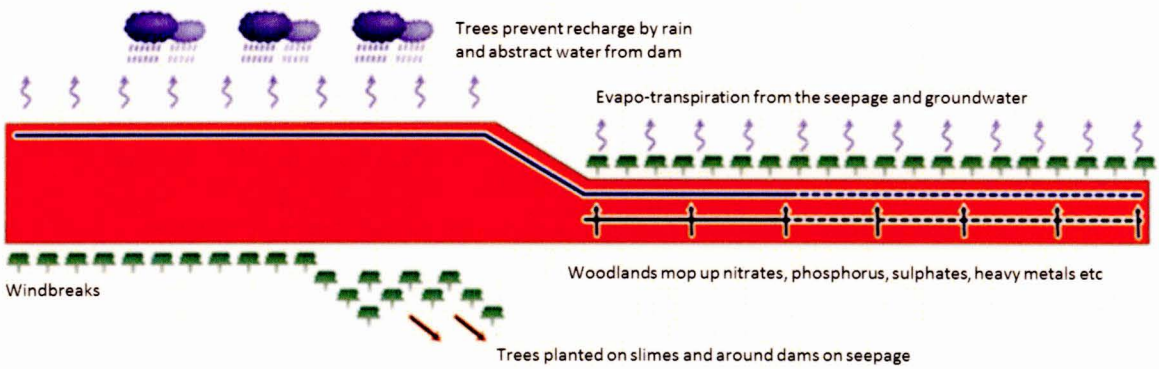

Figure 2: Conceptual model of woodlands plantation comprising plants like *Eucalyptus* to control the seepage and groundwater contamination by acid mine drainage in the Witwatersrand Basin (AngloGold Ashanti Report to Society, 2004).

2.3.5 Economic opportunities for *Eucalyptus* trees under the MWP

Growing *Eucalyptus* trees presents several economic opportunities in the continent of Africa and elsewhere. Timber can be used for pulp and paper production, charcoal production, solid wood products, plywood and veneer, poles for construction, firewood and biomass for energy (Couto *et al.*, 2003). The leaves of some species can be used for the production of essential oils, while the whole plant can be used for land reclamation, wind breaks, stabilization, control of AMD and carbon sequestration (Couto *et al.*, 2003; Mokgalaka *et al.*, 2009). Both international and local studies proved that *Eucalyptus* trees perform better in accumulation of contaminants, productivity, growth and evapotranspiration when grown in

contaminated waste sites compared to other species like *Acacia nilotica, Dalbergia sissoo* and *Phragmites australis* (Singh *et al.,* 2010; Shukla *et al.*, 2011; Morris, 1984; Sun *et al.,* 1994, Zohar and Schiller, 1998; Dye, 2008a). Some communities rely on the forests for their survival. Areas where there is poor infrastructural development and government services are normally prone to have communities that rely on trees in these forests (Shackleton *et al.*, 2007). The use of these forests for bioenergy can also be beneficial for poverty alleviation in these communities (Shackleton *et al.*, 2007).

E. camaldulensis is also used in a number of pharmaceutical applications for the treatment of skin ailments (Mabona and Van Vuuren, 2013). Its bark is used as a pharmaceutical product to wash pimples (Hutchings, 1996; Babayi *et al.*, 2004; Ayepola and Adeniyi, 2008 and Musa *et al.*, 2011). Eucalypts are also a good source of essential oils which are obtained from their leaves (Boland *et al.* 1982; Weston, 1984). The leaves when crushed emit a peppermint aroma which can be used to synthesise peppermint flavours (Weston, 1984). Some foliage from trees such as poplar is also used for manufacturing of animal feed products (Balatinecz and Kretschmann, 2001). The focus of this project is the use of *Eucalyptus* trees for fuelwood to local communities for domestic heating, cooking and recreational purposes e.g. braai.

2.4 Risk assessment of *Eucalyptus* trees grown on acid mine drainage for use as fuelwood

Risk assessment can be viewed as the natural human intelligence process that a person consciously or subconsciously uses every day to protect himself/herself from harm (Lee, 1999). Risk assessment is a valuable tool that attempts to improve environmental planning and business outcomes (Lee, 1999). From the current scenario, *Eucalyptus* trees have the potential to provide biofuels when grown around TSFs as part of MWP to control AMD, surface and groundwater pollution. During AMD control, nutrients and toxic metals could be accumulated by the plants. It has been proven that planting trees at contaminated sites such as landfills and tanneries has the potential to decrease the environmental impacts associated with leachate (Duggan, 2005; Shukla *et al.*, 2011). The accumulation of metal/loids and radioactive elements in the tree biomass might also be of concern. Thus there might be a risk of metal/loids accumulated by *Eucalyptus* being released into the atmosphere during combustion of the woody biomass or contained in the left-over ash.

While the *Eucalyptus* tree species are used in this study for hydraulic control on AMD through consumption of seepage and evapotranspiration, it must also be noted that there are parts of the trees that are consumed by human beings without government regulations. This involves the use of *Eucalyptus* leaves in medicines that can be bought over the counter. *Eucalyptus* leaves are a traditional herbal remedy (Chevallier, 1996). The essential oil found in the leaves, which is also a common ingredient in many over-the-counter cold remedies, is a powerful antiseptic and is used all over the world for relieving coughs and colds, sore throats and other infections (Chevallier, 1996). The most risk to human health is that these can also be obtained from the tree by making incisions in the trunk (Grieve, 1971; Lassak and McCarthy, 1983).

Some of the other environmental impacts that can be experienced by the use of *Eucalyptus* trees for fuelwood use can vary according to the following:

i. Water quantity impacts caused by the use of exotic species that have a high evapotranspiration rate;
ii. Soil quality positive impacts caused by the improvement of soil organic matter if trees are planted on degraded land;
iii. Air quality impacts experienced when harvesting trees for fuelwood can be as a result of increased dust emissions and also release of some contaminants (depending on the land used for planting these trees) into the environment via combustion. The other air quality impacts could be the release of greenhouse gas emissions from logging equipment used during harvesting.

A human health risk assessment follows four basic steps (USEPA, 1991) which are illustrated in **Figure 3** below. The same method was used in this study to identify the risk associated with the harmful effects of domestic use of contaminated trees:

i. Hazard identification was calculated directly from the concentration or content of metals and metalloids found in the *Eucalyptus* trees; and
ii. Exposure assessment, dose-response assessment and risk characterization was assessed through various literature sources.

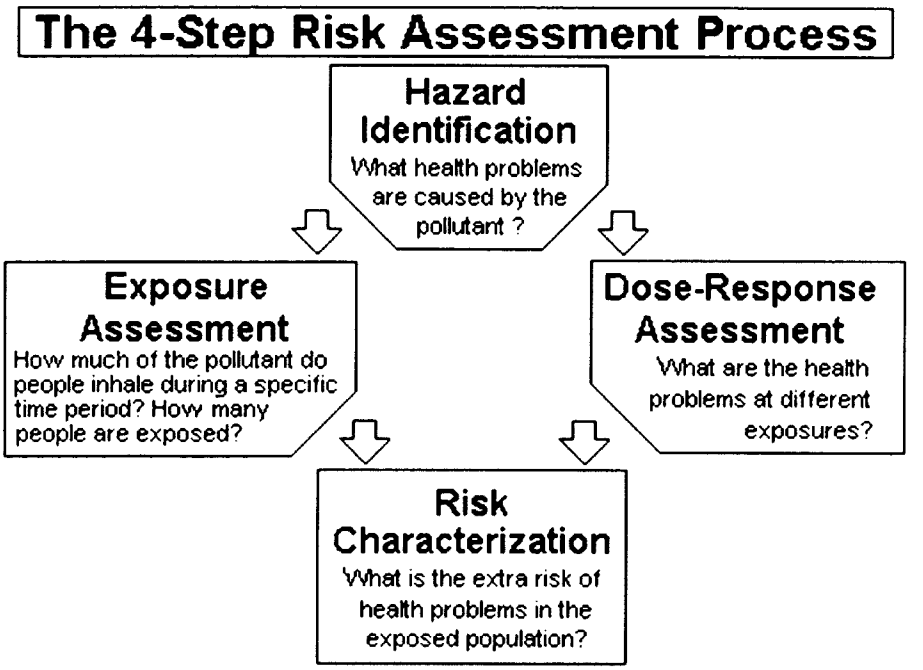

Figure 3: The 4-step Risk Assessment Process (USEPA, 1991)

The equation Risk = Hazard x Exposure was then used following the above highlighted process.

CHAPTER 3: MAIN AIMS AND SPECIFIC OBJECTIVES

3.1 Main Aims

- The aim of this study was to determine concentration and content levels of metals and metalloids in selected *Eucalyptus* trees grown on acid mine drainage in the Witwatersrand Basin and to determine which species produces the most benign wood for combustion and also which species produces the riskiest wood.

3.2 Specific Objectives

The objectives for this study were,

a) To determine and compare metal/loids concentrations and content among three *Eucalyptus* tree species;
b) To compare the metal concentration between tissue types (wood, bark, leaves, branches and twigs) for each species;
c) To determine whether metal/loids concentrations are within the WHO MPLs for plants;
d) To determine which species produces the most benign wood for combustion.

3.3 Research Questions

a) What is the concentration and content of metals in the wood of three *Eucalyptus* tree species/hybrid clones cultivated on acid mine drainage?
b) Do elemental allocations vary with tissue types and with species?
c) Which species will be less harmful when used as fuelwood?

CHAPTER 4: MATERIALS AND METHODS

4.1 Site Description

The study was conducted on site-species trials that were established at AGA's West Wits mine operation near Carletonville (26°26'12"S 27°21'12"E) and AGA's Vaal River mine operation near Klerksdorp (26°59'57"S 26°46'28"E) as part of the broader Mine Woodlands Project. The study locations, Vaal River (VR) and West Wits (WW) mine operations are 112 kilometres away from each other (**Figure 4, Figure 5 and Figure 6**). At Carletonville (WW mine), there are two sites named Red soil and Madala. At Klerksdorp (VR mine), there are two sites named Mispah and West Complex.

4.1.1 Rainfall

The area where VR operation is located normally receives about 657 mm of rain per year, with most rainfall occurring mainly during mid-summer (Herbert, 2008). **Figure 7** below shows the average rainfall values for the study area per month. It receives the lowest rainfall (4 mm) in July and the highest (120 mm) in January. The monthly distribution of average maximum temperatures (**Figure 8**) for the location ranges from 18.5°C in June to 29.1°C in January. The region is coldest during July where the mercury drops to 3.6°C on average.

The WW area normally receives about 703 mm of rain per year, with most rainfall occurring mainly during mid-summer. The chart below (**Figure 9**) shows the average rainfall values for the location per month. It receives the lowest rainfall (2 mm) in July and the highest (129 mm) in January. The monthly distribution of average maximum temperatures (**Figure 10**) for this area ranges from 17.5°C in June to 26.9°C in January. The area is the coldest during July when the mercury drops to -1.4°C on average.

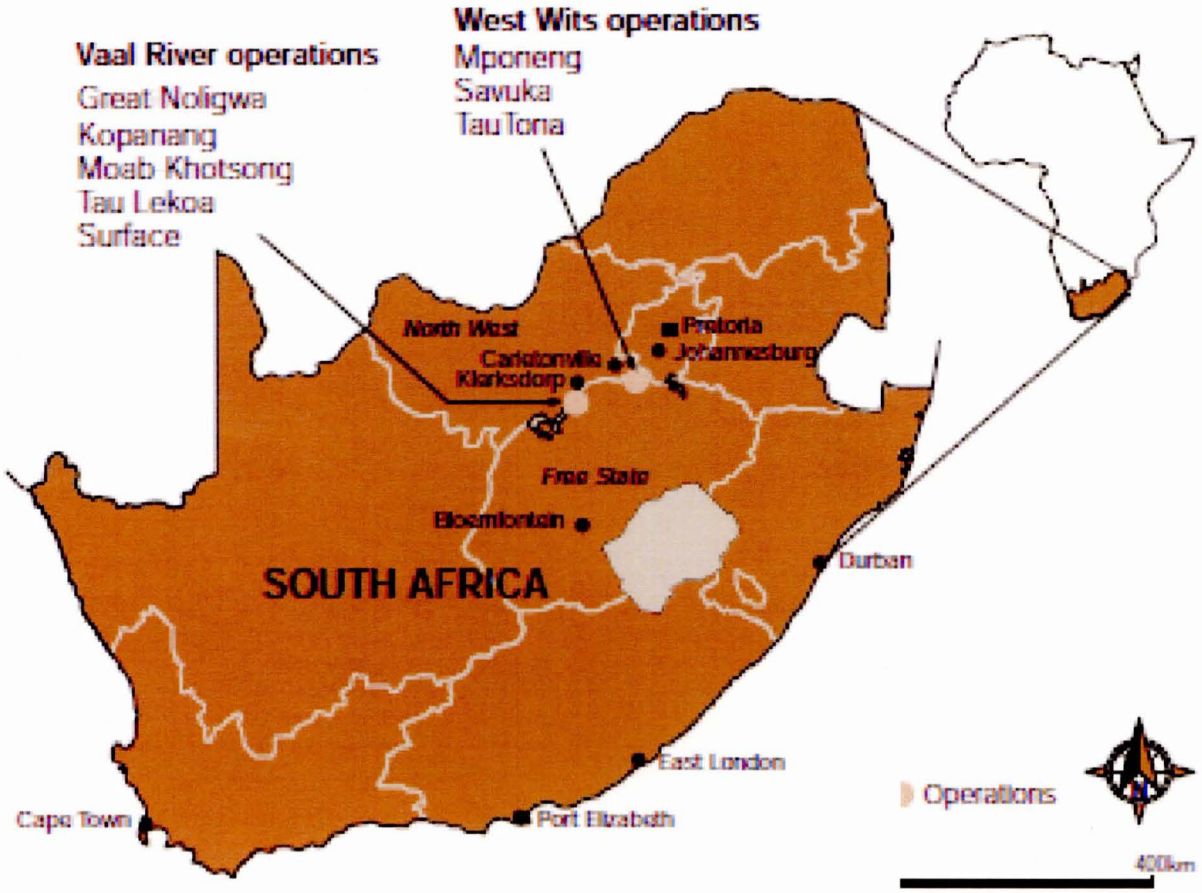

Figure 4: Map of South Africa showing the AGA's Vaal River operation and West Wits operation.



Figure 5: Location of the WW's Madala and Red Soil sites



Figure 6: Location of woodlands trials at the VR Mispah site

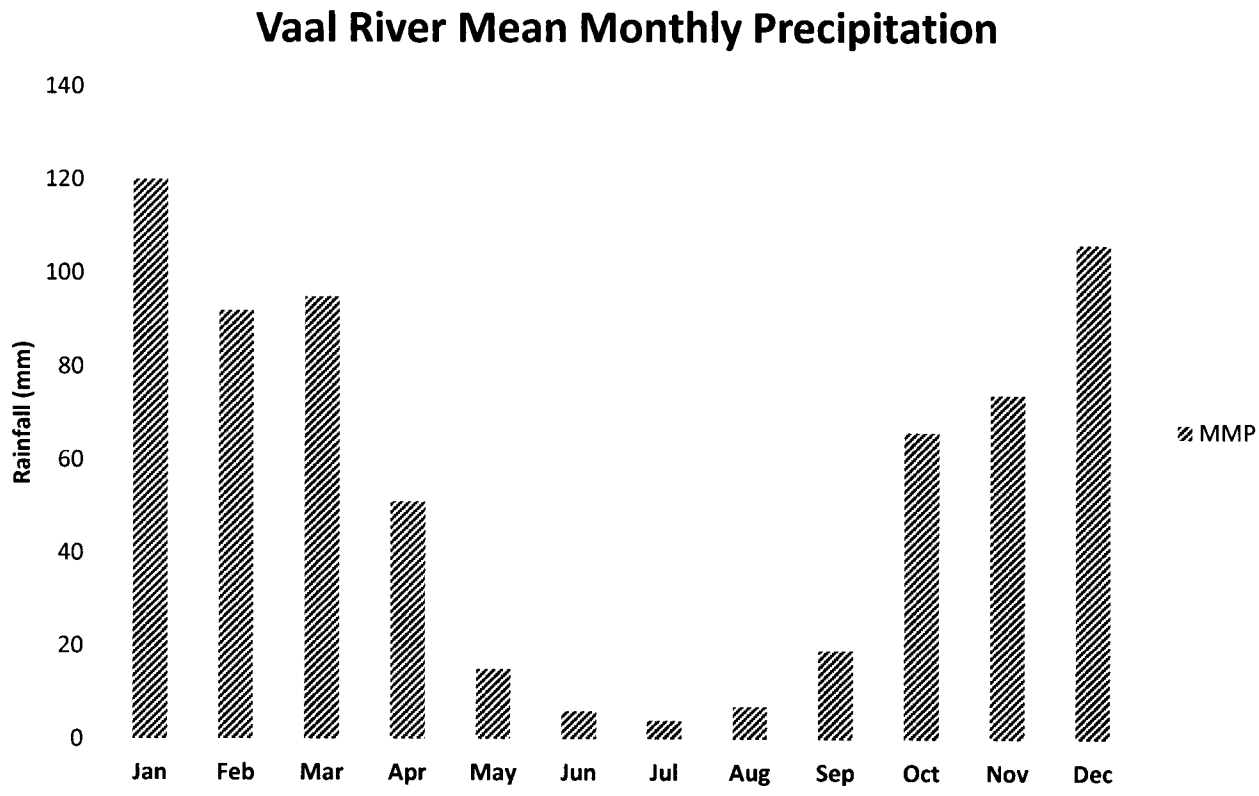

Figure 7: Long-term mean monthly rainfall for AGA's VR operation near Klerksdorp (Herbert, 2008).

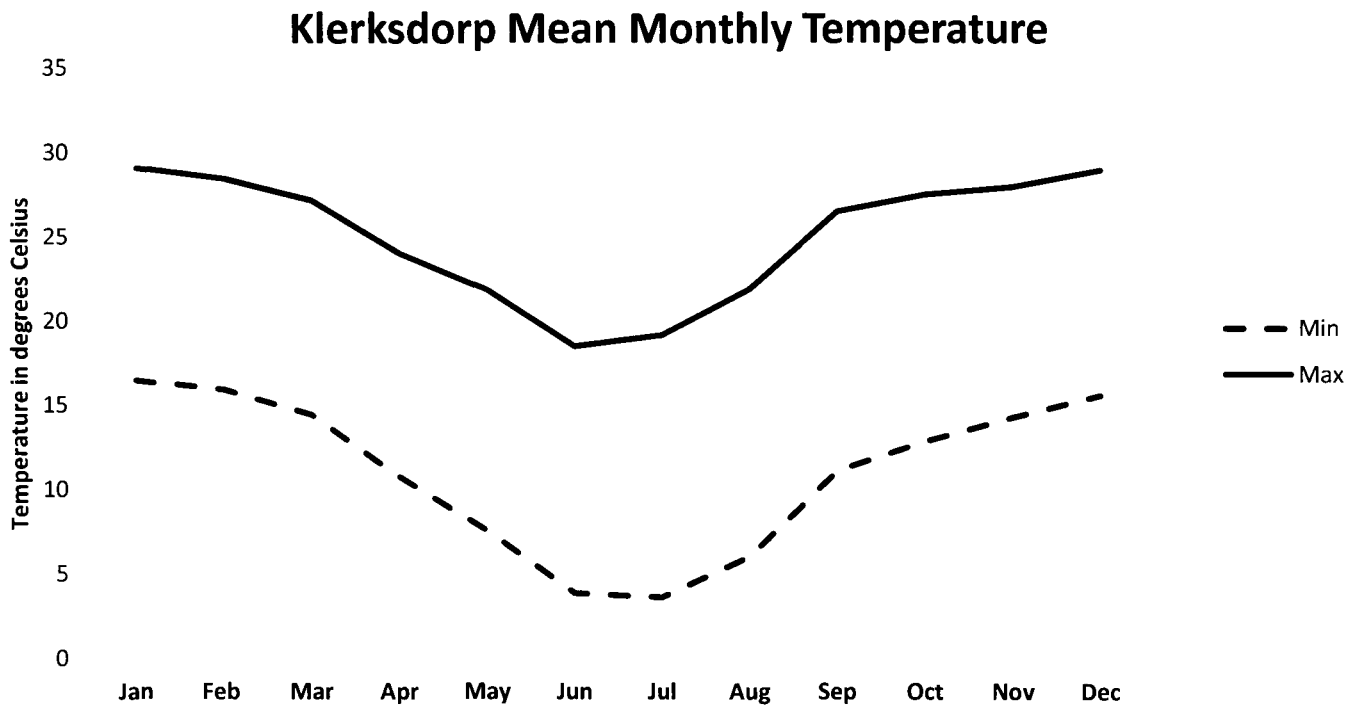

Figure 8: Mean monthly minimum and maximum temperature for AGA's VR operation near Klerksdorp (Herbert, 2008).

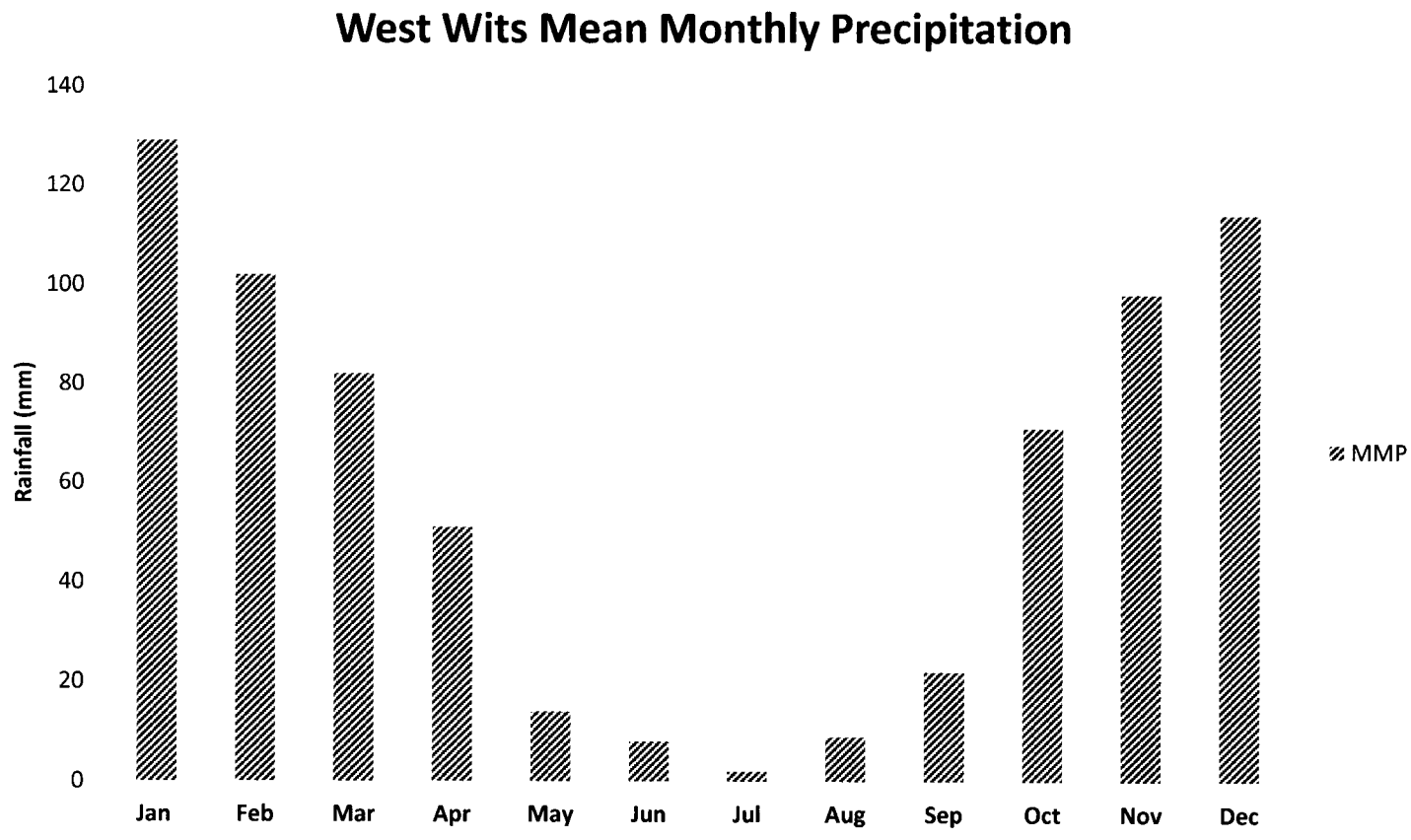

Figure 9: Long-term mean monthly rainfall for AGA's WW operation near Carletonville (Herbert, 2008).

