Phytoremediation: a tool for restoring land degraded due to opencast coal mining

Mansi A Desai (2013)

Phytoremediation: A tool for restoring land degraded due to opencast coal mining

Mansi Anilkumar Desai

Thesis submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy at Oxford Brookes University

Faculty of Humanities and Social Sciences

Department of Social Sciences

October 2013

Dedication

To my parents Hema and Anil Desai, my brother Milind, my sister-in-law Forum and my Husband Vivek, all of whom supported me throughout,

ABSTRACT

This research set on a former reclaimed opencast coal spoil in Varteg Hills, South Wales, UK explores the phytoremediation potential of *Alnus glutinosa* (Alder), *Betula pendula* (Roth) (Birch) and *Larix decidua* (Larch), which are commonly used in UK land reclamation, to sequester metals: Zn, Cd, Mn, Pb and Cu, from mine spoils. This study also compares the abilities of newly planted trees to sequester metals on a new experimental site, within the landscape and compares these with those from older plantations on adjoining land. This chronosequence is used to evaluate the overall impact of forestation on metals levels in soils over two decades. Metal concentrations in soil were measured over three years and those in tree leaves were measured over two years.

The majority of the samples examined lie between the UK defined thresholds for contamination but below levels that trigger immediate action and treatment. Samples within this range are placed within a new 'critical soil' category. Mapping the spatial distribution of contaminants across selected test plots shows the presence of micro-scale contamination hotspots on these sites. These show that while such sites may, on average, have sub-critcial levels for metal contamination, they may still contain sections where contaminants reach very severe levels.

Planting trees in mixes is beneficial because different trees selectively remove different metal contaminants while some species benefit the soil in other ways. For example, while Alder leaves showed average concentrations of Cd, Zn and Mn, their roots also aid nitrogen fixation. Of the five metals selected for this study, two are known to be highly mobile (CD, Zn), two relatively immobile (Pb, Cu) and one is intermediate (Mn). Foliar analysis of Birch shows consistency in accumulation of Cd and Zn in leaves, while young Larch needles accumulated the highest concentrations of Mn and Pb. Correlation between movement of metals in soil and leaves over time, discovered that the uptake of Mn in Alder and Birch foliar on all plots and Larch on the new experimental plot is positively correlated to the presence of Zn in the soil.

Studies of the general effects of forestation on metal concentrations in soil showed that metal levels declined significantly even on the most recently planted test sites. On four year old plots a 14-18% decrease in Mn concentrations and about 8% decrease in Cd and Zn concentrations were observed. Observations of the soils on the full 18 year chronosequence found that metal levels declined very significantly ($p = 0.003$ to 0.0002) through time. Based on the reduction of metal concentration in soil and uptake in leaves, it is possible to project 40 – 45 years for concentration of Mn to reach normal soil levels and about 20 – 25 years for Cd level to reach normal soil levels, provided the soil is not further disrupted by physical, chemical or biological activities which may recontaminate the spoil. This demonstrates, conclusively, that forestation is an effective means of metal remediation on the moderately contaminated lands produced by opencast coal-mining in Wales.

Keywords: Opencast coal mining; Phytoremediation; contaminated land; Heavy metal uptake; Alder; Birch.

ACKNOWLEDGEMENT

My immense gratitude goes to my director of studies Prof Martin Haigh and my supervisor Dr Helen Walkington for their constant support, excellent advice, kindness, encouragement and patience throughout the course of this research at Oxford Brookes University. I owe it all to you.

Heartfelt thanks to the Cradle for Nature team at Varteg for their help with planting, sampling, maintenance, advice and friendship.

Many thanks to the Department of Life Sciences, OBU for letting me use the labs and machines. The lab technicians at Brookes were always ready to help and understood my impatience and panic with great fortitude. Dr. Andrew Rundall, for the needed guidance on the use of the Atomic Abosrption Spectrophotometer. Dr. Hooshang Izadi for statistical advice.

Special thanks to Dr. Santosh Tungare in India for his invaluable help and advice throughout the course of this project.

To all my colleagues and lovely friends at OBU to whom I owe a great deal. Your help and support, be it small chats in the corridors or a drink at Kazbar were the perfect distractions.

A special thanks to my beloved friends in India and UK for always being there to listen, guide and praise me at the smallest achievement.

This research would have not been possible without the confidence and trust my parents, brother and sister-in-law had in me. Thank you for your constant encouragement, love and blessings. You are the reason why this has been possible. A big thank you to the assembled masses of the Desai family.

And finally to my husband, Vivek for being the best distraction from work and supporting me in every possible way throughout the whole production.

LIST OF ABBREVIATIONS

This list should be used for reference in conjunction with textual descriptions.

General Terms

AAS - Atomic Absorption Spectrophotometry

AMD – Acid Mine Drainage

DEFRA - Department of the Environment, Food and Rural Affairs

EA - Environment Agency

EAA – European Environment Agency

EPA – Environment Protection Agency

EDTA - Ethylenediaminetetraacetic Acid

ICRCI - Interdepartmental Committee on the Redevelopment of Contaminated Land

IFPRI – International Food Policy Research Institute

MPG – Mineral Planning Guidance

SGV - Soil Guideline Value

USEPA - United States Environmental Protection Agency

WDA – Welsh Development Agency

Experimental Site abbreviations (refer to section 3.5 for a full description)

PA-93 – Pan Plot planted in 1993

TA-94 – Titania Plot planted in 1994

SH-97 – Shiela Plot planted in 1997

CA-03 – Cariad Plot planted in 2003

MI-04 – Mike Plot planted in 2004

MD-07 – Mansi Plot planted in 2007

TABLE OF CONTENTS

Dedication ii

Abstract iii

Acknowledgement v

List of abbreviations vi

Contents vii

List of tables xii

List of figures xiv

List of plates xv

1 Introduction 1

1.1 General 1

1.2 Coal mining in the UK 3

1.3 Effects of coal contamination 5

1.4 Remediation of contaminated land 6

1.5 Plants for phytoremediation 7

1.6 Summary 8

1.7 Aims and objectives 10

2 Review of Literature 11

2.1 Introduction to coal mining in UK and EU 11

2.2 Background of opencast coal mining in the UK 14

2.3 History of mining in Wales 16

2.4 Environmental impact of opencast coal mining 17

2.5 Contaminated land 23

2.6 Heavy metals in soil 25
2.7 Metals of interest 29
2.7.1 Cadmium (Cd) – Class 1 contaminant 29
2.7.2 Lead (Pb) – Class 1 contaminant 33
2.7.3 Copper (Cu) – Class 4 contaminant 38
2.7.4 Zinc (Zn) – Class 4 contaminant 40
2.7.5 Manganese (mn) – Class 5 contaminant 43
2.8 Soil properties affecting plant growth on coal spoils 46
2.9 Phytoremediation for successful reclamation 49
2.10 Benefit of planting trees 57
2.11 Uptake and translocation of metals in plants 60
2.11.1 Rhizosphere 61
2.11.2 Uptake by roots 63
2.12 Choice of tree species 69
2.12.1 Alder (*Alnus glutinosa*) 70
2.12.2 Birch (*Betula pendula* Roth) 74
2.12.3 Larch (*Larix decidua)* 78
2.13 Plant and the community 82
2.14 Forestry: Potential land-use policy for land reclamation 83
2.15 Reclamation initiative on opencast coal spoils in Wales 86
2.12 Summary 89

3 Site Description 92
3.1 Location and History of Varteg Hills 93
3.2 Post reclamation site condition 96
3.3 Site description: Problems due to mining 97

3.4 Sites of study 102

3.5 Planting and planting method 103

3.5.1 PA-93 104

3.5.2 TA-94 106

3.5.3 SH-97 107

3.5.4 CA-03 108

3.5.5 MD-07 109

3.5.6 MI-04 111

3.6 Summary 113

4 Materials and Methods 115

4.1 Experimental plot selection 115

4.2 Species selection 116

4.3 Plot preparation and design 118

4.4 Soil compost 119

4.5 Sample collection 120

4.6 Soil physical and chemical analysis 126

4.6.1 Bulk density 126

4.6.2 Soil NPK (nitrogen, phosphorous and potassium content) 126

4.6.3 Laboratory analysis methods 126

4.6.4 Soil pH 127

4.6.5 Loss on ignition 127

4.6.7 Chemical analysis of soil samples 128

4.6.8 Foliar sample collection and analysis 128

4.6.9 EDTA extraction of soil samples 129

4.6.10 Microwave digestion of foliar samples 131

4.7 Apparatus and working of AAS 132
4.7.1 Flame spectroscopy 133
4.7.2 Atomic Absorption Spectroscopy 134
4.7.3 Components of AAS instrument 135
4.7.4 Calibration 138
4.8 Preparation of standard solutions 139
4.9 Contamination factor (CF) and Pollution load index (PLI) 140
4.10 Contamination contour maps 141
4.11 Tree mortality 141
4.12 Statistical and computational analysis 142

5 Results and Analysis 144

5.1 Contaminant mapping 144
5.1.1 Data analysis and production of contaminant maps 144
5.2 MD-07 contamination maps 146
5.2.1 Cadmium (Cd) in soil at md-07 in 2007 and 2010 149
5.2.2 Zinc (Zn) in soil at md-07 in 2007 and 2010 150
5.2.3 Manganese (Mn) in soil at md-07 in 2007 and 2010 151
5.2.4 Lead (Pb) in soil at md-07 in 2007 and 2010 152
5.2.5 Copper (Cu) in soil at md-07 in 2007 and 2010 153
5.3 Soil metal interaction – All plots 154
5.4 Contamination factor (CF) and pollution load index (PLI) 163
5.5 Foliar metal uptake and interaction – MD-07 169
5.6 Foliar metal uptake and interaction – All plots 179
5.7 Overview of heavy metal uptake by tree leaves on all plots 190
5.8 Annual metal distribution in leaves 193

5.9 Soil metal concentration after last sampling season 195

5.10 Addition of compost 198

5.11 Tree mortality 200

6 Discussion 203

7 Conclusion 239

8 References 250

APPENDIX 1 317

LIST OF TABLES

Table 2.1: Great Britain's saleable open cast coal production in 2008-2009 15

Table 2.2: Great Britain's application for opencast coal mining (approved) 15

Table 2.3: Great Britain's application for opencast coal mining (rejected) 15

Table 2.4: Published standard soil metal concentrations. 25

Table 2.5: Environmental significance of heavy metals in soil. 28

Table 2.6: Braithwaite's classification of metals 29

Table 2.7: Phytoremediation technologies and its application 51

Table 2.8: Metal uptake by 3 tree species by various researchers 88

Table 3.1: Study site summary 103

Table 4.1: Tree species selected for the present study 111

Table 4.2: Number of trees planted on the sub-plots on plot MD-07 119

Table 4.5.1: Sampling schedule at the different study plots. 125

Table 4.7.1: Instrument setting for analysis of 5 metals studied 139

Table 5.1.1: Soil metal concentrations at MD-07 samples collected in 2007. 146

Table 5.1.2: Soil metal concentrations at MD-07 samples collected in 2010. 147

Table 5.2.1: Comparison of Cd in soil at MD-07 in 2007 & 2010 149

Table 5.2.2: Comparison of Zn in soil at MD-07 in 2007 and 2010 150

Table 5.2.3: Comparison of Mn in soil at MD-07 in 2007 and 2010. 151

Table 5.2.4: Comparison of Pb in soil at MD-07 in 2007 and 2010. 152

Table 5.2.5: Comparison of Cu in soil at MD-07 in 2007 and 2010. 153

Table 5.3.1: Descriptive statistics of metal contamination on MD-07 155

Table 5.3.2: Descriptive statistics of metal contamination on all plots in 2009 156

Table 5.3.3: Descriptive statistics of metal contamination on all plots in 2010 157

Table 5.4.1: Contamination factor for heavy metals 164

Table 5.5.1: Descriptive statistics of metal in foliage on MD-07 in 2009 169

Table 5.5.2: Descriptive statistics of metal in foliage on MD-07 in 2010 170

Table 5.5.3: Pearson's correlation at MD-07 178

Table 5.6.1: Descriptive statistics of metal in Alder – All plots in 2009 180

Table 5.6.2: Descriptive statistics of metal in Alder – All plots in 2010 181

Table 5.6.3: Descriptive statistics of metal in Birch – All plots in 2009 184

Table 5.6.4: Descriptive statistics of metal in Birch – All plots in 2010 185

Table 5.6.5: Pearson's correlation all plots 188

Table 5.7: T-test results for annual variability in metal uptake by leaves........ 194

Table 5.8: Difference in soil metal concentration from 2007 to 2010........ 196

Table 6.1: List of research questions 205

Table 6.2: Standard published values for soil metal concentration 211

Table 6.3: Soil metal concentrations at BL-07 and MI-04........ 215

Table 6.4: Mean foliar metal concentration on site MD-07 217

Table 6.5: Foliar metal concentrations reference data 218

Table 6.6: Problems of mined land, long-term treatments & suitable trees 233

LIST OF FIGURES

Figure 2.1: Schematic representation of Opencast Coal mining in UK 12
Figure 2.2: Vertical-section of the root showing the Rhizosphere region................ 61
Figure 2.3: Root structure with the different tissue layers 64
Figure 2.4: Distribution of metals (Cottenie et. al. 1982):. 66
Figure 2.5: Benefit of woodland on contaminated lands 84
Figure 3.1: UK political map and Wales road map. 93
Figure 3.2: Historic Map of Varteg in 1960. 96
Figure 3.3.1: Geological map of Varteg and location of the site of study 99
Figure 3.3.2: Plot plan with names of all reclaimed and experimental plot 101
Figure 4.1: 9 sub-plots on the Experimental plot MD-07 118
Figure 4.2: Schematic representation of HCL 135
Figure 4.3: Schematic diagram of a Flame Atomizer 137
Figure 4.4: Schematic representation of an AAS 138
Figure 5.2.1: Contamination map for Cd in soil in 2007 and 2010 149
Figure 5.2.2: Contamination maps for Zn in soil in 2007 and 2010 150
Fig. 5.2.3: Contamination map for Mn in soil in 2007 and 2010 151
Figure 5.2.4: Contamination map for Pb in soil in 2007 and 2010 152
Figure 5.2.5: Contamination map for Cu in soil at 2007 and 2010. 153
Figure 5.3.1: Mean metal concentrations in soil on all plots in 2009 & 2010 158
Figure 5.3.2: Scatter plots of metals in soil against age of planting 162
Figure 5.4.1: Pollution Load Index (PLI) 164
Figure 5.5.1: Concentration of metals in foliage on MD-07 in 2009 & 2010. 171
Figure 5.5.2: Concentration of metals in soil on MD-07 in 2009 & 2010 175
Figure 5.6.1: Concentration of metals in soil and Alder leaves – All plots. 183
Figure 5.6.2: Concentration of metals in soil and Birch leaves – All plots 187
Figure 5.7: Difference in soil metal concentration between Year 1 & 3 197
Figure 5.8: Effect of compost on metal concentration in foliage at MD-07 199
Figure 5.9: Percentage mortality of trees on MD-07 201
Figure 6.1: Comparison of Mn and Cd in soil between BL-07 and MI-04. 214
Figure 6.2: Distribution of metals in leaves against metal in soil 219
Figure 6.3: Ranks of metal accumulation in trees at MD-07 in 2009 and 2010 220
Figure 6.4: Scatter graphs of Mn (Alder and Birch)and Cd (Alder) in foliar 223

LIST OF PLATES

Plate 3.1: Varteg Hill and area of the proposed Varteg Hill Opencast Mine 95
Plate 3.2: Google image of the area of study 99
Plate 3.3: Satellite image of Varteg Hill with the study plots superimposed 101
Plate 3.4: Site PA-93 during its initial planting in November 1993........................ 105
Plate 3.5: Site PA-93 after 18 years of planting in July 2010................................ 105
Plate 3.6: Site TA-94 after four years of planting in 1998 106
Plate 3.7: Site TA-94 after 17 years of planting in July 2010................................ 106
Plate 3.8: Site SH-97 after one year of planting in 1998. 107
Plate 3.9: Site SH-97 after 14 years of planting in July 2010 107
Plate 3.10: Site CA-03 before and after ground preparation in 2003.................... 109
Plate 3.11: Site CA-03 after seven year of planting in July 2010.......................... 109
Plate 3.12: View of plot MD-07 before planting in 2005. 111
Plate 3.13: View of plot MD-07 after four year of planting in 2011........................ 111
Plate 3.14: Plot MI-04 after ploughing and tree plantation in 2010........................ 112
Plate 3.15: Six year tree above the ploughed trench at MI-04.............................. 112
Plate 4.1: Flame Atomic Absorption Spectrophotometer...................................... 133

CHAPTER 1

INTRODUCTION

1 INTRODUCTION

This chapter outlines the background information on opencast coal mining in the UK and worldwide, its impacts on the environment and the pressing need to reclaim these spoils. It introduces the widely used concept of phytoremediation, the benefit of planting trees for reclaiming mine spoils and finally sets out the aims of this research.

1.1 General

Plants have a natural ability to absorb nutrients from the medium they grow in. Many of these plants possess specific mechanisms to refine chemicals and contaminants present in these soils and water. For over three centuries an extensive amount of research has established the effectiveness of plants in not only cleaning up these contaminants, but also stabilising the environment. There are many ways of defining this process termed '*Phytoremediation*'. One of the definitions by Schnoor et al. (1995) is

"*The use of plants in situ to contain, sequester, remove, or degrade organic or inorganic contaminants in soils, sediments, surface water and groundwater*" (Page, 318).

Soil is an imperative part of the environment which has been extensively disturbed by human activities which in turn has resulted in adapting the technique of phytoremediation to manipulate and curb the harmful effects of contaminants in the environment.

Mining is one such human activity which has led to the disruption of soil leading to poor soil conditions and heavy metal contamination. This research explores the ability of trees planted for remediating a former open-cast coal mine site contaminated with a variety of heavy metals at different concentrations. The research also looks at the different characteristics presented by such sites and the ability of trees to sustain and advance under adverse conditions and discuss the benefits in plausibly applying the technique to achieve a healthy woodland cover as a land use strategy for such lands.

The present study examines the capacity of three of the most commonly used tree species in land reclamation in UK (Moffat and McNeill, 1994; Moffat, 1995; Bending, 1999), Alder (*Alnus glutinosa*), Birch (*Betula pendula*) and Larch (*Larix europaea*). These trees have been tested for their phytoremediation potential of heavy metal contamination in field but have mainly concentrated on brownfield contaminated sites, fertilizer residue, sewage sludge or specific soil chemicals. *Larix* spp, once the Forestry Commission's desired tree species for mining reclamation, was used in the past but not to the extent that this research presents.

This thesis looks at a combination of components such as opencast coal mining, heavy metal contamination, phytoremediation and uptake of metals by trees and the role of trees in reclamation and plant uptake. For this, it is first and foremost essential to understand the basis of each of these components to better outline the aims and objectives of the research.

1.2 Coal Mining in the UK

The UK has a rich history of mining. Coal mining in the UK began in the early 18th century with the oldest mines being in Durham, South Wales, Scotland and the Midlands. The extraction of coal from opencast mining is a simple and usually favoured mining process in the UK. The UK alone is said to produce nearly 10 million tonnes of coal ever year (BGS, 2009). Planning the aftercare of a mine site is as important as the initial mining plan. In 1996 the Mineral Planning Guidance 7 (MPG7) which deals with the consultation, policies and conditions relevant to the achievement of effective reclamation of mineral workings outlined that, the restoration plan for reclamation and aftercare of mine sites must form an essential part of the initial planning application (DoE, 1997). The main objective of reclamation of these coal spoils is to shelter the restoration of the soil layers and materials in such a way that the land is brought back to the condition where it can self-sustain. This usually includes top and subsoil replacements in a way that the soil structure and layers are maintained in a correct sequence to facilitate optimum growth of plant and micro-organisms.

According to the Mineral Planning Guidance (DoE, 1997) planners must be able to demonstrate that a site can be reclaimed to an acceptable after-use standard, which involves any form of plant growth i.e. forestry, agriculture or any form of nature conservation, the plan needs to involve the following key stages:

- "Stripping of soils and soil-making materials and either their storage or their direct replacement/ restoration on another part of the site;
- Storage and replacement of overburden;

- Achieving the landform and landscape objectives for the site; including filling operations if required, following mineral extraction;
- Proper restoration, including soil placement, relief of compaction and provision of surface features;
- Aftercare; (MPG-7: DoE, 1997; pg 12, pt 27)
- In cases where forestry is the intended after-use, consultations on its appropriateness and on aftercare requirements should be with the Forestry Commission" (MPG-3: DoE, 1999; pg 21, pt 60).

There has been a growing concern about the risks of opencast coal sites being left unrestored or where the restoration process has been severely delayed due to lack of responsibility or an event of financial failure of a coal operator (DoE, 1997). In 2005 the Environment Agency estimated about 300,000 ha of land contaminated in England and Wales alone and a large proportion of this was a result of mining activities. In their progress review from 2000-2007 under part 2A of the EPA, the Environment Agency in March 2007 noted that 781 sites had been determined as contaminated in England and Wales, including 35 sites which are designated as 'special sites' in need of immediate attention. The cost of remediating these contaminated and special sites in early 2007 was around £20.5 million, with costs anticipated to rise to around £60 million for all of the sites currently determined under part 2A. The successful planning, reclamation and post-mining uses of land is now required by law in most countries (Vance, 2009). However, prior to 1970 before sufficient legislation was in place, sites which were reclaimed were not necessarily checked over a period of time to ensure they were still achieving self-sustainability (Countryside Commission, 1993). Whether the present day legislation is being successfully followed remains a big question.