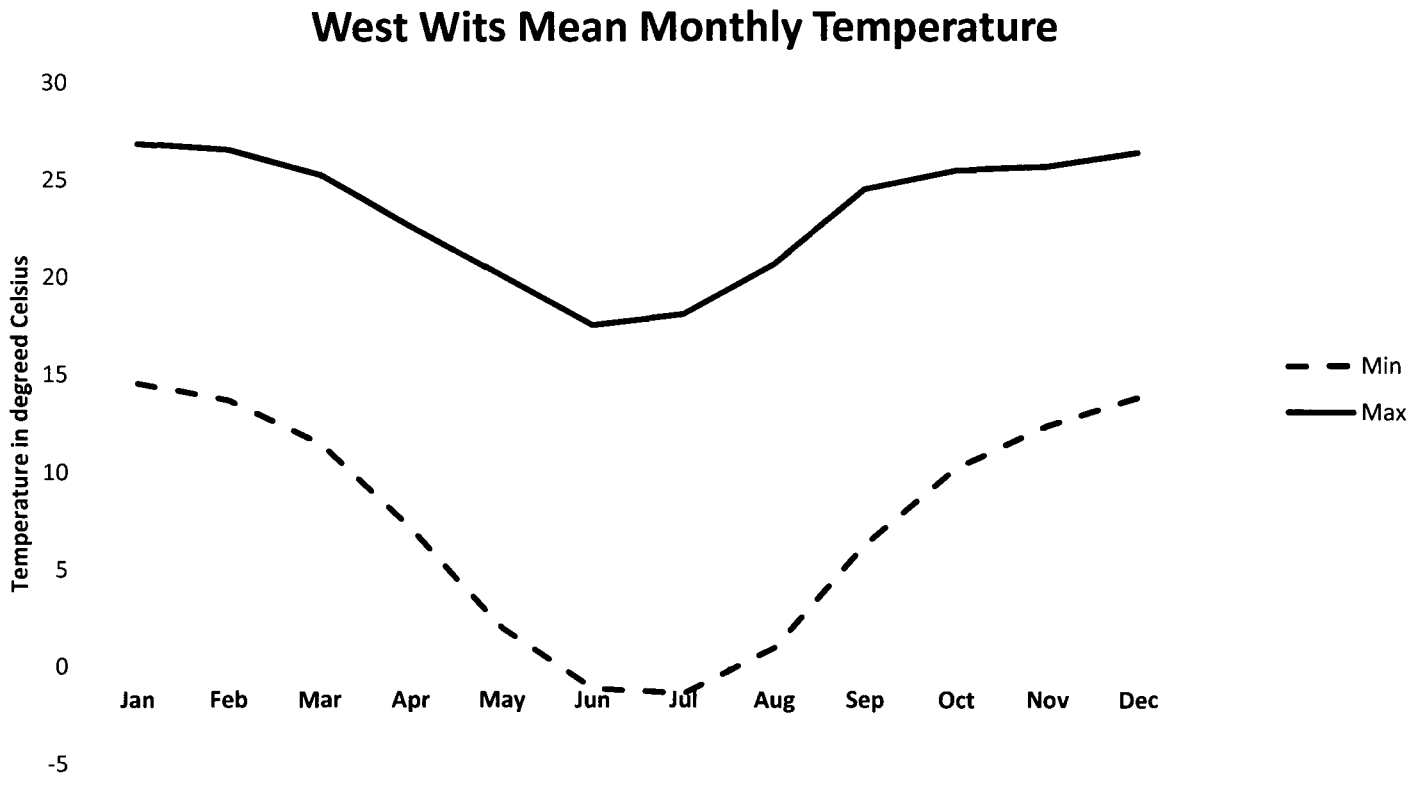

Figure 10: Mean monthly minimum and maximum temperature for AGA's WW operation near Carletonville (Zuurbekom station between Soweto and Carletonville) (Herbert, 2008).

4.1.2 Soils

The soils at VR's Mispah site consist of well drained deep red Hutton and Mispah soils (**Figure 11**) ideal for growing *Eucalyptus* trees (Herbert, 2008). These are shallow, red, sandy loam soils with flat to gentle slopes and 1-10% exposed dolomitic surface rock (WSP, 2009a).

At WW's Red Soil site there are deep reddish brown soils (**Figure 12**) (greater than 200 cm depth) well drained clay loam to clay textured Hutton soils originating from weathered diabase (Herbert, 2008; McLeroth, 2015). The soils' parent material is diabase (dominant) and shale and has an orthic A-horizon with 20-35% clay and a red apedal B-horizon with 50-30% clay (McLeroth, 2015).

At WW's Madala site, the soil profile was classified as yellow brown apedal soils with a shale (or occasionally ferricrete) parent material with a midslope and a very gently concave slope (McLeroth, 2015). The area is dominated by Clovelly soil forms with an orthic A-horizon with 20-35% clay and apedal B-horizon with 20-35% clay (**Figure 13**) (McLeroth, 2015).

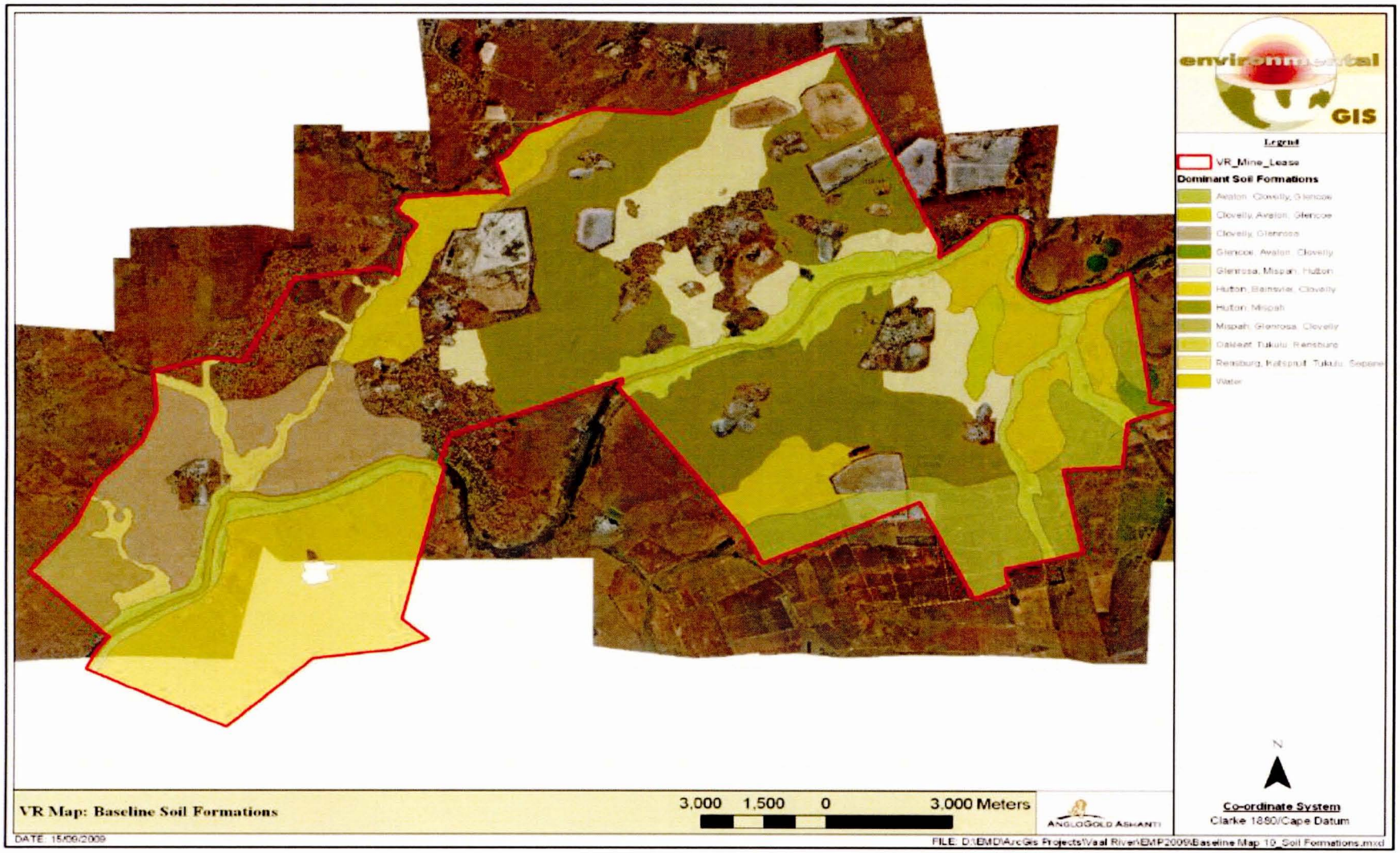

Figure 11: Soil Map for Vaal River Mine (WSP, 2009a)

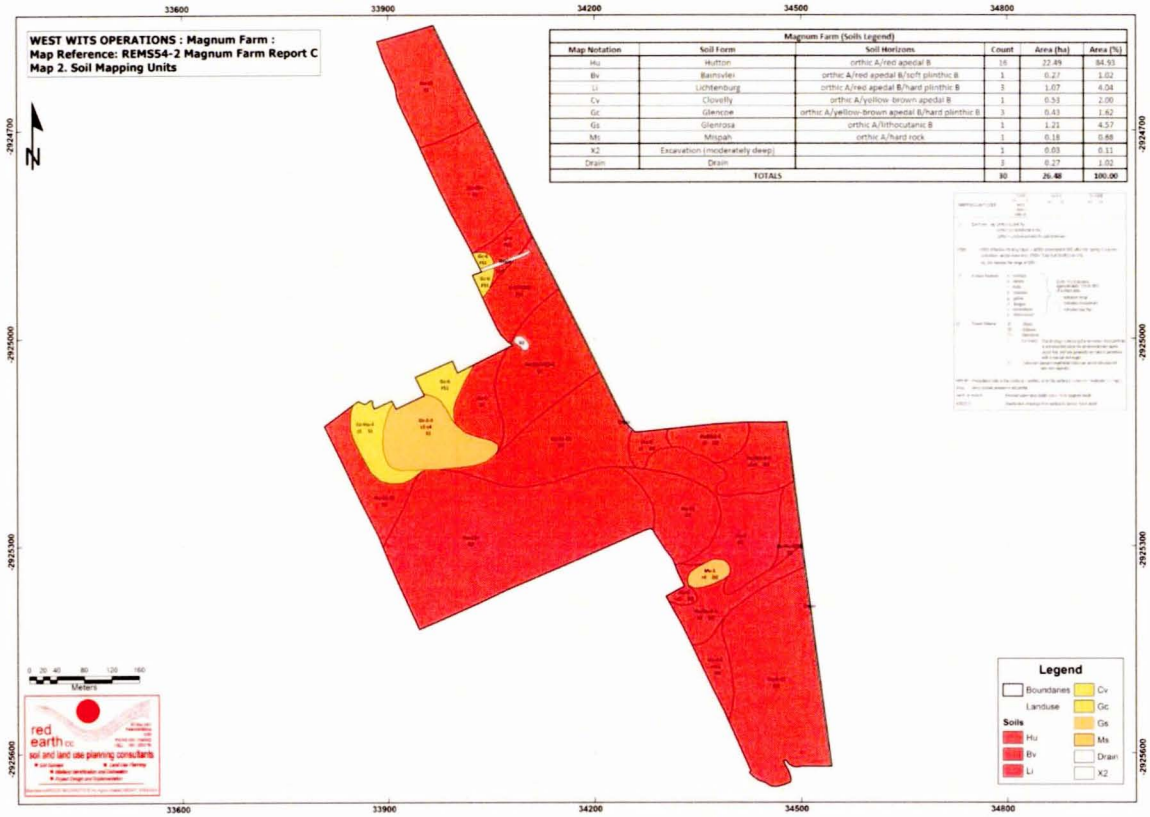

Figure 12: Soil Map for WW's Red Soil site (McLeroth, 2015)

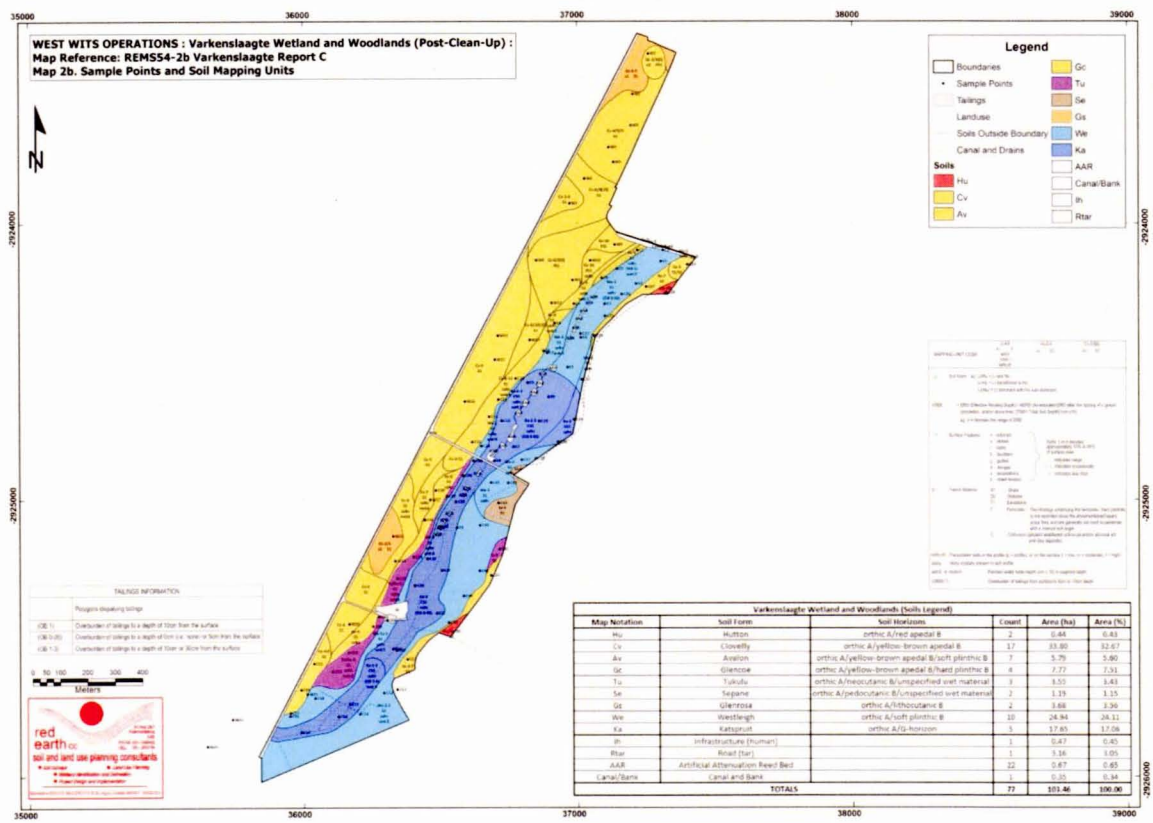

Figure 13: Soil Map for WW's Madala site (McLeroth, 2015)

4.1.3 Groundwater

The VR's Mispah site's groundwater was modelled (**Figure 14**) and showed that polluted groundwater (total dissolved solids (TDS) >1000mg/L) originated from the Mispah and Great Noligwa areas (WSP, 2009a).

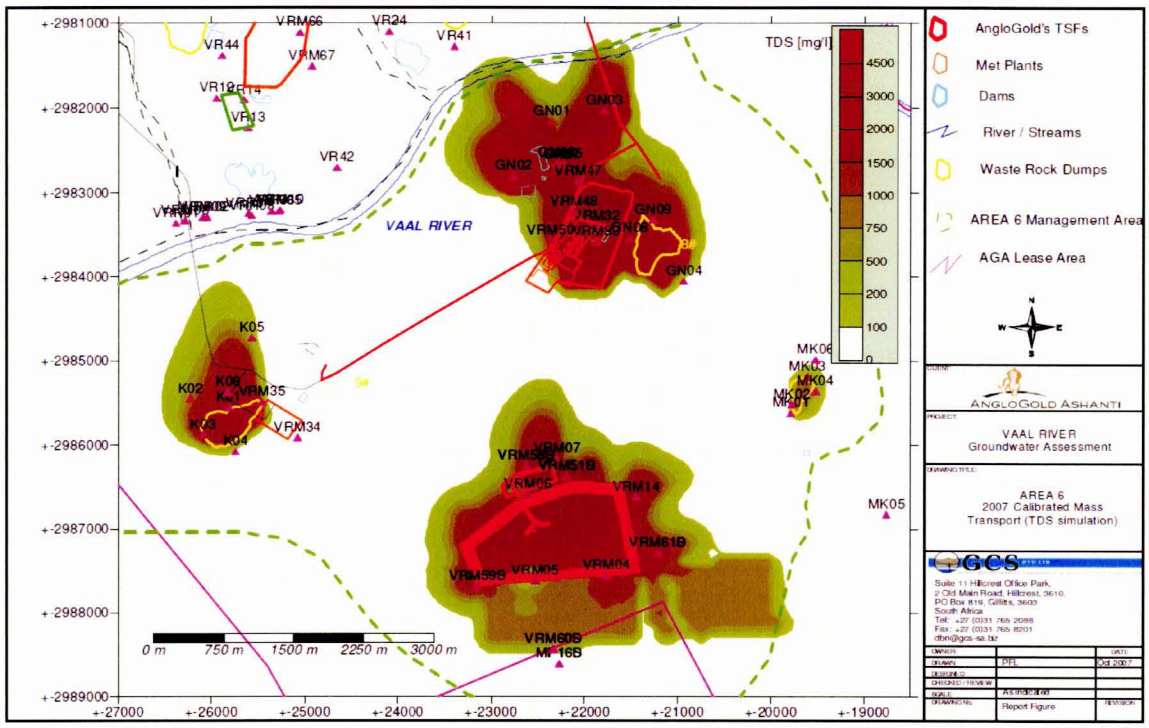

Figure 14: Vaal River South Mass Model from 2007 (TDS in mg/L) (WSP, 2009a)

At WW's Madala and Red Soil sites, the groundwater flow and elevation is depicted in **Figure 15** below [Water Levels (WL) in blue]. The aquifers present in the areas are defined as a 15 to 25 m semi-confined to confined weathered and fractured rock aquifer system (WSP, 2009b). The weathered aquifer system is situated above an inter-bedded impervious shale bedrock aquifer system that extends down to approximately 75 to 250 metres above mean sea level (WSP, 2009b). The TDS is illustrated in **Figure 16** which shows polluted water (above 3200 mg/L TDS) at point MBH44 and MBH18/MBH37.

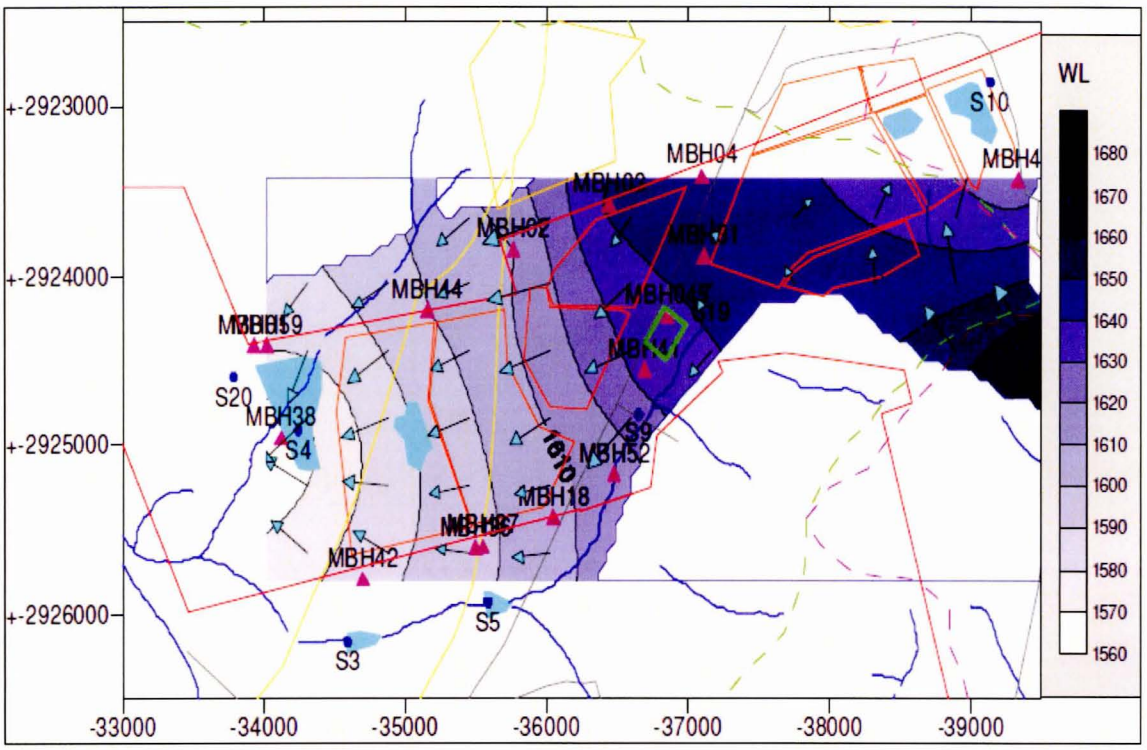

Figure 15: Interpolated groundwater elevation and flow direction (WSP, 2009b)

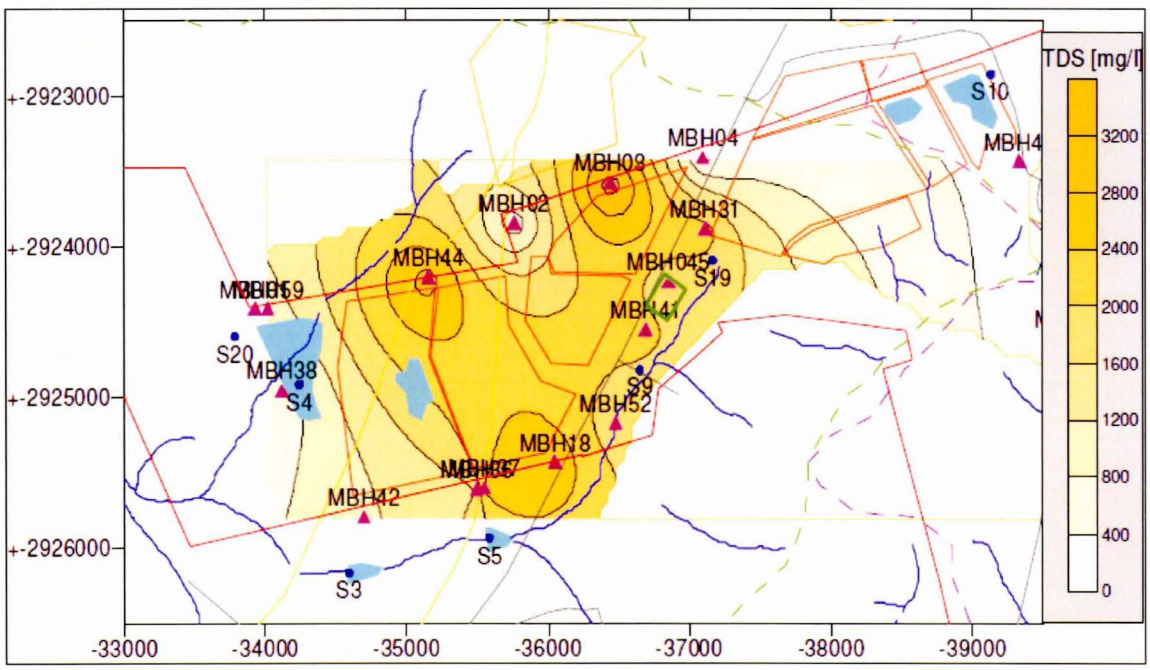

Figure 16: Interpolated groundwater TDS concentrations (WSP, 2009b)

4.2 Study Species

There were at least seven species of *Eucalyptus* grown in the woodlands trials as part of the Mine Woodlands Project (MWP). The exotic species trials were established in 2004. This study focused on three of these species; *E. camaldulensis, E. dunnii* and *E. grandis x camaldulensis hybrid* clone.