1.3 Effects of Coal contamination

Mining is said to contribute the largest deposits of heavy metal contamination in the world (Pulford, 2008). The main anthropogenic activities causing metal entry to soil and atmosphere are: Metalliferous mining, refining and smelting, agriculture and sewage sludge disposal, combustion of fossil fuels, metallurgical and specialist industries and disposal of waste material (Alloway, 1995a). Metal contaminants considered by these processes are Ag, As, Au, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Se and Zn. In terms of quantities released and distribution in the environment the most significant are Cd, Cu, Zn, Pb and Ni (Alloway 1995b). These elements are referred to as heavy metals or toxic metals because they possess negative, acute or chronic physiological effects on plants, animals, humans and aquatic life. The specific contaminants studied in this research are Cadmium (Cd), Lead (Pb), Copper (Cu), Zinc (Zn) and Manganese (Mn) which are described in the forthcoming chapter. The disintegration of soil layers by mining leads to the oxidation of heavy metals, which further leads to elevated bioavailability. Making them available and mobile in water within the coal strata means they can easily drain into aquifers and clear water under mining conditions (Roberts, 2007). Heavy metals present in high concentrations can be phytotoxic. Poor plant growth and soil cover, caused by metal toxicity, can lead to metal mobilization in runoff water and subsequent deposition into nearby water bodies (Lasat, 2002). Bare contaminated soil is also more vulnerable to erosion and spread of contamination by dust. Contaminated and derelict lands can be dangerous; hazards include tip failure, crumbling of quarry faces or exposure of dangerous chemical contaminants (Moffat & McNeill, 1994). Mining also presents challenges like soil compaction, soil contamination, infertility, high acidity, salinity and hostile temperature regimes, etc. (Haigh, 1995; Bending, 1999; Haigh, 2000a).

The scars of mining from the distant past reveal that the altered physical, chemical and biological systems are not repaired by nature alone in a reasonable time, or to a desirable state (Ramani, 1990). These need suitable planning and restoration activities by those carrying out the mining processes in the first place to set these distorted lands to a healthy, safe and self-sustainable state, a state where these lands can develop and flourish without any external support or aid.

1.4 Remediation of Contaminated lands

"Remediation technologies have been constantly focussing on ways in which contaminants can be destroyed, or removed sensitively with low risk of secondary environmental problems, taking a holistic and life cycle analysis approach" (Lynch, 2005, p217).

Different methods and techniques are devised for effective restoration/ remediation of degraded lands and one such method used extensively for restoration is 'Phytoremediation'. Phytoremediation is the use of green plants for *in situ* risk reduction and/or removal of contaminants from contaminated soil, water, sediments and air. This technique uses specially selected metal-accumulating plants to remediate soil contaminated with metals and other substances (Blaylock, 2000). It is applied at sites with shallow contamination of nutrient, organic or metal pollutants. These pollutants are mostly removed by one of the following applications: Phytotransformation, Rhizosphere Bioremediation, Phytoextraction, Phytostabilization or Rhizofiltration (Schnoor, 1997; Jing et al., 2007). Phytoremediation of metals that are usually perennial in the environment is said to be a low cost and environmentally friendly substitute to chemical methods and therefore has attracted growing interest in the last decade (Baker et al., 1998; Blaylock et al., 1997 & Wang et al., 2004).

1.5 Plants for Phytoremediation

Plants have a remarkable capacity to survive in the most unfavourable conditions on the planet (Larcher, 1995). Metal accumulating trees are capable of concentrating most heavy metals like Zn, Cd, Co, Mn, Pb, Ni up to 100 times to those taken up by non-accumulators. Such trees are grown on metal contaminated sites, which extract metals from the soil and store them in the above ground tissues like the stem, leaves and fruits. Planting trees has been suggested as a feasible and preferred option for remediation of contaminated and derelict/degraded lands and this has been widely adapted in Wales and across the UK (Moffat and McNeill, 1994; Moffat et al., 2008). Some trees are capable of removing heavy metals from soil like hybrid *Populus* species, *Thlaspi* species, *Alyssum*, etc. (Brooke et al., 1979; Baker et al., 2000; Brown et al., 1994; Mertens et al., 2006), whereas some are good at immobilising these metals at the root levels like *Salix* species, *Acer* species, *Alnus* species, etc (Huang et al., 1997; Cooper et al., 1999; Merten et al., 2006; Shi et al., 2012).

Establishment of woodland on contaminated and derelict lands has been one of the top preferences of the England Forestry Strategy (Hutchings, 2002). Tree planting offers many benefits today besides just timber production. These include the prevention of soil erosion, soil creation and enhancement of landscape and provision of important habitats for forest flora and fauna (Forestry Commission, 1994). The roots help to stabilise the soil and substrate. Leaf litter also contributes to a considerable amount of organic matter to the surface of the soil. On mine spoils where soil compaction is a common problem planting deep rooting native trees helps to break down the compact soil layers thereby making establishment of trees possible. Trees with the potential of remediation can either extract the chemicals from the soil through phytoextraction or could immobilise the chemicals and stabilise the soil through the technique termed as phytostabilization.

1.6 Summary

Mining is carried out on a large scale, now more than ever. Although coal is very important, the process of removal is very harsh on the environment and it is imperative to reclaim these lands after mining is carried out. Mining activities not only disrupt the aesthetics of the area but also disturbs the important soil components such as soil profile and layers, structure, microbe populations and the various nutrient cycles that are essential for a healthy ecosystem (Kundu & Ghose, 1997; Sheoran et al., 2010). Mine wastes can have multiple adverse effects such as erosion, air, soil and water pollution, heavy metal toxicity, environmental disasters, depletion of biodiversity and great economic loss (Wong, 2003; Ghose, 2005; Sheoran et al., 2009). Mine spoils consist of loosened piles of spoils, overburden surfaces, stripped top layers, waste rocks and degraded land areas which present challenging conditions for successful restoration (Sheoran et al., 2010).

Conservation of land after mining is imperative in order to restore the productivity of the affected area. Reclamation through phytoremediation is a well-known process through which contaminated, derelict and degraded lands are brought back to abundance, and their productivity and biotic processes are restored and renewed by planting trees capable of removing or stabilizing these contaminants. Long term mine spoil reclamation requires and demands the foundation of stable nutrient cycles from microbial processes and plant growth (Singh et al., 2002; Lone et al., 2008; Sheoran et al., 2010; Kavamura and Esposito, 2010). Restoration of healthy vegetation cover on these degraded and contaminated coal spoils can accomplish the objectives of pollution control, visual aesthetics, soil stability and removal of harmful threats to the environment (Wong, 2003). Mine spoils pose difficult soil conditions like acid mine drainage (AMD), chemical hotspots, varied pH levels, high bulk density, lack of moisture etc. which makes establishment of plants difficult.

Forestry is one land use strategy, which has been used to bring back these lands to productive use. It is not only aesthetically pleasing but also shows potential for decontaminating these lands. However, it is not very clear how tree performance is affected by contamination. There is a need to identify tree species which are local and will be effective in not only establishing themselves on these degraded lands which pose various soil problems, but also show potential to reclaim these lands by dealing with the problem of contamination.

This further poses a need to model this process over time to evaluate the progress of reclaimed coal-lands towards that condition where they may be converted safely and sustainably to forestry.

1.7 Aims and Objectives

The main aims of the research are to: use the technique of phytoremediation to test the ability of trees, to accumulate metals from coal contaminated spoils; and to evaluate the progress of reclaimed coal-lands towards a condition where they may be converted, safely and sustainably, to forestry.

The research also aims to:

- Determine the amount and variety of contaminants on a former reclaimed coal mine site.
- Determine the performance of trees under varied levels of mixed contaminants.
- Use previously forested test plots to establish a time series that will help examine the uptake capacity of these tree species over a longer timescale, as compared to the new test plots.
- Contribute to the debate surrounding whether the forest reclamation of former coal-lands is the best land use technique for areas bound to return to non-urban/industrial uses.

The next chapter looks at the topics visited this chapter in much more depth. An in-depth review of literature will be carried out to identify the gaps in the literature and present an argument towards selection of this project and help identify the specific research questions.

CHAPTER 2

REVIEW OF LITERATURE

2 REVIEW OF LITERATURE

This in-depth literature review provides the background information on this particular project and a recapitulation of opencast coal mining, its reclamation, remediation strategy, and selection of tree species.

Initially, the literature on coal mining with specific reference to opencast coal mining in the UK and EU, will be reviewed. Additionally, details about mining in Wales, its history and the environmental impact of opencast coal mining and the after use of such lands will be explored.

2.1 Introduction to Coal Mining in UK and EU

Coal is an important natural resource in the UK and around the world. The extraction of coal from opencast mining is favoured to underground mining in terms of coal recovery, coal quality, safety, operational flexibility, productivity and costs (Vance, 2009). Coal is a combustible, organic sedimentary rock which occurs in the rock strata, in veins or layers known as coal beds or seams (EA, 2011). It also acts as a large sink for minerals, organic and inorganic materials. Mining of mineral resources results in considerable damage of the soil, which causes alternation of soil layers and disruption of microbial communities. It also leads to alteration of vegetation cover, which leads to the downfall of vast land covers.

Coal is excavated by two main processes; Open cast 'Surface' mining and underground 'deep' mining. Surface or opencast mining is viable when the coal seams are near the top surfaces (BGS, 2006). This process helps recover a high proportion of coal as compared to deep mining as it covers a vast area as most of the coal seams are utilized. Figure 2.1 below shows the process of Opencast 'surface' mining.

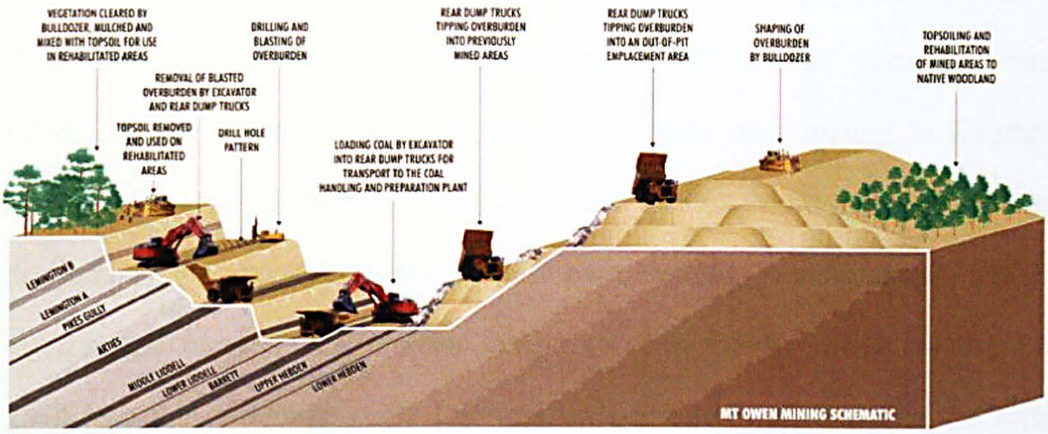


Figure 2.1: Schematic representation of the process of Opencast Coal mining in UK. Source: coal.infomine.com/commodities

Opencast coal-mining has been through some modern recuperation in the EU and the UK; this is mainly because of the rise in global gas and coal prices. Mining companies and power stations are progressively turning to opencast mines to provide a cheaper supply of coal (CPRE, 2008). According to the IEO2009 reference case, consumption of coal around the world is said to increase by 49 percent from 2006 to 2030 and coal's share of world energy consumption is said to increase from 27% to 28% in 2030 (EAA, 2009). The peak coal production in the UK was around 228 million tonnes in 1952 (Kerai, 2013). Due to an increase in the UK energy there was an increase in the demand for coal in the country. By 1970 most of the coal produced was used for electricity generators which on average consumed almost 70% of the coal produced in the UK. Hence to meet growing demands, the UK has become a key major importer of coal from other countries (Kerai, 2013). In 2006 the major coal-consuming countries in Europe included Germany, United Kingdom, Poland, Spain, Turkey, Italy, and Czech Republic (International Energy Outlook, 2009). Almost 50% of the British coal is extracted by opencast coal mining methods. In 2005, the total production of coal in the UK was 20m tonnes of which 9.6m tonnes came from underground mining production and 10.4m tonnes from opencast mining (Vidal, 2008). In the last two years, open cast mining in England alone has risen by 50% from 1.2 to 1.8 million tonnes. More than 80% of coal demand in the world is currently met by open-cast mining (Maiti, 2007); disturbance of land is therefore inevitable. The claims made by the UK government to become world Leaders on combating climate change have been undermined by the rapid increase of coal production through opencast coal mines (Carrel, 2009). There have also been reports on ministers and council authorities granting opencast coal mining permissions in an attempt to revert the decline in coal production in the UK (Carrel, 2009). There are reports of mining companies having access to nearly 71m tonnes of coal from licensed opencast mines as opposed to 51m tonnes in 2007. The Guardian reported that the mining industry was likely to gain licence to mine an additional 15m tonnes from across the UK (Carrel, 2009). The figures are obtained from the Historical Coal data produced by the Department for Energy and Climate Change (2013). Britain's decision to expand its coal mining industry has also generated increased concern amongst environmentalists and the general public, and that the move will lead to an increase in CO_2 emissions and in turn harm the local environment and communities (Vidal, 2008).

2.2 Background of Opencast coal mining in the UK

Large scale coal mining first developed in the UK in the 18th century during the Industrial Revolution. Coal was also the main source of energy for developing industries and transportation across the UK until mid-1950's (Hatcher, 1993). During the 1980's there was a big surge in opencast coal mining throughout the UK. Annual production of coal produced by opencast mines increased by 50% to 19m tonnes between 1979 and 1992, of which only 16m tonnes were produced by British Coal and the remaining by small scale private industries (Hatcher, 1993; Trigg and Dubourg, 1993; Chadderton et al., 2011). The Council for the Protection of Rural England once regarded Opencast coal-mining as "one of the most environmentally destructive activities being carried out in the UK" (Trigg and Dubourg, 1993; Chadderton et al., 2011). Many local communities living near these opencast mines complained about noise pollution and traffic congestion, adverse visual impact, dust and the damage caused to the local rural areas after restoration of these lands (Trigg and Dubourg, 1993). Indeed opencast mining was found to have adverse effects on the quality of life for the local communities. Despite this, due to its abundance and lower costs, coal continues to remain a major source of energy when compared to other fossil fuels.

The United Kingdom alone is said to produce approximately 10 million tonnes of coal every year now, just from opencast coal mining. Table 2.1 shows UK's saleable opencast coal production in 2008-2009 alone.

Table 2.1: Great Britain's Saleable Open Cast coal production in 2008-2009

	2008		2009	
	Tonnes	Operational Sites	Tonnes	Operational Sites
England	2138568	9	2137294	13
Wales	1632730	8	1597907	7
Scotland	5655143	18	6036769	18

Source: Coal Authority and Local planning authority;
Opencast Coal Statistics, BGS

In 2009 Britain applied for more opencast mines to be opened to meet its coal requirement. The following 2 tables show the number of applications made for new opencast mines to be opened and the number of applications rejected.

Table 2.2: Great Britain's Application for Opencast coal which have been granted planning permission during 2009

	Application's approved	Total Hectares	Total reserves Tonnes
England	4	367	3090000
Wales	2	51	400000
Scotland	8	624	4675000

Source: Coal Authority and Local planning authority; Opencast Coal Statistics, BGS

Table 2.3: Great Britain's Application for Opencast coal which have been refused planning permission during 2009

	Application's declined	Total Hectares	Total reserves Tonnes
England	2	166	1015000
Wales	1	unknown	unknown
Scotland	0	0	0

Source: Coal Authority and Local planning authority; Opencast Coal Statistics, BGS

According to a news article in the Guardian (Carrel, 2009) authorities in London, Edinburgh and Cardiff are discounting the concerns and objections of the local communities in order to push through applications for opening new opencast mines in the areas. The Guardian report claims that

that since the general election in 2005, applications for a total of 54 mines have been approved across the UK and only 4 have been rejected, although there are no official documents available to verify these claims.

2.3 History of mining in Wales

Coal mining in Wales was considered an important source of income in the 19th and early 20th century. Large quantities of coal, Pb, Cu and Au were extracted from various mines all around Wales along with smaller quantities of Zinc and silver. The common practice was to continue mining until the resources were exhausted, at which time the mine would be abandoned. This practice left large areas of abandoned coal mines which have not yet been restored, leading to large patches of unreclaimed, infertile and unproductive land. According to the Coal and Local Planning Authorities (BGS, 2009) between 1985 and 2009, coal production in Wales was highest in 1994, with a total production of over 3000 tonnes, after which there was a steady decline until 2002. However, there has been a constant increase thereafter (BGS, 2009). The Welsh Development Agency (WDA) was allocated the task of improving the environment, thereby rejuvenating the economy of Wales. The agency under the WDA Act 1975 (amended by 1982 Derelict land Act) is granted funds for the reclamation of derelict land and is also empowered to grant aid projects of environmental improvement (Griffiths, 1992). Reclamation in Wales was prompted after the disaster in Aberfan in October 1966, but this reclamation was only seen as a necessary means of clearing hazardous wastes and eradicating visual scars of industrial activities (Griffiths, 1992). Reclamation in Wales according to WDA has however "moved from being the last step in the first industrial revolution in the UK to the first step in the regeneration process" (Griffiths, 1992). This reclamation however came at a very high price, to date over £200 million have already been spent on the reclamation of land in Wales (Griffiths, 1992) and yet not all of the landscape has been reformed to its original undisturbed beauty.

The Varteg Hill, in South Wales, UK is an area of open upland where mining began as early as 1802, and was exploited largely through open cast mining for its iron and coal between 1948 and1963. This mining site is located south west of the UNESCO World Heritage site of Blaenavon which opened in 1789 as the Blaenavon ironworks and lead to the establishment of the Varteg ironworks at c. NGR SO263055. The Varteg Hill Colliery opened in 1860 which comprised of the Varteg Hill pit (SO 261063) and Mine Slope (SO 262067). A detailed review of the study site in Varteg, South Wales is described in the forthcoming chapter.

2.4 Environmental Impact of Opencast Coal Mining

Opencast coal mining is different from other types of mining and does not require any form of deep extractions which would require tunnelling into the earth's crust. Instead this type of mining occurs on the top surfaces and is said to have detrimental effects on the soil and environment. Some of these include destruction and degradation of the landscape and ecology, deterioration of water and air quality, land subsidence, etc. (Ramanathan et al., 2002; Dutta and Agarwal, 2003; Nwachukwu and Pulford, 2008). Mining is found to release enormous quantities of metal species which are highly volatile in nature and large quantities of dust particles into the atmosphere (Thornton, 1996; Nwachukwu and Pulford, 2008).

The environmental impact of opencast mining has a variety of elements, and these are highly influenced by the nature and magnitude of the local environment and site conditions. According to Tomlinson (1982) the factors that influence the nature and scale of the impacts of opencast mining are as follows:

- Population density – as the degree of impact may depend on the numbers affected, e.g. noise.
- Topography – as the local terrain would influence the propagation of dust and noise and the visibility of a site.
- Weather – especially rainfall and wind as they affect the magnitude and range over which the pollutants are dispersed.
- Economic and Social factors – the perspective of the local communities to mining being generally conditioned by the local economy and the nature of the community.

Tomlinson (1983) also listed the main environmental impacts that are caused by opencast mining which are: visual impact, blasting vibrations, noise, coal transportation impacts, water pollution, air pollution and the deep impacts on agriculture. Mining also affects human and animal health and plant flora and fauna in and around the mining area (Bradshaw and Chadwick, 1980; Sengupta, 1993; Ghose and Majee, 2000). Failure to successfully reclaim a coal spoil leads to major environmental concerns which affect air, water and land:

Air pollution: Mining itself may not be the dominant contributor of pollution to air but the various processes involved in mining such as coal and oil burning, dust rising from over burden material, blasting in surface mines, screens at processing plants etc. can also lead to air pollution (Ramani, 1990; Vance, 2009). The loosening of the coal layers exposes the heavy metals contained within them, which add to air pollution.

Water pollution: Mining has led to challenges with water pollution. The disturbance of the overlaying rocks and compaction, affects the normal drainage patterns and the pollutants easily seep into the rain water and drainage pipes. Water pollution can result from the dissolution of the minerals and metals in the coal ore and the associated strata (Ramani, 1990). Acid mine drainage is a major problem posing an environmental pollution threat at these mine sites (McGinness, 1999). The quality and amount of drainage can be affected by the chemical composition of the rocks in and around the ore, their association and breakdown due to oxidation, the rate of infiltration and the bacterial composition at the mine site (EPA, 1999).

Land pollution: Opencast coal mining can lead to problems such as soil compaction and soil contamination, which in turn can lead to land degradation (Haigh, 2000b). Soil compaction makes rooting difficult and establishing vegetation on spoils produced from coal mining often presents challenging problems. These include compaction, infertility, high acidity, salinity and hostile temperature regimes, etc. (Bending, 1999). Also after the removal of coal, the land which otherwise is home to many organisms, is destroyed hence breaking the link in the ecosystem. The problem is one of world-wide dimensions. It is more clearly seen within highly developed, industrialised nations (Bradshaw and Chadwick, 1980).

Within the mining industry it is well known that in order to reclaim mined sites the surface needs to uncompact, however some areas end up being compacted due to traffic, operating machinery and on site storage units which are essential for mining. There has been some study on the problem of soil compaction as a result of mining activities (Phelps and Holland, 1987; Bending, 1999; Haigh, 2000). Sweigard et al. (2007) in their experiment for the Forestry reclamation approach (FRA) tried ripping the compacted soil to facilitate planting. According to their research ripping did loosen the spoil and facilitate planting to some extent but it also brought large boulders and stones to the soil surface, plus the use of large rippers ended up compacting the spoil in areas. What the study did not point out is the mobility of metals and contaminants due to loosing of the spoils and their subsequent pollution of soil and air.

Soil degradation is an issue of concern in the UK and EU. According to the EU legislative published by Europa (2002), the following threats to soil have been identified in Europe: Soil erosion, decline in organic matter, soil contamination, soil sealing, soil compaction, and decline in soil biodiversity; landslide and flooding. The impacts of mining are said to have negative effects on the surrounding environment as well. Some of these include: destruction of landforms, habitat fragmentation, air pollution, siltation, contamination and negative aesthetics (Haigh, 2000; Maiti, 2007; CPRE, 2008). Opencast mines not only drastically alter landscapes, disrupt the biodiversity and ecosystem stability but an increase in opencast mining leads to more carbon emissions, which could be the cause for climate change and global warming (CPRE, 2008). Restoration of vegetation cover can fulfil, to some extent, the objectives of reclaiming these coal spoils, pollution control, visual aesthetic development and environmental cleanup.

The soils that are recreated on opencast sites after mining activities are finished are a manmade habitat that in turn presents challenging problems. According to the National Coal Board Opencast Executive (1982), opencast mining leads to high soil bulk density along with a poorly structured weak soil especially in the earlier years. There are also often zones of compactions in the soil profile. As confirmed by many researchers compaction leads to restricted rooting, impeding drainage and generally the land becomes difficult to work with (Bradshaw and Chadwick, 1980; Tomlinson, 1983; Haigh, 2000; Maiti 2007). The NCBOE (1982) in their report also added that increased stoniness hinders farm machinery and there is also the issue of shallower depths of top soil and subsoil above the overburden.

Mine sites often leave a legacy of spoil heaps or spoil tips contaminated with metals (Dowing et al.,1998). Although the main objective of coal mining is the removal of coal there is always some portion that is left behind. A greater proportion of what is left behind could be locked up in mineral fragments which could become available due to processes such as weathering and acid mine drainage (Bradshaw and Chadwick, 1980). Metal pollution can be released into soils in large amounts via natural weathering processes of parent rock materials (Borovik, 1989). Sedimentary rocks such as clay and shale contain higher quantities of metal ions from absorption

(Brooks, 1987). These rocks are faster weathering and soils formed upon them may have naturally elevated levels of heavy metals. A great proportion of accessory metals are found to be associated with coal mining which were never meant to be extracted. Heavy metals in mine spoils can originate from different backgrounds. These can be a part of the initial minestone composition, native to the region, a result of past industrial activities and equipment used, smelting, due to transport vehicles, or addition of soil amending substances like fertilizers as a part of initial reclamation after mining (Haigh, 1995; Kabata-Pendias and Pendias, 1989; Lasat, 2002). These metals contaminate the soil and environment and enter the food chain. Elevated levels of these metals can be toxic and prevent plants colonising (Bradshaw and Chadwick, 1980; Maiti, 2007).

Establishing vegetation cover on mine spoils and maintaining it can be problematic. Problems include soil compaction, heavy metal contamination, low soil fertility, high acidity, salinity and hostile temperature regimes (Bending, 1999; Haigh, 2000; Lasat, 2002). These adverse physico-chemical properties tend to hinder soil forming processes and plant growth. Apart from the non-productive aspect of derelict land, there are other problems as well. These include acidity, high metal concentrations, and high content of stone with less fine soil for successful plant growth, lack of organic matter, low soil moisture holding capacity and unhelpful soil forming materials all of which inhibit the development of a healthy vegetation cover (Bradshaw and Chadwick, 1980; Maiti & Saxena, 1998; Maiti, 2007). These lands can also be dangerous; hazards include tip failure, crumbling of quarry faces or exposure of dangerous chemical contaminants (Moffat & McNeill, 1994). In any case, it can be very difficult to attain successful restoration on such nutrient-poor degraded soils and not all attempts made for the renewal of these degraded lands have been successful. Restoration aims to upgrade damaged land, or to re-create land that has been destroyed and to bring it back into beneficial use, in a form where the biological potential is restored (Bradshaw and Chadwick, 1980). Biological potential of the soil is the capacity of soil and the organisms within to carry out a range of processes to maintain the soil health and fertility. These soil organisms are responsible for the decomposition and recycling of soil organic matter and soil structure, prevention of diseases, enhancing nutrient availability and degrading pollutants (DAFF, 2011).

Forestry has been identified as a land-use offering many benefits besides timber production. These include the prevention of soil erosion, soil creation and enhancement of landscape and provision of important habitats for forest flora and fauna. The afforestation of degraded lands also increases carbon sequestration and hence somewhat reduces the effect of human accelerated global warming caused by burning of the coal that was excavated by the mine (Faeth, 1994; Forestry Commission, 1999).

2.5 Contaminated land

The situation where an area of land is affected with one or more contaminants is usually referred to as 'contaminated land' (Environment Agency, 2009). In the UK, a site may be termed 'contaminated' according to the new legislation under the Environmental Protection Act Part IIA (1990). The revised definition of contaminated land under Section 78A (2) is:

"any land that appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on or under the land, that (1) Significant harm is being caused or there is a significant possibility of such harm being caused; or (2) Pollution of controlled waters is being, or is likely to be caused" (DEFRA 2008; page 2)

Across the UK there are large areas that have been termed contaminated due to previous industrial processes or activities where waste products or remaining residues present a hazard to the general environment (Environment Agency, 2006). Where sites have been remediated this has mainly been through excavation and off-site disposal of material, which involves high costs. Over the years the Environment Agency, Department for Environment, Food and Rural Affairs (DEFRA), Inter Departmental Committee on Redevelopment of Contaminated Land (ICRCL) and various soil scientists (Davies & Roberts, 1978; Kelly, 1980; Bending, 1999) have published soil guideline values for uncontaminated soils, threshold or screening level soils and action or treatment level soil. Soils which belong to this action or treatment levels are classed as 'special soils or contaminated soils'. However, soils that belong to threshold or screening levels pose equal threat to the environment if left untreated. Natural process like weathering and oxidation, can cause chemical levels in these soils to go beyond the threshold limit and soon reach action levels. Table 2.4 outlines the soil guideline values published by various sources for the different metals under study in this research.

Table 2.4: Standard soil metal concentration published by various authors (mg/kg).

Standard Published Soil Values for UK	Mn	Cd	Cu	Pb	Zn
Normal soil*	<500	<1	<40	<200	<100
Contamination threshold*	500-1500	1-2	80	320	250
Action / Treatment levels^	>2000	>8-10	>300	>1000	>500

*Standard values from Davies & Roberts (1978); Kelly (1980); ICRCL (1987); Bending (1999); DEFRA (2004); Environment Agency (2009). ^ Action/Treatment Levels from Kelly (1980).

2.6 Heavy metals in the soil

The following section reviews the literature on the presence and distribution of heavy metals and soil, their effects on the soil and surrounding environment, methods of reclaiming these lands polluted with heavy metal with the tool of Phytoremediation. The role of plants in taking up these chemicals from the soil and also a detailed review of the literature on the tree species selected for this particular research.

"Being at the interface between the atmosphere and the earth's crust, as well as the substrate for natural and agricultural ecosystems, the soil is often open to imputs of heavy metals from many sources" (Alloway, 1995a pp.5). Traditionally heavy metals are normally defined as elements with metallic properties which have a specific density >5 g/cm^3 (Lasat, 2002; Jarup, 2003). There has been constant mention in literature about the potential hazards and the frequent occurrence of heavy metals in contaminated soils. These metals are namely Chromium (Cr), Zinc (Zn), Cadmium (Cd), Lead (Pb), Copper (Cu), Iron (Fe), Arsenic (As), Manganese (Mn) etc. (Roberts and Johnson, 1978; Alloway, 1995a; Lasat, 2002; Akoto et al., 2008; Suciu et al., 2008; Mmolawa et al., 2011). The conditions of the

soil into which heavy metals have been deposited will have a substantial effect on their behaviour, fate and ultimately interaction with plant systems. Physical and chemical soil factors such as pH, organic matter concentration, nutritional status and particle size distribution will affect the behaviour of metals present in the soil (Ernst, 2000). There have been reports of mobility of metals at lower pH values. Effective mobilisation of Cd, for example is found to occur at pH 6.0 – 5.5, whereas Zn, Ni and Cu are found to mobilise at 5.0-5.5 (Blake and Goulding, 2002; Schulte, 2004). Blake and Goulding (2002) also found the mobility of Cd to increase by 60% -90% at pH 4.0.

The nature and chemical form of metal contaminants, the presence and concentration of other elements and abiotic factors such as weathering of minerals and groundwater hydrology will also affect contaminant behaviour (Welch et al., 2000). Further biotic influences such as the interaction of plant roots, soil bacteria and fungi and soil fauna such as earthworms or other macro invertebrates can affect soil contaminant behaviour. It is the vast number of physical, chemical and biological interactions that make modelling and predicting the availability, fate and transport of these metals within the soil-plant system complex. Exposure to heavy metals, like Hu, Cd, As and Pb pose a major risk to human and plant health. International organisations like WHO have extensively studied some of these metals and their effects on the ecosystem (Jarup, 2003).

Soils may contain naturally-derived heavy metals, or elevated concentrations due to anthropogenic inputs. Heavy metals such as Pb, Zn, Cd, Cu, As, etc. are transmitted from the earth's crust into the soil, water and air by anthropogenic sources such as non-ferrous metal industries, mines or non-renewable energy utilisation (Menon et al., 2002; EU, 2003). Background levels of metals in soils in an undisturbed state and soils from urban environments have been well documented (Angelone and Bini, 1992; McGrath and Loveland, 1992; Dickinson, 2000; Dickinson et al., 2000; Kabata-Pendias, 2001). In soil, contamination caused by Ni, Cu and Zn caused by mine wastes and smelters are known to be phytotoxic to sensitive shrubs and plants (Chaney et al., 1997; Lasat, 2002). Bhuiyan et al. (2010) showed that heavy metal pollution from coal mines had an adverse effect on the agricultural soils in northern Bangladesh. Results from soil chemical analysis of the agricultural soil showed significant levels of Ti, Zn, Pb, Mn, As, Fe, Nb and Sr which indicated inputs from mining activities.

There have been reports of heavy metals causing serious risk to plant, human and animal health (Chernykh, 1991; Kabata Penias and Pendia, 1992; Alloway, 1995a; EPA, 1999; USDA, 2000). Elements like Cd easily accumulate in the crops and enter the food chain causing toxicity (Vazquez et al., 1992; Alloway 1995a, Lasat, 2002). Zn, an essential element, which otherwise has a lower toxicological risk to human and animals, had been found to readily disperse into the environment and accumulate in soil and eventually into the food chain causing metal contamination (Alloway, 1995a; Reichman, 2002; Smolders and Degryse, 2002). Similarly Cu and Mn which are considered to be essential to plant metabolism, when present in high concentrations of bioavailable forms have the potential of becoming phytotoxic (McLean and Bledsoe, 1992; Reichman, 2002).

According to the Alloway (1995a), EPA (1999) and USDA (2000) the most problematic positively charged metals in the soil, are Hg, Cd, Pb, Ni, Cu, Zn, Cr and Mn. As, Mo, B and Se, on the other hand are negatively charged elements in soil. Each of these metals is present in soil in different concentrations and poses toxicity above a certain level. With regards to toxicity due to the quantities released and distribution in the environment, Alloway (1995a) listed Cd, Cu, Zn, Pb and Ni to be the most significant of the metals. Table 2.5 outlines some of the toxic metals (mg/kg) generally related to coal and metal mining industry, their normal and critical levels in soil and the specific problems they pose.

Table 2.5: Environmental significance of heavy metals in soil.

Metals in Soil	Normal soil	Critical Levels	Associated problems	Source of pollution	Class
As	1.0 - 40	10	poisonous and carcinogenic	Metal working, coal smelting and combustion and manure application	1
Cd	0.01- 2.0	>3	Highly toxic to plants and animals	carboniferous black shale, coal mining, smelting of sulphide ores (Pb & Zn)	1
Cr	100	200	Toxic at high levels	through application of lime and fly ash, coal combustion and iron and steel industries	1
Cu	10-40	130	essential macronutrient however toxic at low pH levels	mining, fly-ash and fertilizer application, smelting,	4
Hg	0.01-0.5	1	Moderately poisonous, toxic to humans and animals	Mining and smelting ores (Cu & Zn), burning fossil fuels (coal), Methyl mercury occurs naturally in soil in association with fulvic acid	2
Mn	20-1000	>1500	Important plant nutrient, toxic at high levels	sewage sludge, coal spoils	5
Pb	10-100	320	Poisonous. Long term accumulation in food chain	generated by mining and smeltering, vehical exhausts	1
Zn	50-200	250	essential macronutrient however toxic at high levels	coal combustion, metal works, manure application, sewage sludge	4

Adapted from Kelly, 1980; Alloway, 1995a; Haigh, 1995 and Braithwaite, 1999

Table 2.6: Braithwaite (1999) classification of metals based on contamination level

Class 1	Contaminants should be considered in ever non-specific contaminated land investigation as their presence cannot be ruled out
Class 2	Significant contamination but less likely to be encountered
Class 3	rare contaminants, however industrial specific contamination may occur
Class 4	Contaminants are phytotoxic at higher levels
Class 5	Contaminants can be ignored unless present at critically high concentration

2.7 Metals of Interest

As this research looks at the contamination and remediation of degraded coal mine spoil, metals that are specifically related to and pose a threat due to coal mining industry have been considered. These have been listed in detail in the sequence of its classification based on the contaminant levels created by Braithwaite (1999) in order of their severity and toxicity in soil.

2.7.1 Cadmium (Cd) - Class 1 contaminant

Cadmium is a relatively rare metal found mainly in the earth's crust with no essential biological function, but when present in the soil is highly toxic to plants, humans and animals (Heinrichs et al., 1980; Alloway, 1995b; EPA, 1999; Milton et al., 2003). Cd is found to be closely associated with Zn in its geochemistry and mainly occurs in combination with Zn and Pb (Wan Ngah and Fatinathan, 2010). Other major sources of Cd contamination in soil are mining, smelting and ore-dressing (Alloway, 1995b). With the exception of a small number of carboniferous black shales, natural occurrence of Cd in the soil by mineral weathering processes is extremely low, typically less than 1 mg kg^{-1} (Alloway 1995b). Cadmium emissions are mainly affiliated with non-ferrous metallurgy and fuel ignition (Jarup, 2003). Anthropogenic sources consist of mining (as a by-product from Zn smelting) and waste disposal activities, either from products containing cadmium such as batteries, plastics and galvanized metals or aerial deposition from smelting, burning fossil fuels and tyre ware (DEFRA and Environment Agency 2002). The EU usage of Cd decreased exponentially during the 1990's, largely because of the gradual reduction in usage of Cd products other than Ni-Cd batteries and the application of EU environmental legislation (Directive 91/338/ECC; Jarup, 2003). Despite these reductions in EU, Cd consumption, production and emissions have continued to rise worldwide drastically during the 20th century (DEFRA, 2002, Jarup, 2003; DEFRA, 2008; Wan Ngah and Fatinathan, 2010). World production of Cd is estimated at around 14.000 tonnes per year, the main producing countries being Canada, along with the USA, Australia, Mexico, Japan and Peru (Biswas, 2010).

In the soil, Cd is normally found to be concentrated in the surface horizon (Alloway, 1995b), which may be because this is the zone which has the highest content of soil organic matter which retains this metal either as a result of addition of fertilizers or through biomass production. In the soil Cadmium also enter as waste streams from industrial activities. Cadmium waste is also found to enter into the atmosphere due to the combustion of (household) waste and burning of fossil fuels (DEFRA, 2008). Cadmium is most commonly found in the soil as free Cd^2 ions and is therefore one of the most mobile heavy metals, although it may be adsorbed onto clay or mineral surfaces and adsorption increases with soil pH (Douben, 1994). Cd is primarily zootoxic and represents a risk to human health via oral and inhalation pathways. The latter representing carcinogenic and genotoxic risk (Defra and Environment Agency 2002).

Cadmium is said to be strongly adsorbed by the organic matter in soil and can be extremely dangerous, as it is readily available for uptake by plants, hence the threat to enter into the food chain (Roberts and Johnson, 1978; Alloway, 1995a; Cooke and Johnson, 2002). One of the top factors regulating the availability of Cd in soil and their subsequent uptake in plants is pH and composition of soil solution (DEFRA, 2008). Cadmium uptake is inversely proportional to soil pH, in plants Cd can be amplified at lower pH levels (Jarup, 2003). The increase in Cd content in leaves of swiss chard as a result of lowering of soil pH were studied by Page et al. (1981). Wang et al. (2004) studied the effects of pH on the uptake of Cd and Zn in *Thlaspi caerulescens* and found that the soluble form of both Cd and Zn metals greatly increased with a reduction in soil pH, thus making them more available for plant uptake. This in turn is a potential danger to the animals that are dependent upon the plants for survival. Earthworms and the other essential soil organisms are extremely susceptive to cadmium poisoning. They can die at very low concentrations (Europa, 2011) and this can have adverse effects on the soil structure, especially for compact soils. Soil processes of microorganisms are also influenced under high concentrations of Cd and can act as a potential threat to the soil ecosystem. According to Braithwaite (1999) Cd can be accumulated in large proportions in plants without the plant showing any signs of stress, although Lamoreaux (1978) suggested that the *Plastochron Index*, a numerical index to identify the developmental age of a plant, would be able to indicate the inadequate growth and development of the plant.

The presence of other elements in soil also affects the uptake of Cd in plants. An excess of Cu, Ni, Se, Mn and P are found to reduce the uptake of Cd in plants (Lepp, 1981; Cheng, 2003). Although this is not the case for Zn. Zn is found to have a negative effect on Cd uptake when Cd is present in low concentrations in soil (Page et al.,1981), however in a contrast observation Smilde et al. (1992) observed synergistic effect where Zn uptake was increased with an increase in soil Cd. Pb on the other hand is found to have a positive effect on the uptake of Cd, mainly due to the preferred adsorption of Pb, hence leaving behind more Cd in soil ready for uptake (Alloway, 1995b)

Cadmium contents in mine spoils have been reported to be much higher than background levels (Hutton et al.,1978; Casado et al., 2008). Mines are said to be significant sources of Cd contamination especially from those which exploit Zn and Pb ores and coal. Zinc ore is said to contain the maximum amount of Cd, but of course the mining, topography and drainage system of the area affects the intensity of contamination (Bradshaw and Chadwick, 1980). This problem is however not restricted to active mines, many abandoned mines still act as reserves of Cadmium (Hutton et al.,1978). One of the main problems of mining is the waste tips and deposits which create hostile environments due to elevated exposure to metals due to weathering of rocks and acid mine drainage (AMD) (Nwachukwu and Pulford, 2008). There are reports of mine spoil heaps being discharged into the local rivers decades after mining practices (Hutton et al., 1978; Bending, 1994). Merrington and Alloway (1994) found high levels of up to 3.3 kg on wind-blown Cd deposits per year, in soil which were at a distance of 300m from a historic Zn and Pb mines in the UK. Extremely high concentrations of Cd have also been reported in soils which are as far as 40km from a metal mine in South Wales, UK (Alloway, 1995b).

Although Cd is present in low concentrations in soil, its highly available behaviour in soil especially at contaminated sites makes it a high risk metal for humans and the environment.

2.7.2 Lead (Pb) – Class 1 contaminant

Pb is a component of igneous rocks and is neither essential nor of any benefit to plants or animals, although it is known to be poisonous for mammals (Alloway, 1995a). Soil is known to act as a sink for anthropogenic Pb of which the primary sources are mining, smelting, sewage sludge and aerial deposition from vehicle exhausts (Alloway and Jackson, 1991; Alloway, 1995a; Davies, 1995). The amount of Pb in nature is limited. This metal is presently found in the soil in mixtures with copper, zinc and silver and it is derived in combination with these metals. The anthropogenic emissions of Pb around the 80's was found to be more than 20 times greater than natural emissions, this was much greater than any other trace metal (Nriagu and Pacyna, 1988). During the 20th Century emissions of Pb to the atmosphere have drastically polluted the environment and over 50% of Pb emission originated from petrol and coal (Jarup, 2003). As a consequence the amount of Pb deposited into the atmosphere is a great addition to the biogeochemical cycle, especially in urban areas, although this was substantially reduced with the phasing out of Pb in petroleum products (Watmough and Hutchinson, 2004).

After continual complaints of soil infertility from framers in Aberystwyth, Wales, UK, Griffith (1992) investigated and concluded that the main reason for soil infertility was due to the appreciable presence of Pb originating from the Pb mining industries of the 19^{th} century. Alloway and Davies (1995) continued the research for issues related to Pb toxicity, and found elevated levels of Pb near abandoned mines as compared to control sites. Similar results were found by Thornton (1989) and more recently by Lee et al. (2001). Thornton (1980) reported that nearly 4000 km^2 of land in the UK is contaminated with Pb due to past mining and smelting industries.

Different estimates of the normal concentration of Pb in soil have been recorded. In 1988, Nriagu and Pacyna, recorded a mean level 17 mg/kg in soil, whereas Ure and Berrow (1982) recorded it to be 29 mg/ kg. In more recent times in Davies (1995) recorded normal surface soil (0 – 15cm) level of 42 mg/ kg. Pb in soil occurs in Pb (II) and Pb (IV) oxidation states both of which are stable, and typically accumulates in the top of soil profiles (Davies 1995). However, most Pb concentrations that are found in the environment are a result of human activities. Smith et al. (2009) stated that elevated levels of Pb can affect the environment by, ingestion of Pb through grazing cattle on Pb contaminated open lands, thereby entering the food chain; seepage of Pb contaminated water into surface and ground water through mine workings and tailings; human health through exposure and consumption of water (Ghosh and Singh, 2005a); the natural ecosystem and cause agricultural hazards. Human occupational exposure to Pb is said to occur mainly in mines and smelters along with exposure from working at Pb welding industries and battery plants (Jarup, 2003). In nature Pb has no biological function and hence its elevated presence is toxic and carcinogenic and when discharged in to the soil or atmosphere it has an exceptionally long residence time (Braithwaite, 1999). Pb is found to majorly affect the soil functions near farmlands, local communities and highways. Humans, animals and soil organisms have suffered casualties' from poisoning of Pb, as it is free from microbial degradation and found to accumulate not only in individual organisms, but also into the food chains (Koeppa, 1981; Alloway, 1995a, Davies, 1995). Many believe that there is a positive relationship between the concentration of Pb in soil and in plants (Colbourn and Thornton, 1978; Davies, 1995; Ghosh and Singh, 2005a). Although only a small proportion of the soil Pb is actually available to for plant uptake. Pb is not readily lost through leaching in the soil and remains in an insoluble state in the surface layers (Davies et al., 1988). Because of its insoluble nature Pb is not readily taken up by plants and remains in a toxic state in the soil environment. In soils with a pH range of 5.5 - 7.5, Pb solubility is controlled by phosphate and carbonate precipitates thus very little is available to plants (Blaylock et al., 1997). Lagerwerff et al. (1993) studied the uptake of Pb on maize and found that plant Pb content reduced by 21% when the pH of soil was increased from 5.5 – 7.2.