E. camaldulensis is also called Red River gum and is a tall-growing perennial, single stemmed tree with an average mature height of 30 m (Bren and Gibbs, 1986), although some authors (e.g. Boland, 1984; Brooker *et al.*, 2002) record trees to 45 m. They often thrive in areas with mean annual rainfall of 1000 mm. According to Hunter (2001), the species cannot adjust easily to prolonged drought. However, it can tolerate reduced periods of low rainfall, inundation and salinity (Van der Moezel *et al.*, 1989). *E. camaldulensis* is a hardy, fast growing gum that is tolerant of salinity, waterlogging, drought and frost, with a range of amenity and wood uses. It commonly grows on riverine sites, whether of permanent or seasonal water (Brooker *et al.*, 2002). This eucalyptus species is the most extensive on grey heavy clay soils along river banks and on floodplains subject to frequent or periodic flooding, preferring deep moist subsoils with clay content (Costermans, 1989). *E. camaldulensis* is a free producer of seeds (Cunningham *et al.*, 1981). According to Jacobs (1955) river red gum could reach ages of 500 to 1000 years.

E. dunnii is commonly known as Dunn's White Gum, Killarney Ash, or White Gum. The species is tall (~50 m) and fast-growing (especially in fertile soils) with rough brown and flaky barks (Trueman *et al.*, 2013). According to Trueman *et al.* (2013), *E. dunnii* is reasonably frost tolerant and adapted to a range of soil types, but prefers moist, deep, fertile and well-drained soils.

E. grandis x camaldulensis is a hybrid obtained by in-vitro rooting of clones of *E. grandis* (Hill ex. Maiden) and *E. camaldulensis* (Dehnh.). The hybrid was developed to combine good growth with drought tolerance traits that both species share.

Eucalyptus camaldulensis	*Eucalyptus dunnii*
	
Eucalyptus grandis x camaldulensis	
	

Figure 17: Typical examples of three *Eucalyptus* species; *E. camaldulensis*, *E. dunnii*, *E. grandis x camaldulensis hybrid*.

4.3 Experimental Design

The study was conducted in three sites. A fourth trial at the VR site could not be included in the experimental design due to destruction by fire of trees resulting in uneven replication. The plan of study looked at the difference between the species and tissues rather than the variation between sites as there were not enough trees harvested to create replicated species within each site. Therefore, the experimental design factorial for the *Eucalyptus* trees at each site is; 3 *Eucalyptus* species x four tissue types (leaf, stem wood, bark, branches/twigs) = 12 samples.

4.4 Sampling procedure

The experiment included three *Eucalyptus* tree species/ hybrids of the same age. Nine trees were destructively sampled in 2012 at three sites as it was important to minimise damage to the plots (i.e. one tree per species per site). The diameter at breast height (dbh) of each tree on each site was recorded so as to select a representative sample for each species (i.e. each site

saw one tree per species felled; one small from site 1, one medium from site 2, one large from site 3). Samples of leaves, stem wood, bark, and twigs/branches from three tree species at each site were harvested within the same week in late-growing season in June 2012. Tree felling was performed and harvesting of leaves, branch/twig, bark and stem wood occurred immediately after felling. A representative sample of approximately 25 leaves (North, West, East and South aspects of the tree) was collected from the top of the canopy. The stem samples were collected in two or three disks per tree and the bark was removed and stored separately to be air dried. The branches/twigs samples were collected for both a branch sample and twig sample (three each). The branches/twigs samples were washed with distilled water and air dried. After the collection of the leaf samples, they were washed with distilled water and freeze dried. Dry sample mass was recorded. These samples were later prepared for the metal analysis.

4.5 Sample preparation for chemical analysis

The samples were collected, cleaned and prepared for analysis according to Tan (2005). The tree samples of wood, bark, leaves and branches/twig were analysed using ICP-OES to determine the concentration of the following elements: Aluminium (Al), Barium (Ba), Calcium (Ca), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Nickel (Ni), Phosphorus (P), Sulphur (S), Strontium (Sr), Titanium (Ti) and Zinc (Zn). Data were expressed in mg/kg.

The metals and metalloids were analysed using ICP-MS for Vanadium (V), Chromium (Cr), Cobalt (Co), Copper (Cu), Arsenic (As), Barium (Ba), Gold (Au), Mercury (Hg), Lead (Pb) and Uranium (U). Data were expressed in μg/kg.

The dried and ground samples were prepared for elemental analysis using the ICP-OES technique (Tan, 2005). Approximately 0.250 ± 0.010 g (weighed mass in Appendix G) of the ground sample was weighed into clean microwave digestion tubes. The samples were microwave digested using a mixture of concentrated hydrogen peroxide (4 ml) and nitric acid (16 ml) at 800 W for about 30 min. Blanks and certified reference materials were prepared using the same procedures. The samples were cooled to room temperature and transferred to 50 ml polyurethane volumetric flask where they were diluted to the mark using de-ionized water (Millipore, USA). The diluted samples were then kept in a fridge until analysis. The concentrations of elements in the samples were determined on an ICP- OES instrument (Spectro Genesis, Germany).

Elements, which read over their calibration limits, were serially diluted to read within the limits by 10x, 20x, 50x and 100x. The CHNS 932 Leco elemental analyser was used to

analyse the ground samples for the light elements C and N (LECO Instruments, St. Joseph, USA). The instrument was calibrated by analysing a series of blanks prior to analysing the calibrating standards. The calibrating standards used were protein standards. Approximately 0.2g of the ground sample was encapsulated within a tin capsule for the analyses of C, N and ash. The samples were combusted at temperatures between 950° - 1000° C. Data were expressed in % dry mass.

The ash content was determined by using the TAPPI standard T 211 om-85 (Munalula and Meincken, 2009). Wood samples were weighed before they were placed in a furnace at 575^0C for 3 hours. Data were expressed in % dry mass.

4.6 Risk assessment methodology

The risk assessment was designed using the USEPA (1991) methodology:

- Hazard was measured from metals and metalloids in the tree biomass;
- Exposure was determined from literature.

The exposure was calculated by using transfer factors from literature to estimate the amount of metals or metalloids that could be accumulated by various vegetables planted in soil fertilised with contaminated ash. The calculation was:

Concentration of metal/loids in vegetables = transfer factor x concentration found in *Eucalyptus* plants (this is assuming the concentration of metal/loids was still the same for ash as that found in *Eucalyptus* plants)

Then daily intake of metal/loids through consumption of vegetables = daily vegetable consumption x concentration of mean vegetable metal concentration (Jolly *et al.*, 2013).

4.7 Calculations

The concentration of metals from the original dry tissue (leaf, branch/twig, stem wood, bark) was calculated as follows,

Dilution factor (DF) = Total volume/Total mass digested and analysed

Metal concentration = DF x Metal reading from ICP-OES or ICP-MS x further dilutions = mg/kg (Dennis, 2008).

To convert µg/kg to mg/kg, data was divided by 1000.

The final data was expressed in mg/kg (ppm) or µg/kg (ppb) dry mass of tissue. For graphs, all data was presented in mg/kg.

To calculate whole plot biomass, the Schumacher and Hall equation, published in the South African Forestry Handbook, 2000 by Bredenkamp, was used to estimate the stem volume (Equation 1).

$$lnV = b_0 + b_1 \ln(dbh + f) + b_2 \ln H \qquad \textit{(Equation 1)}$$

where: *ln* = natural logarithm to the base e

V = stem volume (m^3 under bark), usually to 75mm tip diameter

dbh = breast height diameter (cm – over bark)

f = correction factor

H = tree height (m)

b_0, b_1, b_2 = coefficients (**Table 8**)

The volume was calculated by using the coefficients as indicated in **Table 8**.

Table 8: Coefficients for the estimation of standing tree volume using the Schumacher and Hall model (Bredenkamp, 2000).

Species	b_0	b_1	***F***	b_2	**Author of original equation**
E. grandis x camaldulensis	-10.6435	1.9185	0	1.1494	Du Plessis, 1996

The volume for *E. camaldulensis* and *E. dunni* was estimated by using the *E. grandis* generic equation 2 by Schumacher and Hall (1933).

$$\log volume = -4.584 + \log dbh + \log ht \qquad \textit{(Equation 2)}$$

To calculate the dry mass of trees harvested, the standard industry conversion factors for volume to dry mass (Other *Eucalyptus* species) as detailed by Bredenkamp (2000) were used and are presented in **Table 9** below.

Table 9: Standard industry conversion factors for roundwood

Species	tonne/m^3	m^3/tonne
Eucalyptus grandis	0.68	1.47
Other *Eucalyptus* species	0.83	1.25

4.8 Data Analysis/ Statistics

In this chapter the statistical methods used in the data analysis are described.

The mean, standard deviation, number of samples (n), standard error, and 95% confidence interval formed part of the descriptive statistics which were calculated using Microsoft® Excel Data Analysis. Data was checked for normality using MiniTab® 17.1.0 software. Where normal distribution of all data was observed, wood, bark, branch/twig and leaf, and chemical data of the three *Eucalyptus* plants growing in three different sites was analysed using the Tukey's Honest Significant Difference (HSD) to statistically conduct the mean separation test. It was used in conjunction with a one way ANOVA to find means that are significantly different from each other.

The hypothesis set up was:

Null hypothesis:	All means are equal (H_0: $v_1=v_2=v_3$)
Alternative hypothesis:	At least one mean is different (H_1: $v_1\neq v_2$/ $v_2\neq v_3$/ $v_1\neq v_3$)
Significance level:	$\alpha = 0.05$

The experimental design used one tree species per site (three different sites) therefore a paired t-test was not possible in the statistical analysis. The metal/loids and nutrients found in highest concentrations in wood were subjected to the Tukey HSD test; i.e. Ba, As measured by ICP-MS and Ca measured by ICP-OES.

The ash content was determined by using the TAPPI standard T 211 om-85. The mean, standard deviation, number of samples (n), standard error, 95% confidence interval formed part of the descriptive statistics which were calculated using Microsoft® Excel Data Analysis.

The mean concentration of potentially harmful metals in wood of the different *Eucalyptus* species was compared to the MPLs in plants. A risk assessment was then conducted using the methodology in section 4.6.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 Results

The measured results were checked against the CRM's known concentrations. For ICP-MS, results with an agreement of 80-120% were accepted (Downer, 2008). For ICP-OES, results with an agreement of 90-110% were accepted (Downer, 2008). Only those measurements that fall within this acceptable range were discussed. The results of elements without a certified reference value for Orchards Leaves (1571) were not discussed. The calculations of CRM agreements are indicated in **Table 10** below.

Table 10: CRM agreements to measured values

Element	Measured value	Certified Value	% agreement
ICP-MS results in µg/kg			
V	432.9±17.2	No reference value	NA
Cr	14969±14.57	2300 ±300	No agreement
Co	190.1±5.25	2000*	10%
Cu	9283±203.8	12000±1000	77%
As	9936±872.3	10000±2000	99%
Ba	41737±2130.9	44000*	95%
Au	10±4.78	No reference value	NA
Hg	109.9±5.6	155±15	71%
Pb	26583±340.9	45000±3000	59%
U	7.25±0.7	29±5	No agreement
ICP-OES results in mg/kg			
Al	309.33±17.66	No reference value	NA
Ba	59.03±4.21	44*	75%
Ca	21980.61±484.97	20900±300	95%
Fe	332.55±9.02	300 ± 20	90%
K	7327.90±128.93	14700±300	50%
Mg	7311.29±215.92	6200±200	85%
Mn	89.00±2.24	91 ± 4	98%
Na	529.54±103.19	82 ± 6	15%
Ni	20.25±0.04	1.3 ± 0.2	6%

Element	Measured value	Certified Value	% agreement
P	7741.47±553.07	2100±100	27%
S	1962.54±415.37	1900*	97%
Sr	32.70±0.35	37 ± 1	88%
Ti	14.58±0.22	No reference value	NA
Zn	49.12±1.96	25 ± 3	51%

*Published non certified values

This section specifically reports the concentration of metal/loids in wood of *Eucalyptus* tree species as wood is the tissue that is primarily used for fuel. The comparison of the concentration of metal/loids in other plant tissues (leaves, branches /twigs, bark) was also done. The elements were grouped and are presented as follows:

- Nutrients: nitrogen (N), phosphorus (P), potassium (K); calcium (Ca), sulphur (S), magnesium (Mg);
- Metals and metalloids: manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), nickel (Ni) cobalt (Co), chromium (Cr), lead (Pb), arsenic (As), barium(Ba), mercury (Hg);
- Carbon and Ash content.

The metals which reported the highest concentrations in wood were subjected to the Tukey HSD test; i.e. As measured by ICP-MS and Ca measured by ICP-OES. The results (**Table 11**) show that there was no significant difference noted between the concentrations of As and Ca in three different *Eucalyptus* species.

Table 11: Statistical comparison of concentrations of metals in three *Eucalyptus* species Tukey HSD, The hypothesis where H_0: $v_1=v_2=v_3$and H_1: $v_1\neq v_2$/ $v_2\neq v_3$/ $v_1\neq v_3$.

Element	F- value	P-value	Tukey HSD Test
As	2.40	0.171	$v_1=v_2=v_3$; no significant difference noted between the concentration of arsenic in 3 *Eucalyptus* species
Ca	2.10	0.204	$v_1=v_2=v_3$; no significant difference noted between the concentration of calcium in 3 *Eucalyptus* species

5.1.1 Nutrients

Only the measured result of Ca and S had an agreement of above 90% when compared to the CRM. All other nutrients were either below or above the 90%-110% acceptable range.

The mean concentration ± SD of Ca and S in wood dry mass of the *Eucalyptus* species are presented graphically in **Figure 18**. Grouping information using the Tukey Method and 95% Confidence Interval showed that the means were not significantly different between the three species for calcium and sulphur but were significantly different for magnesium ((*E. dunnii* ≠ (*E. cam* = *E. gxc*)).

The mean concentration ± SD of Ca and S dry mass in leaves, branches/twigs, bark and wood of the *Eucalyptus* species are presented graphically in **Figure 19** and in Appendix E.

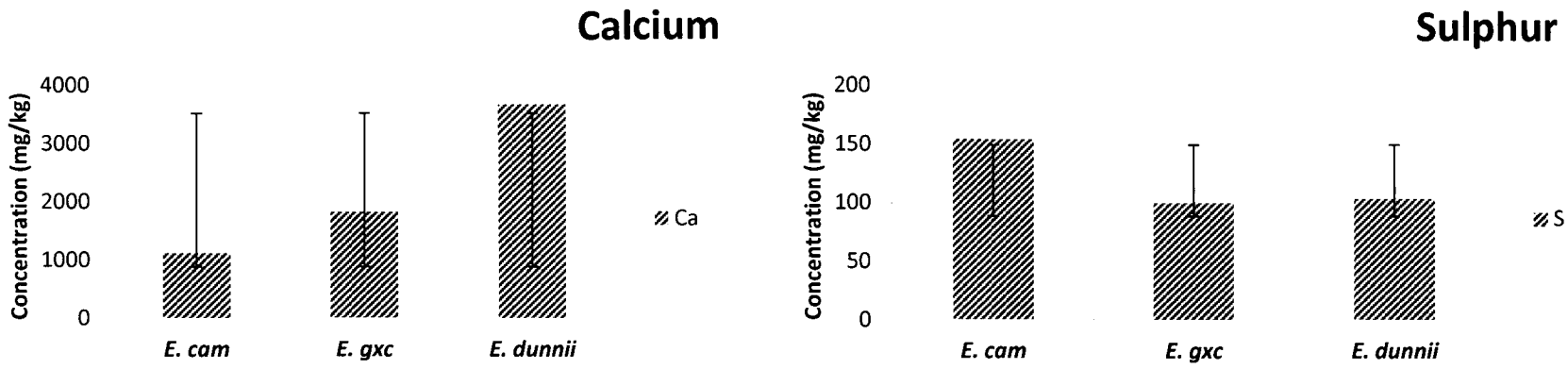

Figure 18: Comparison of nutrients Ca (left) and S (right) concentration in wood averaged across all three *Eucalyptus* species.

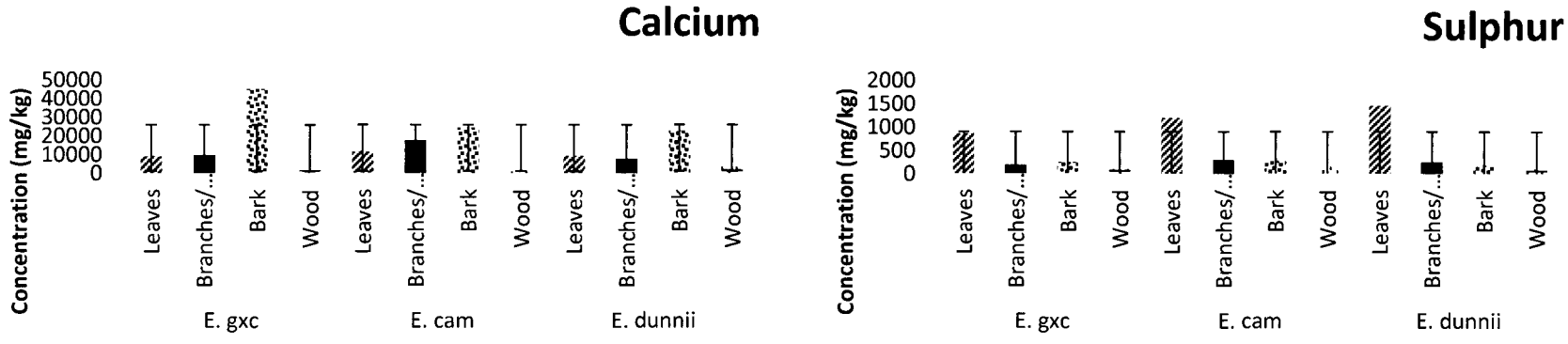

Figure 19: Comparison of nutrients Ca (left) and S (right) concentration in all tissues averaged across all three *Eucalyptus* species.

5.1.2 Metals and Metalloids

Only the results of Mn, Fe, As and Ba had an agreement of above 80% when compared to the CRM. These elements are the only ones discussed. The mean concentration ± standard deviation (SD) of Mn, Fe, As and Ba in wood dry mass of the *Eucalyptus* species are presented graphically in **Figure 20**. Grouping information using the Tukey Method and 95% Confidence Interval showed that the means were not significantly different between the three species for Mn, Fe, As and Ba.

The mean concentration ± SD of Mn, Fe, As and Ba dry mass in leaves, branches/twigs, bark and wood of the *Eucalyptus* species are presented graphically in **Figure 21** and in Appendix E.

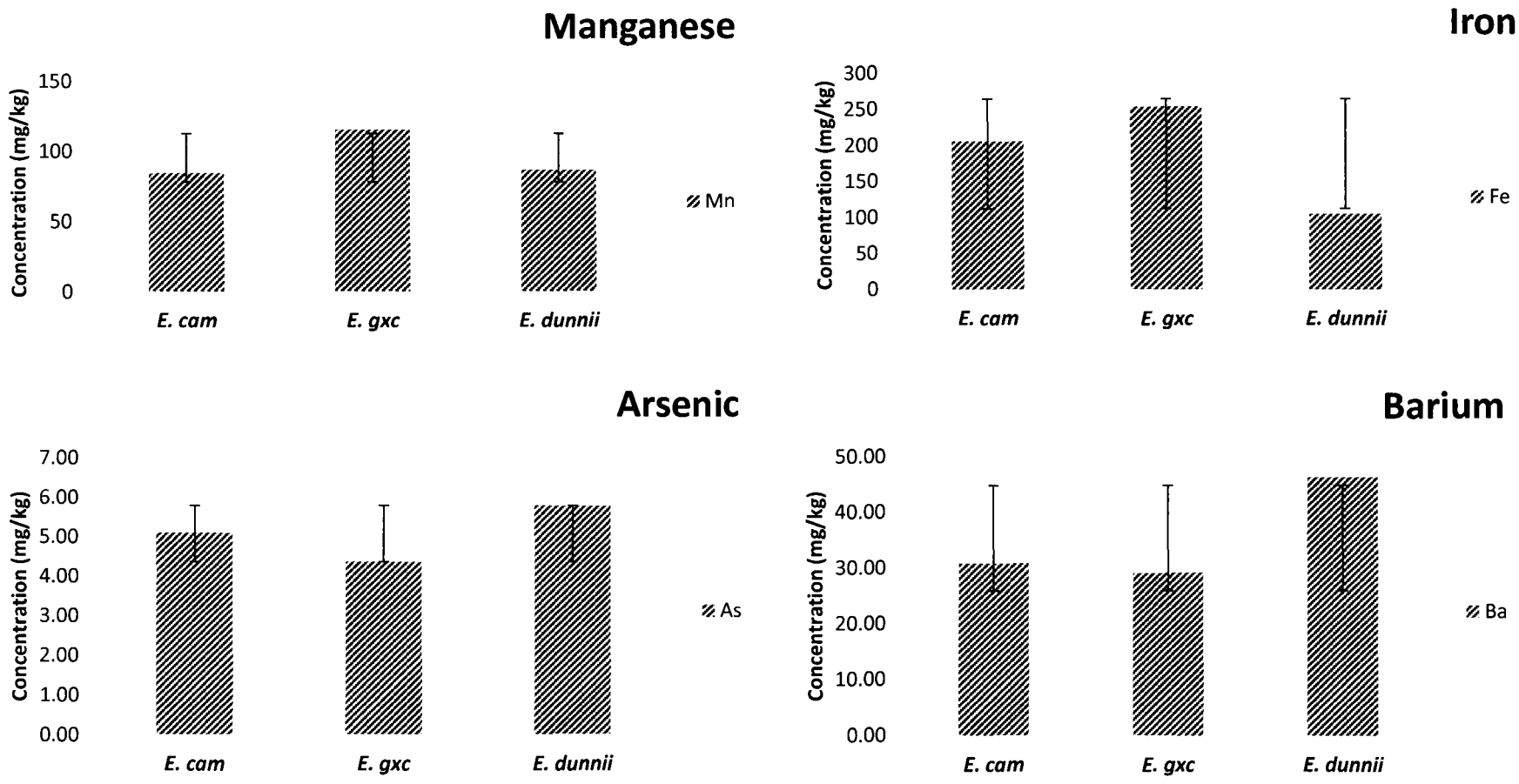

Figure 20: Comparison of metal/loids Mn (top left), Fe (top right), Ba (bottom left) and As (bottom right) concentration in wood across *Eucalyptus* species.