Soil organic matter is also responsible for the bioavailability of Pb. According to Davies (1995) presence of organic matter strongly helps bind the Pb in the soil, whereas, Pichtel and Salt (1998) believed that high organic matter led to the formation of soluble organometallic complexes which increased the solubility of Pb. Slow oxidation of Pb sulphides during weathering, lead to the formation of carbonates and its incorporation into iron, clay and manganese oxides thereby reducing the mobility of Pb in water along with its bioavailability (Kabata – Pendias and Pendias, 1992; Ghosh and Singh, 2005b).

Pb is highly unavailable to plants however some species are known to take up some amount of available Pb from the soil (Dudka, 1992). Within the plant body the highest concentration of Pb in found in the roots. Root uptake of Pb in Norway spurce on Pb spiked soil was studied by Hovmand et al. (2009). Results showed 2% of Pb accumulation in roots and leaves whereas the remaining 98% of Pb accumulated was through atmospheric deposition. Results concluded that the uptake by roots, translocation and deposition by litter to the forest floor had no significant contribution as compared to atmospheric deposition of the metal. Pahlsson (1989) found that low concentrations of Pb in soil stimulated plant growth, despite of Pb being a non-essential element. He also stated that roots accumulated considerably high amounts of Pb, although almost none of it was translocated into the upper shoot. Similarly Vedagiri and Ehrenfeld (1991) tested the bioavailability of Pb and Zn in red maple and cranberry seedlings. They too found Pb to be highly unavailable for plant uptake. This immobility and atmospheric deposition of Pb in soil and environment causes metal toxicity which in turn affects the plants, humans and animals.

Metals can also influence the activity of microbes in the soil thereby reducing the productivity of soil (Alloway and Jackson, 1991; Alloway, 1995a). Pb in soil which has no biological role is found to be toxic to microorganisms. Liang and Tabatabai (1978) found Pb to inhibit N mineralisation rates in acidic soil in Sweden. Similarly Chang (1992) reported that N mineralisation and nitrification was inhibited by Pb toxicity. In a recent experiment, Sobolev and Begonia (2008) tested the effects of high and low Pb concentration on soil microbial communities. Results showed that even at the lowest concentrations Pb had detectable effects on the diversity of the microbial community.

"Pb is one out of four metals that have the most damaging effects on human health. It can enter the human body through uptake of food (65%), water (20%) and air (15%)" (Wan Ngah and Fatinathan, 2010, p. 963).

Much is known about human health effects of increased exposure to Pb which can result in reduced cognitive development in children and reduced sperm counts (DEFRA and Environment Agency 2002). Exposure to Pb is known to give rise to proximal renal tubular damage and kidney damage (WHO, 1981; Mortada et al., 2001).

Pb is widespread in the environment and enters into the soil from various mediums. Due to lack of solubility and mobility in soil it is unavailable for greater uptake making its residence time in soil so long that it can be regarded as a permanent element in the soil (Alloway, 1995a). It can easily enter into the food chain and cause potential risk to health. Due to its negative effect on microbial communities it prolongs the time period needed for soil stabilisation.

2.7.3 Copper (Cu) – Class 4 contaminant

Copper is one of the most essential and important element to plants and animals and naturally occurs in the environment (Alloway, 1995a). Cu has been used extensively by humans in a variety of industries and agricultural activities. The production of copper has lifted over the last decades and due to this the amount of copper in the environment has expanded (Braithwaite, 1999). Elevated Cu concentrations may be found in the soil surface as a result of smelter deposition or mining wastes (Alloway, 1995a). Cu smelters release large quantities of As and Zn and Zn smelters release high amounts of Zn, Cu, Cd and Pb, all of which pollute the soil and environment (Alloway, 1995a; Baker and Senft, 1995). These metals from mining enter the water streams though erosion and effluents, which leads to elevated levels of Cu toxicity in fish and other aquatic life forms as well as alluvial soils downstream, thereby entering and contaminating the food chain and environment (Baker and Senft, 1995). Cu enters the air through the combustion of fossil fuels and remains in the air for a significant amount of time and returns to the soil in the form of rain (Baker and Senft, 1995). In the UK, the annual deposition of Cu from dust is found between 100 g/ha to 480 g/ha (Alloway, 1995a; Bernard et al., 2009). On agricultural land Cu inputs may originate from fertilizers, pesticide use, the use of sewage sludge, or litters and manures from animals fed with Cu compounds (Alloway, 1995a). When copper enters into the soil it attaches itself to the organic matter and mineral and a result its mobility is reduced and it hardly ever enters groundwater (Bernard et al., 2009). As copper is released both naturally and through human activities such as mining it is widespread in the environment and can be harmful to soil, environment, animals and humans (Braithwaite, 1999). Copper is said to be easily accumulated in plants. The abundance of essential micronutrients in plants are generally ranked in the order of Fe>Mn>B>Zn>Cu>Mo>Cl (Baker and Senft, 1995). Cu was an important element of reference and was confirmed as an essential element in 1939 by Arnon and Stout. The accumulation of Cu differs in plant species. On copper-rich soils only a limited number of plants have a chance of survival, resulting in low plant diversity near copper-disposing factories (French, 2004). Cu strongly associates with organic matter and is the primary source of its retention within the soil, but may also complex with Fe and Mn oxides and silicate clays. As an essential plant micronutrient Cu is utilized as an activator and also in some enzyme systems, although absorption into plants is among the lowest of plant essential elements. Increased quantities of Zn^{2+} ions pose hostile conditions for Cu uptake as the mechanisms are thought to be similar (French, 2004).

Cu in excessive concentrations is known to be phytotoxic. Common visual symptoms like foliar chlorosis in *Casuarina distyla* (she-Oak) and *Eucalyptus eximia* (Yellow bloodwood) in elevated Cu toxicity have been reported (Taylor and Foy, 1985; Mitchell, 1988; Alloway, 1995a) Toxicity of Cu has serious effects on the growth of roots, even before any evidence of negative effect on the above ground tissues (Minnich et al., 1987; Reichman, 2002). Seeds of dicotyledonous plants under Cu toxicity showed short, dark coloured and blunt radicles with fungal depositions (Patterson and Olson, 1983). In another study by Arduini et al. (1996), seedlings of *Pinus* trees showed thickened root apicies. Similarly *Betula payyrifera* and *Lonicera tatarica* produced lesser number of root hairs and stunted roots under the influence of Cu toxicity (Patterson and Olson, 1983)

The main factor controlling Cu retention and mobility in the soil is the quantity of organic matter; Cu forms stable ligands with COO- groups in the solid and liquid phases of organic matter. This has been identified as the cause of Cu deficiencies, but also in the decrease of Cu toxicity in high organic matter soils (Baker and Senft, 1995). There is a debate on the effect of excess Cu on the increase or decrease of uptake of Zn in plants (Alloway, 1995a; Reichman, 2002). Reichman (2002) believes that if Cu in excess does have some negative effect on Zn uptake by plants then some of the Cu toxicity symptoms could be a result of deficiency of Zn.

Cu is used widely in multiple applications and is available in abundance in soil resulting in greater than normal levels in the soil (Braithwaite, 1999). Due to its fixed nature in soil it is one of the least mobile metals in soil. pH plays an important role in the availability of the metal and if not maintained at 6.0 – 7.0 range is likely to cause problems. Baker and Senft (1995) pointed out that the chemistry of Cu and Pb in soil is fairly similar, in that both are specifically fixed in the soil.

2.7.4 Zinc (Zn) – Class 4 contaminant

Zinc is found to occur naturally in the environment and is one of the essential elements for humans, higher plants and animals. Apart from its natural occurrence in air, soil and water, elevated levels of Zn are also attributed to human activities (Kiekens, 1995) such as coal mining, combustion of waste products and production and processing of steel and steel products (Alloway, 1995a; Braithwaite, 1999). Zn is also found to contaminate areas where there is an increased use of fertilizers produced by sewage sludge (Kabata-Pendias et al., 1992; Merten, 2006). The normal range of Zn in soil is 10 – 250 mg/kg with an average of 50 mg/kg (Kiekens, 1995; Braithwaite, 1999). The worldwide distribution of Zn is found in most of the developed nations like America, Australia, Russia, Canada and Peru and its production goes up by nearly 12 million tonnes/ year (Wan Ngah and Fatinathan, 2010). More than 30% of the world's need for zinc is met by recycling. Burning fossil fuels, metal smelting and sewage sludge are all anthropogenic sources of Zn (Wan Ngah and Fatinathan, 2010).

The world's zinc production is continuously rising, which means that more and more zinc ends up in the environment. Zn is present in the soil in large amounts and is extremely mobile and bioavailable in soil, existing predominantly as a divalent cation below pH 7.7 (Kiekens, 1995) and is adsorbed mainly onto organic matter and clay minerals. Factors affecting Zn availability and solubility in soil are mainly total content, pH, organic matter, microbial activity and factors such as moisture, climatic conditions and presence of other nutrients in soil (Lucas and Davis, 1991; Kiekens, 1995; Schulte, 2004). Of these, soil pH is one of the most important factors affecting its availability. pH is inversely proportional to the uptake of Zn therefore deficiency of Zn normally occurs where pH is greater than 6.5

(Schulte, 2004). Merten (2006) observed the solubility of Zn in degraded sediments started to increase for pH lower than 6.

Zinc commonly interacts with Phosphorus in the soil system, and high P may result in a decrease in Zn uptake and availability (Kiekens, 1995). Due to its high bioavailability plant tissue concentrations generally raise with zinc soil concentrations (Kiekens, 1995). On zinc-rich soils only a limited number of plants have a chance of survival (Reiman and de Caritat, 2000). This is one reason why plant diversities near zinc-disposing factories are found to be minimal. Like Cu Zn toxicity is primarily visible through leave chlorosis (Harmens et al., 1994; Fontes et al., 2000; Reichman, 2002). Ren et al., (1993) found plants under Zn toxicity exhibited smaller leaves as compared to control. He also found the roots under stress to exhibit reduction in growth of the parent root, short and fewer lateral roots and root hairs and in some cases discolouration of the root structure. Zinc has also been found to impede soil microbial activity as it reduces the breakdown of organic matter present in soil by earthworms and microorganisms (Harmens et al., 1994, Schulte, 2004).

The absorption and accumulation of Zn in plants mainly depends on its presence in the soil. An increase in soil metal concentrations causes an increase in the metal uptake by the plant (Kiekens, 1995; Mertens, 2006; Mertens et al., 2011) studies the growth and uptake of Zn and Cd in five tree species in a greenhouse experiment. Of the five trees studies an increase in Zn concentration in the soil, increased the concentration in the leaves of poplars and alder trees, although the concentration in alder was half the concentration in poplar trees.

Zinc is widely used and found in the environment in elevated concentrations especially near mining industries. Due to its abundance in soil it is readily available for plant uptake. Zn along with Cd is found to be highly mobile and bioavailable in the soil which easily accumulates in plants and humans. At very high concentrations this phytotoxic metal inhibits plant growth (Braithwaite, 1999) People who work in coal mines, refineries, nonferrous metal industries and even those who live near these sites are found to be exposed to high levels of Zn which is toxic and carcinogenic to human health (ATSDR, 2005).

2.7.5 Manganese (Mn) – Class 5 contaminant

Manganese is present in the earth's crust in large amounts and is widely distributed in the soil, rocks, sediments, water and biological materials (Smith and Paterson, 1995). The largest sources of Manganese via man-made activities are through production of steel, alloy and iron products (Howe et al., 2004). Manganese is released into the atmosphere from industrial emissions, coal and fossil fuel combustion and erosion of soils containing Mn (ATSDR, 2008). It is also widely spread through mining activities and manufacturing of pesticides and fertilisers. Mn is also found to be widely distributed in the air and this can cause harmful effects on plants and animals (ATSDR, 2008).

Manganese was identified as a hazardous waste in nearly 869 sites out of a total of 1699 sites that have been recommended for inclusion on the EPA National Priorities list (NPL) (HazDat, 2008; ATSDR, 2008) and comprises of about 0.1% of the earth's crust. Normal soil levels range from

40-900mg/kg (Schroeder, 1987; Rope et al., 1988; Eckel & Langley, 1988; Barceloux, 1999; EPA, 1999; ATSDR, 2008) and the maximum value of Mn documented is 7000 mg/kg (Eckel & Langley, 1988). Accumulation of Mn typically occurs in the sub-soil layer (WHO, 1981). In many soils Mn may be oxidised by bacteria making it more available to plants (Johnston & Kipphut, 1988; Alloway, 1995a; ATSDR, 2008).

The main scientific interest in the abundance of the element in soil is due to its role in plant and animal systems. The most common biological toxicity effect is generally observed in plants that are exposed to excess amounts of natural Mn in soil and this effect is visibly different from other plants (Smith and Paterson, 1995; Kasraei et al., 1996). On leaves the first symptoms of Mn contamination occurs in the form of necrotic brown spots and these are also visible on stems and branches and another condition known as 'crinkle leaf' effect which affects the young leaves, branches and stem and causes chlorosis (Bachman and Miller, 1995; Wu et al., 1999; Reichman, 2002). Roots that are affected due to Mn toxicity turn brown in colour and under extreme toxicity these roots have been found to crack (Le Bot et al., 1995; Foy et al., 1995). Essential plant processes like photosynthesis, respiration, nucleotide synthesis are greatly affected by Mn contamination in soil and plant (Davey, 1999; Schulte and Kelling, 2004).

In trees the rate of Mn accumulation is greatly dependent on the tree species. Davey (1999) noted that two trees of different varieties on a Mn contaminated soil varied 10 fold in the amount of Mn accumulated. Mn in acidic soil is greatly affected by soil pH and soil redox reaction (Sparks, 2003). The availability of Mn and hence the subsequent toxicity is reduced when soil pH is raised to 6.0 (Slattery and Ronnfeldt, 1992, Smith and Paterson, 1995). In other words Mn toxicity is common in acidic soils with a pH below 5.5 (Schulte and Kelling, 2004).

Because of its mobility and bioavailability, Mn is readily taken up by plants. The polar concentrations of Al, Mg, Si, P, Ca, Fe and Mn metals in *Carya galbra* (Pignut Hickory) leaves was studied by Brownridge and Shafroth (2003). Results showed that at high concentrations in soil, highest amounts of Al and Mn were accumulated in the leaves. On the other hand in a study by Machova et al. (2007) hybrid *Populus tremula* (Aspen) and *Sorbus aucuparia L.* (Rowan) trees were used to study the uptake of Cd, Pb and Mn. Results showed that the uptake of Cd and Pb in roots of both species was much higher than the shoot. Whereas, the amount of Mn accumulation was much lower than that for Cd and Pb in both the trees. This difference in uptake could be due to difference in plant species. Another reason is also that some elements like Ca, Fe, Mn, B and Cu, having entered the plant, fix their position and become immobile (Davey, 1999).

Manganese is found to cause manganese poisoning in mammals which results in neurological damage (Alloway, 1995a). Exposure to high levels of Mn can lead to a range of motor and psychiatric disorders (Uren, 1991). Chronic Mn poisoning is also reported as a hazard from mining and welding industries (WHO, 1981). In areas of manganese processing plants Mn was reported to be present in the air as Mn Oxides which causes respiratory disorders and these oxides are also added to the soil with rain water (WHO, 1981).

Study of the various metals has shown that different plants accumulate different amounts of metals under different soil conditions. Hence it is essential to select plants which are able to survive on such coal contaminated and degraded soils. The following section looks at some of the best available techniques in reclaiming such soils.

2.8 Soil properties affecting plant growth on coal spoils

Soils, according to Brevik (2011), are ecosystems that are dynamic and support a range of life forms, making them appropriate to be thought about in terms of vitality, health and biological productivity. In order to facilitate healthy plant growth it is imperative for the soil beneath to be healthy. Plants have an important role in the conservation of the soil surfaces from processes like accumulation of fine particles and erosion (Conesa et al., 2007; Lasat 2011). These plants can reverse the process of degradation by stabilizing the land through the growth and development of a healthy root system, which in turn helps increase soils organic matter, lowers bulk density, stabilizes pH and enables the plant to take up the nutrients and water from the deep soil layers (Lasat, 2011). Brevik (2011) pointed out that

"the challenge is to manage soils such that they are able to perform the various uses they are put to without degradation of the soils and the environment" (Brevik, 2011, pg.2).

For successful establishment of plant growth, ideal physical, chemical and biological properties of the soil are essential. Some of these include pH, fertility, soil texture, bulk density, soil moisture and microbial content. Vegetation attains proper growth under sufficient soil fertility and a neutral pH. Mine spoil pH usually ranges from 5.5 to 7.5 which are ideal for plant growth (Gould, et al., 1996; Sheoran, et al., 2010). At a pH below 5 the bioavailability of toxic metals such as lead, nickel and cadmium increases (Maiti, 2007). Furthermore, in mine spoils major micronutrients such as nitrogen, phosphorus and potassium are found to be deficient (Coppin and Bradshaw, 1982; Moffat and McNeil, 1994; Sheoran et al., 2009). These can be compensated for by additional natural fertilizers or by planting trees with nitrogen fixation properties. Organic matter is another important source of soil nutrients. Organic carbon greater than 0.75% usually indicates good fertility of the soil (Ghosh et al., 1983; Sheoran et al., 2010). Fe, Cu, Mn and Zn are said to be important nutrients for plant growth, however, in mine spoils and acidic soils these metals are found to dissolve readily to form toxic concentrations that inhibit plant growth (Donahue et al., 1990; Barcelo and Poshenrieder, 2003; Maiti and Ghose, 2005; Das and Maiti, 2006; Sheoran, 2010). During their work on the restoration of coal overburden in India, Maiti & Ghose (2005) claimed that it was imperative to increase soil pH and organic matter for sustainable reclamation of coal spoils. Some fast growing, drought resistant trees are found to be able to establish successfully in acidic soils with nutrient deficiency (Lasat, 2002).

Physical parameters such as soil texture, moisture content, bulk density, rooting depth and compaction are also essential parameters to consider before establishing plant cover on coal spoils. Soil textures are determined by relative amounts of sand, silt and clay particles. Mine soils, which are normally sandy textures, are unable to hold as much water and nutrients as loamy or clay soils (Lasat, 2002). A sand content of 66% and

8.6% of clay content has been reported by Ghose (2005) in mined soils. Normal productive soil has a bulk density ranging from 1.1 to 1.5 g/cm^3. Rooting depths are limited in mine spoils due to higher bulk density. Ballard (2000) noted that some forest soils are unable to assist rooting with a bulk density above 1.2 g/cm^3. Moffat and Bending (1992) suggested that for forestation of disturbed mine spoils in Britain the soil bulk density should be <1.5 gm/cm^3 for a depth of 50cm. Severely compacted mine spoils (with a bulk density more than 1.7g/cm^3), which have a rooting depth of less than 60cm, shallow compact bedrock and large boulders in the upper layers of the soil profile cannot sustain healthy plant life (Lasat, 2002). Barnhisel (1988) coined the term critical soil bulk density as the density at which root growth in soil ceases. The compaction is increased due to repeated movement of heavy mining machineries like haulers, bulldozers loaders forming compacted zones on the mine spoils. It has been suggested that the soil bulk density parameter be included as a crucial indicator while defining successfully reclaimed land (Haigh, 1995).

Mine spoil slopes generally greater than 15% may not be suitable for crop or vegetation production, but ideal for forestry (Lasat, 2011; Sheoran et al., 2010) as establishment of trees would stabilise the soil and also prevent erosion. Flat benches have problems like water logging and due to compact soils the water is unable to penetrate into deep soil layers.

Soil microbes and bacteria are important components for successful soil conservation and plant establishment on these mine spoils. Soil microbes play an important role in the stabilization of the aggregate, which is essential for conserving structural conditions for cultivation and porosity for crop growth (Ghose, 2005). It is essential to consider all of the characteristics outlined above before planning the reclamation of mine spoils.

2.9 Phytoremediation for successful reclamation

Plants can be used in several ways to clean contaminated soil, water and air. The technology of phytoremediation is one that uses either natural or genetically engineered plants to remediate polluted air, soil and water. Phytoremediation technologies currently fall into the following six categories.

Phytoextraction: This is a process in which the metals are taken up from soil and ground water by plant roots and translocated to the different above ground tissues like stem, branches, leaves, flowers and fruits (Henry, 2000). Contaminants are generally removed from the site by harvesting these plants and careful disposal (Lasat, 2000). Different plants have different abilities to uptake metal contaminants and tolerate different levels of pollutants (Schnoor, 1997). Phytoextraction is best done with the help of hyperaccumulator plants. Hyperaccumulators are plants capable of accumulating 100 times more metal than a common non-accumulating plant (Henry, 2000). This is of great importance at sites that are heavily contaminated with one or more types of contaminants.

Rhizofiltration: This technology is very similar to Phytoextraction but is involved with the clean-up of contaminated *surface and ground water* with lower contaminant concentrations rather than soil (Henry, 2000). Here the plants roots are used to absorb, concentrate and translocate metal contaminants from groundwater.

Phytostabilization: This technology refers to immobilisation of contaminants in soil and water. Through this technology the contaminated soil is held in place with vegetation, thereby immobilizing the soil contaminants within (Vangrons-Veld et al., 2009) Phytostabilization prevents or reduces the mobility or even migration of contaminants into the air or groundwater and reduces the bioavailability of the contaminants which would prevent the spread of these contaminants into the food chain (Henry, 2000; Schnoor, 1997).