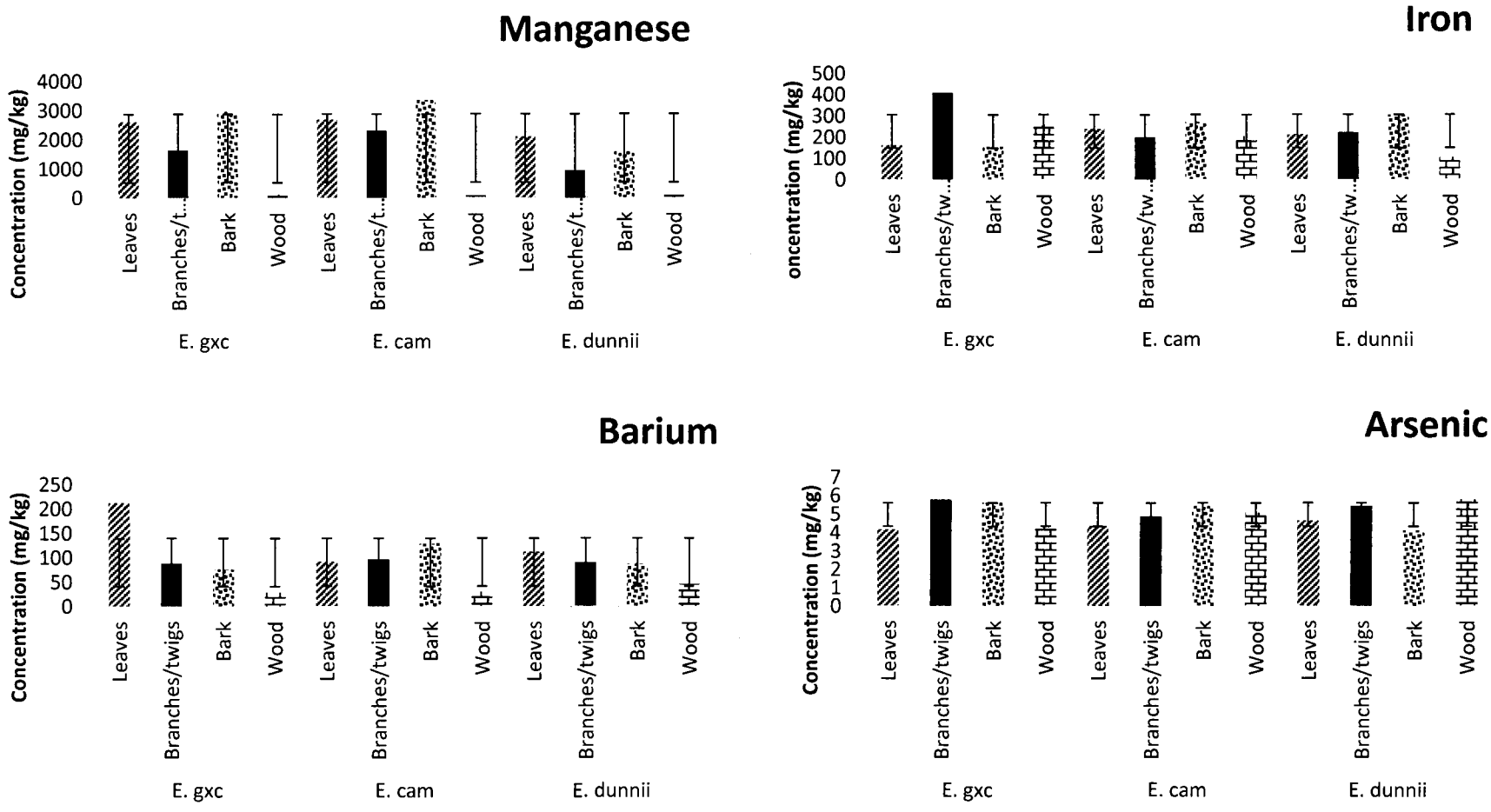

Figure 21: Comparison of metal/loids Mg (top left), Fe (top right), Ba (bottom left) and As (bottom right) concentration in all tissues averaged across all three *Eucalyptus* species.

5.1.3 Carbon and Ash content

There was no CRM value for Carbon and Ash content, therefore this data was not discussed.

5.1.4 Amount of nutrients, metals and metalloids per tree

The concentration of nutrients, metals and metalloids was then scaled up to the whole plot so that the amount of nutrients, metals and metalloids per plot could be estimated. The allometric algorithms as indicated in section 4.7 were used to calculate volumes and mass of wood and content of nutrients, metals and metalloids as indicated in **Table 12** and average content per site indicated in **Table 13** below.

Table 12: Calculated wood volume, wood mass and content of nutrient, metal or metalloid per tree

Tree species	Site	Height (cm)	dbh (cm)	Volume (m3)	Mass (ton)	Mass (kg)	Ca (mg)	S (mg)	Mn (mg)	Fe (mg)	Ba (mg)	As (mg)
E. cam	Mispah	1 013	16.3	0.004	0.004	3.57	4 735.21	697.82	524.43	890.08	125.40	16.63
	Madala	1 790	26.2	0.012	0.010	10.14	13 313.44	1 403.23	7 340.11	1 790.99	348.76	59.32
	Red soil	1 040	9.6	0.003	0.002	2.16	1 543.53	273.73	181.99	410.82	49.71	10.28
E. gxc	Mispah	1 388.2	10.6	0.045	0.038	37.70	74 060.68	3 334.61	5 428.61	3 000.02	699.65	134.57
	Madala	1 500	12.8	0.071	0.059	59.24	52 664.21	5 331.51	4 473.50	6 455.80	2 032.32	291.02
	Red soil	1 830	20.8	0.228	0.189	188.99	493 409.99	22 604.40	36 906.69	108493.59	6 550.70	866.33
E. dunnii	Mispah	2 430	28.6	0.018	0.015	15.03	36 007.58	1 663.85	24 155.08	1 192.75	760.74	97.49
	Madala	1 648	19.9	0.009	0.007	7.09	14 108.58	711.80	11 473.85	650.89	455.26	43.94
	Red soil	1 170	11.1	0.003	0.003	2.81	18 574.79	272.73	3 591.50	403.76	67.70	13.07

Table 13: Average content per site (species combined)

	Ca (mg)	S (mg)	Mn (mg)	Fe (mg)	Ba (mg)	As (mg)
Mispah	38 267.82	1 898.76	10 036.04	1 694.28	528.60	82.90
Madala	26 695.41	2 482.18	7 762.49	2 965.89	945.45	131.43
Red soil	171 176.11	7 716.96	13 560.06	36 436.06	2 222.70	296.56

5.2 Discussion

5.2.1 Nutrients

The calcium concentrations in wood were 1 117.62, 1 821.42 and 3 665.36 mg/kg for *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. The normal concentration range of calcium in plants according to **Table 3** is 1 830.2 to 2 042.5 mg/kg. The concentration of calcium in *E. dunnii* was above this range. The accumulation of calcium in *Eucalyptus* tissues showed the following trend: Bark>branches/twigs>leaves>wood.

In a study conducted on *E. camaldulensis* irrigated with municipal effluent, calcium was recorded as ranging between 11 090-19 950 mg/kg (Singh *et al.*, 2010). This was mainly due to the background Ca soil concentrations in the Singh (2010) study which were on average 12 175 mg/kg. In India, a study of *E. tereticornis* grown in bauxite mining soils showed an increase in Ca uptake (3 480 mg/kg) in the shoot when the soil was inoculated with mycorrhizal bacteria (Khosla and Sudhakara Reddy, 2008). Two of the studied species (*E. cam* and *E. gxc*) were below the findings of these previous studies and one species (*E. dunnii*) was above the normal range of calcium in plants. This study recorded a range of 22 841 to 45 332 mg/kg for stem bark. This might lead to calcium depletion as seen in other *Eucalyptus* stand studies (Spangenberg *et al.*, 1996) in soils as the bark gets removed from site.

Concentrations of sulphur in wood ranged from 99 to 153 mg/kg. The sulphur concentrations were 153.48, 99.35 and 102.70 mg/kg for *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. The accumulation of sulphur in *Eucalyptus* tissues showed the following trend: Leaves>branches/twigs>bark>wood.

The sulphur concentrations recorded for the leaves in this study ranged from 865.15 to 1465.50 mg/kg. In a study conducted in Australia on *E. cladocalyx* seedlings grown on gold tailings, the accumulation of sulphur in leaves was approximately 4 000 mg/kg (Madejón *et al.*, 2012). This is four times more than what was recorded in this study and it could be attributed to the addition of biosolids to the tailings substrate which had background sulphur concentrations of 22 600 mg/kg (Madejón *et al.*, 2012). The sulphur concentrations of the wood can therefore be assumed to be safe for health as they fall below the normal range concentration in plants of 1 000 mg/kg as indicated in **Table 3**.

5.2.2 Metals and Metalloids

Manganese concentration in wood ranged from 84 to 115 mg/kg. The manganese concentrations were 84.35, 115.20 and 86.73 mg/kg for *E. camaldulensis, E. grandis x*

camaldulensis and *E. dunnii* respectively. The accumulation of manganese in *Eucalyptus* tissues showed the following trend: Bark>leaves>branches/twigs>>wood.

The critical concentration levels for manganese in plants ranges from 300 to 500 mg/kg (Kabata-Pendias and Pendias, 1992; Khan *et al.*, 2008). The levels in wood were much lower than this limit although the concentration found in bark, leaves and branches/twigs samples was above this level. In a study conducted on *E. camaldulensis* irrigated with municipal effluent, manganese was recorded as ranging between 134 to 458 mg/kg (Singh *et al.*, 2010). The normal concentration ranges for manganese are 15 to 100 mg/kg. The concentration of manganese that was reported for leaves was however much higher than this and was averaged at 2 475 mg/kg. Excessive levels of manganese can cause damage to the brain, liver, kidneys, the developing foetus etc. (Khan *et al.*, 2008). Care must be taken to ensure these leaves are not supplied to third parties and are disposed of properly.

Iron concentrations in wood were 205.32, 254.21 and 104.94 mg/kg for *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. The accumulation of iron in *Eucalyptus* tissues showed the following trend: Bark>leaves>branches/twigs>>wood.

According to Table 4, the normal concentration ranges for iron in plants are between 640 to 2 486 mg/kg. The wood concentrations are within this range. All the tissue samples also fell within this range. In a study conducted on *E. camaldulensis* irrigated with municipal effluent, iron was recorded as ranging between 570-959 mg/kg (Singh *et al.*, 2010).

In the wood of the *Eucalyptus* trees sampled in this study, the concentration of arsenic was between 4.36 and 5.78 mg/kg. The *Eucalyptus* tree species planted in this study accumulated almost the same amount of arsenic in the wood. Arsenic concentrations of 5.09, 4.36 and 5.78 mg/kg were accumulated in the wood of *E. camaldulensis, E. grandis* x *camaldulensis* and *E. dunnii* respectively. These arsenic levels were considerably higher than the 1 mg/kg maximum permissible limit (Rauf *et al.*, 2011). *E. camaldulensis* and *E. dunnii* were observed to have concentrations above the normal concentration range of arsenic in plants which is 5 mg/kg. The accumulation of arsenic in *Eucalyptus* tissues showed the following trend: Branches/twigs>wood>bark>leaves.

In a case study conducted in Australia on a gold mine tailings facility, it was found that the concentration of arsenic in various types of *Eucalyptus* species was 0.29-5.14 µg/g in mature leaves and 0.11-0.61 µg/g in the stem (King *et al.*, 2008). This was significantly much lower than what was recorded in this study. In another study on plants growing on mine waste in south west England, arsenic ranged from 350 to 2 040 mg/kg (Porter and Peterson, 1975).

Both these studies did not report similar results as were found in this study and this could be due to soil chemistry.

The highest concentration of barium in wood was measured for *E. dunnii* at 46.29 mg/kg. The highest recorded barium concentration in bitter orange tree leaves in a study conducted in Spain was 100 mg/kg (Oliva *et al.*, 2007). Barium concentration in most plants ranges from 4-50 mg/kg (Chaudhry *et al.*, 1977). The concentration measured in this study is lower than both these literature sources.

5.2.3 Amount of nutrients, metals and metalloids per tree

The amount of fuelwood used per household in a study conducted in Bushbuckridge in Mpumalanga was recorded as 3 285 kg/annum (Matsika *et al.*, 2013). This could be estimated at 273.75 kg/household/month (assuming constant usage throughout the year, which might not be the case). This can be further broken down to 9.13 kg/household/day. If contaminated *Eucalyptus* wood is used in this household, the result would be as indicated in **Table 14.**

Table 14: Average content of nutrients, metals and metalloids in *Eucalyptus* species consumed and available in ash per household per day (assuming no volatilisation during combustion)

	Wood Mass (kg)	**Ca (mg)**	**S (mg)**	**Mn (mg)**	**Fe (mg)**	**Ba (mg)**	**As (mg)**
E. camaldulensis	9.13	10 203.83	1 401.30	770.09	1 874.61	281.53	46.45
E. gxc	9.13	16 629.55	1 057.18	347.17	1 158.68	441.07	57.13
E. dunnii	9.13	33 464.77	907.07	1 051.76	2 320.93	266.37	39.76

5.2.4 Comparison to maximum permissible levels

It can be seen from **Figure 22** that arsenic was above four times the WHO permissible levels for plants for all three *Eucalyptus* species. Care must thus be taken when harvesting these trees and selling them as fuelwood.

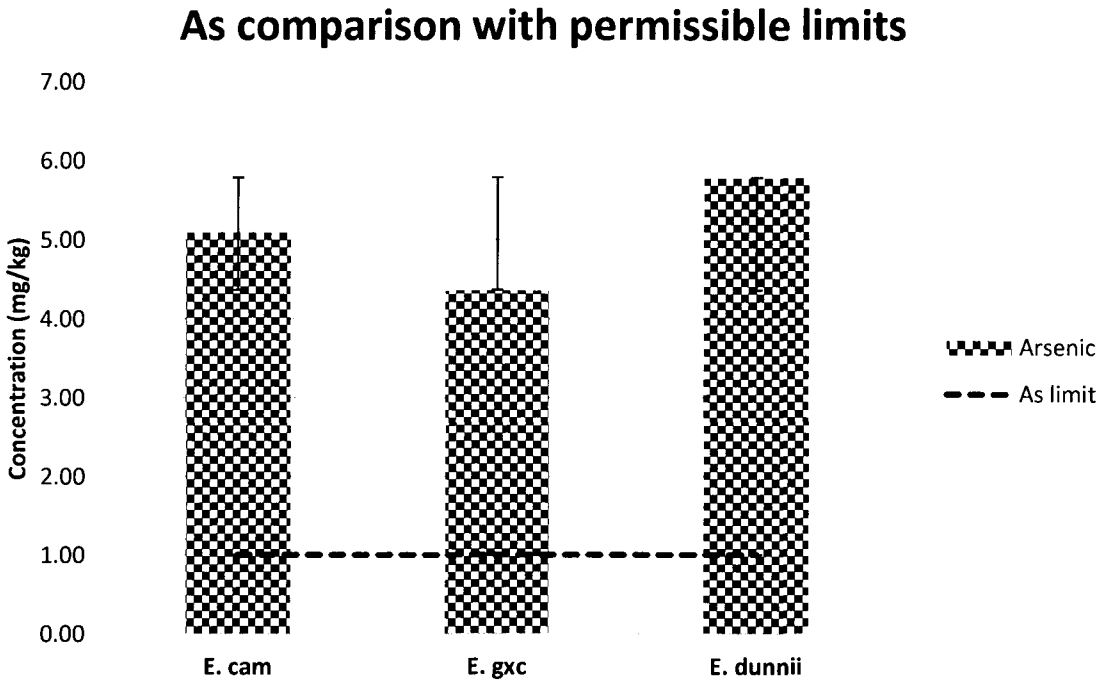

Figure 22: Comparison of metal/loids to the WHO maximum permissible levels

5.2.5 Risk Assessment

The methodology for the risk assessment was highlighted in chapter 4. The hazard as determined in the results chapter is indicated in **Table 15** below. The exposure was determined by assuming that the pathway of these metals and metalloids is through human consumption of vegetables grown in gardens fertilised with ashes from the *Eucalyptus* wood grown in the AMD contaminated sites. The exposure was then calculated and estimated by using literature. According to Madubansi and Shackleton (2005), the average amount of collected wood in 2002 in different settlements in Limpopo was 44.9 kg/person/month (or 1.50 kg/person/day). Assuming the ash content is 2.38% (Munalula and Meincken, 2009), the ash produced from burning this wood would be 1.069 kg/person/month. A transfer factor of metals from soil to plants was adapted from Jolly *et al.* (2013) and is presented in **Table 15** below for the common vegetables. It only covers Mn, Fe and As (there was no reference for Ba found). The estimated averages of metal/loids found in typical garden vegetables are indicated in **Table 16** below.

Table 15: Transfer Factor from soil to vegetables (Jolly *et al.*, 2013)

Elements	Transfer Factor					
	Spinach	Tomato	Radish	Bean	Cauliflower	Carrot
Mn	-	-	-	0.038	-	-
Fe	0.006	0.001	0.001	0.003	0.002	0.002
As	-	0.001	0.001	0.001	0.001	0.001

Table 16: Average concentration of metal and metalloids in typical vegetables found in gardens

Species	Concentration of metal/loids in vegetables						
	Spinach	Tomato	Radish	Bean	Cauliflower	Carrot	Average
Mn							
E. camaldulensis	-	-	-	3.21	-	-	**0.53**
E. gxc	0.69	0.12	0.12	0.35	0.23	0.23	**0.29**
E. dunnii	-	0.09	0.09	0.09	0.09	0.09	**0.07**
Fe							
E. camaldulensis	-	-	-	7.80	-	-	**1.30**
E. gxc	1.53	0.25	0.10	0.76	0.51	0.51	**0.61**
E. dunnii	-	0.10	0.10	0.10	0.10	0.10	**0.09**
As							
E. camaldulensis	-	-	-	0.19	-	-	**0.03**
E. gxc	0.03	0.00	0.00	0.01	0.01	0.01	**0.01**
E. dunnii	-	0.01	0.01	0.01	0.01	0.01	**0.00**

WHO (1998) guidelines specify that the required vegetable daily intake for a human diet is 300 g/person. Assuming an average person of 70kg, then the daily intake is as presented in **Table 17.**

Table 17: Daily intake of metal/metalloids per *Eucalyptus* species

Species	Conc. in wood (mg/kg)	Conc. in vegetables (mg/kg)	Daily intake of metal/loids (mg/d)	Oral reference Dose (mg/d)	Reference for oral reference dose
Mn					
E. camaldulensis	84.35	0.53	0.16	0.5-5.0	WHO, 1993
E. gxc	115.20	0.29	0.09	0.5-5.0	WHO, 1993
E. dunnii	86.73	0.07	0.02	0.5-5.0	WHO, 1993
Fe					
E. camaldulensis	205.32	1.30	0.39	10.0-60.0	WHO, 1993
E. gxc	254.21	0.61	0.18	10.0-60.0	WHO, 1993
E. dunnii	104.94	0.09	0.03	10.0-60.0	WHO, 1993
As					
E. camaldulensis	5.09	0.03	0.01	0.05	WHO, 1966
E. gxc	4.36	0.01	0.00	0.05	WHO, 1966
E. dunnii	5.78	0.00	0.00	0.05	WHO, 1966

To complete the risk assessment, I have rated both the hazard and the exposure as follows:

- For hazards: 1 for least concentration of metal/loid in wood and 3 for most concentration of metal/loid in wood;
- For exposure in Mn: 10 for ≤0.5; 20 for 0.5-2.5; 30 for 2.5-5 and 40 for >5 with reference to the oral reference dose;
- For exposure in Fe: 10 for ≤10; 20 for 10-30; 30 for 30-60 and 40 for >60 with reference to the oral reference dose;
- For exposure in As: 10 for <0.05; 20 for 0.5; 30 for 0.5-1.0 and 40 for >1 with reference to the oral reference dose.

The following equation was used to calculate the risk. The risk assessment is indicated in **Table 18** below.

Risk = Hazard x Exposure (USEPA, 1991).

Table 18: Risk Assessment

Species	Hazard	Exposure	Risk
Mn			
E. camaldulensis	1	10	10
E. gxc	3	10	30
E. dunnii	2	10	20
Fe			
E. camaldulensis	2	10	20
E. gxc	3	10	30
E. dunnii	1	10	10
As			
E. camaldulensis	2	10	20
E. gxc	1	10	10
E. dunnii	3	10	30
Final Risk Assessment Score		***E. camaldulensis***	**50**
		E. gxc	**70**
		E. dunnii	**60**

From the risk assessment done above, there was no evidence that there will be adverse effects caused by supplying fuelwood from these contaminated *Eucalyptus* trees. Even though high arsenic concentrations were recorded in this study, if the wood is used as fertilizer in a vegetable bed, the transfer of the arsenic to the common vegetables is below the daily oral reference dose. All the calculated results for the daily intake of Mn, Fe and As fall below the oral reference dose recommended. This, however, does not rule out harm that might be caused by other metals or metalloids that were not assessed in this section due to the results that were not in agreement with the CRMs. It is suspected that the CRM used was old.

CONCLUSION AND RECOMMENDATIONS

The results of this study highlight the importance of evaluating the environmental impact that can be caused by selling or supplying contaminated wood to the public. It also highlights the different health impacts that can be realised should the ash from the wood be directly or indirectly ingested by the public over a long period of time. While the nutrients accumulated varied, the metal/loids accumulated were almost the same for all the *Eucalyptus* species studied. Concern was raised in regards to the exceeded permissible levels in plants for arsenic for the *Eucalyptus* species studied. Arsenic concentrations of 5.09, 4.36 and 5.48 mg/kg were accumulated in the wood of *E. camaldulensis, E. grandis x camaldulensis* and *E. dunnii* respectively. The concentrations exceeded the WHO MPL of 1 mg/kg for both chromium and arsenic. Arsenic has also been found to volatilise at temperatures below 400°C in hyperaccumulators such as *Pteris vittata* containing high As content (1 170 mg/kg). This causes a threat to human health as As is a Group 1 carcinogen associated with cancers of the lung, bladder, kidney, skin, liver and prostate.

Arsenic has been known to cause a lot of ailments following prolonged exposure. The use of ash resulting from burning this *Eucalyptus* wood for healing purposes or as a fertilizer for a long duration, might result in health impacts to end users. Two species had arsenic levels above the normal concentration range in plants of 0.02 to 5 mg/kg i.e. *E. camaldulensis* and *E. dunnii.* All the species however were above the WHO MPL in plants of 1 mg/kg which seems to suggest that all plant species concentration are unsafe. However, when this arsenic is transferred as ash to vegetables, it was found to be below the daily oral reference dose. Taking into account all the determining properties, it was seen from the risk assessment that the *E. camaldulensis* species was the preferred fuelwood followed by *E. dunnii.* To answer the research objectives;

- The accumulation of metals varies with plant tissues in *Eucalyptus*, with leaves accumulating the majority of metals;
- There was variation in metals accumulated by the different *Eucalyptus* species, with *E. camaldulensis* hybrid accumulating the least amount of harmful metals;
- Arsenic was above the WHO permissible levels in plants for all three *Eucalyptus* species;
- A risk assessment performed found that there was no evidence that there will be adverse effects caused by supplying fuelwood from these contaminated *Eucalyptus* trees. Even though high arsenic concentrations were recorded in this study, if the

wood is used as fertilizer in a vegetable bed, the transfer of the arsenic to the common vegetables is below the recommended daily intake;

- The best option for fuelwood was the *E. camaldulensis* hybrid clone, although there is a concern about other metal/loids that were not evaluated due to non-agreement with CRM.

A considerable amount of metal/loids was accumulated in the leaves. Normally, the stem is the most frequently used part of the tree to fuel fires. A consideration on what will happen to the leaves if they are not combusted will also have to be investigated. The major question raised by this study is how the environmental and health impacts will be managed when selling/supplying this contaminated wood to the public. The other major concern is if arsenic in the wood of these *Eucalyptus* tissues volatilises, is it at all safe to supply this wood to the community. The crucial task will be to ensure that the contamination is reduced before the wood is supplied or to ensure that the ash is treated accordingly. As a future study it would be interesting to also see the relationship between the metal/loids in the soil and those accumulated by the trees. It would also be interesting to check if there is variability between the sites.

REFERENCES

Abbasi, T., and Abbasi, S.A. 2010. Biomass Energy and the Environmental Impacts Associated with its Production and Utilization, Renewable and Sustainable Energy Reviews, 14:919–937.

Aber, J., Nadelhoffer, K., Steudler, P. and Melillo, J. 1989. Nitrogen Saturation in Northern Forest Ecosystems. Bioscience, 39: 378–386.

Aber, J.D., Magill, A., McNulty, S.G., Boone, R.D., Nadelhoffer, K.J., Downs, M., Hallett, R. 1995. Forest Biogeochemistry and Primary Production Altered by Nitrogen Saturation. Water Air and Soil Pollution 85: 1665-1670.

Abo-Hassan, A., Kandeel, S. A. and Kherallah, I. E. 1988. New Eucalypt Species Introduction in the Saudi Arabia Central Zone. In Proceedings of the International Forestry Conference, Australian Bicentenary, 5: 1–16.

Ahmad, S.A. 2000. Arsenic: Water Contamination and Health Hazards, 1st edition. Udayan Press, Rajshahi, Bangladesh, p. 48.

Akcil, A., and Koldas, S. 2006. Acid Mine Drainage (AMD): Causes, Treatment and Case Studies, Journal of Cleaner Production, 14: 1139–1145.

Álvarez-Ayuso E., Otones, V., Murciego A., García-Sánchez A. and Santa Regina I., 2012. Antimony, Arsenic and Lead Distribution in Soils and Plants of an Agricultural Area Impacted by Former Mining Activities, Science of the Total Environment 439: 35-43.

AngloGold Ashanti Report to Society. 2004. Environment, South Africa, 7.11. www.anglogoldashanti.com (Access on 2 April 2013).

AngloGold Ashanti Case Study. 2008. Growing Vegetation to Reduce Environmental Damage, South Africa, www.anglogoldashanti.com (Access on 2 April 2013).

AngloGold Ashanti Sustainability Review. 2010. South Africa, www.anglogoldashanti.com (Access on 2 April 2013).

Aronsson, P. and K. Perttu (Eds.). 1994. Willow Vegetation Filters for Municipal Wastewaters and Sludges, A Biological Purification System, Swedish University of Agricultural Sciences, Uppsala, p. 230.

Assareh, M.H., Shariat, A. and Ghamari-Zare, A. 2008. Seedling Response of Three *Eucalyptus* Species to Copper and Zinc Toxic Concentrations, Caspian Journal of Environmental Science, 6 (2): 97–103.

Ayepola, O.O. and Adeniyi, B.A. 2008. The Antibacterial Activity of Leaf Extracts of *Eucalyptus camaldulensis* (Myrtaceae), Journal of Applied Sciences Research 4, 1410-1413.

Babayi, H., Kolo, I., Okogun, J.I. and Ijah, U.J.J. 2004. The Antimicrobial Activities of Methanolic Extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* Against Some Pathogenic Microorganisms, Biokemistri 16, 106-111.

Baker, A.J.M., McGrath, S.P., Sidoli, C.M.D. and Reeves, R.D. 1994. The Possibility of In Situ Heavy Metal Decontamination of Polluted Soils Using Crops of Metal-Accumulating Plants, Resources, Conservation and Recycling, 11(1):41–49.

Balatinecz, J.J. and Kretschmann, D.E. 2001. Properties and Utilization of Poplar Wood, NRC, Research Press National Research, Council of Canada, On KIA OR6, Canada, pp. 277-291.

Becquer, T., Quantin, C., Sicot, M. and Boudot, J.P. 2003. Chromium Availability in Ultramafic Soils from New Caledonia, The Science of the Total Environment 301: 251–261.

Begonia, G.B.; Davis, C.D.; Begonia, M.F.T. and Gray, C.N. 1998. Growth Responses of Indian Mustard [Brassica juncea (L.) Czern.] and its Phytoextraction of Lead from a Contaminated Soil, Bulletin of Environmental Contamination and Toxicology, vol. 61, pp. 38-43.

Bennett, D. 2003. Combustion economics, Short Rotation Crops for Bioenergy: New Zealand, Report, pp. 185- 190.

Benstead. P. 2003. Bioenergy in New Zealand- The way forward, Short Rotation Crops for Bioenergy: New Zealand, Report, pp. 33–38.

Bhati, M. and Singh, G. 2003. Growth and Mineral Accumulation in *Eucalyptus camaldulensis* Seedlings Irrigated with Mixed Industrial Effluents, Bioresources Technology 88, 221-228.

Blaylock, M.J.; Salt, D.E.; Dushenkov, S.; Zakharova, O.; Gussman, C.; Kapulnik, Y.; Ensley, B.D. and Raskin. I. 1997. Enhanced Accumulation of Pb in Indian Mustard by Soil-Applied Chelating Agents. Environmental Science and Technology, vol. 31, no. 3, pp..860- 865.

Boland, D. J, Brophy, J. J., Flynn, T M. and Lassak, E. V. 1982. Phytochemistry 21, 2467

Boland, D.J., Brooker, M.I.H., Chippendale, G.M., Hall, N., Hyland, B.P.M., Johnston, R.D., Kleinig, D.A. and Turner, J.D. 1984. Forest Trees of Australia. Nelson and CSIRO, Melbourne.

Bouwer, H. 2000. Integrated Water Management: Emerging Issues and Challenges, Agricultural Water Management, 45(3), pp. 217-228.

Brady, N.C. and Weil, R.R. 2008. The Nature and Properties of Soils, 14th edition, Pearson Education Inc., New Jersey.

Bredenkamp, B. 2000. Section 4.5, Volume and Mass of logs and standing trees, South African Forestry Handbook., Department of Forest, University of Stellenbosch, Pg 167-174.

Bren, L.J. and Gibbs, N.L. 1986. Relationships between Flood Frequency, Vegetation and Topography in a River Red Gum Forest. Australian Forest Research 16, pp. 357-370.

British Herbal Medicine Association, British Herbal Pharmacopoeia, 1996.

Brooker, M.I.H., Connors, J.R., Slee, A.V. and Duffy, S. 2002. EUCLID: eucalypts of southern Australia, CSIRO Publishing, Collingwood.

Brown, G.E., Foster, A.L. and Ostergren, J.D. 1999. Mineral Surfaces and Bioavailability of Heavy Metals: A Molecular-Scale Perspective. Proceedings of the National Academy of Science. 96: pp. 3388-3395.

Brown, P.H., Welch, R.M. and Cary, E.E. 1978. Nickel: A Micronutrient Essential for Higher Plants. Plant Physiology 85: 801-803.

Cao, X., Wahbi, A., Ma, L., Li, B. and Yang, Y. 2009. Immobilization of Zn, Cu and Pb in contaminated soils using phosphate rock and phosphoric acid. Journal of Hazardous Materials 164: 555-564.

CCA, Bill Hinkley Center for Solid and Hazardous Waste Management, University of Florida, (http://www.ccaresearch.org/) (Accessed 04 January 2015).

Chamber of Mines of South Africa. 2004. Facts and figures –plants, edition 2, part 2, Memoirs of the Botanical Survey of South Africa No. 56.

Chaudhry, F.M., Wallace, A. and Mueller, R.T., 1977. "Barium toxicity in plants," Communications in Soil Science and Plant Analysis, vol. 8, no. 9, pp. 795–797

Chenery EM. 1948. Aluminum in the Plant World. Part I, General Survey in Dicotyledons. Kew Bull, 2: 173–183.

Chenery EM. 1949. Aluminium in the Plant World. Part II. Monocotyledons and Gymnosperms. Kew Bull, 4: 463–466.

Chevallier, A. 1996. The Encyclopedia of Medicinal Plants, London: Dorling Kindersley, pp 141-273.

Chrysochoou, M., Dermatas, D. and Grubb, D.G. 2007. Phosphate application to firing range soils for Pb immobilization: The unclear role of phosphate. Journal of Hazardous Materials 144: 1–14.

Costermans, L.F. 1989. Native Trees and Shrubs of South-Eastern Australia, Weldon, Sydney.

Couto, L., Nicholas, I., Muller, M.D. and Barcellos, D.C. 2003. SRC with *Eucalyptus* in Brazil, Short Rotation Crops for Bioenergy, New Zealand, pp. 279–281.

CSIR, 2010. Council for Scientific and Industrial Research, Division of Natural Resources and the Environment Annual Research Report, NRE, Pretoria, South Africa, pg45.

Cunningham, G.M., Mulham, W.E., Milthorpe, P.E., and Leigh, J.H. 1981. Plants of Western New South Wales, Soil Conservation Service of New South Wales, Australia.

Curtis, R. 1998. "Arsenic and Old Wood", This Old House Magazine, March/April 1998.

Dare, P., Gifford, J., Hooper, R. J., Clemens, A.H., Damiano, L.F., Gong, D. and Matheson, T.W. 2001, Combustion Performance of Biomass Residue and Purpose Grown Species, Biomass and Bioenergy 21: 277–287.

Dennis, A. 2008. Salt Glands and Elemental Concentrations in Leaves of *Tamarix Usneoides E.May ex Bunge* (Tamaricaceae) Grown on Seepage from Highveld Gold Mines, Honours Project, Ecological Engineering and Phytoremediation Programme, School of Animal, Plant and Environmental Sciences (AP&ES). University of the Witwatersrand (unpublished).

Department of Minerals and Energy (DME). 2007. E. Swart, Director of Mine Rehabilitation, Department of Minerals and Energy of South Africa, cited in: The Star Daily Newspaper, Johannesburg, 23rd May.

Department of Tourism, Economic and Environmental Affairs of the Free State Province. 2002. The Waste Lekgotla Conference, Bloemfontein.

Dickinson, N M. 1997. Rehabilitation and Remediation of Metal-Contaminated Soils Using Trees. 4th International Conference on the Biogeochemistry of Trace Elements, Berkeley, California, pp. 437-8.

Dickinson, N.M. 2000. Strategies for Sustainable Woodland on Contaminated Soils. Chemosphere, 41: pp. 259–263.

Djavanchir, K., and Mossadegh, A. 1973. *Eucalyptus*. Tehran University Press. 435p.

Dovie, D.B.K., Witkowski, E.T.F., and Shackleton, C.M. 2005. Monetary Valuation of Livelihoods for Understanding the Composition and Complexity of Rural Households, Agriculture and Human Values 22, pp. 87–103.

Downer, N.R. 2008. Method Development for the Digestion and Analysis of Four Common Sedimentary Lithologies using ICP-OES and ICP-MS, University of Johannesburg, Faculty of Science, Master of Science in Analytical Chemistry Dissertation (unpublished).

Dube, A., Zbytniewski, R., Kowalkowski, T., Cukrowska, E. and Buszewski, B. 2001. Adsorption and Migration of Heavy Metals in Soil. Polish Journal of Environmental Studies 10: 1-10.

Duggan, J., 2005, The Potential for Landfill Leachate Treatment Using Willows in the UK—A Critical Review, Resources, Conservation and Recycling 45, pp. 97–113.

Du Plessis, M. 1996. Tree Volume and Taper Equation for *Eucalyptus grandis* (*Eucalyptus camaldulensis* GXC. Internal Report, HL&H Mining Timber, Tree Improvement Centre, White River.

Dutchinse, 2014. Website article dated 8/05/2014, Very Rare South Africa 5.3M Earthquake – Location: Vaal Reefs Mine – 6.2miles depth http://i2.wp.com/dutchsinse.tatoott1009.com/wp-content/uploads/2014/08/sa_ops.gif (Accessed 5 March 2015)

Dye, P.J., Jarmain, C., Oageng, B., Xaba, J., and Weiersbye, I.M. 2008a. A.B. Fourie, M. Tibbett, I.M. Weiersbye, P.J. Dye (eds). The Potential of Woodlands and Reed-beds for Control of Acid Mine Drainage in the Witwatersrand Gold Fields, South Africa, Mine Closure 2008 –Australian Centre for Geomechanics, Perth, ISBN 978-0-9804185-6-9.

Dye, P.J. and Weiersbye, I.M. 2010. The Mine Woodlands Project in the Witwatersrand Basin Gold Fields of South Africa: Strategy and Progress, Ecological Engineering and Phytotechnology Programme, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg.

Dye, P.J. 2010. Modelling The Effects of Salinity (Chlorides) on Water Uptake by Trees Growing on Sites Affected by Seepage from Gold Tailings Dams, Ecophysiology Research Group Natural Resources and the Environment, CSIR Interim Report (unpublished).

Ebbs, S.D. and Kochian. L.V. 1998. Phytoextraction of Zinc by Oat (*Avena sativa*), Barley (*Hordeum vulgare*), and Indian Mustard (*Brassica juncea*). Environmental Science and Technology, vol. 32, no. 6, pp. 802-806.

Ebbs, S.D. Lasat, M.M.; Brandy, D.J.; Cornish, J.; Gordon, R. and Kochian, L.V. 2010. Heavy Metals in the Environment: Phytoextraction of Cadmium and Zinc from Contaminated Soil. Journal of Environmental Quality, 1997, vol. 26, pp. 1424-1430.

Ellis, R.W. and Eslick, L. 1997. Variation and Range of Mercury Uptake into Plants at a Mercury-Contaminated Abandoned Mine Site, Bull. Environmental Contamination and Toxicology 59: pp. 763-769.

Epstein, E. 1965. Mineral Metabolism. In Plant Biochemistry (J. Bonner and J.E. Verner, eds), Academic Press, London and Orlando, pp. 438-466.

Epstein, E. and Bloom, A. 2005. Mineral Nutrition of Plants: Priciples and Perspectives. 2nd Edition. Sinauer Associates, Inc. Publishers, Sunderland, MA, ISBN 0-87893-172-4

Estrellan, C.R. and Iino, F. 2010. Toxic Emissions from Open Burning, Chemosphere, 80: 193–207.

Evans, J., 1992. Plantation Forestry in the Tropics, Second edition, Oxford Science Publication. USA, p. 403.

FAO. Ugalde L. and Pérez, O. 2001. Mean Annual Volume Increment of Selected Industrial Forest Plantation Species, Forest Plantation Thematic Papers, Working Paper 1. Forest Resources Development Service, Forest Resources Division. FAO, Rome (unpublished).

Feikema, P.M. and Baker, T.G. 2011. Effect of Soil Salinity on Growth of Irrigated Plantation *Eucalyptus* in South-Eastern Australia, Agricultural Water Management, 98:1180–1188.

Fendorf, S.E. 1995. Surface Reactions of Chromium in Soils and Waters. Geoderma. 67: 55-71.

Fendorf, S.E. and Li, G. 1996. Kinetics of Chromate Reduction by Ferrous Iron. Environmental Science and Technology. 30: 1614-1617.

Fine, P., Paresh, R., Beriozkin, A., and Hass, A. 2014. Chelant-enhanced Heavy Metal Uptake by *Eucalyptus* Trees under Controlled Deficit Irrigation. Science of the Total Environment, 493: 995–1005.

Gansauer, G. 2012. Trees in Phytoremediation. Biology Lecture Notes, BZ 572, Colorado State University, Fort Collins.

Garnett, T. and Smethurst, P. 1999. Ammonium and Nitrate Uptake by *Eucalyptus nitens*: Effects of pH and Temperature. Plant and Soil, 214: 133–140.

Garnett, T.P., Shabala, S.N., Smethurst, P.J. and Newman, I.A. 2003. Kinetics of ammonium and nitrate uptake by eucalypt roots and associated proton fluxes measured using ion selective microelectrodes. Functional Plant Biology 30: 1165–1176.

Glimmerveen, I (Ed.). 1996. Heavy Metals and Trees, Institute of Chartered Foresters, Edinburgh, p. 206.

Grieve, M. 1971. A Modern Herbal: The Medicinal, Culinary, Cosmetic and Economic Properties, Cultivation and Folk-Lore of Herbs, Grasses, Fungi, Shrubs, & Trees with All Their Modern Scientific Uses (Vol. 2). Courier Corporation.

Guzzi, G. and La Potta, C.A. 2008. Molecular Mechanisms Triggered by Mercury. Toxicology 244: 1-12.

Hajar, E.W.I., Sulaiman, A.Z.B. and Sakinah, A.M.M. 2014. Assessment of Heavy Metals Tolerance in Leaves, Stems and Flowers of *Stevia rebaudiana* Plant. Procedia Environmental Sciences 20: 386 – 393.

Hänsch, R. and Mendel, R.R. 2009. Physiological Functions of Mineral Micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl), Plant Biology 12: 259–266.

Harborne, J.B. 1993. Introduction to Ecological Biochemistry. Gulf Professional Publishing.

Hawkins, H.J., Hettaschb, H., Mesjasz-Przybylowiczc, J., Przybylowicz, W. and Cramera, M.D. 2008. Phosphorus Toxicity in the *Proteaceae*: A problem in Post-agricultural Lands, Scientia Horticulturae 117: 357–365.

Hazell, P. and Wood, S. 2008. Drivers of Change in Global Agriculture. Philosophical Transactions of the Royal Society B: Bio. Sci., 363 (1491): 495–515.

Herbert, M., 2008, Mine Woodlands Research Project Annual Report: 2007- 08. Restoration and Conservation Biology Research Group, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa (unpublished).

Hillis, W. E. and Brown, A. G., 1984, Eucalypts for Wood Production, CSIRO/Academic Press, London, Pg434.

Huang, J.W., Chen, J. and Cunningham, S.D. 1997. Phytoextraction of Lead Contaminated Soils: In: Phytoremediation of soil and water contaminants. ACS Symposium Series, Kruger, E.L., Anderson T.A. and Coats J.R. (eds), 1997a, p. 283-298.

Huang, J.W., Chen, J.J., Berti, W.R. and Cunningham, S.D. 1997. Phytoremediation of Lead Contaminated Soils: Role of Synthetic Chelates in Lead Phytoextraction. Environmental Science and Technology, 1997b, vol. 31, no. 3, p. 800-805.

Hunter, I., 2001. Above Ground Biomass and Nutrient Uptake of Three Tree Species (*Eucalyptus camaldulensis, Eucalyptus grandis and Dalbergia sissoo*) as affected by Irrigation and Fertiliser, at 3 years of age, in Southern India, Forest Ecology and Management, 144: 189–199.

Hutchings, A. 1996. Zulu Medicinal Plants, University of Natal Press, Pietermaritzburg.

International Agency for Research on Cancer (IARC). 2012. IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans, Vol 100 C, Arsenic, Metals, Fibers and Dusts; IARC: Lyon,France.

Jacobs, M.R. 1955. Growth Habits of the Eucalypts. Forestry and Timber Bureau, Canberra, Australia, ISBN 0959459243.

Jain, R.K. 1994. Fuelwood Characteristics of Medium Tree and Shrub Species of India, Bioresource Technology, 47:81–84.

Jansen S, Broadley MR, Robbrecht E. and Smets E. 2002. Aluminum Hyperaccumulation in Angiosperms: A Review of its Phylogenetic Significance. Botanical Review; 68: 235–269.

Jolly, Y. N., Islam, A. and Akbar, S., 2013. Transfer of metals from soil to vegetables and possible health risk assessment, SpringerPlus 2013 2:385.

Kabata-Pendias, A. and Pendias, H. 1984. Trace Elements in Soils and Plants. CRC Press, Boca Raton, Florida.

Kabata-Pendias, A. and Pendias, H. 1992. Trace Elements in Soils and Plants. 2nd edn. CRC Press, London. pp. 227–233.

Kanias, G.D., Tsitsa, E., Loukis, A. and Kilikoglou, V. 1993. Determination and Statistical Analysis of Trace Element and Active Constituent Concentrations in the Medicinal Plant *Eucalyptus camaldulensis* Dehnh (E. Rostratus Schlecht), Journal of Radioanalytical and Nuclear Chemistry, Vol 169, No. 2, p. 483-491.

Khan, S.A., Khan, L., Hussain, I., Shah, H. and Akhtar, N. 2008, Comparative Assessment of Heavy Metals in *Euphorbia helioscopia* L., Pakistan Journal of Weed Science Research 14(1-2): 91-100.

Khosla, B. and Sudhakara Reddy, M. 2008, Response of Ectomycorrhizal Fungi on the Growth and Mineral Nutrition of *Eucalyptus* Seedlings in Bauxite Mined Soil, American-Eurasian Journal of Agriculture and Environmental Science 3:123-126.

King, D.J., Doronila, A.I., Feenstra, C., Baker, A.J.,M. and Woodrow, I.E. 2008. Phytostabilisation of Arsenical Gold Mine Tailings Using Four *Eucalyptus* Species: Growth, Arsenic Uptake and Availability after Five Years, Science of the Total Environment 406: 35-42.

Krämer, U. 2010. Metal hyperaccumulation in plants, Annual Review, Plant Biology 61:28.1–28.18

Kumar, P.B.A.N; Dushenkov, V.; Motto, H. and Raskin, I. 1995. Phytoextraction: The Use of Plants to Remove Heavy Metals from Soils. Environmental Science and Technology, 1995a, vol. 29, no. 5, p. 1232-1238.

Kumar, P.B.A.N.; Motto, H. and Raskin, I. 1995. Rhizofiltration: The Use of Plants to Remove Heavy Metals from Aqueous Streams. Environmental Science and Technology, 1995b, vol. 29, no. 5, p.1239-1245.

Labrecque, M; Teodorescu, T and Daigle, S. 1998. Early Performance and Nutrition of Two Willow Species in Short-Rotation Intensive Culture Fertilized with Wastewater Sludge and Impact on the Soil Characteristics, Canadian Journal of Forestry Research 28. 1621-1635.

Lamprecht, H. 1990. Silviculture in the Tropics: Tropical Forest Ecosystems and their Tree Species-Possibilities and Methods for their Long-Term Utilization, GTZ, Eschborn, Germany, Pg 296.

Larcher, W. 2003. Physiological Plant Ecology: Ecophysiology and Stress Physiology of Functional Groups. Springer.

Lasat, M. M. 2002. Phytoextraction of Toxic Metals: A Review of Biological Mechanisms. Journal of Environmental Quality 31, p 109–120.

Lassak, E.V., and McCarthy, T. 1983. Australian Medicinal Plants. Methuen, Australia.

Lavilla I, Filgueiras AV, Bendicho C. 1999 Comparison of Digestion Methods for Determination of Trace and Minor Metals in Plant Samples. Journal of Agriculture and Food Chemistry; 47(12), pp. 5072-5077.

Leco Corporation, 2002. CNS-2000 Elemental Analyzer, CNS-2000 Instruction Manual.

Lee, M. 1999. Risk Assessment Framework for the Management of Sulfidic Mine Wastes, Australian Centre for Mining Environmental Research, Kenmore, 1–20.

Leslie, A.D., Mencuccini, M., and Perks, M. 2012. The Potential for *Eucalyptus* as a Wood Fuel in the UK, Applied Energy, 89:176–182.

Little, K.M., Schumann, A.W., Noble, A.D., 2002, Performance of a *Eucalyptus grandis x E. camaldulensis* Hybrid Clone as Influenced by a Cowpea Cover Crop, Forest Ecology and Management, 168:43-52.

Lockitch, G. 1993. Perspectives on lead toxicity. Clinical Biochemistry 26(5):371-81.

Lyons G.J., Lunny, F., and Pollock, H.P. 1985. A Procedure for Estimating the Value of Forest Fuels. Biomass 8:283–300.

Ma, L.Q., and Rao, G.N. 1997. Chemical fractionation of cadmium, copper, nickel and zinc in contaminated soils. Journal of Environmental Quality 26: 259–264.

Maathius, F. 2009. Plant Physiology of Mineral Macronutrients, Plant Biology 12:250–258.

Mabona, U. and Van Vuuren, S.F. 2013. Southern African Medicinal Plants Used to Treat Skin Diseases, South African Journal of Botany 87:175-193.

Madejón, E., Doronila, A.I., Madejón, P., Baker, A.J.M. and Woodrow, I.E. 2012. Biosolids, Mycorrhizal Fungi and Eucalypts for Phytostabilization of Arsenical Sulphidic Mine Tailing, Agroforestry Systems 84:389–399.

Madubansi, M. and Shackleton, C.M., 2005. Changing Energy Profiles and Consumption Patterns Following Electrification in Five Rural Villages, South Africa, Energy Policy 34 (2006) 4081–4092.

Maestri E, Marmiroli M, Visioli G. and Marmiroli N. 2010. Metal Tolerance and Hyperaccumulation: Costs and Trade-Offs Between Traits and Environment. Environmental Experimental Botany 68:1–13.

Mahmood, K., Morris, J., Collopy, J. and Slavich, P., 2001, Groundwater Uptake and Sustainability of Farm Plantations on Saline Sites in Punjab Province, Pakistan, Agricultural Water Management, 48:1–20.

Mandal, B.K. and Suzuki, K.T. 2002. Arsenic Round the World: A Review. Talanta: 58, 201-235.

Manirul, M.I.; Mayda, U.; Mehraj, H.; Shiam, I.H. and Jamal Uddin, A.F.M. 2015. Evaluation of Arsenic Accumulation by *Colocasia esculenta* (Kochu) from Soil, Journal of Soil Nature 8(2): 1-4.

Marcar, N.E., 1993. Waterlogging Modifies Growth, Water Use and Ion Concentration in Seedlings of Salt-Treated *Eucalyptus camaldulensis, E. tereticornis, E. robusta and E. globulus.* Australian Journal on Plant Physiology. 20:1–13.

Marschner, H. (1995). The Mineral Nutrition of Higher Plants, 2nd edition. Academic Press, London.

Marufu, L., Ludwig, J., Andreae, M.O., Meixner, F.X. and Helas, G. 1997. Domestic Biomass Burning in Rural and Urban Zimbabwe- Part A, Biomass and Energy, 12 (1):53–68.