Phytotransformation (Phytodegradation): This technology of Phytoremediation refers to uptake of different types of *organic* contaminants from the soil and ground water and its subsequent metamorphosis by the plants (Schnoor, 1997). With this technology the contaminant is removed by plant enzymes where the aromatic rings of the contaminants are degraded by these enzymes (Dec and Bollag, 1994; Susarla et al., 2002). These contaminants are directly taken up from the soil and groundwater and the metabolites are accumulated in the different plant tissues (Burken and Schnoor, 1999). The direct uptake of the chemicals in the different parts of the plant thorough the root system depends on the efficiency of uptake, rate of transpiration and the concentration of chemicals in the soil water (Burken & Schnoor 1997, Schnoor 1997).

Phytovolatization: Phytovolatization involves uptake of water soluble contaminants by plants which are then released into the air as the plants transpire (USEPA, 2006). These contaminants are modified along the path from the time they are absorbed by the roots, as the water travels from the plants vascular system from roots to leaves, where the contaminants finally evaporate (Henry, 2000).

Rhizodegradation: In the soil are present soil-dwelling microbes which breakdown the organic contaminants present in the soil. This process of contaminant degradation in the Rhizosphere is known as Rhizodegradation (EPA, 2000). Certain organic pollutants such as solvents and fuels are digested by the microbes dwelling in the soil, and broken down into harmless products through a process known as *Bioremediation*. Plant root exudates such as organic acids act as carbohydrate facilitators for the growth of these microbes (EPA, 2000). Table 2.7 shows the various phytoremediation applications used at different sites for remediating contaminated soil.

Table 2.7 Phytoremediation Technologies and its application (adapted from Schnoor 1997 and French 2004).

Application	Site	Contaminants
Phytoextraction	Soil, mine wastes, Brownfield's, sediments	Metals (Zn, Cd, Pb, As, Cu, Se, U)
Phytotransformation	Soil, Groundwater, Wastewater, Agricultural runoff	TCE, PCA (1,1,2,2-tetrachloroethane),Herbicides, Nutrients (NO_3 NH_4), TNT, RDX, Atrazine, Nitrates
Phytovolatilization	Refinery Wastes, Agricultural soils	Se
Phytostabilization	Smelter, barren land, mine wastes	Metals (As, Cd, Pb, Zn, Cu, Cr, U)
Rhizofiltration	Groundwater, Energy wastes, Wastewater, Landfill leachate	Nitrates, Radionuclides Metals (U, Zn, Ni) ^{137}Cs, ^{90}Sr
Rhizosphere Bioremediation	Soil, Wastewater, Sediment, Agricultural waste land	Organic Contaminants (PAH's, Herbicides, Pesticides)

Just like any other technology, Phytoremediation has advantages and disadvantages and it is very important to take all of these into consideration when selecting a plant variety for a particular site.

Advantages of Phytoremediation:

Phytoremediation is a cost effective and economic technology. It has a wide range of aesthetic advantages and long-term applicability (Baker et al., 1998; Blaylock et al., 1997). This technique uses specially selected metal-accumulating plants to remediate soil contaminated with metals and other substances (Blaylock, 2000). Because of its *in situ* nature of treatment it can be applied to large field sites where other technologies can prove to be costly and also no disposal sites are needed. This technology is applicable to extensive areas where there is limited contamination. Phytoremediation has a capability to address multiple objectives, including erosion control, ecological restoration, site maintenance and secondary production of biomass, energy and industrial raw materials. It has a great attractiveness as a green technology. This technique is less disruptive to the environment and provides great potential to treat sites polluted with more than one type of pollutant. It avoids excavation and transport of polluted media, thus reducing the risk of spreading the contamination.

Disadvantages of Phytoremediation:

This technology does have some limitations and it is important to consider these before applying this technology to a site for remediation. Some of these include phytotoxicity to many of the contaminants on site, *in situ* application makes it relatively slow for many applications and hence it becomes difficult to predict completion times. Contaminants present below the rooting depth cannot be extracted and the plant or tree may not be able to grow in the soil at every contaminated site due to toxicity (Suthersan, 1997). There is the possibility of uptake of contaminants into leaves and release and deposition on ground during litter fall. This technology also shows an inability to assure clean-up below action levels in a short period of time. At sites with heavy toxicity it becomes difficult to establish good vegetation cover. Also some regulatory policies do not support the use of phytoremediation technology due to factors like plant species, use of amendment, time taken for remediation, etc. (Miller 1996, Schnoor 1997, Glass 2000, Marmiroli and McCutcheon 2003).

For successful reclamation of land, it is important to know the main cause of contamination and attempts should be made to eliminate them.

"Remediation technologies have been constantly focussing on ways in which contaminants can be destroyed, or removed sensitively with low risk of secondary environmental problems, taking a holistic and life cycle analysis approach" (Lynch and Moffat, 2005, p2).

Different methods and techniques are devised for effective restoration/remediation of degraded lands and one such effective method is 'Phytoremediation'. This technique uses specially selected metal-accumulating plants to remediate soil contaminated with metals and other substances (Blaylock, 2000). The concept of phytoremediation was first applied in the 18th century followed by more exhaustive research in the 19th and 20th century and continues to be a popular choice to commercial remediation (Smith and Bradshaw, 1972; Bradshaw and Chadwick, 1980; Dushenkov et al., 1995; Kumar et al., 1995; Chaney et al., 1997; Burken and Schnoor, 1997 & 1999; Raskin and Ensley, 2000; Terry and Banuelos, 2000; Pulford and Dickinson, 2003; Vangronsveld et al., 2009). Phytoremediation of metals that are usually perennial in the environment is said to be a low cost and environmentally friendly substitute to chemical methods and therefore has attracted growing interest in the last 2 decades (Baker et al., 1998; Blaylock et al., 1997 & Wong, 2003). The development of phytoremediation is primarily driven by the high costs involved in many of the other remediation technologies, along with a desire to use a 'green', sustainable process (Pulford and Watson, 2003).

Initially there was a great interest on hyperaccumulator plants which are known to be capable of taking up potentially phytotoxic elements to very high concentrations as compared to non-hyperaccumulators (Chaney et al., 1997; Salt et al., 1998; Raskin and Ensley 2000; Pulford and Watson, 2003). These plants are said to accumulate heavy amounts of metals, 100 times more than non-hyperaccumulators, in their shoots as compared to their roots (Chaney et. al., 1997; Pulford and Watson, 2003). According to research by Baker et. al (1998) and Brown et al., (1994) the species of *Thlaspi* (Brassicaceae) is found to accumulate more than 0.5% Pb, 0.1% Cd and 3% Zn in its shoots. Also according to a research by Brooks et al. (1979) some species of *Thlaspi* are found to accumulate over 1% of Ni. Even though this plant can hold heavy amounts of metals, its application and use in the field is considerably low as the plant is very small and takes a long time to grow (Ebbs and Kochian, 1997). Planting hyperaccumulators may also result in producing biomass which can be hazardous to the soil (Banuelos and Ajwa, 1999). It would be ideal to plant species which have a good capacity of taking up the chemicals from the soil and produce biomass. Ideally for any particular soil and site conditions, some species would be better suited than others and it is essential to select species which are native to the soil and environment as this makes it easier for the tree to acclimatise to the environment (Moffat, 2006)

However, phytoremediation does have some limitations, as previously explained, for example contaminants present below the rooting depth cannot be extracted and the plant or tree may not be able to grow in the soil at every contaminated site due to toxicity (Suthersan, 1997). Hence, plants which have a good rooting system and are appropriate for the different contaminants present in the soil must be used (Pulford and Watson, 2003). Additional factors such as soil acidity, nutrient deficiency, poor physical and soil structure, heavy metal contamination, and their subsequent interactions restrict successful plant growth and establishment (Pichtel and Salt, 1998). In addition, the remediation process can take a very long time for contaminant concentrations to reach regulatory levels and thus require a long-term commitment to maintain the system (Suthersan, 1997).

Baker et al. (2000) proposed phytoremediation as an environmental cleanup technology for the remediation of metal-contaminated soil. The identification of metal accumulators demonstrates that some plants have the genetic potential to clean up contaminated soil (Lasat, 2002). The idea is that, plants capable of accumulating high concentrations of different metals, are grown on metal contaminated sites. These plants extract metals from the soil and store them in the above ground tissues like the stem, leaves and fruits where these metals are biodegrade and bio-transform into inert forms in their tissues (Sinha et al., 2004). Some metals, like Cu, Zn, Mn, Fe, are nutrients to the plants. However, when such metals are present in excess they are harmful, both for the development of soil in terms of soil fertility and also for healthy plant growth. The choice of plants, in this case tree species, is very important in the reclamation of sites affected by mining activities (Moffat and McNeil, 1994; Moffat, 1995; Moffat et al., 2008). It is important to identify species that are suited to planting conditions. Many plant species can take up metals and eventually accumulate them, but different species have different capacities to take up different metals so it is necessary to examine the potential of different species to accumulate metals and evaluate their capabilities to sequester usually suitable amounts of key metals at different stages in their life cycles. Site conditions that influence the choice of tree species include availability of topsoil, soil chemistry, soil wetness, pollution level, local climatic conditions and exposure (Moffat & McNeill, 1994). The selection criteria must include selecting drought-resistant fast growing tree species which can take up the specific metals and which can grow in compact, acidic, nutrient lacking and metal contaminated mine spoil.

2.10 Benefit of Planting Trees

Planting trees has been suggested as an appropriate option for remediation of contaminated and derelict/degraded lands and for recycling land for forestry by various researchers (Moffat, 1992; Dobson and Moffat, 1993; Bending, 1994; Forestry Commission, 1994; Hodge, 1995; Arduini et al., 1996; Kozlov et al, 1996; Moffat, 1997; Pisano and Rockwood, 1997; Kozlov et al., 2000; Prasad and Freitas, 2003; Pulford and Watson, 2003; Rosselli and Bashi, 2003; Brunner et al., 2008). This is because trees provide a number of benefits. Many tree species can take up metals and eventually accumulate them, but different species have different accumulation potentials and different tolerance levels to the different metals. A study of trees with phytoremediation properties suggested that these trees could break down metals into simpler forms at the root level and take up large amounts of these metals into their above ground tissues and help in cleaning up contaminated soils (Brooks et al., 1979; Chaney et al., 1997; Brooks, 1998). Another benefit of trees is their genetic variability factor. Many fast-growing trees are genetically diverse such as *Salix* and *Populus* species; hence there is the opportunity to select particular genotypes with traits for high or low metal uptake or for resistance to higher metal concentrations (Pulford & Dickinson, 2003). French et al. (2006) reported the economic yield of *Salix* and *Populus* to be viable and the potential of these short-rotation coppices in community forestry. Similar results were also found by Rockwood et al. (2001), while testing the potential of short-rotation woody trees like *Populus* and *Salix* for phytoremediation and agroforestry. The lack of reported toxicity symptoms in trees indicates the high tolerance mechanism of trees might permit them to cope with high levels of metal concentrations then regular agricultural crops (Riddell-Black, 1993; Pulford and Watson, 2003). Poplars have been very successful in remediation and have been used in both *in vivo* and *in vitro* trials for metal tolerance (Dix et al., 1997; Schnoor et al., 1995; Schnoor, 1997; Pulford and Watson, 2003; Laureysens et al., 2004; Lingua et al., 2005; Franchin et al., 2007). Similarly Willows have been very successful in remediation of land contaminated with a variety of metals and many studies are now being focused on the ability of willows and poplars in phytoextraction (Labrecque et al., 1995; Erriksson and Ledin, 1999; Klang-Westin and Perrtu, 2002; Lasat, 2002; Robinson et al., 2005; Jensen et al., 2009; Capuana, 2011; Evangelou et al., 2013). However it is essential to select trees that are native to the area; require low maintenance and which provide a long-term benefit. The expectation of a reclamation programme should not be to immediately revert land to its original natural ecosystem (Moffat, 2006). This is a long process and requires many years. Reclaiming coal spoils puts forth various challenges, some of which are, soil compaction, poor soil quality, low fertility, loss of top organic layer, contaminated soil with hot spots, water logging and summer drought conditions etc. These conditions, when considered, demand a need to plant trees which are *most suited* to tackle these issues. This does not necessarily mean planting native species (Moffat, 2006) however, some native species might be capable of providing a solution. Research has shown that 'pioneer species' like poplars and birches perform well on reclaimed land (Good et al., 1985; French, 2004; Jensen et al., 2009). A study by Migeon et al. (2009) tested the ability of 25 woody trees to accumulate metals at a Zn, Cd and Pb contaminated site in France. Results showed maximum metal accumulation in *Salicaceae* family trees. Birch and Larch showed maximum concentrations of Mn in leaves as compared to any other trees. Alders too have been successfully tested for its role in metal accumulation and remediation (Pulford et al., 2001; Mertens et al., 2004; French et al., 2006) would be a suitable plant due to its N-fixation which adjoining trees can benefit from (Densmore, 2005, Moffat, 2006).

Tree planting offers many benefits today besides just timber production. These include the prevention of soil erosion, soil creation and enhancement of landscape and provision of important habitats for forest flora and fauna (Forestry Commission, 1994). The roots help to stabilise the soil and substrate (Dawson et al., 1984). Leaf litter also contributes to a considerable amount of organic matter to the surface of the soil. Planting trees on contaminated and degraded lands has a greater public acceptance and is well accommodated into the rural and urban landscape (Forestry Commission, 1994; Moffat, 2006). Urban regeneration in the UK and elsewhere in the EU is transforming post-industrial and derelict landscapes using multipurpose forestry to increase lowland forest cover in these urban areas (Pulford and Dickinson, 2003). In South Wales, UK large areas of land previously used for opencast mining have been restored to forestry and a large proportion of these trees are conifer species (Broad, 1979; Scullion, 2006). Research has shown that trees can survive and grow in harsh contaminated soils with remarkably high levels of a variety of metals (Kahle, 1993; Watmough and Dickinson, 1995; Glimmerveen, 1996; Dickinson et al., 2000; Pulford and Watson, 2003). Trees have also shown to the ability to survive and grow and have shown to have large capacity for phenotypic adjustment to metal stress (Watmough and Dickinson, 1995; Dikinson et al., 2000). Seedlings have shown to be more sensitive to metal contamination as oppose to saplings or young trees (Patterson and Olson, 1983; Lehn and Bopp, 1987).

Regardless of the capability of the trees to survive in harsh chemical conditions there has been evidence that in some specific soil conditions even the toughest of trees find it difficult to grow up to the standards of commercial forestry. The best evidence is from conditions where trees have been planted on reclaimed mine sites (Borgegard and Hakan, 1989). Soil compaction, waterlogging and elevated amounts of metals in these mines make it difficult for trees to survive.

2.11 Uptake and translocation of metals in plants

Plants need to take up water and essential nutrients for their growth and development for which it needs to not only take up macronutrients (N, P, K, Ca, Mg) but also micronutrients (Fe, Zn, Mo, Cu, Ni and Mn). Plants have developed specific mechanisms for the uptake, translocation and storage of these nutrients (Lasat, 2011). This section examines the soil-plant interactions and the different factors determining the translocation and accumulation of these metals in plants. The movement of metals from soil to plants usually follows 5 principle steps or pathways which include i) Adsorption or Dissolution, ii) Diffusion, iii) Sorption, iv) Absorption by roots, v) Translocation in plants. These pathways include passive and active uptake through the root system, direct contact between soil and plant tissue and gaseous and particulate deposition (EA, 2006). The plant root area is the most essential and desired site for chemical uptake (Bell, 1992). Plant roots are capable of taking up water, nutrients and chemicals from water and soil mediums. This uptake is initiated in the crucial region of the Rhizoshpere. Plant roots with the help of chelating agents produced by the plant and plant induced pH changes are able to solubilize and take up the micronutrients from minute levels within the soil (Tangahu, et al., 2011). Plants have also developed specific mechanisms to take up macronutrients and it is these mechanisms that are responsible for the uptake, translocation and storage of chemicals.

2.11.1 Rhizosphere

Rhizosphere is the region of soil about 1mm wide, in direct influence of the root, where the soil biology and chemistry are influenced by the roots (Abbott and Murphy, 2003a). The rhizosphere is known as the zone of the highest interrelationship between plants and microorganisms and the zone that has the highest macrobiotic activities (Grayston et al., 1997).

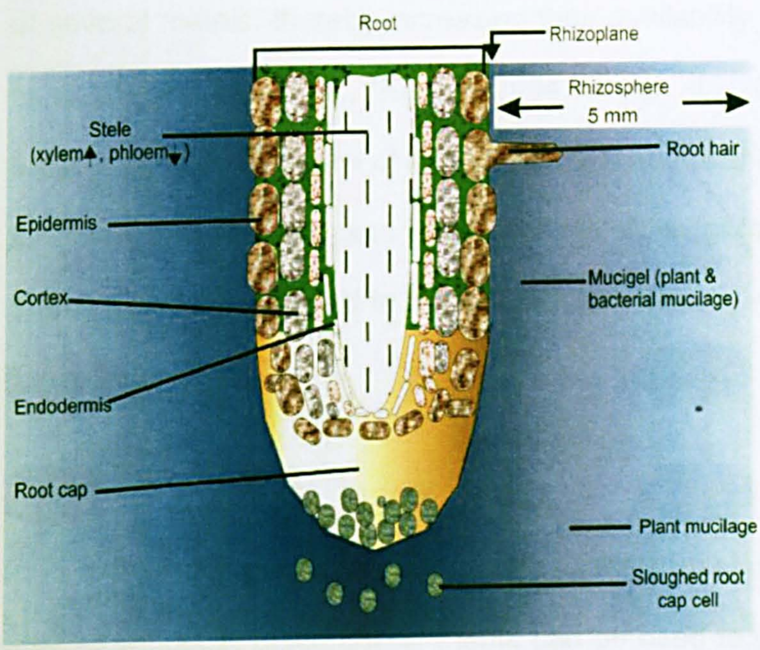


Figure 2.2: Vertical-section of the root showing the Rhizosphere region. (Maier, et. al. 2009)

The rhizosphere region also plays an important role in the bioavailability of metals and nutrients, not only to the plant, but to bacteria in and around the region and the mychorrhizal fungi (McNear Jr., 2013). The roots secrete water and compounds broadly known as exudates, which include organic and amino acids, carbohydrates, vitamins, mucligae and proteins (Abbott and Murphy, 2003b), which have a direct or indirect role on the nutrient availability for plant growth. These exudates provide maintenance and lubrication of root tips, protection from desiccation, adsorption and storage of ions and stabilization of soil micro-aggregates (Griffin et at., 1976; Bengough, 1997; Hawes et al., 2000; Walker et al., 2003). Root exudates also provide nutrition to the microbes in the rhizosphere, thereby increasing the microbiological activity in the area which exhilarates plant growth (Marschner and Baumann, 2003). Researchers have shown the rhizosphere to have the capability to increase the solubility of several metals, thereby increasing their availability to the plant (Buckland et al., 1995; Tao et al., 2003 & 2004; Su et al., 2004). Factors like pH, organic carbon, presence of and amount of cations and anions are all said to influence the solubility and uptake of metals in plant roots (Lasat, 2011). These metals are diluted in the soil solution thereby making them available for uptake.

2.11.2 Uptake by roots

The study of the uptake of contaminants by plants has gained a lot of interest amongst researchers. Plants can be used to optimize the factors to improve the uptake capacity of plants (Tangahu et al., 2011). Sinha et al., (2004), believe plants to act as excluders and accumulators. The accumulator plants are good at storing contaminants in their aerial parts where they bio-transform or biodegrade these contaminants in inert form in their tissues, whereas the excluders are known to restrict these contaminants uptake into their biomass (Tangahu et al., 2011). Chemicals enter the roots through the rhizosphere for soil water solution or directly from water in aquatic plants. The transport of water and nutrients from the root within the plant body is known as translocation (Bell, 1992; EA, 2010). The majority of the transport within the plant is through the bulk flow of water through xylem resulting from a pressure gradient, caused by evapotranspiration of water vapour from the foliage to the atmosphere (Paterson et al., 1990; Bell, 1992). In soil, contaminants are broken down into simpler forms by microbial activity which is amplified by the root zone, this process is known as rhizodegradation (Tangahu et al., 2011). For chemicals absorbed into the root to reach the xylem they must diffuse through various plant tissues like the epidermis, cortex, endodermis and pericycle. The movement of the chemicals through the first endodermal layer is dependent on the chemical aversion. These chemicals may bind or get metabolised in the endodermal layer before entering into the xylem (Paterson et al., 1990). Once into the xylem the chemicals are partitioned into the different plant tissues and may react or get degraded before reaching the storage cells and some may be preferentially bound to root or shoot membranes (Paterson et al., 1990; Tangahu et al., 2011). Figure 2.3 shows the root structure with the different tissue layers involved in the uptake of chemicals.

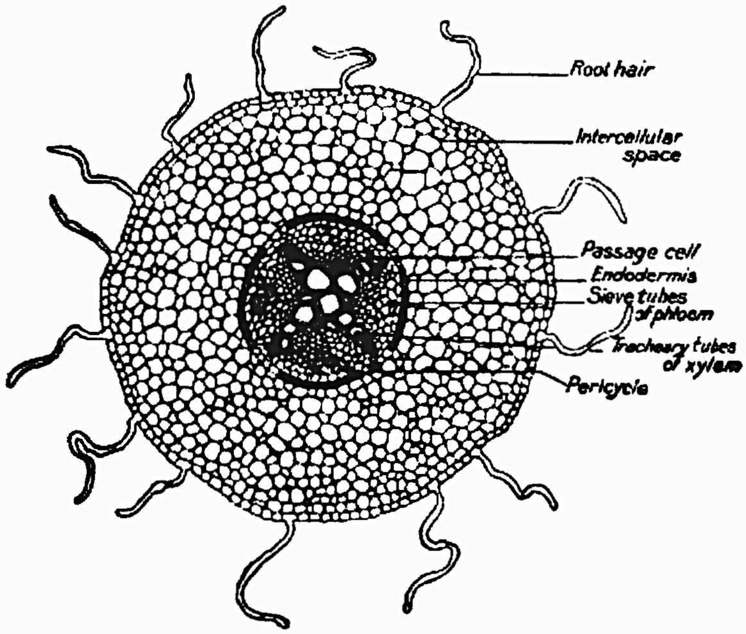


Figure 2.3: Root structure with the different tissue layers. (Weaver, 1982)

Once inside the plant cells, metals not essential for growth, or essential metals which are absorbed in quantities greater than required for growth, can have adverse effects on the plants. These metals can inhibit growth and reproduction of the plants in many ways. Heavy metals can hugely disrupt many of the physiological processes due to their ability to bind strongly with enzymes and substitute essential metals (Van Assche and Clijster, 1990; French, 2004).

Plants require mineral nutrition for optimum growth and metabolic activity. These can be grouped in essential macronutrients, essential micronutrients and those having only beneficial or restricted use (Alloway, 1995a). All plants require essential macronutrients; N, P, K, S. Ca and Mg in large quantities, typically 1000 mg/kg or more dry weight (Alloway, 1995a). Essential micronutrients are limited to just 10 trace metals; Fe, Cu, Mn, Zn, Co, Mo, Ni, V, Na and Rb (Kieffer, 1991) at concentrations equal to or less than 100 mg kg' dry weight (Mehra and Farago 1994). Al, Sn, Cr and Sr are recognised as being beneficial to plants (Phipps, 1981). Elements such as As, Ag, Cd and Pb may be required at extremely low concentrations but generally serve no recognised biological function and can be toxic at higher concentrations (French, 2004). The mechanisms used to translocate and utilise nutrients are the products of several millennia of evolution. Such mechanisms are selective, acquiring some metals in preference to others.

In the process of phytoremediation the point of entry into the living tissues by the plants root cells is a step of utmost importance. Once the metal ions have been absorbed by the roots, and have been transported to the xylem, its movement throughout the plant body is possible (Alloway, 1995b). However, for this process to materialise it is important that the metal is transported from the roots to the shoot area. The extent and rate of movement of metals within the plant depends mainly on the plant body, the metal under consideration and the age of the plant (Chaney et al., 1988). Cottenie et al. (1982) illustrated the levels at which different metals were translocated in to the aerial parts of the plant. The distribution of metals in the above ground plant body, compared to the metal concentration in soil illustrated in figure 2.4. This shows that metals like Cd and Zn for example have the maximum uptake levels followed by Mn and the least uptake is for metals like Cu and Pb.

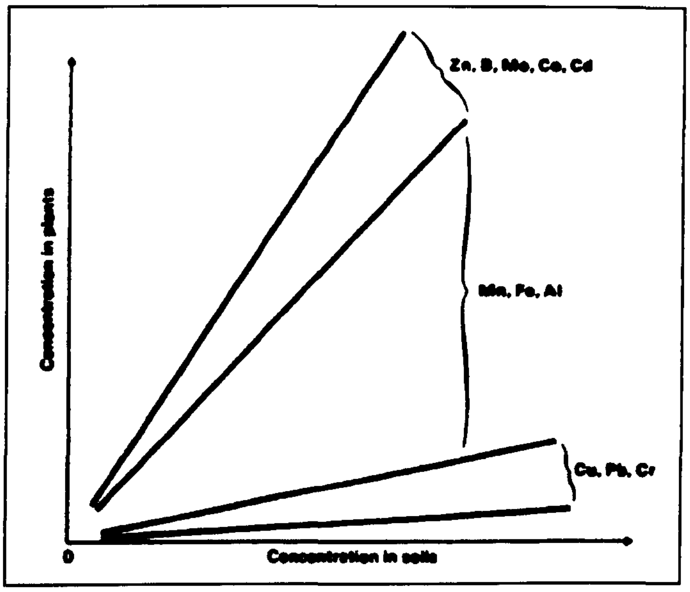


Figure 2.4: Distribution of metals into aerial parts against the metal concentration in soil (Cottenie et al.,1982)

"Movement of metal-containing sap from the root to shoot, termed translocation, is primarily controlled by two processes: root pressure and leaf transpiration" (Lasat, 2002, p. 111).

There are several factors that considerably affect the extent of metal extraction by plants. According to Henry (2000) these include:

- Metal bioavailability within the plant rhizosphere area
- Rate of metal uptake by roots
- Proportion of metal 'fixed' within the roots
- Rate of loading in xylem and translocation to the shoot area
- Cellular tolerance to toxic metals

In order for these to be attainable, the plants must be capable of a) extracting large quantities of heavy metals into their roots (b) translocating the heavy metal to the surface biomass and break down and store the metals in their stem and leaves (Brennan and Shelley, 1999; Henry 2000). In order to solubilise and extract metal ions from the soil environment, plant roots can modify the soil environment in several ways by exuding protons to change soil pH, or exuding other compounds such as amino acids and hydroxycarboxylic acids to complex trace metals (Cataldo et al., 1987). Soil biochemical activity in the rhizosphere, stimulated especially by carbon-based plant root exudates, can mobilise metals though acidification and organic complexion (Pahlsson, 1989). Conditions within the rhizosphere can therefore differ dramatically from the bulk soil component due to these rapidly changing and dynamic processes. Trees vary in their potential to absorb and translocate heavy metals from root to the shoot (Pulford and Watson, 2003). Absorption of metals directly into the leaf can occur as a result of deliberate deposition (such as the use of fertilisers) or accidental deposition (such as anthropogenic aerosol sources). Species type, leaf age, cuticle thickness and position and nutritional status will all influence the rate of foliar absorption (Alloway, 1995b). In many cases the waxy cuticle will prevent translocation into the leaf; especially in the case of Pb, and metals become bound into the leaf surface. Metals in solution may penetrate the leaf directly though the stomata where they can be transported to other parts of the tree via the phloem. Aerial deposition may also increase soil metal levels as they are washed down the trunk and accumulate in the soil around the base of the tree (Watmough and Dickinson 1995).

The bioavailability of metals to trees and the accumulation of these metals in the tissues depend on the source of the metal contamination and the site of the contamination. Moffat and Bending (1999) studied the relationship of tree establishment and growth with the physical, chemical and hydrological properties of mine spoils. The growth of these trees was affected by physical characteristics such as compaction and infertility (mainly N deficiency), salinity and poor water-holding capacity and chemical characteristics like Zn and Mn contamination. In another study the distribution of heavy metals in the axial and radial growth rings in Oak trees was studied by Queirolo et al. (1990). The concentration of four heavy metals namely Cd, Pb, Cu and Zn were determined in the oak growth rings. The radial distribution for all the metals was found to be the same. The more toxic metals Cd and Pb were found to accumulate to the lower parts of the trees. Also young saplings were found to accumulate more amounts of the four metals than older trees.

Ang et al., (2010) studied the phytoremediation of Cd and Pb by four tropical timber species grown on an abandoned tin mine in Malaysia. Results showed that variation in species affected the concentration of heavy metals in soil and trees. Higher concentrations of metals were found in the stem and leaves as compared to the roots. A team of international researches investigated the effects of heavy metals in soil on young forest ecosystems as a part of an initiative of the European Commission (2007). They used 32 replicates of a built model ecosystem which included four European forest tree species and herbaceous plants from different regions of the EU. After 3 years of processing and analysis the results showed that for 3 out of the 4 tree species tested, fine root biomass decreased and so did the total leaf area because of metal contamination. The results also showed that the efficiency of the trees to use water for their growth was also significantly reduced. Thus showing the drastic effects heavy metals can have on plants.

2.12 Choice of Tree Species

The choice of tree species is very important in the reclamation of sites affected by mining activities. Site conditions which influence the choice of tree species include availability of topsoil, soil chemistry, soil wetness, pollution level, local climatic conditions and exposure (Moffat & McNeill, 1994). Hence, it is important to identify the species which are most likely to suit planting conditions. In the soil, metals are present in a variety of chemical species. Many are present in a dynamic equilibrium which is governed by the soil's physical, biological and chemical properties (Chaney, 1988). Only a fraction of metals that are present in the soil are readily available (bioavailable) for uptake by plants (Lasat, 2002). It is important to select trees that are good at taking up these metals from the soil and transferring them into their above ground tissues, especially in their leaves.

Since the 19th century (McGeorge, 1995; Rees et al., 1998), plants have been identified, which have the capability of accumulating high levels of metals (Lasat, 2002). A great deal of research has gone into the understanding of the physiology and biochemistry of metal accumulation in plants. Based on a background study, it was felt appropriate to study tree species which help tackle the problems associated with open cast coal mining reclamation; provide a healthy ecosystem for other biological life forms, are pioneering species, and species that provide stability to the soil and environment. Bearing this in mind, for this specific research project, three tree species were used, which in some way address one or more of the issues. These have been described below.

2.12.1 Alder (*Alnus glutinosa*)

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Family: *Betulacae*

Genus: *Alnus*

Botanical Name: *Alnus glutinosa*

Common Name: Alder, Common Alder, Black Alder

Place of Origin: Ireland, Britain and temperate zones in Europe and across Russia and Siberia

Alder is a member of the Birch family and typically reaches heights of 25 to 30m. It is one of the pioneering species, quick growing and short-lived. The maximum age is approximately 150 years (Featherstone, 2004), maturity is attained at about 60 years, producing a long trunk and a narrow crown (Mitchell, 2006). Alders are said to grow on most soil types except poor acidic peats. As these are deep-rooted species they help control soil erosion and also help maintain soil at river banks. The leaves which fall as litter are said to make good compost (Mitchell, 2006).

There have been reports of some serious threats to the survival of alders in the UK and EU. One of the fungus Phytophthora sps grows from the bottom of the tree upwards and kills the roots and bark. This has been widely identified in England and Wales (Zavitkovski and Newton, 1971; Webber et al., 2010). Another problem that has been reported to affect alders is crown dieback. In this the tree dies from the top downwards. This condition was first noted in the 1980s in Scotland and since then there have been reports of similar problems in other parts of the UK and Europe (Bakonyi et al., 2003, Featherstone, 2005).

Alders belong to the 'actinorhizal' group of tree species and are a popular choice in forestry-based restoration due to their nitrogen fixing properties through a symbiotic relationship with the micro-organism *Frankia* spp. which form root nodules (Fitter and Hay, 1981; Moffat and McNeill, 1992; Moffat and McNeill, 1994; French, 2004). These trees fix atmospheric nitrogen with the help of root nodules that contain the enzyme nitrogenase that can convert N_2 from air to NH_3 (Tao et al., 2004) Approximately 47 species of lichens and mycorrhizal fungi have been reported to grow on Alders. A study by Roy et al. (2007), confirmed that the nitrogen fixation property of Alders, helps improve soil quality and also acts as a Phytoremediator. In another study by Densmore (2005) *Alnus virdis* seedlings were planted in combination with *Salix* and *Populous* species on a placer mine spoil in Alaska. Results showed that after 10 years the Alnus treatment plots had denser stands of *A. viridis* approximately 1-2m tall. Results also showed that Salix plants mixed with *Alnus* plants had higher levels of foliar nitrogen as compared to control. This study highlights two important points a) alders colonise quickly and b) Alders not only fixes nitrogen in soil but also donates it to adjoining trees.

There is additional satisfactory evidence that nitrogen-deficient species benefit nutritionally if planted in mixtures with alders on infertile soils. Findings from analysis on Japanese larch needles showed improvement in nitrogen nutrient content when planted in combination with Alder and Birch trees (Moffat and Roberts 1988; Moffat, 1994; Mofatt and McNeill, 1994). The basis for introduction of a "nurse" tree species was the deciduous nature and nitrogen-fixing capacity of Alder trees, which in turn would increase the organic matter in mine spoils and also improve nitrogen capacity (Plass, 1977; Rietveld et al., 1983).

Nitrogen fixation is one of the key selling points of Alnus species especially when considered for a reclamation programme. Nitrogen deficiency is a major factor that limits plant growth on mine spoils along with site conditions. Dawson et al., (1998) studied the nodulation in Alnus seeds in soil from different topographic positions at an Illinois spoil bank. After 10 weeks of germination, spoil material from level terrace showed 26% nodulation and material from the slope below the level terrace showed 13% nodulation whereas steep upslope only showed 3% nodulation. These results confirm the speculations that level terraces provide suitable infiltration and water retention, thus favouring microbial and plant growth.

Although Alders are often found close to water, they tend to grow better in soils that are not permanently waterlogged. This is another reason why Alders have been a favourite for the purposes of phytoremediation to the local authorities in UK, as seasonal water logging is a very common problem at opencast mining sites due to compaction. However there have been casualties after a few years of establishment, probably due to the lack of water during summer months in such mine spoils (Moffat, 2006). In a study by Rosselli, & Bashi, (2003), the ability of 5 woody tree species to extract heavy metals (Cu, Zn & Cd) from polluted soil to their above ground tissue was tested. Metal content in leaf and twigs was determined using an Atomic Absorption Spectrophotometer (AAS). Results have showed that *Salix viminalis* (Willow) and *Betula pendula* (Birch) transferred Zn and Cd to leaves and twigs and *Alnus incana* (Alder), *Fraxinus excelsior* (Ash) and *Sorbus mougeotii* (Whitebeam) took them up to above tissue level, into the stem. This illustrates the ability of these woody trees to be good phytoremediators. Although *Alnus* and *Sorbus* species did not accumulate high levels of metals as compared to the other species the author pointed out that these two species can be considered as heavy metal excluders. He suggested that these trees may have a mechanism to avoid the uptake of metals by stabilising it in the rhizosphere. This is in accordance with Mertens (2006) findings, where he suggested Alders to be used for Phytostabilization.

In a similar study by Mertens et al. (2004), the metal uptake capacity of young trees from brackish sediments was tested. This team selected five tree species which include *Alnus glutinosa* L., *Acer pseudoplatanus* L., *Fraxinus excelsior*, *Robinin pseudoacacia* and *Populus alba*. These trees were planted on a mound constructed of dredged sediments, which originated from a brackish river mouth and was slightly polluted with heavy metals. This study also showed that poplars accumulated large amounts of

Cd and Zn. It concluded that, Ash, alder and maple were, also suitable for reclamation of dredged sediment mounds. Results have also showed that Alder and Robinia rapidly reduced pH because of their nitrogen fixing capacity.

In section 2.6 it was outlined that for most of the metals under consideration soil pH plays an important role in their uptake into the plant. Mine spoils exhibit a variety of soil pH and Alder through its N-fixation reduces the soil pH making it possible for metals to be bioavailable and readily taken up by trees. However, reducing the soil pH may also lead to soil acidification which may negatively affect the soil and plant environment and lead to destruction of the microbial community.

2.12.2 Birch (Betula pendula Roth)

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Family: *Betulacae*

Genus: *Betula*

Botanical Name: *Betula pendula (Roth)*

Common Name: Birch, Silver, European White.

Place of Origin: Native to Britain and to West and central Ireland, Northern Europe.

Birches are rapid-growing trees and they readily colonise open grounds. These trees normally reach a height of 25 m and they are short lived with a life span of 70 to 90 years. There have been reports on the occurrence of Birch trees with other forest trees, such as oak woods (*Quercus spp.*) and pine (*Pinus sylvestris*) and as largely predominant (Featherstone, 2005).

As one of the prime species birch trees fulfil an important function of improving the soil. The roots have mychorrhizal associations with fungi. This is a symbiotic association in with both the trees and fungi benefit from their interactions. Arbuscular mychorrhiza have been known to be actively involved in the uptake of metals from the soil and their presence has reported significant effects on the response of plants to metal stress (Charvat and Pawloska, 2004; Capuana, 2011). A great source of literature is available on the effects of mychorrhizal association on plants growing on metal-polluted soils and their possible role in remediation (Khan et al., 2000; Gohre and Paszkowski, 2006; Audet and Charest, 2007).

Birches are deep rooted trees and this helps them take up the nutrients from the soil and translocate them into the leaves and stems. Some of these nutrients are given back to the soil when the leaves fall during winter months and become available for other soil micro-organisms (Featherstone, 2005). Birch support a large community of invertebrates and insects and this supports the growth a forest ecosystem. There has been a growing interest of Birch as a source of timber species in the UK (Malcom and Worrell, 2001). In a study by Morin (1981) metal concentration in maple, ash, pine, birch and cottonwood trees grown on sludge-amended mine spoil was studied. Results showed that one-year old trees exhibited high metal accumulation patterns. Third year samples did not show an increase in concentration however this does not mean that there was a decline in the accumulation of metal over time but rather this was due to higher biomass production and accumulation in litter layer (Morin, 1981).

Birches have an association with fungi but not all associations are symbiotic. The 'Witches' broom fungus (*Taphrina betulina*) is parasitic on birch trees and causes a growth of small abnormal twigs (Featherstone, 2005). There have been reports of extensive Birch dieback in many of the woodland projects in Scotland (Green, 2005). In cold regions the twigs of the birch trees suffer from cold drying winds.

For successful plantation on compacted soils of degraded sites, it is very important that the tree species selected has a good rooting system along with remediating properties. To this end, Borgegard & Hakan (1998) studied biomass, root penetration and heavy metal uptake in Birch in a soil cover over Cu tailing. Results showed that even with 1 m thick layer of compact soil fine roots of Birch trees which were less than 4 years old had penetrated into the soil. When the leaves were tested, contents of Zn, Pb and Cd were higher than the published values for uncontaminated soil. These plants showed a good rooting system and deep root penetration. Uptake of elements was found to be high and showed a correlation between soil and leaf concentration for the elements. Similarly Kuznetsova et al., (2009) studied the survival of Black alder, Silver birch and Scots pine from a reclaimed oil shale mine. Results showed that the tree roots improved mineral nutrition by the fine-root morphological adaptations and the roots increased in the following order Scots pine (62) < black alders (172) < silver birch (314).

In a study by Pulford et al. (2001), Birch and Salix trees were tested for the uptake of Cr and Zn from a chromite processing waste site. Leaves, wood and bark were tested for translocation of the two metals in the tree species. Cr was poorly taken up into the aerial parts of both, Birch and Salix trees, however, Birch showed a good uptake of Zn into its leaves. Baltrenaites & Butkus (2007) modelled the transport of Cu, Ni Zn, Mn and Pb in Alder, Birch and Pine leaves by adapting the Hung and Muckay generalized model, of contaminant uptake by plants. Results showed that for both Alder and Birch the concentration of Mn and Zn increased from roots upwards with a maximum concentration in leaves.

Another study by Eltrop et al. (1991) tested metal tolerance to Zn and Pb in birch and willow populations. Results showed that willow grew in soil with a total concentration of 170mg/kg of Pb of which it accumulated 40 mg/kg Pb. Whereas Birch grew in concentrations of 290 mg/kg of Pb of which it accumulated 70 mg/kg. The measurement of metal concentrations in plant tissues have been commonly carried out in order to estimate the effectiveness of tree species and studies by Gallagher et al. (2008), Kopponen et al. (2001) and Utriainen et al. (1997) on the metal concentration in *Betula sp* has attracted great interest (Pulford and Dikinson, 2003). Betula Pendula has been well documented as being able to tolerate increased levels of heavy metals and grow on acidic soils and also as a hardy prime species (Moffat and McNeill 1994, Utriainen et al.1997; Kozlov et al.2000). In a study by Goransson and Philippot (1994) Betula pendula was identified as a 'metal collector' for Cadmium uptake and 100% of Cd added via modifications was taken up by seedlings. This however does pose the problem of the return of the metals into the soil in the form of litter. Birch is called one of the pioneering species however the issue of high biomass production on heavy contaminated sites need an appropriate harvest plant in case of accelerated metal accumulation.

2.12.3 Larch (*Larix decidua)*

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Pinopsida

Family: *Pinaceae*

Genus: *Larix*

Botanical Name: *Larix decidua*

Common Name: Larch tree.

Place of Origin: Native to mountainous areas of Northern and southern Europe, Swiss and Italian Alps and Siberia.

Larches are deciduous coniferous trees native to Europe and the European larches are most preferred for planting and have been considerably planted in England. The trees grow rapidly to a height of 10 to 45 m. The leaves are soft and thin evergreen needles with two pale stripes on the lower side. The wood is hard, heavy and decay resistant (Forestry Commission, 1999). These trees grow in a range of soil conditions but are light demanding. In UK larches are one of the first trees introduced for its timber in early 1620s and also became one of the first conifers to be planted on large scale plantations (Osmond and Upton, 2012). *Larix* when planted in a mixture with other trees as *Betula* and *Alnus* species have shown promising results as the latter provides the necessary nitrogen through mychorrhizal association (Moffat and Roberts, 1988; Moffat, 1994; Mofatt and McNeill, 1994).

Tree survival and performance was tested by the Forestry Commission in 1960 on a former opencast coal mine in South Wales (Moffat, 1994). Analysis showed that in the first year of planting some of the pines suffered high mortality rates. However species like Alders, Sitka spruce, Larch and false Acacia were most successful. Japanese larch showed a great variation in height and had the least mortality rate on site. Similarly another study was set up by the Forestry commission in 1973 (Moffat, 1994), to test the nutrition and early growth of four conifer species (*Japanese larch, Corsican pine, Sitka spruce and Lodgepole pine*) on a former coal mine. Larch and Corsican pine showed the most successful growth while the other two tree species showed a sharp decline. Foliar analysis suggested that all the species heavily lacked Nitrogen and also Phosphorus to some extent. However, Larch and Corsican pine adapted and utilised the low available N and P supplies, thus supporting the observations of many of adaptability and survival of both these species (Fourt and Best, 1983, Bending, 1993; Bending & Moffat, 1999).