Matsika, R., Erasmus, B.F.N. and Twine, W.C., 2013, Double Jeopardy: The Dichotomy of Fuel Wood Use in Rural South Africa, Energy Policy 52: 716-725.

McLeroth, B.B., 2015. Soil Survey, Land Capability, Wetland Delineation, Present Land Use, Vegetation Communities, Recommendations (Indigenous Tree Potential, I.E Compartment Divisions, and Land Preparation), and Location of Sites of Cultural and Archaeological Interest, of Tau Tona Waste Rock Dump Footprint (Post-Clean-Up), Varkenslaagte Wetland (Post-Clean-Up) and 'Woodlands', and Magnum Farm, West Wits Mining Operations, prepared for University of the Witwatersrand, Johannesburg and Anglogold Ashanti S.A. Region, Survey Reference: REMS54-Areas A-C Combined - version 1 (unpublished)

McMahon, L., George, B. and Hean, R., 2010b. *Eucalyptus melliodora.* Primefacts- for Profitable, Adaptive and Sustainable Primary Industries, www.dpi.nsw.gov.au/primefacts. (Accessed 20 January 2015).

Medhurst, J.L., Battaglia, M., and Beadle, C.L. 2002. Measured and Predicted Changes in Tree and Stand Water Use Following High-Intensity Thinning Of An 8-Year-Old *Eucalyptus nitens* Plantation. Tree physiology. 22(11): 775-784.

Meers, E., Du Laing, G., Tack, F.M.G. and Verloo, M.G., 2009. Heavy Metal Displacement by Exchangeable Bases (Ca, Mg, K, Na) in Soils and Sediments. Soil Science 174:202-209.

Mengel, K. and Kirkby, E.A. 1987. Principles of Plant Nutrition. 4th Edition. International Potash Institute, IPI, Bern, Switzerland. 685p.

Mengel, K., Kirby, E.A., Kosegarten, H. and Appel, T. 2001. Principles of Plant Nutrition. ISBN-13: 978-1402000089 ISBN-10: 1402000081. 5th Edition

Menzies, N.W., Donn, M.J. and Kopittke, P.M., 2007. Evaluations of Extractants for Estimation of the Phytoavailable Trace Metals in Soils. Journal of Environmental Pollution. 145:121-130.

Meskimen, G. and Francis, J., 2012. *Eucalyptus Grandis* Hill ex Maiden. Eucalipto Rosado, United States Forest Service, http://www.na.fs.fed.us/pubs/silvics_manual/volume_2/eucalyptus/grandis.htm (Accessed on 12 September 2014).

Misra SG, Mani D. 1991. Soil Pollution. Ashish Publishing House, Punjabi Bagh.

Mokgalaka, N.S., Combrinck, S., Lepule, S.P., Regnier, T.C., Weiersbye, I.M. 2009. A.B. Fourie, M. Tibbett (eds). Essential Oil Profiles of *Lippia scaberrima* Sond. from Highveld Gold and Uranium Mines, Mine Closure 2009. Pg 529-537.

Morris, J.D. 1984. Establishment of Trees and Shrubs on a Saline Site Using Drip Irrigation. Australian Forestry, 47:210–217.

Mulligan, C.N., Yong, R.N., and Gibbs, B.F. 2001. Remediation Technologies for Metal-Contaminated Soils and Groundwater: An Evaluation. Engineering Geology, 60(1), 193–207.

Munalula F. and Meincken, M. 2009. An evaluation of South African Fuelwood With Regards to Calorific Value and Environmental Impact, Biomass and Bioenergy, 33:415–420.

Musa, D.A., Nwodo, F.O.C. and Ojogbane, E. 2011. Phytochemical, Antibacterial and Toxicity Studies of the Aqueous Extract of *Eucalyptus camaldulensis* Dehnh, Asian Journal of Plant Science and Research 1, 1-10.

NARSTO, 2004. Particulate Matter Science for Policy Makers: A NARSTO Assesment, edited by P.H. McMurry, M. Shepherd, and J. Vickery, Cambridge University Press, Cambridge, UK, pp 510.

National Academy of Sciences (NAS), 1980, Firewood Crops: Shrub and Tree Species for Energy Production. National Academy Press, Washington, D. C, Pg 237.

Nengovhela, A.C., Yibas, B. and Ogola, J.S. 2006. Characterisation of Gold Tailings Dams of the Witwatersrand Basin with Reference to their Acid Mine Drainage Potential, Johannesburg, South Africa. Water SA. 32 (4): 499–506.

Newman, D. 1981. Third Year Growth of the Species in the *Pulpapel Arboretum*. Research Report, Investigación Forestal, Cartón de Colombia, No. 66. 7p.

Oliva, S.R., Valde´s, B. and Mingorance, M.D., 2008. Evaluation of some pollutant levels in bitter orange trees: Implications for human health, Food and Chemical Toxicology 46: 65–72.

Organisation for Economic Co-operation and Development (OECD). 2014. Consensus Document on the Biology of *Eucalyptus* spp. Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Series on Harmonisation of Regulatory Oversight in Biotechnology No. 58, Pesticides And Biotechnology, www.oecd.org/chemicalsafety/ (Accessed 22 March 2015).

Otarola, A. and Ugalde, L. 1989. Tablas de volumen para *Eucalyptus camaldulensis* en Nicaragua. Proyecto IRENA-CATIE-ROCAP No. 596-0089. Turrialba, C. R. 14 p.

Pallardy, S.G. 2008. Physiology of Woody Plants. 3rd edition. Academic Press publications. ISBN: 978-0-12-088765-1.

Parraga-Aguado, I., Querejeta, J-I., González-Alcaraz, M.N. and Conesa, H.M. 2014, Metal(Loid) Allocation and Nutrient Retranslocation in *Pinus halepensis* Trees Growing on Semiarid Mine Tailings, Science of the Total Environment, 485-486:406-414.

Pawlisz, A. V. 1997. Canadian Water Quality Guidelines for Cr, Environmental Toxicology and Water Quality, 12(2), 123–161.

Peltzer, K. 1999. Faith Healing for Mental and Social Disorders in the Northern Province (South Africa). Journal of Religion in Africa. 387-402.

Peuke, A.D., and Rennenberg, H. 2005. Phytoremediation with Transgenic Trees. Naturforsch C, 60, 199–207.

Ponce, S. C., Prado, C., Pagano, E. and Prado, F.E. 2015. Effect of Solution pH on the Dynamic of Biosorption of Cr (VI) by Living Plants of *Salvinia minima*, Ecological Engineering 74, p. 33-41.

Porter, E. K. and Peterson, P. J. 1975. Arsenic Accumulation by Plants on Mine Waste (United Kingdom). The Science of the Total Environment 4, 365-371

Pradhan, S.K., Holopainen, J.K., and Heinonen-Tanski, H. 2009. Stored Human Urine Supplemented with Wood Ash as Fertilizer in Tomato (*Solanum lycopersicum*) Cultivation and its Impacts on Fruit Yield and Quality. Journal of Agriculture and Food Chemistry. 57(16): 7612-7617.

Prosser, I. P., and Williams, L. 1998. The Effect of Wildfire on Runoff and Erosion in Native *Eucalyptus* Forest. Hydrological Processes, 12(2): 251–265.

Pulford, I.D. and Dickinson, N.W. 2006. In Prasad, m. N. V., Sajwan, K. S. and Naidu, R. (Eds). Phytoremediation Technology Using Trees, Elements in the Environment: Biogeochemistry, Biotechnology and Bioremediation, CRC Press, NW. 375–395.

Pyatt, F.B. 2001. Copper and Lead Bioaccumulation by *Acacia retinoides* and *Eucalyptus torquata* in Sites Contaminated as a Consequence of Extensive Ancient Mining Activities in Cyprus. Ecotoxicology and Environmental Safety 50:60-64.

Rauf, M.A., Hakim, M.A., Hanafi, M.M., Islam, M.M., Rahman, G.K.M.M and Panaullah, G.M. 2011. Bioaccumulation of Arsenic (As) and Phosphorous by Transplanting Aman Rice in Arsenic-Contaminated Clay Soils. Australian Journal of Crop Science 5(12): 1678-1684.

Raungsomboon, S., Chidthaisong, A.; Bunnag, B., Inthorn, D. and Harvey, N.W. 2008. Removal of Lead (Pb2+) by the *Cyanobacterium gloeocapsa* sp. Bioresource Technology 99: 5650–5658.

Riddell-Black, D. 1994. In. P. Aronsson, K. Perttu (Eds.). Heavy Metal Uptake by Fast Growing Willow Species: Willow Vegetation Filters for Municipal Wastewaters and Sludges. A Biological Purification System, Swedish University of Agricultural Sciences, Uppsala, 145–151.

Robb, L.J., and Robb, V.M. 1998a. In: Wilson MGC and Anhaeusser CR (eds.), Environmental Impact of Witwatersrand Gold Mining, The Mineral Resources of South Africa: Handbook, Council for Geoscience. 16: 14–16.

Robb, L.J., and Robb, V.M. 1998b. In: Wilson M.G.C. and Anhaeusser C.R. (eds.). Gold in the Witwatersrand Basin, The Mineral Resources of South Africa: Handbook, Council for Geoscience. 16, Pg 294–349.

Rockwood, D., Alker, G.R., Cardellino, R.W., Ma, L.G., and Tu, C. 2001. Phytoremediation of Contaminated Sites Using Wood Biomass, Florida Center for Solid and Hazardous Waste Management, University of Florida, Gainesville, New York, Report # 01-03, April 2001. 1–96.

Rockwood, D.L., Naidu, C.V., Carter, D.R., Rahmani, M., Spriggs, T.A., Lin, C., and Segrest, S.A. 2004. Short-rotation Woody Crops and Phytoremediation: Opportunities for Agroforestry. In New Vistas in Agroforestry, 51–63.

Sakthivel, V. and Vivekanandan, M. 2009. Reclamation of Tannery Polluted Soil through Phytoremediation, Physiological and Molecular Biology of Plants, 15(2), p. 175-180.

Salt, D.E.; Blaylock, M.; Kumar, N.P.B.A.; Dushenkov, V.; Ensley, D.; Chet, I. and Raskin, I. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology, 1995a, vol. 13, p. 468-474.

Salt, D.E.; Prince, R.C.; Pickering, I.J. and Raskin, I. 1995. Mechanisms of Cadmium Mobility and Accumulation in Indian Mustard. Plant Physiology, 1995b, vol. 109 p. 1427-1433.

Sánchez, S. A., 1994, Crecimiento de Eucalyptus deglupta y E. grandis bajo tres sistemas de plantación a nivel de finca en la zona de Turrialba, Costa Rica, Master's Thesis, CATIE, Pg 95.

Schumacher, F.X., Hall, F.S.D., 1933, Logarithmic Expression of Timber Tree Volume, Journal of Agricultural Research, Washington, v.47, pp 719-734.

Shackleton, C.M., Shackleton, S.E., Buiten, E., and Bird, N., 2007, The Importance of Dry Woodlands and Forests in Rural Livelihoods and Poverty Alleviation in South Africa, Forest Policy and Economics, 9:558– 577.

Sheoran, A.S., and Sheoran, V. 2006. Heavy Metal Removal Mechanism of Acid Mine Drainage in Wetlands: A Critical Review. Minerals Engineering, 19(2): 105–116.

Shukla, O.P., Juwarkar, A.A., Singh, S.K., Khan, S., and Rai, U.N. 2011. Growth Responses and Metal Accumulation Capabilities of Woody Plants During the Phytoremediation of Tannery Sludge, Waste Management, 31 (2011): 115–123.

Sims, R.E.H., Senelwa, K., Maiava, T., and Bullock, B.T. 1999. *Eucalyptus* Species for Biomass Energy in New Zealand- Part II: Coppice Performance, Biomass and Bioenergy, 17:333-343.

Singh, G., Bhati, M., Rathod, T. 2010. Use of Tree Seedlings for the Phytoremediation of a Municipal Effluent Used in Dry Areas of North-Western India: Plant Growth and Nutrient Uptake, Ecological Engineering, 36:1299–1306.

Smit, H.C., and Meincken, M. 2012. Time/temperature Combustion Profiles of Various Wood-Based Biofuels, Biomass and Bioenergy, 39:317–323.

Smith, C.W.; Kassier, H.; Morley, T. 2006. The Effect of Initial Stand Density on the Growth and Yield of Selected *Eucalyptus grandis* Clonal Hybrids in Zululand. Pietermaritzburg: Institute for Commercial Forestry Research. (ICFR Bulletin 04/2006).

Smith, K.R. 1987. Biofuels, Air Pollution, and Health (Plenum, N.Y.)

Snowball, K. and A.D. Robson. 1991. Nutrient Deficiencies and Toxicities in Wheat: A Guide for Field Identification, Mexico, D.F.: CIMMYT. ISBN 968-61 27-48-8.

Soghoian, S. and Sinert, R. 2008. Toxicity, heavy metals. http://emedicine.medscape.com/article/814960-overview (Accessed 3 March 2015)

Soil Classification Working Group. 1991. Soil classification: A Taxonomic System for South Africa. Memoirs on the Agricultural National Resources of South Africa, 15:1–57.

Spangenberg, A., Grimm, U, Silva, J. and Fölster, H. 1996. Nutrient Store and Export Rates of *Eucalyptus urograndis* Plantations in Eastern Amazonia (Jari). Forest Ecology and Management, 80: 225-234.

Sparks, D.L, 2003. Environmental soil chemistry, 2nd edition, Elsevier Science, USA.

Sun, D., Dickinson, G.R., Bragg, A. 1994. The establishment of *Eucalyptus camaldulensis* on Tropical Saline Site in North Queensland. Australia Agricultural Ecosystem Environment, 48: 1–8.

Sun, D. and Dickinson, G.R. 1995. Salinity Effects on Tree Growth, Root Distribution and Transpiration of *Casuarina cunninghamiana* and *Eucalyptus camaldulensis* Planted on a Saline Site in Tropical North Australia, Forest Ecology and Management 77, Pg 127–138.

Susarla, S., Medina, V.F., and McCutcheon, S.C. 2002. Phytoremediation: An Ecological Solution to Organic Chemical Contamination. Ecological Engineering, 18(5): 647–658.

Tan, K.H. 2005. Soil Sampling, Preparation and Analysis, CRC press.

Trueman, S.J., McMahon, T.V., and Bristow, M. 2013. Production of Cuttings in Response to Stock Plant Temperature in the Subtropical Eucalypts, *Corymbia citriodora* and *Eucalyptus dunnii*. New Forests, 44(2): 265–279.

Ulrich, S.M., Tanton, T.W. and Abdrashitova, S.A. 2001. Mercury in the Aquatic Environment: A Review of Factors Affecting Methylation, Critical Review Environmental Science and Technology 31: 241-293.

United States Environmental Protection Agency (USEPA). 1991. Risk Assessment for Toxic Air Pollutants: A Citizen's Guide. EPA450/3-90-024. Washington, DC.

United States Environmental Protection Agency (USEPA). 1997. Cleaning Up the Nation's Waste Sites: Markets and Technology Trends. EPA/542/R-96/005. Office of Solid Waste and Emergency Response, Washington, DC.

United States Environmental Protection Agency (USEPA). 2015. Sulphur Dioxide. Health. Website (http://www3.epa.gov/airquality/sulfurdioxide/health.html) (Accessed 25 September 2015).

US Department of Health and Human Services. 1999. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Asbestos. Update. Agency for Toxic Substances and Disease Registry.

US EIA, International energy statistics, Energy Information Administration, United States. Available from ⟨http://www.eia.gov/cfapps/ipdbproject/IEDIndex3.cfm⟩; 2013 [accessed 6 September 2013].

Van den Broek, R., Faaij, A., van Wijk, A., 1996. Biomass Combustion for Power Generation, Biomass and Bioenergy, 11(4): 271–281.

Van der Moezel, P.G., Watson, L.E., Pearce-Pinto, G.V.N. and Bell, D.T. 1988. The Response of Six *Eucalyptus* Species and *Casuarina obesa* to the Combined Effect of Salinity and Waterlogging. Australian Journal of Plant Physiology 15, Pg 465–474.

Van der Moezel, P.G., Watson, L.E., Bell, and D.T., 1989. Gas Exchange Responses of Two *Eucalyptus* Species to Salinity and Waterlogging. Tree Physiology 5, Pg 251–257.

Van der Moezel, P.G., Gladys, V.N., Pearce-Pinto, G.V.N., and Bell, D.T. 1991. Screening for Salt and Waterlogging Tolerance in *Eucalyptus* and *Melaleuca* species. Forest Ecology Management 40, Pg 27–37.

Van Horen, C., Nel, R., and Terblanche, P., 1996. Indoor Air Pollution from Coal and Wood Use in South Africa: An Overview, Energy for Sustainable Development, Volume III No. 1: 38–40.

Vasquez, W. and Ugalde, L., 1995, *Tablas de volumen y productos de Eucalyptus grandis en Costa Rica*, CATIE, Turrialba, Costa Rica, Pg 17.

Vivier, J.J.K. 2005. Mine Woodlands Project: Integrated Mass Balance Model, Unpublished report submitted to the University of the Witwatersrand, Johannesburg and Anglogold Ashanti Ltd. AGES (Africa Geo-environmental Services).

Wang, J., Feng, X., Anderson, C.W.N., Xing, Y. and Shang, L. 2012. Remediation of Mercury Contaminated Sites - A Review, Journal of Hazardous Materials 221-222, 1-18.

Weaver, R. W., J. R. Melton, D. S. Wang & R. L. Duble. 1984. Uptake of Arsenic and Mercury from Soil by Bermuda Grass *Cynadon dactylon*. Environmental Pollution 33: 133-142.

Weiersbye, I.M., Cukrowska, E.M., Margalit, N., Feingersh, T., Revivo, G., Braun, O., Stark, R., and Zur, Y., 2006. Acid Mine Drainage (AMD) and Phreatophyte Vegetation: Cycling of Pollutants and Implications for Land-Use Planning, Invited Paper Presented at the Natural Woodlands and Forests Symposium IV, Summerstrand, Port Elizabeth 2006, Department of Water Affairs and Forestry.

Weiersbye, I.M., Margalit, N., Felingersh, T., Revivo, G., Stark, R., Zur, Y., Heller, D., Braun, O. and Cukrowska, E. M. 2006a. Use of airborne hyper-spectral remote sensing (HSRS) to focus remediation and monitor vegetation processes on gold mining landscapes in South Africa. In A.B. Fourie and M. Tibbett (eds), Proceedings of the 1st International Seminar on Mine Closure, Australian Centre for Geomechanics, Perth.

Weiersbye, I.M., Witkowski, E.T.F., and Reichardt, M. 2006b. Floristic Composition of Gold and Uranium Tailings Dams, and Adjacent Polluted Areas, on South Africa's Deep Level Mines. Bothalia. 36 (1): 101–127.

Weiersbye, I. M. 2007. In A, Fourie, M. Tibbett. and J. Wiertz [Eds]. Global review and cost comparison of conventional and phyto-technologies for mine closure. Mine Closure 2007, Australian Centre for Geomechanics, Perth, ISBN 978-0-9804 185-0-7.

Weiersbye, I. M. and Witkowski, E.T.F. 2007. In J. J. Bester., A. H. W. Seydack., T. Vorster., I. J. Van de Merwe. and S. Dzivhani (Eds). Impact of Acid Mine Drainage on the Regeneration Potential of Highveld Phreatophytes. Multiple Use Management of Natural Forest and Woodlands: Policy Refinements and Scientific Progress IV. Department of Water Affairs and Forestry of South Africa, Pg 224–237.

Weston, R.J. 1984. Composition of Essential Oil from Leaves of *Eucalyptus delegatensis*, Phytochemistry, Vol 23, No 9, pp 1943- 1945.

Williams, A., Jones, J.M., Ma, L., and Pourkashanian, M. 2012. Pollutants from the Combustion of Solid Biomass Fuels, Progress in Energy and Combustion Science, 38:113–137.

Winde, F., Wade, P., and Van der Walt, J. 2004a. Gold Tailings as a Source of Waterborne Uranium Contamination of Streams. The Koekemoerspruit (Klerksdorp goldfield, South Africa) as a Case Study Part I of III: Uranium Migration Along the Aqueous Pathway, Water SA Vol. 30 No. 2, pp 219-225.

Winde, F. and Van der Walt, J. 2004b. Gold Tailings as a Source of Waterborne Uranium Contamination of Streams - The Koekemoerspruit (Klerksdorp goldfield, South Africa)

as a Case Study Part II of III: Dynamics of Groundwater-Stream Interactions, Water SA Vol. 30 No. 2, pp 227-232.

Winde, F., Wade, P. and Van der Walt, J., 2004c. Gold Tailings as a Source of Water-Borne Uranium Contamination Of Streams - The Koekemoerspruit (South Africa) as a Case Study Part III of III: Fluctuations of Stream Chemistry and their Impacts on Uranium Mobility, Water SA Vol. 30 No. 2, pp 233-239.

Witkowski, E.T.F. and Weiersbye, I.M. 1998. Establishment of Plants on Gold Slimes Dams: Characterization of the Slimes and Adjacent Soils. Plant, Plant Ecology and Conservation Series No. 6, p3.

Witkowski ETF, Lamont BB.1996. Disproportionate Allocation of Mineral Nutrients and Carbon Between Vegetative and Reproductive Structures in *Banksia hookeriana.* Oecologia 105:38–42.

World Health Organization (WHO). 1966. Specification for the identity and Purity of Food Additives and their Toxicological Evaluations: Some Emulsfiers and Stabilizers and Certain other Substances, Technical Report Series No. 373, Tenth Report of the Joint FAO/WHO Expert Committee on Food Additives (Geneva: 11-18 October 1966), Switzerland.

World Health Organisation (WHO), 1993. Evaluation of Certain Food Additives and Contaminants. In: Forty-First Report of the joint FAO/WHO Expert Committee on Food Additives. WHO, Geneva, Switzerland, WHO Technical Series, 837

World Health Organization (WHO). 1996. Permissible Limits of Heavy Metals in Soil and Plants, (Geneva: World Health Organization), Switzerland.

World Health Organization (WHO). 1998. Health Guideline for the Use of Wastewater in Agriculture and Aquaculture. Report of WHO Science Group, World Health Organization, Geneva, Switzerland, Technical Report Scientific, p778:10.

World Health Organization (WHO). 2010. Exposure to Arsenic: A Major Public Health Concern, (Geneva: World Health Organization), Switzerland.

WSP Environmental (Pty) Ltd, 2009a, Vaal River Operations Environmental Management Programme, AngloGold Ashanti, File reference number 1919ES/ EMP.

WSP Environmental (Pty) Ltd, 2009b, West Wits Operations Environmental Management Programme, AngloGold Ashanti, File reference number 1919ES/ EMP.

Wymer, D. 2001. The Impact of Gold Mining on Radioactivity in Water and Foodstuffs. Proceedings, Chamber of Mines Of South Africa (2001): Conference on Environmentally Responsible Mining in Southern Africa. Muldersdrift/Johannesburg, 25-28, September, 1: 219–230.

Xie Z.M. and Huang C.Y. 1998. Control of Arsenic Toxicity in Rice Plants Grown on an Arsenic-Polluted Paddy Soil. Communications in Soil Science and Plant Analysis 29: 2471–2477.

Xu Q.S., Hu J.Z., Xie, K.B., Yang H.Y., Du K.H., Shi G.X. 2010. Accumulation and Acute Toxicity of Silver in *Potamogeton crispus* L. Journal of Hazardous Materials; 173:86–193.

Yan X.L., Chen T.B., Liao X.Y., Huang Z.C., Pan J.R., Hu T.D., Nie C.J. and Xie H. 2008. Arsenic Transformation and Volatilization during Incineration of the Hyperaccumulator *Pteris vittata* L., Environmental Science Technology 42(5):1479-84.

Yoon, J. 2005. Phosphate-induced Lead Immobilization in Contaminated Soil. Doctoral dissertation, University of Florida, Gainsville, FL.

Zayed A, Terry N. 2003. Chromium in the Environment: Factors Affecting Biological Remediation. Plant Soil 2003; 249:139–56.

Zhang, W., Cai, Y., Tu, C., and Ma, L.Q. 2002. Arsenic Speciation and Distribution in an Arsenic Hyperaccumulating Plant. Science of the Total Environment, 300(1): 167-177.

Zheng, N., Wang, Q. and Zeng, D. 2007. Health Risk of Hg, Pb, Cd, Zn and Cu to the Inhabitants Around Huludao Zinc Plant in China via Consumption of Vegetables. Science of the Total Environment. 2007a, 383: 81-89.

Zheng, N., Wang, Q.C. and Zeng, D.M. 2007. Transfer Characteristics of Mercury, Lead, Cadmium, Zinc and Cuprum from Soil to Vegetable Around Zinc Smelting Plant. Huan Jing Ke Xue. 2007b, 6:1349-1354.

Zohar, Y. and Schiller, G., 1998, Growth and Water Use by Selected Seed Sources of *Eucalyptus* Under High Water Table and Saline Conditions, Agriculture, Ecosystems and Environment 69 (1998), Pg 265-277.

APPENDIX A

SAMPLE CODES

Date	Plot	Tree No.	GPS co-ordinates	Location & species	Sample Type
28/08/2012	3	T17	26°59'21.06"S 26°46'33.55"E	Mispah cam	Branches + Twigs
28/08/2012	3	T17	26°59'21.06"S 26°46'33.55"E	Mispah cam	Wood
28/08/2012	3	T17	26°59'21.06"S 26°46'33.55"E	Mispah cam	Bark
28/08/2012	3	T17	26°59'21.06"S 26°46'33.55"E	Mispah cam	Leaves
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Branches + Twigs
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Leaves
29/08/2012	18	T47	26°25'53.27"S 27°22'6.13"E	Madala gxc	Branches + Twigs
29/08/2012	18	T47	26°25'53.27"S 27°22'6.13"E	Madala gxc	Leaves
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Wood
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Branches + Twigs
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Leaves
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Wood
29/08/2012	18	T47	26°25'53.27"S 27°22'6.13"E	Madala gxc	Wood
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Wood

Date	Plot	Tree No.	GPS co-ordinates	Location & species	Sample Type
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Wood
29/08/2012	27	T29	26°25'53.27"S 27°22'6.13"E	Madala dunnii	Bark
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Wood
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala Cam	Bark
29/08/2012	21	T47	26°25'53.27"S 27°22'6.13"E	Madala cam	Bark
29/08/2012	18	T47	26°25'53.27"S 27°22'6.13"E	Madala gxc	Bark
30/08/2012	28	T47	26°26'25.35"S 27°20'40.55"E	Red Soil cam	Branches + Twigs
30/08/2012	28	T47	26°26'25.35"S 27°20'40.55"E	Red Soil cam	Leaves
30/08/2012	17	T48	26°26'25.35"S 27°20'40.55"E	Red soil dunnii	Branches + Twigs
30/08/2012	17	T48	26°26'25.35"S 27°20'40.55"E	Red soil dunnii	Leaves
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Wood
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Leaves
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Branches + Twigs
30/08/2012	17	T48	26°26'25.35"S 27°20'40.55"E	Red soil dunnii	Wood
30/08/2012	28	T47	26°26'25.35"S 27°20'40.55"E	Red Soil cam	Wood

Date	Plot	Tree No.	GPS co-ordinates	Location & species	Sample Type
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Wood
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Wood
30/08/2012	28	T47	26°26'25.35"S 27°20'40.55"E	Red Soil cam	Bark
30/08/2012	46	T47	26°26'25.35"S 27°20'40.55"E	Red Soil gxc	Bark
30/08/2012	17	T48	26°26'25.35"S 27°20'40.55"E	Red soil dunnii	Bark
31/08/2012	13	T17	26°59'21.06"S 26°46'33.55"E	Mispah dunnii	Branches + Twigs
31/08/2012	13	T17	26°59'21.06"S 26°46'33.55"E	Mispah dunnii	Leaves
31/08/2012	13	T17	26°59'21.06"S 26°46'33.55"E	Mispah dunnii	Bark
31/08/2012	10	T32	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Branches + Twigs
31/08/2012	10	T32	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Leaves
31/08/2012	13	T17	26°59'21.06"S 26°46'33.55"E	Mispah dunnii	Wood
31/08/2012	10	T32	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Wood
31/08/2012	10	T32	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Bark
31/08/2012	32	TA	26°59'21.06"S 26°46'33.55"E	Mispah gxc	Wood

APPENDIX B

DATA FROM ICP-MS

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
NSPS 1	416.0	3.7	14985.8	1.8	184.8	4.8	9054.1	1.9	8960.1	1.8	39668.2	0.4	15.3	13.2	114.7	4.0	26194.2	1.0	7.4	59.5
NSPS 2	432.5	2.6	14961.9	1.3	190.2	1.5	9443.9	1.3	10210.6	2.4	41619.4	2.7	8.7	20.5	111.1	3.2	26825.9	1.7	6.5	19.8
NSPS 3	450.3	4.6	14959.5	0.9	195.3	5.2	9352.7	1.8	10638.8	3.7	43925.2	1.2	6.1	16.2	103.8	3.0	26731.8	0.7	7.9	26.6
Plot28 T24 Branches & Twigs	104.2	11.3	12800.6	0.2	171.5	5.1	5411.1	2.1	3765.3	2.6	47812.8	2.1	7.3	29.6	85.8	5.0	412.0	2.8	8.5	28.3
Plot28 T47 Branches & Twigs	146.5	7.4	13919.4	1.8	686.3	3.4	6129.6	3.1	6323.0	2.4	106007.2	0.9	9.7	14.8	85.8	3.2	460.1	1.0	8.1	26.5
Plot3 T17 Wood	96.2	3.4	12457.8	1.0	163.7	1.7	2275.2	1.3	4655.2	1.9	35109.2	3.1	54.2	24.2	104.6	3.2	205.5	3.8	0.0	N/A
Plot3 T17 Bark	94.7	3.1	12355.3	3.1	392.5	1.4	2178.3	0.8	6303.8	0.8	163117.1	0.2	39.0	8.4	97.4	1.0	400.7	2.5	7.5	9.0
Plot18 T47 Bark	127.1	11.4	13089.0	0.7	698.3	0.3	2656.9	1.5	5906.6	0.6	52274.0	2.1	40.2	8.9	109.0	3.9	506.7	2.7	67.2	2.1
Plot47 T24 wood	80.2	1.6	12051.6	0.4	155.5	5.0	2106.3	1.9	5938.4	1.6	52678.1	1.2	48.3	11.9	102.7	2.3	2722.5	2.1	3.9	9.5
Plot21 T47 Bark	414.7	2.7	12889.4	2.1	641.1	5.6	3148.4	0.7	4934.1	1.3	68471.1	1.5	71.2	11.2	103.0	2.2	684.9	2.8	125.5	1.6
Plot46 T47 Bark	203.1	2.1	11946.2	1.7	812.4	2.2	3089.1	1.6	5340.7	0.2	53845.0	2.3	47.5	18.9	106.7	2.4	215.1	2.2	17.5	12.7
Plot46 T47 Branches & Twigs	135.8	3.0	13135.5	2.3	665.3	1.9	5955.3	1.8	6696.9	1.8	87977.0	1.1	52.6	14.2	98.2	3.1	973.7	1.1	143.5	6.5

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
Plot21 T47 wood	115.1	2.2	11979.3	2.3	148.7	6.2	2352.1	3.4	4759.5	1.7	23017.3	1.5	27.7	12.1	97.0	1.7	254.0	2.6	0.0	N/A
Plot47 T24 Branches & Twigs	184.0	0.2	11627.4	2.1	303.2	0.7	3292.6	1.5	5058.6	1.6	62908.4	1.3	22.7	13.1	88.3	1.9	861.3	1.4	17.7	29.5
Plot18 T47 wood	65.2	10.2	11927.1	0.6	225.7	6.5	2466.4	2.3	3569.5	2.2	18558.7	2.4	22.2	14.6	99.6	2.6	435.7	2.0	0.0	N/A
Plot17 T48 wood	64.2	26.1	11630.3	2.4	174.5	1.6	1641.4	2.5	6485.3	0.3	50603.9	3.6	18.8	16.3	87.6	2.9	933.0	1.9	0.0	N/A
Plot25 T17 wood	67.8	18.0	12155.3	1.0	129.1	3.7	2001.4	2.4	6576.8	1.9	43942.2	1.6	16.3	12.4	107.7	2.8	226.4	3.7	0.0	N/A
Plot46 T47 wood	86.8	0.8	11584.3	1.7	179.4	1.9	1904.5	3.2	4912.3	3.9	34304.1	2.1	16.8	8.8	93.0	5.3	527.0	1.8	1.0	158.4
Plot17 T48 Branches & Twigs	127.0	11.6	12264.9	3.3	218.6	2.2	4048.6	0.8	7043.9	3.1	12399.4	1.9	14.9	12.6	90.6	2.1	260.3	3.9	12.7	13.4
Plot10 T32 Bark	99.4	4.2	12401.8	1.3	581.2	3.0	2924.9	2.1	5605.9	1.6	12304.6	0.9	16.2	10.3	92.0	2.8	616.0	3.6	11.7	9.4
Plot28 T47 wood	97.2	8.8	11632.3	0.9	354.6	1.8	2695.0	2.6	5847.7	1.0	34379.0	1.3	17.5	9.5	88.1	0.6	277.6	1.0	7.6	30.6
Plot10 T32 Branches & Twigs	119.6	13.4	11748.4	0.2	267.6	3.1	5520.3	2.3	6062.0	1.5	14113.5	1.6	15.1	25.4	87.5	3.5	216.2	1.4	21.1	17.6
Plot27 T29 wood	58.2	19.9	10344.6	1.0	268.6	0.6	1480.2	1.7	6194.3	1.2	64175.9	1.4	15.7	11.7	87.6	4.5	494.5	1.0	0.0	N/A
Plot27 T29 Bark	107.9	9.2	10678.0	0.3	433.3	0.7	2239.5	3.5	4704.2	2.2	63660.4	1.4	11.6	12.4	91.8	1.7	299.3	1.6	18.1	3.6

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
Plot17 T48 Bark	157.9	7.3	11283.5	0.2	1268.0	0.6	2699.8	2.8	4659.1	1.1	88863.3	0.2	8.7	5.7	133.5	1.6	310.2	1.0	11.8	25.2
Plot13 T17 Bark	159.9	3.5	11516.9	1.6	531.5	1.7	2493.4	1.5	2956.2	1.8	10876 4.3	0.6	12.5	5.0	132.4	2.7	247.7	4.6	30.9	1.3
Plot26 T24 Bark	160.2	5.8	11024.1	1.1	193.6	2.0	2851.0	2.8	4549.6	0.6	74162 .8	1.3	9.7	2.4	116.8	1.5	3093 .8	0.5	35.9	3.4
Plot47 T24 Bark	155.9	4.5	10959.2	1.7	466.9	3.4	2380.4	1.1	3883.9	1.1	10951 9.0	0.8	5.1	17.5	95.0	3.4	223.4	2.4	19.9	10.4
Plot13 T17 wood	67.2	7.2	10763.4	1.7	152.1	2.4	1381.5	2.1	4652.7	1.4	24099 .5	0.7	15.3	3.2	87.4	3.7	241.6	2.1	0.0	N/A
Plot10 T32 wood	119.7	2.6	11241.2	3.2	206.8	1.5	2675.1	1.7	4584.1	0.0	34662 .3	1.3	8.1	14.7	88.1	1.7	443.1	1.5	21.3	19.2
Plot3 T17 Branches & Twigs	93.0	3.7	10614.6	1.5	253.3	4.8	7772.6	1.5	3937.9	0.2	15337 0.6	1.5	16.5	8.4	92.6	2.2	546.7	0.4	8.8	12.7
Plot27 T29 Branches & Twigs	114.5	1.8	10750.9	2.3	247.2	2.0	3540.6	0.2	4895.2	1.1	43767 .8	1.9	4.8	7.5	85.9	1.9	283.9	5.5	7.3	27.6
Plot13 T17 Branches & Twigs	128.7	3.6	10955.7	1.6	232.4	0.8	3601.3	0.6	4335.9	1.6	10188 0.4	0.6	12.1	20.7	87.7	0.8	358.4	1.4	27.5	5.7
Plot18 T47 Branches & Twigs	72.2	8.3	10849.3	2.4	233.9	7.5	5374.5	0.6	4610.9	0.6	35973 .8	0.2	82.5	4.4	81.1	3.7	186.2	2.7	4.9	24.5
Plot25 T17 Branches & Twigs	129.2	8.2	10733.6	2.4	197.9	2.8	2355.8	2.3	4633.4	1.9	72816 .0	1.7	33.8	9.0	93.3	1.0	343.6	1.0	32.7	9.2

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
Plot25 T17 Bark	182.8	2.6	10753.4	0.8	254.8	2.4	1792.9	3.0	4251.1	0.6	67613.6	0.9	16.6	13.2	92.2	3.6	916.2	2.3	9.3	4.9
Plot21 T47 Branches & Twigs	152.0	7.7	10124.9	2.4	222.1	1.7	4745.8	1.9	4397.8	3.2	30686.3	1.3	8.3	22.3	88.2	2.6	411.4	1.0	20.8	2.5
Plot28 T47 Bark	183.4	6.7	12313.8	1.3	1807.9	2.0	3626.1	1.4	5023.8	0.4	15580 8.3	2.1	7.2	16.9	89.6	2.3	276.0	0.8	31.4	2.6
Plot26 T24 Leaves	45.2	8.5	10724.3	1.1	616.0	4.1	2051.4	3.2	4027.2	3.3	30547.3	5.1	10.6	4.0	103.8	3.2	351.5	4.1	52.5	4.9
Plot47 T24 Leaves	142.8	4.6	10038.9	1.2	646.0	1.8	4151.3	1.1	4561.6	0.6	56782.1	2.2	22.9	2.2	108.4	2.7	313.0	3.2	65.6	2.9
Plot28 T47 Leaves	261.7	10.9	19585.8	1.0	1172.8	1.0	5352.3	1.9	4435.3	3.0	16259 8.8	1.2	10.4	5.6	103.2	2.4	831.0	1.0	40.2	5.6
Plot13 T17 leaves	326.0	3.3	11077.7	1.1	651.0	3.9	3986.6	3.7	4472.8	2.1	59527.1	2.6	39.3	4.8	108.7	5.5	726.8	5.2	199.3	3.2
Plot27 T29 leaves	159.9	1.1	17578.7	2.3	657.2	4.7	5375.3	3.3	4846.1	3.5	14264 8.8	0.7	9.7	5.0	124.5	3.8	1093.3	3.8	60.7	3.0
Plot3 T17 Leaves	137.4	4.6	1721.8	4.2	550.3	1.7	5056.7	2.3	4126.7	2.3	10047 8	1.2	9.5	3.3	109.8	3.6	702.6	2.4	75.3	5.2
Plot46 T47 Leaves	58.2	1.9	10614.6	1.3	433.3	2.2	2355.8	0.6	4097.8	3.9	48272 5	1.3	8.1	5.0	80.1	3.6	299.3	2.8	32.7	5.1
Plot21 T47 Leaves	107.9	9.2	10849.3	2.4	641.1	1.1	3540.6	0.2	4135.9	1.2	53118	1.6	7.9	9.4	89.0	2.2	310.2	1.1	33.8	4.5
Plot18 T47 Leaves	114.5	1.8	10955.7	0.8	394.5	1.8	3601.3	1.3	4251.1	1.7	46250	1.4	9.7	4.7	88.9	1.7	247.7	2.1	35.6	6.2
Plot25 T17 Leaves	128.7	3.6	10750.9	2.4	589.2	0.6	4740.1	0.9	4344.8	2.9	52287	1.4	9.1	4.9	84.7	1.5	343.6	1.7	47.4	2.8

Sample Name	V		Cr		Co		Cu		As		Ba		Au		Hg		Pb		U	
	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD	[µg/kg]	%RSD
Plot10 T32 Leaves	159.9	3.5	135441	1.1	694.3	1.1	5374.5	1.3	4110.9	1.3	109789	0.2	14.6	3.9	118.6	0.8	616.0	1.5	82.5	2.4
Plot17 T48 Leaves	160.2	5.8	109629	1.9	696.4	1.4	5472.3	3.0	4533.4	2.1	135400	0.6	21.0	3.3	107.7	1.0	861.3	0.6	87.7	1.7

APPENDIX C

DATA FROM ICP-OES

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Blank	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
0.1 ppm	0.106	0.109	0.099	0.098	0.101	0.098	0.098	0.101	0.103	0.107	0.097	0.098	0.095	0.099
0.5 ppm	0.493	0.502	0.502	0.502	0.497	0.505	0.508	0.495	0.503	0.506	0.494	0.507	0.501	0.507
1 ppm	0.995	1.003	1.004	0.998	0.993	1.003	0.995	0.997	0.996	1.002	1.002	1.006	0.96	1.002
NSPS 1	325.651	54.910	22114.228	322.144	7274.549	7234.469	87.675	415.832	19.840	7134.269	1482.966	32.665	14.830	50.301
% RSD	5.624	0.435	1.073	0.455	0.364	0.195	0.924	0.378	1.615	0.698	0.671	1.130	4.236	0.450
NSPS 2	311.751	58.853	21442.846	338.129	7234.213	7144.285	87.730	555.556	20.284	7873.701	2196.243	32.374	14.488	46.863
% RSD	1.274	0.467	0.921	0.230	0.792	0.404	0.252	1.157	1.276	7.403	0.722	0.701	2.418	0.540
NSPS 3	290.581	63.327	22384.770	337.375	7474.950	7555.110	91.583	617.234	20.641	8216.433	2208.417	33.066	14.429	50.200
% RSD	2.479	0.186	0.194	0.471	0.678	0.453	0.568	3.305	1.052	6.850	0.601	1.033	4.616	1.690
Plot 17 T48 Leaves	428.287	134.861	11907.371	204.183	4785.857	4272.908	1792.829	677.291	35.259	7599.602	1304.781	27.590	13.745	18.625
% RSD	1.594	0.225	0.397	0.425	2.873	2.891	0.395	2.338	0.484	8.152	0.495	1.325	1.182	1.722
Plot 10 T32 Leaves	470.000	197.800	10930.000	160.500	2930.000	3065.000	3370.000	510.000	34.900	6945.000	720.000	13.400	9.400	25.600
% RSD	1.242	1.437	0.507	0.289	2.244	0.704	0.684	1.775	1.944	4.449	1.361	0.560	1.069	0.983
Plot 25 T17 Leaves	405.487	52.062	7188.626	279.936	5416.500	2142.571	770.925	460.553	29.836	7644.173	1271.526	5.707	11.213	23.528
% RSD	2.020	0.733	0.014	0.517	2.730	0.221	0.310	3.097	0.552	5.522	1.471	0.545	3.396	1.422
Plot 18 T47 Leaves	389.377	46.126	8391.573	164.736	5710.863	4048.522	2481.030	494.209	25.060	9135.383	1098.243	9.385	9.185	18.870
% RSD	0.286	0.302	4.043	0.498	0.337	0.506	0.247	0.954	1.739	2.097	0.406	0.180	0.630	0.666
Plot 21 T47 Leaves	552.789	52.888	11663.347	358.566	3351.594	3819.721	2544.821	1180.279	25.498	8779.880	1200.199	10.359	16.534	31.474

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
% RSD	1.257	1.204	1.285	1.149	4.479	0.416	0.675	4.330	2.775	6.917	1.375	1.854	4.906	3.597
Plot 46 T47 Leaves	383.619	48.027	8499.402	159.426	4593.464	3143.683	2032.682	562.973	32.084	8260.263	777.202	9.466	9.566	17.238
% RSD	2.102	0.185	1.460	0.288	2.536	0.493	0.225	3.144	0.029	4.710	0.379	1.322	0.512	2.660
Plot 3 T 17 Leaves	409.509	147.623	11616.061	184.778	3995.206	2781.662	2337.195	1747.903	27.068	10037.954	1692.968	12.385	11.087	26.968
% RSD	2.375	0.609	1.113	0.142	4.708	0.574	0.884	3.243	1.097	5.891	0.149	1.563	3.329	2.238
Plot 27 T29 Leaves	463.332	38.362	6755.680	239.139	4424.073	4244.719	2625.548	1220.606	22.220	10123.555	1310.283	7.573	12.953	29.892
% RSD	0.635	0.450	0.045	0.282	0.976	0.455	0.245	1.178	0.833	2.950	0.905	0.254	9.829	0.455
Plot 13 T17 Leaves	925.741	72.658	10018.014	190.152	3763.010	3167.534	1911.529	810.649	25.620	9517.614	1781.425	9.107	8.006	22.318
% RSD	2.380	0.840	0.706	1.048	2.648	0.215	0.190	2.836	0.941	5.181	0.483	1.033	2.326	2.692
Plot 28 T47 Leaves	285.342	185.523	11869.243	173.809	2783.340	2002.403	3183.821	555.667	35.443	6783.140	690.829	34.341	7.609	45.855
% RSD	0.705	1.056	0.754	0.879	1.892	0.328	0.366	2.236	1.401	4.534	0.927	1.572	2.282	1.476
Plot 47 T24 Leaves	460.000	66.500	10645.000	270.000	3555.000	2595.000	695.000	350.000	24.100	7045.000	310.000	16.500	16.600	21.400
% RSD	2.337	0.654	1.022	0.830	3.585	0.858	1.235	3.028	2.408	7.965	1.231	1.543	2.667	2.592
Plot 26 T24 Leaves	220.441	44.589	8211.423	185.972	5746.493	2570.140	1277.555	445.892	27.455	8962.926	921.844	7.615	6.613	16.733
% RSD	1.513	0.811	1.966	0.378	2.452	0.296	0.230	2.869	0.754	4.167	0.324	0.453	6.235	2.954
Plot 28 T47 Bark	148.078	184.121	5756.908	220.264	3349.019	4019.824	3519.223	525.631	29.936	8159.792	165.198	136.964	4.505	22.527
% RSD	3.851	0.582	1.505	0.036	1.783	0.764	0.952	2.901	0.959	4.683	0.953	1.229	3.421	1.749
Plot 21 T47 Branches + Twigs	102.405	33.467	9539.078	221.944	1803.607	3396.794	1663.327	480.962	19.840	7284.569	225.451	15.832	3.407	26.453
% RSD	2.554	0.985	2.591	0.617	2.597	0.284	0.418	2.714	1.387	4.693	1.145	0.455	1.610	1.845

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Plot 25 T17 Bark	213.716	75.558	16836.124	172.648	1744.418	5806.419	1191.188	448.565	20.534	7441.188	203.648	19.936	4.585	15.451
% RSD	1.159	0.672	0.758	0.586	2.543	0.542	0.768	2.509	1.380	4.797	0.853	0.944	2.742	1.546
Plot 25 T17 Branches + Twigs	128.797	84.333	23171.463	238.809	2058.353	4456.435	1658.673	459.632	20.683	7683.853	429.656	31.175	3.897	22.982
% RSD	2.490	0.798	0.874	0.207	2.787	0.430	0.366	2.509	1.056	4.202	0.839	0.763	4.993	1.035
Plot 18 T47 Branches + Twigs	75.010	39.453	7001.598	232.421	2786.656	1363.364	1468.238	484.419	20.176	8235.118	214.243	12.185	2.697	11.586
% RSD	1.357	0.822	0.042	0.435	2.625	0.499	0.065	2.410	2.621	4.314	0.304	0.882	22.538	1.356
Plot 13 T17 Branches + Twigs	177.255	113.327	11372.745	215.431	2439.880	4784.569	1367.735	681.363	20.140	8096.192	335.671	18.236	4.509	21.142
% RSD	1.107	0.671	0.719	0.661	2.760	0.102	0.306	2.891	2.143	5.129	0.563	0.503	4.548	1.729
Plot 27 T29 Branches + Twigs	83.267	37.675	4478.958	235.772	2730.461	1813.627	1252.505	991.984	23.347	2885.772	177.756	12.625	4.208	13.627
% RSD	1.367	0.919	0.615	1.311	1.916	1.130	1.036	2.549	0.472	6.881	4.600	0.760	2.874	2.177
Plot 3 T 17 Branches + Twigs	84.764	127.696	21670.327	205.272	2391.174	2885.383	3549.321	888.578	25.160	4437.899	369.010	38.239	4.193	43.630
% RSD	2.316	1.619	0.454	1.205	2.718	0.830	1.116	2.635	2.180	9.886	2.885	0.780	1.059	2.363
Plot 10 T32 Wood	434.305	31.450	2610.823	574.081	1652.356	195.288	172.025	798.722	23.163	5321.486	119.609	8.087	12.280	16.274
% RSD	2.465	1.004	1.314	1.480	3.623	1.499	1.518	3.219	2.286	7.277	1.765	1.380	1.711	2.620
Plot 13 T17 Wood	67.519	21.974	6612.066	143.728	1977.627	1278.466	27.068	978.825	22.573	6132.641	97.083	8.490	3.596	6.692
% RSD	2.821	0.954	0.751	1.472	3.263	1.270	1.052	3.669	2.645	7.656	6.521	1.229	2.718	2.071
Plot 47 T24 Bark	120.555	99.780	44796.245	304.634	5453.456	5957.851	1563.124	848.981	25.170	5768.078	277.367	115.461	4.694	15.481
% RSD	2.696	1.054	3.536	1.018	2.292	0.748	0.782	2.254	1.809	5.443	1.350	0.864	1.111	2.839