Wang and Jia (2010) studied the adsorption and remediation of heavy metals by larch and poplar trees in a contaminated site in Japan. Results showed that Poplars were 2 to 4 times better at remediating soil contaminated with Cd, Cu and Zn as compared to Larch trees. However, larch did accumulate some amount of each of the metals studied. In contrast Moudouma et al. (2013) studied the ability of hybrid larch species to accumulate Cd in their above ground tissues. Results showed that larch translocated good amounts of Cd into its shoot thus showing a potential for phytoremediation. Poplars have been established as hyperaccumulators (Fernandes et al., 1997; Pulford and Watson, 2003; Mertens et al., 2004; French, 2004) and hence it should come as no surprise that the tree accumulates greater amounts of metals in their above ground tissues, mainly shoot and leaves. The positive observation here is the ability of Larch to accumulate metals in their above ground biomass.

This has been confirmed by Bending & Moffat (1999) while studying the performance of *Larix leptolepis* (Larch) on restored opencast coal mine spoils in the South Wales coalfields. The relationship between tree growth and the mine spoil's nutritional, physical and chemical factors was studied. The foliar concentration of Nitrogen and Phosphorus were positively related to tree growth. High levels of Magnesium were found in bark and leaves. Larch roots were found to penetrate heavily compacted soil with a bulk density of 1.7 g/cm^3. The uptake capacity of Larch has been demonstrated in a study by Myre and Camire (1996) which tested the distribution of nutrients (Mn, Mg, Zn, Ca, P & K) in stems of European Larch and Tamarak. The research involved a ring-by-ring study of the distribution pattern of nutrients in the stems of the 18 year old trees. The distribution observed showed maximum concentration of nutrient in the bark. The concentration of mobile nutrients (P & K) was found to be highest in the inner xylem where as that of the immobile nutrients (Ca, Mg & Mn) was the lowest. Zn concentrations were found to be similar in both zones.

A trial of revegetation practices with larch species under different environmental conditions was studied by Kioke et al. (2003). The research studied physiological ecology of larch species in relation to global climate change. It was proposed that Larch was one of the most suitable candidate species for reforestation in cool temperate regions. Various researchers have found Larix sps to be suitable candidates for rehabilitation of degraded lands, revegetation and reforestation after wood harvesting in Northeast Asia (Gower and Richards, 1990; Kioke et al., 2003; Ryu et al., 2009).

A study by Moffat (2000) tested the potential of planting nitrogen-supplier alder species in a mix with Larch species on a restored opencast coal spoil. Performance of six alder species inoculated with *frankia* in a nursery experiment was tested on a field over a period of six years. The effect on growth and foliar nutrition of Larch was also studied. Although the Nitrogen took a long time to accumulate in the growth substrate, alders were able to significantly improve growth rate of Larch. This showed that Larch can grow well on coal spoils when mixed with Nitrogen-providing tree species. Mertens et al. (2004) also found that conifers namely larch in this instance, tolerated infertile and acidic soil conditions.

However some of the drawbacks of these trees are high mortality rate in the initial years of establishment (Bending and Moffat, 1999; Rawlinson et al., 2000; Karpati et al., 2011), susceptibility to pathogens like phytophthora which is currently widely spreading in the UK, especially Wales (BBC, 2011) and inability to survive under extreme infertile conditions, although the trees show good vitality when plated in mixture with trees 'nurse trees' like alder.

In summary all, the three species selected for this study help tackle most of the issues outlined, related to reclaiming mine spoils. All three trees especially, Birch and Larch are good phytoremediators. Alder provides stability to the soil and acts as a 'nurse tree' and supports establishment of birch and larch. Birch is a pioneering tree with deep rooting, which is capable of metal accumulation and has been found to be a successful tree in reclamation. Larch a conifer; is tolerant to infertile and acidic soil and is a low demanding tree and most importantly all of the three trees documented to be suitable for forestry.

2.13 Plants and the community

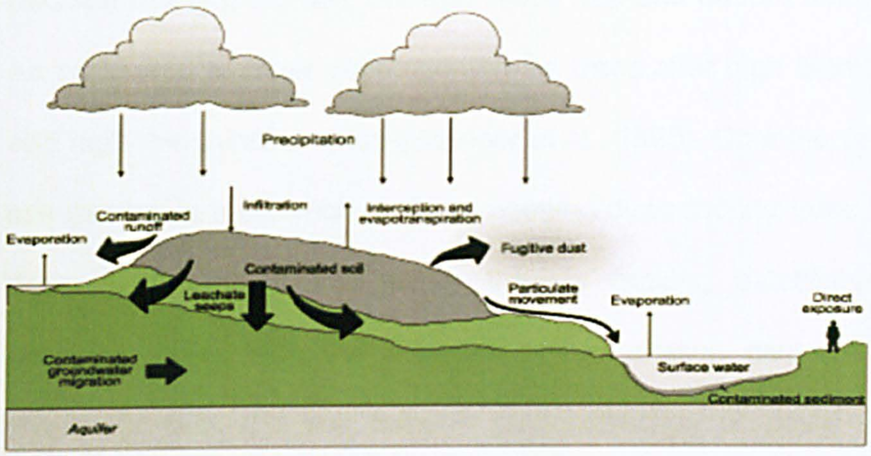
A lot of emphasis is given on the biology and chemistry of phytoremediation; however it is important to consider the psychology and social significance of remediating sites. Investigations have found significantly positive benefits linked to tree planting on the human health and well-being (Westphal and Isebrands, 2001). Many degraded and contaminated sites are in the vicinity of distressed neighbourhoods that are in need of social and economic restoration. Degraded sites which tend to have an adverse effect on the physical health of the environment, can also have a negative effect on the people living in the vicinity. These effects could be health related and psychological. Planting trees as a part of remediation are likely to have a positive effect on society. Through their research

Westphal and Isebrands (2001) believed that residents who view planting as a part of remediation could benefit from reduction in stress, increase in productivity and develop greater capabilities for coping with stress.

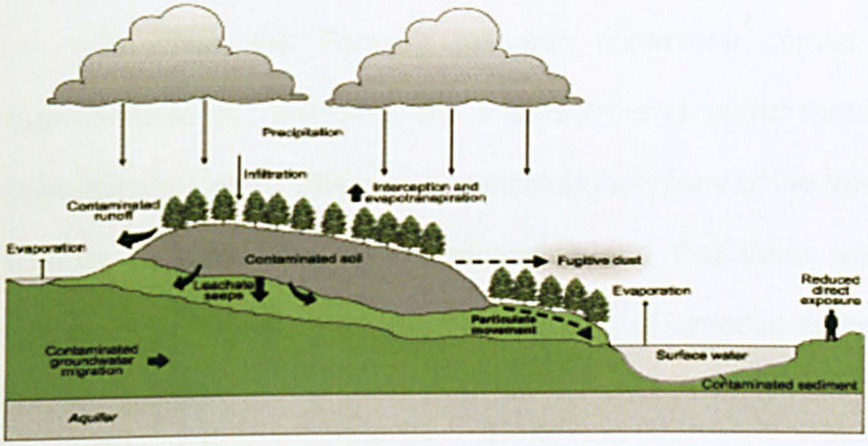
2.14 Forestry: Potential land-use policy for Land reclamation

Forestry as a land use policy to reclaim contaminated and/or degraded land has many social, environmental and economic benefits and presents opportunities for sustainable development. Woodland is an important element of the modern landscape, as it plays a vital role in improving the appearance and also contributes to the economic growth of the region (Forestry Commission, 2003). The establishment of woodland on contaminated and derelict lands has been one of the top preferences of the England Forestry Strategy (Hutchings, 2002). Different approaches for best utilizing trees on derelict lands have been proposed by some researchers. Ward (1996) suggested three different approaches: i) landscape approach which is not commercially viable as this would mean large areas of reclaimed lands not being used; ii) forestry approach via trees planting which is economic, easy to manage but providing slow results; iii) ecological approach which is low input, low cost and non-productive. However, Ward did not consider other vital factors such as time-scales for remediation nor tree species suitable for the specific site and contaminant. Besides, forestry could also be used for recreational purpose and a form of community engagement through community forestry (Handley and Perry, 1998) and a source of employment.

The first step towards any reclamation programme is the identifying and eliminating risk to the environment and individuals. Hutchings (2002) in his report of the Forestry Commission suggested assessment of lands using predictive risk assessments before planting trees for reclamation of contaminated lands. The benefits of woodland on contaminated lands have been illustrated by the UK forestry commission (Figure 2.5). The thickness of the arrows indicates the extent of the problem.



a) Effects of contaminants on the natural ecosystem before planting trees



b) Effects of contaminants on the natural ecosystem after tree planting.

Fig: 2.5 Benefit of woodland on contaminated lands. (Hutchings, 2002).

Trees with the potential for phytoremediation tend to offer an inexpensive, *in situ* and healthy option to remove or stabilise soil contaminants. Trees help prevent or restrict leaching, erosion by wind, surface water erosion and runoff and weaken pollutant linkages (Fig 1.4). Planting trees on degraded/ contaminated lands increases soil stability and retains soil with roots and provides organic matter by leaf senescence. Planting trees for phytoremediation not only helps in stabilising and decontaminating the soil, but also helps trap and absorb airborne pollutants. As compared to other vegetation types, trees offer high biomass production and high transpiration rates (Schnoor et al., 1995). On mine spoils where soil compaction is a common problem planting deep rooting trees helps to break down the compact soil layers thereby making establishment of trees possible. Trees with the potential of remediation can either extract the chemicals from the soil through phytoextraction or could immobilise the chemicals and stabilise the soil through the technique termed as phytostabilization.

In 1998, the Forestry research committee carried out a postal questionnaire to determine the frequency and performance of trees on contaminated lands. The survey indicated that many of the tree species were tolerant to some form of contamination and that there were reasonable grounds to support the re-establishment of woodlands on such lands (Hutchings, 2002).

2.15 Reclamation initiatives on opencast coal spoil in Wales

Opencast coal mining in Wales has been active since 1942 during wartime, which later increased steadily as a means of extracting coal (Moffat and McNeil, 1994). As opencast coal mines present a variety of problems some of which are unsuitable soil, lack of soil, soil compaction, waterlogging, adverse climatic conditions, adverse overburden characteristics, it becomes very difficult for tree establishment and maintenance of growth and such lands demand high standards of restoration and aftercare (Moffat and McNeil, 1994). The Forestry Commission's active participation in reclaiming former opencast coal mines in the region has been longstanding. Apart from being land owners of these opencast mine sites, the Commission was also solely responsible for planting of these restored sites. In recent times, many more private land owners have taken over these areas and are actively involved in the aftercare and reclamation of these mines. Establishing tree growth on mine spoils brings challenging problems. The Forestry Commission appointed many groups of district staff for carrying out research on restored lands in South Wales. Up until 1988, a vast portion of the research was aimed at finding solutions and alternatives to the problem of poor growth on these minespoils. From their work, it was recommended that Alder species should be interplanted as a 'nurse' crop with conifers on restored grounds, as they provide nitrogen to the soil through their mychorrhizal association in the root area (Bending, 1994).

Opencast mining also brings the problem of auto-compaction which is found to be a very common problem in the coal fields in South Wales. Haigh et al. (2008) studied the effects of different planting methods on the growth of

Alder and Oak trees in compacted opencast coal minespoils in South Wales. The study explored planting methods which would be suitable for use by local community volunteers who seek to improve the local environment by restoring lands affected by compaction after opencast mining and low soil nutrition. To this end, three planting methods, namely notch planting, pit planting and trench planting were tested to explore tree survival and growth of the two species. Results showed that both species showed highest growth for trench planting followed by pit planting and the least for notch planting. The study concluded that in order to improve growth and survival rates on opencast coal minespoils, trees should be planted in loose, low density soils with an adequate amount of substrate for improved rooting (Haigh et al., 2013).

The Welsh Development Agency (WDA) established in 1976 was responsible in regenerating the economy of Wales by improving the environment. The Agency spent millions of pounds in funding large-scale land reclamation initiatives in Wales, but was itself abolished in 2006 (WDA, 2009). The current environmental condition of most South Wales opencast coal fields is a consequence of the deep damage caused by short-sightedness and poor planning initiatives. It is only fair to restore the lands, which once supported the Welsh coal economy, back to a condition where they can self-sustain and be aesthetically pleasing.

Based on the review of literature, three tree species namely Alder, (*Alnus glutinosa*), Birch (*Betula pendula*) and Larch (*Larix europaea*), have been selected for this particular research. Table 2.8 shows the selected species against the different metals they are capable of uptake.

Table 2.8: Metal uptake by the 3 selected tree species by various researchers

Species		
Alder	Birch	Larch
Mn, Cu, Fe, Ca, Mg Taleshi & Dhumal (2009)	Ca, Mg Hytonen & Saarsalim (2009)	Pb, Zn, Ni, Cu Dickson & French (2006)
Zn, Pb, Cu, Ni, Mn Baltrenaite, Butkus (2007)	Zn Gallagher, et. al (2008)	Cu, As French et al. (2006)
Ca, Mg Hytonen & Saarsalim (2007)	Zn, Pb, Cu, Ni, Mn Baltrenaite, Butkus (2007)	Zn Gardner (1999)
Cd, Cu, Zn PWT (2007)	Pb, Zn, Cu Dickson & French (2006)	Pb, Mn, Mg Bending (1994)
As, Cd, Cu, Ni, Pb, Zn Dickson & French (2006)	Zn, Cd Rosseli & Bosch (2003)	Ca, Mn, Mg, Zn Myre & Camire (1994)
Cd, Cu, Pb, Zn Mertens et. al. (2004)	Zn Pulford et al. (2001)	N Malcome & Carlyle (1986)
Zn, Cd Rosseli & Bosch (2003)	Cd, Mn, Cu Goransson & Philippot (1994)	Cu Van Den Burg (1983)
Ni Pulford et. al. (2001)	Pb Eltrop & Brown (1991)	Ca Stone (1966)
	Zn, Pb, Cd Borgegard & Rydin (1989)	
	Zn Denny (1987)	
	Mn, Mg, Ca Lobersli & Steinnes (1987)	
	Zn Brown, Wilkins (1985)	

2.16 Summary

Coal mining is a widespread industry not only in the UK but worldwide. It is the responsibility of the mining agency to restore the land back to a condition where it can self-sustain. However, large proportions of the so called 'reclaimed lands' still face the problem of degradation and poor or no plant growth which are mainly affected by soil compaction, waterlogging, gully erosion, lack of nutrients and the presence of heavy metals. The problem is widely known but not much studied, as these problems tend to become apparent only in the years after the mining agencies have relinquished legal responsibility for the lands and because they affect lands that are commonly returned to low-value uses such as mountain grazing. Considerable money has already been spent in reclaiming these lands but expensive techniques are not always the favoured methods for treatment. A desired cost-effective method for reclaiming these lands is through the use of trees for forestry. Forestry is seen as a land-use offering many benefits including enhancement of the environment, timber production and provision for growth of flora and fauna. However, care needs to be taken to select the most appropriate variety of tree species and planting them in combinations so that they benefit from each other and also support healthy 'forest like' environment. The literature refers to some widely used hyperaccumulators and transgenic plants like hybrid poplars, salix and willows for the purpose of reclamation of contaminated land but these trees are not the favoured choice for forestry. Also these trees, being hyperaccumulators need constant harvesting, which require additional costs. At opencast mine sites trees that are well equipped to cope with hostile conditions, such as deep rooting, drought tolerant and metal resistance should be planted. According to the Forestry Commission (1999) report, Alders are suitable for plantation on degraded lands affected by mining as they are able to establish quickly on a range of infertile soils and are good phytoremediators. They are also tolerant to heavy metals, acidic soils and air pollution. Birches are tolerant to heavy metals and have deep rooting system and a good uptake capacity and Larches have also shown tolerance to heavy metals, acidic soils and exposure. Also these are three of the most desired and commonly used tree species in land reclamation in the UK. All of the literature considered suggests that Alder, Birch and Larch would prove beneficial in the remediation of the coal spoil as they meet the basic criteria for selection of tree species for remediation of mine spoils, which are, selected species should be good phytoremediators, they should have deep rooting system, must be heavy metal tolerant, should have nitrogen fixing properties, and most importantly should be suitable for forestry. After careful review, it was decided to use Alder, (*Alnus glutinosa*), Birch (*Betula pendula*) and Larch (*Larix europaea*) for this research. In spite of being generally considered desirable species, these trees are not widely studied, especially on coal spoils where they are extensively for used by local authorities. Time is an essential factor for measuring the success of reclamation. While reclamation of degraded sites is gaining interest, there are very few studies which investigate long-term effects of reclamation. Despite being widely used for land reclamation there is no study that looks at the long term potential of these three trees in reclaiming degraded coal spoils in the UK over time.

This research looks at the legacy of past mining activities and the management of those lands, the potential metal contamination present at these spoils and the threats caused by specific metal contaminants: Cd, Zn, Mn, Pb and Cu in soil. It also aims to test the phytoremediation properties of three plant species Alder (*Alnus glutinosa*), Birch (*Betula pendula*) and Larch (*Larix europapaea*) which are commonly used in land reclamation in UK. These trees will be tested for their ability to sequester metals into leaves from mine spoils and compares the abilities of newly planted trees and relate them to the longer term plantations on adjoining plots using a sequence of related soils. These soils only differ from one another in certain properties primarily as a result of different age of plantation for trees planted regularly over two decade.

CHAPTER 3

SITE DESCRIPTION

3: SITE DESCRIPTION

Opencast mining has been a favoured method for extraction of metals and coal in the UK and this, has had detrimental effects on the environment. Before the introduction of the mine closure, reclamation and best practice guidelines in the 1970's, mines were often left abandoned without being reclaimed or decommissioned as per new standards. Abandoned or poorly reclaimed mines posed problems of environmental and safety hazards (Walley, 1994). The area of study, an open upland on the Varteg Hill in South Wales, UK, was exploited largely through open cast mining for its iron and coal in the 1948-1963. Evidence of this is still visible today with large coal seams laying close to the surface and compacted and contaminated infertile derelict soil. Numerous small iron and metal quarries are scattered throughout the region (GGAT, 2008), which were extensively exploited for their metal and ores, adding to the pollution of this region. The area has been characterised by the Glamorgan Power Ltd. as a post-medieval industrial site with extractive industrial landscape mainly comprising of open moorlands with patches of regenerating areas of mining waste (GGAT, 2008). Today this area of Wales is still littered with unreclaimed spoil tips and is viewed as a '100km belt of ecological scar tissue', (Walley, 1994, pg 22).

For the present study, an area of former reclaimed coal spoil was selected on Varteg Hill, in Torfaen, South Wales, UK and experimental test plots were laid. In addition to the new test plot, five adjoining plots where also selected for study which were said to be 'reclaimed' by local authorities in 1963 mainly through grass seeding. On these adjoining plots tree planting has been carried out since 1991 by a group of volunteer (CradleforNature.org (CfN) researchers. Care was taken in selecting plots with similar topography, tree species and planting styles. The following chapter gives a detailed overview of the town of Varteg, history of mining in Varteg and the study plot itself. It also outlines the various CfN Test Plots studied as a part of this research.

3.1 Location and history of Varteg Hills

Varteg is located in south wales, UK. Figure 3.1 shows the detailed map of UK and Wales.

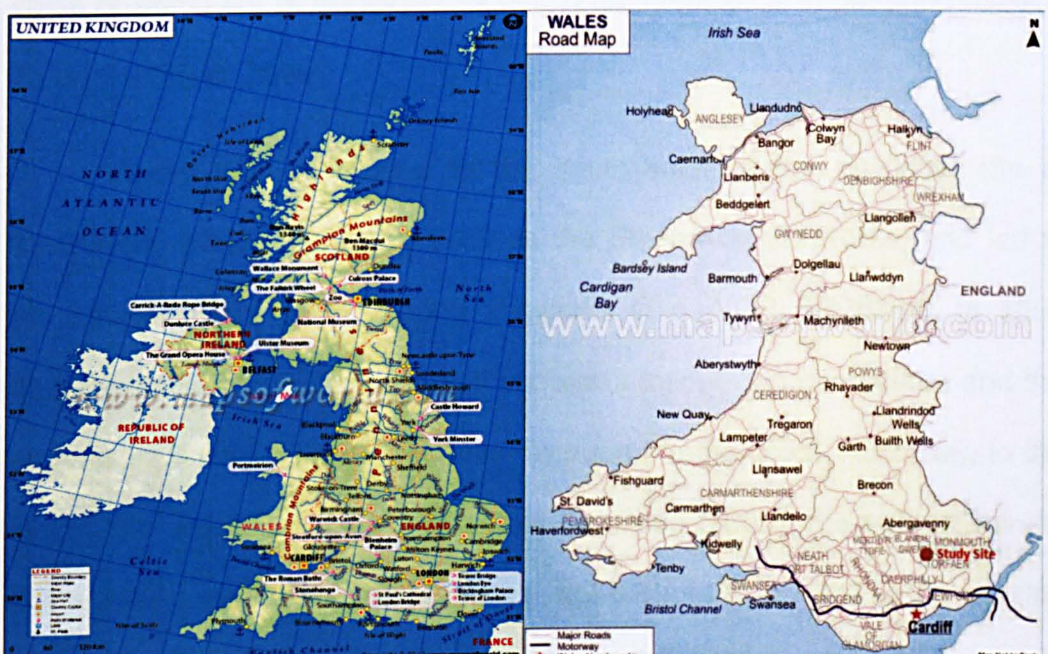


Figure 3.1: UK political map and Wales road map.

Situated 19 miles north of Cardiff and 39 miles east of Swansea, the town of Varteg is located in the county borough of Torfaen in South Wales, UK. It lies near Abersychan on the hills above the valley of the Afon Lwyd, between Pontypool and Blaenavon (Maunsell, 2006). Cwm Afon in Welsh means river valley and Lwyd (meaning brown) may be a reference to the brown river water caused by the mining activities and pollution through acid mine drainage (AMD) (Munsell, 2006).