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Plot 26 T24 Bark	265.000	67.500	25100.000	216.500	5705.000	6200.000	2630.000	745.000	25.200	5535.000	293.100	43.100	5.000	37.400
% RSD	1.393	1.886	0.659	1.488	2.925	1.251	1.267	3.125	3.277	9.044	1.827	1.201	1.118	3.495
Plot 13 T17 Bark	244.012	94.411	28108.782	444.112	3413.174	4625.749	1901.198	1501.996	24.651	6222.555	187.226	43.912	5.788	9.880
% RSD	2.463	1.828	0.907	1.593	3.117	1.130	1.611	2.927	3.968	8.894	3.566	1.737	4.478	3.411
Plot 17 T48 Bark	221.477	77.862	23974.179	236.189	4383.507	5069.055	675.540	970.777	27.322	7055.645	188.851	72.958	5.504	10.709
% RSD	1.803	1.321	1.111	1.531	3.011	1.408	1.197	3.469	1.096	6.894	2.303	1.507	1.835	2.307
Plot 27 T29 Bark	118.558	60.028	16440.272	234.319	1568.118	5433.480	2163.404	1739.912	22.873	4784.259	190.372	44.347	4.095	10.088
% RSD	2.071	1.916	0.852	1.459	3.666	0.899	1.323	2.131	3.339	6.583	3.118	1.549	1.817	2.693
Plot 27 T29 Wood	160.144	56.609	1988.818	91.753	708.866	1617.412	191.693	1152.157	21.366	4772.364	100.339	6.989	3.395	7.288
% RSD	1.863	0.873	1.338	0.661	2.771	1.247	1.123	2.570	2.496	8.261	4.461	1.150	0.919	1.520
Plot 10 T32 Branches + Twigs	144.085	116.707	11111.111	243.006	3727.018	1558.753	1938.449	1059.153	27.378	6804.556	220.823	18.485	4.496	19.285
% RSD	2.288	1.190	0.007	1.471	2.261	0.810	1.156	2.343	2.856	6.396	3.225	1.255	1.690	2.336
Plot 28 T47 Wood	117.964	29.142	1312.375	176.547	2270.459	723.553	113.473	998.004	21.357	7100.798	138.323	4.691	4.491	10.679
% RSD	1.468	1.096	0.691	0.783	3.081	1.023	1.018	2.717	2.535	7.020	3.185	0.541	1.691	2.833
Plot 10 T32 Bark	360.577	104.367	43319.311	110.877	4717.548	3916.266	3876.202	1121.795	27.244	15194.311	230.869	74.820	3.506	19.932
% RSD	1.260	1.257	2.944	1.157	2.849	0.887	0.907	3.286	1.297	5.733	1.501	1.337	1.027	2.461
Plot 17 T48 Branches + Twigs	332.335	97.505	7864.271	216.766	4890.220	1935.130	209.980	1526.946	27.944	3652.695	260.080	26.048	4.790	12.076
% RSD	1.395	1.042	0.053	1.584	2.941	1.123	1.012	2.881	2.662	5.973	0.669	1.301	1.683	2.837
Plot 46 T47 Wood	219.237	29.465	888.933	108.969	1473.232	75.509	35.957	1173.592	19.577	4679.385	89.992	4.195	3.196	8.290

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
% RSD	1.679	1.941	1.639	1.467	2.119	1.190	1.596	2.115	2.215	7.637	2.912	0.731	2.172	1.897
Plot 25 T 17 Wood	111.734	34.441	856.027	97.817	2042.451	173.909	42.151	1141.370	20.224	6512.815	152.683	2.903	3.304	8.510
% RSD	2.668	0.898	1.550	0.716	2.912	1.338	1.447	2.710	1.259	7.772	2.852	1.148	0.377	2.059
Plot 17 T48 Wood	122.255	42.914	2395.210	79.341	1676.647	1606.786	41.417	1127.745	20.659	6007.984	110.679	8.882	3.393	11.078
% RSD	2.691	0.803	0.856	1.874	2.237	0.974	1.501	3.318	3.119	6.368	2.880	1.195	2.791	2.816
Plot 18 T47 Wood	52.653	16.454	1964.499	79.577	2253.690	143.997	137.615	1002.194	21.041	5813.722	88.452	5.684	3.291	7.878
% RSD	1.523	0.851	1.362	1.928	3.519	1.239	1.207	3.093	3.714	7.529	4.647	0.989	7.486	2.738
Plot 47 T24 Branches + Twigs	130.435	53.849	8516.155	198.444	7758.277	2871.959	589.350	996.211	22.138	3340.646	281.213	24.830	5.086	14.659
% RSD	2.277	0.937	0.038	1.251	1.420	1.029	1.078	2.164	2.839	7.136	2.272	0.941	1.646	2.505
Plot 21 T47 Wood	135.446	21.491	714.714	190.224	1839.264	84.266	41.084	1034.586	21.691	5752.699	126.749	3.499	4.398	7.697
% RSD	1.080	1.449	1.557	1.209	2.835	1.043	1.202	2.372	3.478	8.585	2.581	0.722	2.028	1.627
Plot 46 T47 Branches + Twigs	1782.853	79.728	11618.590	749.199	2347.756	1396.234	1500.401	1221.955	28.646	4799.679	155.349	29.147	5.108	15.024
% RSD	1.618	1.253	1.049	1.226	2.502	1.250	0.868	2.499	3.032	7.215	3.137	1.078	1.103	2.716
Plot 46 T47 Bark	136.473	48.397	55781.563	130.561	5611.222	1800.601	2655.311	993.988	23.547	4128.257	294.790	103.607	4.810	8.818
% RSD	2.667	1.816	0.973	1.643	3.620	1.257	1.176	3.662	2.682	7.852	2.862	1.286	4.373	3.131
Plot 21 T47 Bark	334.000	61.900	34190.000	373.000	4240.000	2620.000	3010.000	1420.000	22.500	4110.000	449.000	61.000	9.200	19.400
% RSD	1.096	1.942	1.105	1.228	3.475	1.472	1.117	3.725	1.621	7.234	2.392	1.455	2.583	3.506
Plot 47 T24 Wood	139.200	43.600	2054.000	156.000	944.000	142.700	33.900	962.000	21.800	4770.000	78.900	6.300	3.700	22.000
% RSD	2.256	1.020	0.628	1.769	4.069	0.999	1.028	3.448	5.540	6.762	7.402	1.218	4.247	3.162

Element	Al	Ba	Ca	Fe	K	Mg	Mn	Na	Ni	P	S	Sr	Ti	Zn
Conc. Unit	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Plot 18 T47 Bark	542.217	49.520	36894.758	220.288	2565.026	2132.853	2364.946	894.358	22.709	7122.849	238.195	73.029	4.402	11.505
% RSD	1.339	1.029	2.137	0.833	3.086	0.971	1.060	2.695	2.698	7.693	1.947	1.395	1.871	3.278
Plot 3 T17 Bark	289.652	151.918	34253.895	217.938	3266.081	2936.476	3595.685	1388.334	25.170	8040.352	229.425	72.014	3.895	23.072
% RSD	1.886	1.762	1.824	1.814	3.322	1.341	1.266	3.491	4.390	8.140	3.076	1.456	1.708	3.307
Plot 3 T17 Wood	80.941	31.898	1325.758	249.203	2651.515	146.830	98.485	912.081	21.232	4844.498	195.375	4.187	3.788	10.666
% RSD	2.679	0.970	1.172	1.111	4.126	1.458	0.967	4.158	3.010	8.691	1.929	0.910	1.159	3.088
Plot 28 T47 Branches + Twigs	239.425	105.547	22146.848	174.681	3571.429	2242.618	1739.824	1156.225	24.441	5476.856	297.287	58.360	4.789	26.038
% RSD	1.703	1.651	0.723	1.832	2.646	0.983	1.167	3.382	2.071	5.397	1.646	1.519	1.602	3.847
Plot 26 T24 Branches + Twigs	103.206	47.495	11012.024	220.040	5410.822	2099.198	1706.413	840.681	25.451	4418.838	439.880	19.439	4.409	15.030
% RSD	3.048	1.117	0.112	1.353	3.286	1.135	0.884	3.174	1.239	8.488	2.387	1.152	2.922	3.280

APPENDIX D

DATA FROM CN & ASH

NORTH WEST UNIVERSITY

ECO ANALYTICA

WITS UNIVERSITY (INNOCENT RABOHALE)

21/8/2014

LECO

Sample no.	Plot no.	Tree no.	Site	Species	Sample type	N %	C %	Ash %
1	26	24	MADALA	E. MELLI	BRANCHES+TWIGS	0.32	44.87	95.16
2	28	47	RED SOIL	E. CAM	BRANCHES+TWIGS	0.23	43.35	94.01
3	3	17	MISPAH	E.CAM	WOOD	0.15	45.99	98.92
4	3	17	MISPAH	E.CAM	BARK	0.13	40.05	90.88
5	18	47	MADALA	E.GXC	BARK	0.11	40.75	90.03
6	47	24	RED SOIL	E.MELLI	WOOD	0	45.82	98.49
7	21	47	MADALA	E.CAM	BARK	0.16	40.74	89.74
8	46	47	RED SOIL	GXC	BARK	0.17	39.52	85.24
9	46	47	RED SOIL	G.GXC	BRANCHES+TWIGS	0	44.01	96.43
10	21	47	MADALA	E.CAM	WOOD	0	46.54	-26.80
11	47	24	RED SOIL	E.MELLI	BRANCHES+TWIGS	0.04	44.21	95.71
12	18	47	MADALA	E.GXC	WOOD	0	45.55	99.13
13	17	48	RED SOIL	G.DUNNII	WOOD	0	44.97	99.09

14	**25**	**17**	**MISPAH**	**E.MELLI**	**WOOD**	0.10	45.56	99.13
15	**46**	**47**	**RED SOIL**	**E.GXC**	**WOOD**	0	45.85	99.69
16	**17**	**48**	**RED SOIL**	**E.DUNNII**	**BRANCHES+TWIGS**	0.34	44.16	97.00
17	**10**	**32**	**MISPAH**	**GXC**	**BARK**	0.11	39.43	89.55
18	**28**	**47**	**RED SOIL**	**E.CAM**	**WOOD**	0.04	45.02	99.27
19	**10**	**32**	**MISPAH**	**E.GXC**	**BRANCHES+TWIGS**	0.12	44.01	96.81
20	**27**	**29**	**MADALA**	**E.DUNNII**	**WOOD**	0	45.03	99.26
21	**27**	**29**	**MADALA**	**E.DUNNII**	**BARK**	0.06	41.79	94.78
22	**17**	**48**	**RED SOIL**	**E.DUNNII**	**BARK**	0.07	40.54	92.69
23	**13**	**17**	**MISPAH**	**E.DUNNII**	**BARK**	0.14	40.67	92.34
24	**26**	**24**	**MADALA**	**E.MELLI**	**BARK**	0.18	41.01	92.36
25	**47**	**24**	**RED SOIL**	**E.MELLI**	**BARK**	0.18	39.33	87.75
26	**13**	**17**	**MISPAH**	**E.DUNNII**	**WOOD**	0	44.72	98.32
27	**10**	**32**	**MISPAH**	**E.GXC**	**WOOD**	0	45.06	98.80
28	**3**	**17**	**MISPAH**	**E.CAM**	**BRANCHES+TWIGS**	0.23	42.54	93.79
29	**27**	**29**	**MADALA**	**E.DUNNII**	**BRANCHES+TWIGS**	0.14	44.76	97.69
30	**13**	**17**	**MISPAH**	**E.DUNNII**	**BRANCHES+TWIGS**	0.22	44.28	96.32
31	**18**	**47**	**MADALA**	**E.GXC**	**BRANCHES+TWIGS**	0.05	43.97	97.47
32	**25**	**17**	**MISPAH**	**E.MELLI**	**BRANCHES+TWIGS**	0.29	43.23	95.00
33	**25**	**17**	**MISPAH**	**E.MELLI**	**BARK**	0.12	42.13	94.91
34	**21**	**47**	**MADALA**	**E.CAM**	**BRANCHES+TWIGS**	0.22	44.15	96.12

35	**28**	**47**	**RED SOIL**	**E.CAM**	**BARK**	0.20	38.79	84.83
36	**26**	**24**	**MADALA**	**E.MELLI**	**LEAVES**	1.27	48.47	95.68
37	**47**	**24**	**MADALA**	**E.MELLI**	**LEAVES**	1.01	48.87	95.09
38	**28**	**47**	**RED SOIL**	**E.CAM**	**LEAVES**	1.02	46.35	93.02
39	**13**	**17**	**MISPAH**	**E.DUNNII**	**LEAVES**	1.63	50.33	95.87
40	**27**	**29**	**MADALA**	**E.DUNNII**	**LEAVES**	1.28	49.87	96.06
41	**3**	**17**	**MISPAH**	**E.CAM**	**LEAVES**	1.21	49.49	95.69
42	**46**	**47**	**RED SOIL**	**E.GXC**	**LEAVES**	0.70	49.24	96.46
43	**21**	**47**	**MADALA**	**E.CAM**	**LEAVES**	1.16	50.33	95.31
44	**18**	**47**	**MADALA**	**E.GXC**	**LEAVES**	0.92	47.73	95.91
45	**25**	**17**	**MISPAH**	**E.MELLI**	**LEAVES**	1.19	49.84	96.58
46	**10**	**32**	**MISPAH**	**E.GXC**	**LEAVES**	0.99	47.71	96.03
47	**17**	**48**	**RED SOIL**	**E.DUNNII**	**LEAVES**	1.25	48.93	94.66
48	**26**	**24**	**MADALA**	**E.MELLI**	**WOOD**	0	44.52	98.96
N5P6 1					**ORCHARDS LEAVES**	2.27	43.73	Not enough sample
N5P6 2					**ORCHARDS LEAVES**	2.26	43.49	Not enough sample
N5P6 3					**ORCHARDS LEAVES**	2.27	43.43	Not enough sample

CRM1					**ORCHARDS LEAVES**	2.25	43.58	Not enough sample
CRM2					**ORCHARDS LEAVES**	2.27	43.76	Not enough sample

This laboratory participates in the following quality control schemes:

1. Agricultural Laboratory Association of Southern

Africa.

2. International Soil-Analytical Exchange (ISE), Wageningen, Nederland.

No responsibility is accepted by North-West University for any losses due to the use of this data

APPENDIX E

DATA TABLES FOR RESULTS

Table 19: Mean concentrations of phosphorus, potassium and % nitrogen for wood from three *Eucalyptus* species ± standard deviation

Concentration (mg/kg)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Phosphorus	5899.33 ± 1135.27	5271.53 ± 568.82	5637.66 ± 751.96
Potassium	2253.75 ± 406.38	1793.09 ± 568.82	1454.38± 662.94
% N	0.05 ±0.09	-0.05±0.04	-0.08±0.04

Table 20: Mean concentrations of phosphorus, potassium and % nitrogen for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	Potassium	Phosphorus	Nitrogen
		mg/kg	mg/kg	%
E. gxc	Leaves	4411.44	8113.55	0.87
	Branches/ twigs	2953.81	6613.12	0.23
	Bark	4297.93	8815.14	0.13
	Wood	1793.09	5271.53	-0.05
E. cam	Leaves	3376.71	8533.66	1.13
	Branches/ twigs	2588.74	5733.11	0.23
	Bark	3618.37	6770.05	0.16
	Wood	2253.75	5899.33	0.05
E. dunnii	Leaves	4324.31	9080.26	1.39
	Branches/ twigs	3353.52	4878.22	0.23
	Bark	3121.60	6020.82	0.09
	Wood	1454.38	5637.66	-0.08

Table 21: Mean concentrations of calcium, sulphur and magnesium for wood from three *Eucalyptus* species ± standard deviation

Concentration (mg/kg)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Calcium	1117.62 ± 348.99	1821.42 ± 869.82	3665.36 ± 2560
Sulphur	153.48 ± 36.74	99.35 ± 17.56	102.70 ± 7.10
Magnesium	318.22 ± 352.42	138.26 ± 60.09	1500.89 ± 192.70

Table 22: Mean concentrations of calcium, magnesium and sulphur for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	Ca (mg/kg)	Mg (mg/kg)	S (mg/kg)
E. gxc	Leaves	9273.66	3419.07	865.15
	Branches/twigs	9910.43	1439.45	196.80
	Bark	45331.88	2616.57	254.62
	Wood	1821.42	138.26	99.35
E. cam	Leaves	11716.22	2867.93	1194.67
	Branches/twigs	17785.42	2841.60	297.25
	Bark	24733.60	3192.10	281.21
	Wood	1117.62	318.22	153.48
E. dunnii	Leaves	9560.35	3895.05	1465.50
	Branches/twigs	7905.32	2844.44	257.84
	Bark	22841.08	5042.76	188.82
	Wood	3665.36	1500.89	102.70

Table 23: Mean concentrations in mg/kg of manganese, iron, zinc, copper and nickel for wood from three *Eucalyptus* species ± standard deviation

Concentration (mg/kg)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Manganese	84.53 ± 38.21	115.20 ± 70.75	86.73 ± 91.19
Iron	205.32 ± 38.61	254.21 ± 277.41	104.94 ± 34.16
Zinc	9.68 ± 1.72	10.81 ± 4.73	8.35 ± 2.38
Copper	2.44±0.22	0.20±0.02	0.20±0.06
Nickel	21.43 ± 0.24	21.26 ± 1.80	21.53 ± 0.97

Table 24: Mean concentrations of manganese, iron, zinc, copper and nickel for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	Mn	Fe	Zn	Cu	Ni
		(mg/kg)				
E. gxc	Leaves	2627.90	161.55	20.57	3.78	30.68
	Branches/twigs	1635.70	408.21	15.30	5.62	25.40
	Bark	2965.49	153.91	13.42	2.89	24.50
	Wood	115.20	254.21	10.81	2.35	21.26
E. cam	Leaves	2688.61	239.05	34.77	4.29	29.34
	Branches/twigs	2317.49	200.63	32.04	6.22	23.15
	Bark	3374.97	270.40	21.67	2.98	25.87
	Wood	84.35	205.32	9.68	2.44	21.43
E. dunnii	Leaves	2109.97	211.16	23.61	4.94	27.70
	Branches/twigs	943.41	222.66	15.62	3.73	23.81
	Bark	1580.05	304.87	10.23	2.48	24.95
	Wood	86.73	104.94	8.35	1.50	21.53

-

Table 25: Mean concentrations of chromium, lead and arsenic for wood from three *Eucalyptus* species ± standard deviation

Concentration (mg/kg)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Chromium	12.02 ± 0.41	11.58 ± 0.34	10.91 ± 0.66
Lead	0.25 ± 0.04	0.47 ± 0.05	0.56 ± 0.35
Arsenic	5.09 ± 0.66	4.36 ± 0.07	5.78 ± 0.98

Table 26: Mean concentrations of chromium, lead and arsenic for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	Cr (mg/kg)	Pb (mg/kg)	As (mg/kg)
E. gxc	Leaves	52.34	0.39	4.15
	Branches/twigs	11.91	0.46	5.79
	Bark	12.48	0.45	5.62
	Wood	11.58	0.47	4.36
E. cam	Leaves	13.84	0.62	4.35
	Branches/twigs	11.55	0.47	4.89
	Bark	12.52	0.45	5.42
	Wood	12.02	0.25	5.09
E. dunnii	Leaves	46.10	0.89	4.62
	Branches/twigs	11.32	0.30	5.43
	Bark	11.16	0.29	4.11
	Wood	10.91	0.56	5.78

Carbon and Ash Content:

Table 27: Mean % dry mass of carbon and ash content for wood from three *Eucalyptus* species ± standard deviation

Content (%)	*E. camaldulensis*	*E. g x c*	*E. dunnii*
Carbon	45.85 ± 0.77	45.48 ± 0.40	44.91 ± 0.16
Ash	99.10 ± 0.25	99.21 ± 0.45	98.89 ± 0.50

Table 28: Mean % dry mass of carbon and ash content for leaves, branches/twigs, bark and wood from three *Eucalyptus* species

Species	Tissue Type	% Carbon	% Ash
E. gxc	Leaves	48.23	96.14
	Branches/twigs	44.40	97.01
	Bark	39.90	88.28
	Wood	45.48	99.21
E. cam	Leaves	48.72	94.67
	Branches/twigs	43.35	94.64
	Bark	39.86	88.48
	Wood	45.85	99.10
E. dunnii	Leaves	49.71	95.53
	Branches/twigs	44.40	97.01
	Bark	41.00	93.27
	Wood	44.91	98.89

APPENDIX F

SAMPLE TREE HEIGHTS AND DBH

Site	Species of *Eucalyptus*	Plot	Tree	Total height (cm)	Dbh (cm)
Mispah	*gxc*	10	32	1388,2	11
	cam	3	17	1013	16
	dunnii	13	17	2430	29
Madala	*gxc*	18	47	1500	13
	cam	21	47	1790	26
	dunnii	27	29	1648	20
Red Soil	*gxc*	46	47	1830	21
	cam	28	47	1040	9.6
	dunnii	17	48	1170	11

APPENDIX G

SAMPLE WEIGHED DRY MASS

Sample	Mass (g)
Plot 17 T48 Leaves	0.2510
Plot 10 T32 Leaves	0.2500
Plot 25 T17 leaves	0.2497
Plot 18 T47 Leaves	0.2504
Plot21 T47 Leaves	0.2510
Plot46 T47 Leaves	0.2509
Plot3 T17 Leaves	0.2503
Plot27 T29 Leaves	0.2509
Plot13 T17 Leaves	0.2498
Plot28 T47 Leaves	0.2497
Plot47 T24 Leaves	0.2500
Plot 26 T24 Leaves	0.2495
Plot28 T47 Bark	0.2497
Plot21 T47 Branches +twigs	0.2495
Plot 25 T17 bark	0.2508
Plot 25 T17Branches +twigs	0.2502
Plot 18 T47 branches +twigs	0.2503
Plot 13 T17 Branches + twigs	0.2495
Plot27 T 29 Branches +twigs	0.2495
Plot3 T17 branches + twigs	0.2504
Plot 10 T32 wood	0.2504
Plot 13 T17 wood	0.2503
Plot 47 T24 bark	0.2503
Plot 26 T24 Bark	0.2500
Plot 13 T 17 bark	0.2505
Plot 17 T48 Bark	0.2498
Plot 27 T 29 bark	0.2503
Plot 27 T29 wood	0.2504
Plot10 T32 Branches +twigs	0.2502
Plot 28 T47 wood	0.2505

Sample	Mass (g)
Plot 10 T32 Bark	0.2496
Plot 17 T48 branches + twigs	0.2505
Plot46 T 47 wood	0.2503
Plot 25 T17 wood	0.2497
Plot 17 T48 wood	0.2508
Plot18 T47 wood	0.2507
Plot 47 T24 Branches +twigs	0.2507
Plot21 T47 wood	0.2501
Plot 46 T 47 branches + twigs	0.2496
Plot 46 T47 Bark	0.2495
Plot 21 T47 Bark	0.2500
Plot 47 T24 wood	0.2500
Plot 18 T 47 bark	0.2499
Plot 3 T 17 Bark	0.2503
Plot3 T 17 Wood	0.2508
Plot 28 T47 Branches + twigs	0.2506
Plot 28 T24 branches +twigs	0.2495