"The historic landscape of Mynydd Varteg Opencast defines the extent of quarrying and opencast works largely undertaken by the 1960s, though still ongoing." (GGAT, 2008, [10 March 2009])

The Varteg Opencast is an extractive landscape dominated by modern opencast workings and waste tips. The major characteristics of the area comprised the remains of industrial, mining and quarrying extractions, some of which are of significant industrial archaeological importance (GGAT, 2008, [10 March 2009]).

Varteg Hills is located to the south west of the historical site of Blaenavon, which opened in 1789 as the Blaenavon ironworks, and led to the establishment of the Varteg ironworks at c. NGR SO263055 in 1802 (Faber-Maunsell, 2006). Additional ironworks followed in due course and the areas were linked by the Blaenavon Tramroad to the coast. According to the Glamorgan Power Ltd report by Faber-Maunsell (2006), the earliest mining works in the area (circa 18th century) was thought to involve patch working along various veins for iron to supply to the Varteg Works. Tunnels were laid to then connect these various seams to further excavate the coal. All of this led to the opening of the Varteg Hill Colliery in 1860. This comprised of the Varteg Hill Pit (SO 261063) and Mine Slope (SO 262067) which were built mainly to supply the Varteg Ironworks. An incline was built which linked these collieries to the Monmouthshire Railway Eastern Valley section, which replaced the Blaenavon Tramroad in 1853 (Faber-Maunsell, 2006). Later on the incline was moved by a branch line of the LNWR, built in 1878 linking the colliery to the LNWR Blaenavon-Brynmawr Branch (GATT, 2008, [10 March 2009]). Consequential spoil tips had developed at the Varteg Hill as a result of overburden due to open-cast mining and tramways run over these tips. These are visible even today as unstable heaps of erodible waste. Two Mine Kilns, built in between the Varteg Hill Pit and the Bracy's Pit (Faber-Maunsell, 2006), for iron ore roasting (to separate the ore from the waste) remain. The waste is believed to have been dumped on site adding to the environment pollution.

A Big Baum Box type washing machine was erected on site and the waste was collected in a new tip, which exists even today. The use of big machines and transport vehicles led to the compaction and contamination of the land. The opencast mining operations approximately covered an area of 1.9km NW-SE by 0.6km from Forgeside to Varteg Hills and continued till 1956.

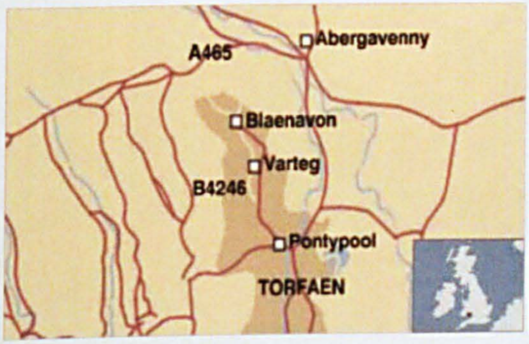


Plate 3.1: Varteg Hill and area showing the site of the proposed Varteg Hill Opencast Mine (BBC, 2011)

3.2 Post reclamation site condition

According to the British Geological Survey, the geology of the site comprises lower to middle coal measure strata (Halcrow, 1986; Faber-Maunsell 2006). These coal measures consist mainly of shales (mudstones) along with secondary siltstone, sandstone and thin coal seams. On site, hard quartzite sandstone casts are widespread. The site investigation showed that the site mainly comprised of light to dark grey soft clayey and occasionally orange, sandy silt soil with prominent sandstones, gravels, cobbles and coal shale. Figure 3.2 shows the historic area of Varteg in 1960 when mining was still active.

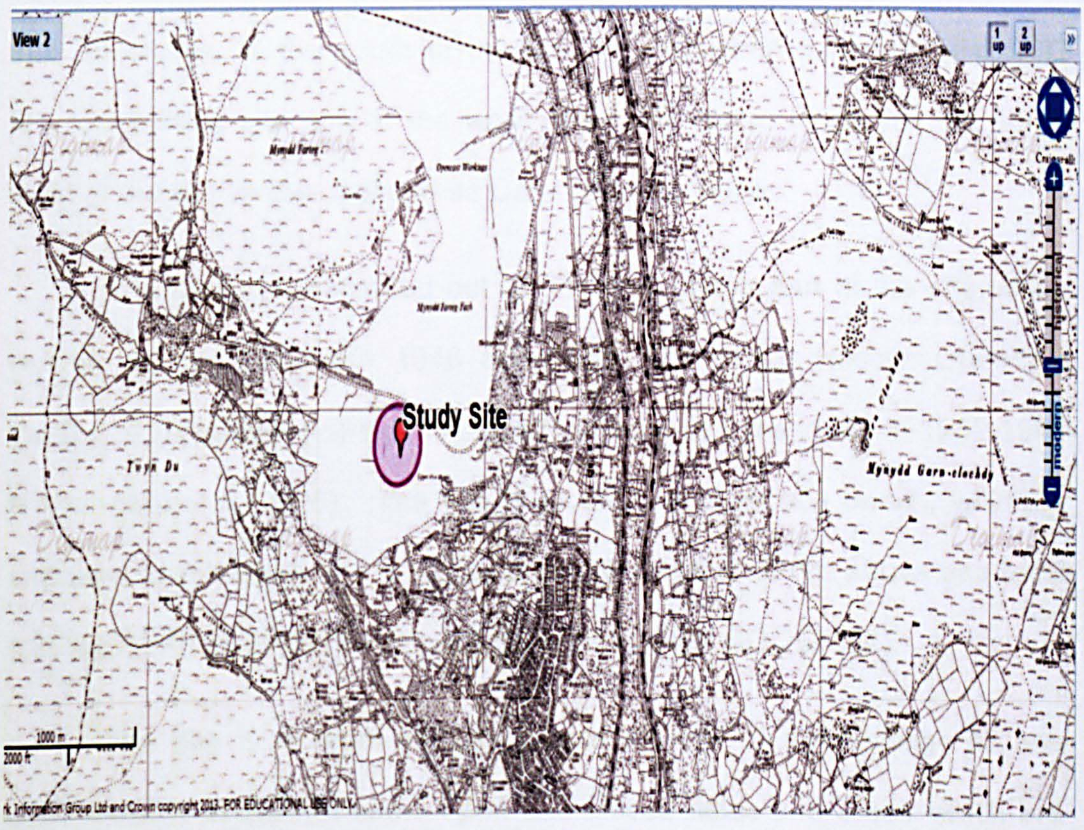


Figure 3.2.: Historic Map of Varteg in 1960.

The site comprising of coal spoil was reclaimed in 1963 under the guidance of the National Coal Board's Opencast Executive, whose aim was to restore the area to open common lands for grazing, rather than the woodland that covered much of the area before industrialisation (Haigh et al., 2008). The site comprises of unenclosed moorland set on reclaimed opencast area and visible spoil tips. Mixed species of mature grass and heather covers most of reclaimed site although visible patches of coal tips are scattered throughout the landscape. Currently most of the reclaimed area is under pastoral or derelict land use.

3.3 Site Description: Problems due to mining

The test site comprises of nearly 5 hectares of land on top of Varteg Hill, which lies to the south of the historic Blaenavon industrial UNESCO World Heritage site and to the west of B4256 Varteg Road. To the south west of the site lie the undisturbed Carnau British woods.

The research is carried out on the final phase part of the site which mainly operated between 1948 and 1956. The Waun Hoscyn Opencast Coalmine Extension (Varteg Hill) was reclaimed and reshaped in 1962-1963 (Faber-Maunsell, 2006). The site is located on a terrace bench, which is exposed to the south and west, at a height of about 370m above sea level ((51°44'44.77"N, 3°4'42.81"W; British National Grid: SO 255057).

The site is a relatively flat terrace on top of the Varteg Hill and consists of a mixture of some open and closed fields and areas which are used for grazing. There are prominent areas of rough spoil tips and remains of former mine work. Across the 5 hectare test are a series of 4 to 18-year-old forest plantation trials sites (Haigh et al., 2008).

Once the open cast mine site was reclaimed in 1963 under the guidance of the National Coal Board's Opencast Executive, the site did not live up to its expectation and soon after the site was reclaimed, problems such as gully erosion and poor grass growth due to compaction were encountered. The compact nature of the spoil was also reported in Faber-Maunsell (2006) report, which resulted in visible water logging. Therefore, in 1970 large sections of land between Varteg and Blaenavon were laid out for grass seeding trials (Haigh et al., 2008). Mixed species of heather and grass were grown as a part of the reclamation plan. Even today the grass patches are visible with prominent coal tips scattered throughout the landscape, although there is no actual data to confirm the outcomes of this work. There is also no evidence of the actual step by step remediation plan for the site anywhere in the literature. However, these trials did not remediate the site completely; as the 'common-land' status of the area meant that it was mainly impossible to prevent cattle overgrazing and public use which led to further auto compaction of the mine spoils (Haigh, 1992; Haigh & Sansom, 1999; Haigh et al., 2008).

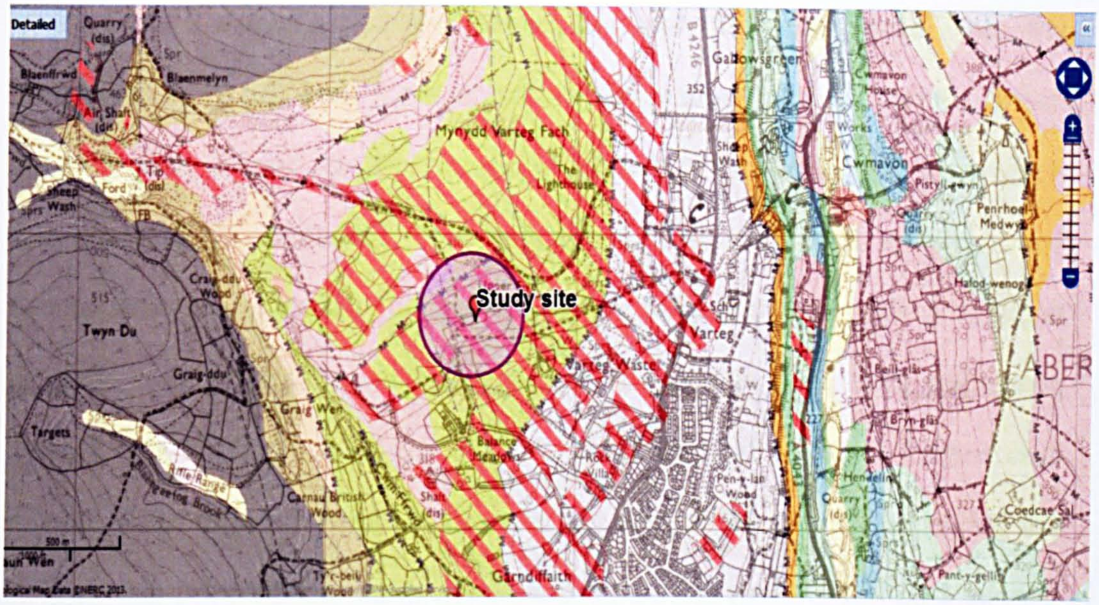
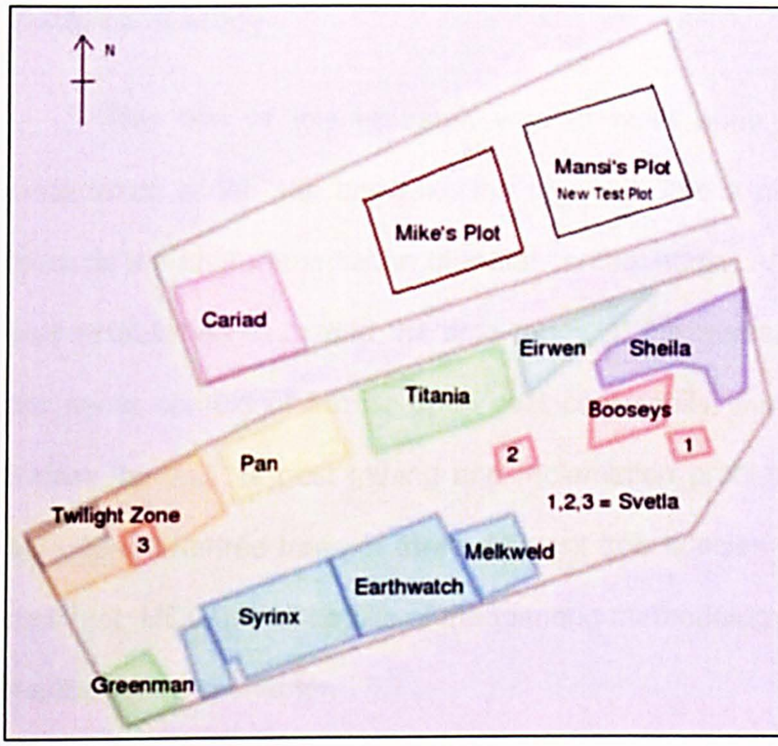


Figure 3.3.1: Geological map of present day Varteg and location of the site of study.

Figure 3.3.1 shows the geological map of present day Varteg. The map shows presence of underlying coal seams and the construction of artificially made ground on which the test site currently sits.

Plate 3.2: Google image of the area of study, showing the new study plot (Experimental Plot) (outlined in black), the older main forestry plots of Cradle for Nature.org (outlined in green) and the natural un-mined woodland (outlined in red).

Plate 2 shows a visible difference between the experimental plots, the CradleforNature.org Test Plots and the natural woodland at Carnau British woods, where mining was not carried out. Much of the Varteg Hill area which was subject to opencast coal mining suffers from soil erosion, soil compaction, metal toxicity, limited vegetation re-growth and severe sections of gully erosion and the officially 'reclaimed' land is constantly degrading (Haigh, 1993; Haigh et al, 2008). It is not dissimilar to other former coal mining areas found in South Wales and the rest of the country as during the time similar remediation plans were adapted across most of the coal sites in the country. The problem was acknowledged by the local government but because of the labour and cost involved, they were resistant to work actively towards solving the problem. Poor economic and environmental conditions have also contributed to a culture of hopelessness, alienation and emigration in local communities (Haigh et al., 2008). To this end, trees were first planted on the CradleforNature.org (CfN) Test Plots in 1991 by Haigh and environmentalist volunteers (previously Earthwatch's Reclaiming the Land' Project) and this was iterated annually until 2004. Their aim was to use a most ancient technology for restoring degraded lands: forest fallowing, which involves planting trees to rebuild, loosen and restore damaged (to a certain degree) contaminated and compacted mine spoils (Haigh et al., 2008). All trees planted on all the CfN plots were supplied by Elmcroft Growers of Newent in Gloucestershire. The figure below locates the main test plots planted on this site since 1991.



Plot Name	Year of Planting
Svetla 1	1991
Svetla 2	1991
Svetla 3	1991
Booseys	1991
TWZ	1992
Fairy Dust Corner	
Pan	1993
Titania	1994
Greenman	1995
Eirwen	1995
Syrinx	1996
Melkwoud	1996
Earthwatch	1996
Shiela	1997
Cariad	2003
Mike	2004
Mansi	2007

Figure 3.3.2: Plot plan with names of all reclaimed and experimental plot on the Varteg Hill (as given by CfN project) along with the year of plantation

Plate 3.3: Satellite image of Varteg Hill with the study plots superimposed.(Source: maps.google.co.uk)

3.4 Sites of study

This aim of this research was to build upon the work previously undertaken at the site and take the research into a new direction, namely towards the phytoremediation of metal contaminants. A new test plot, MD07, was established to extend the time-range of the research while addressing the same context of former open cast coal spoils, mining and reclamation history, as well as post mining and reclamation problems (Section 3.2 and 3.3). Nine hundred trees of three different tree species were planted on this new plot, MD07. Full details of the planting methodology will be discussed in the forthcoming chapter.

In addition to studies carried out on the new test plot, five of the older adjoining experimental plots were also selected for study in order to test the metal uptake capacity of trees over a longer time-span. Each additional test plot selected lies within the same topographic, hydrological and geo-ecological context. These plots were established on exactly the same minespoils on the same bench terrace of the same reclaimed land surface of 1963. The only major difference within these selected plots is the time of initial plantation.

All the plots were originally named by the volunteer group responsible for carrying out the original tree plantation and, without wishing to hurt any sentiments, for the purposes of this study; the plots have been renamed to make labelling simpler, as denoted in Table 3.1.

Table 3.1: Study Site Summary

Original Site Name	Year of Planting	Planting method	Site Code
Pan	1993	Trench/Pit	PA93
Titania	1994	Notch	TA94
Shiela	1997	Notch	SH97
Cariad	2003	Trench/Notch	CA03
Mike	2004	Ploughed/Notch	MI04
Mansi (Experimental Plot)	2007	Notch	MD07

The following section outlines the different plots studied, briefly examining their history, location, planting style and tree species used. All photographs for the adjoining study plots are courtesy of the larger project's "Cradle for Nature" (CfN) group website: www.cradlefornature.org

3.5 Planting and Planting method

Site preparation is an essential step for successful land reclamation (Maiti, 2012). The success of any planting is highly dependent on the ability of the plant to establish itself in the soil, which in turn is dependent on a quality soil preparation. Different planting methods have been advocated for achieving optimum plant growth (Forestry Commission, 1999; Haigh, 2001; Armstrong, 2009; Haigh et al., 2010). In line with the guidance by the Forestry Commission at the experimental site (MD-07) all trees were planted by notch style of planting. However, since 1991, on the other test plots trees have been planted using different planting styles namely pit style, notch style and trench style of planting as outlined in Table 3.1.

3.5.1 PA93

The first Varteg planting was carried out in 1991. Plot PA93 was planted, in November 1993, through a thin layer of snow. The location is a gently sloping surface at the upper edge of the upper convexity of the terrace bench. Tree species on this plot were planted in orchard style, 0.5 metre deep by 0.5 metre wide trenches, cut along the contour, and back filled with the inverted original soil profile. In common with later plantings, the planting scheme involved a mixture of native species. The mixture was dominated by Alder trees (*Alnus glutinosa L.)*, planted as a nursemaid species and because of their tolerance of waterlogged conditions (Haigh et al., 2013). Amongst the *Alnus spp*, were planted Scots Pine (*Pinus sylvestris*) and Durmast (Welsh) Oak (*Quercus petraea*), which was the dominant species of the woodland that preceded industrialisation in this area and which was intended as the final legacy of the planting. Because of their tolerance to windy conditions Rowan (*Sorbus aucuparia*) and Quickthorn (*Crataegus monogyna*) were added to defend the margins of the planting.

Subsequent plantings, plots PA-93, TA-94 and SH-97 were planted according to a similar design. These plots also shared similar topographic positions on the terrace bench; although because of the shape of the land boundary, later plots, especially SH97, were squeezed more on to the upper convexity of the terrace riser. However, to keep consistency with the main study MD-07, for this research, samples were only collected from sites on the flat terrace bench. The plots also retained the same basic mix of species although Silver Birch (*Betula pendula*) and Goat Willow (*Salix caprea*) were added to the mix from TA94 (1994) onward. The Silver Birch trees were planted for their deep rooting and ability to grow in low fertility soil, and Willow trees for their stabilising and land reclamation properties (Kuzovkina et al., 2004; Moffat, 2006). These are also high biomass producing species which are required in such spoils, which lack a basic organic top soil cover. Plate 3.4 shows the initial planting of the site in November 1993 and Plate 3.5 shows the growth of trees at PA-93 in 2010.

Plate 3.4: Site PA-93 during its initial planting in November 1993

Plate 3.5: Site PA-93 after 18 years of planting in July 2010.

3.5.2 TA-94

Plot TA-94 was first planted in November 1994. The tree species used on this plot were Alder, Birch, Oak and Pine. Like PA-93, the site was trenched using a JCB trenching machine and trees were then planted by notch planting method. Plate 3.6 shows plot TA-93 with four old trees and Plate 3.7 shows 17 year old trees at site TA-93.

Plate 3.6: Site TA-94 after four years of planting in 1998

Plate 3.7: Site TA-94 after 17 years of planting in July 2010.

3.5.3 SH-97

Plot SH-97 was planted in November 1997. The tree species used in this plot were Alder, Oak, Pine, Birch and Willow. This site was also trenched and, as before, trees planted into the back-filled trench. Many Alder trees were double planted, one at either edge of the trench. Plate 3.8 and 3.9 show the growth of trees at site SH-97.

Plate 3.8: Site SH-97 after one year of planting in 1998

Plate 3.9: Site SH-97 after 14 years of planting in July 2010.

3.5.4 CA-03

Site CA-03, MI-04 and MD-07 are located on the top end of the study site. These plots are separated from the other study plots by an ephemeral stream and a walkway. Plot CA-03 was planted in 2003 with Alder and Oak trees. Subsequently, some Birch and Willow trees have invaded the test plot as volunteers. Before planting, the plot was divided into a three-by-three Latin Square grid and a total of 900 trees of the three tree species were planted using three different planting methods. These were forestry style notch planting, in which an 'L' shape notch is cut into the soil and held open with the spade while the roots of the plant are inserted vertically down; pit planting, where in a deep and wide pit is dug into the ground wide enough to take the roots; and trench planting, where 0.5 metre deep by 0.5 metre wide trenches, cut along the contour, are dug and trees are planted along the trenches in orchard style. This test was done to identify the best planting method. Plate 3.10 shows the ground preparation before trees were planted in 2003. Notice the soil conditions after disturbance. Plate 3.11 shows seven year old trees on site CA-03.

Plate 3.10: CA-03 before and after ground preparation in 2003.

Plate 3.11: Site CA-03 after 7 years of planting in July 2010.

3.5.5 MD-07

Plot MD-07 is the main experimental plot and lies in the *north-east* corner of the study area on Varteg Hill, across the same ephemeral stream and track from SH-97. The plot contained compacted soils and was covered with a sparse cover of seasonal grass, moss and lichen. In common with its neighbours, its problems included soil compaction, water logging, limited vegetation re-growth and suspected metal toxicity.

Analysis of the soil physical properties before planting showed high soil bulk density of 1.63 g/cm^3 at 35-40cm and 1.81 g/cm^3 at 50 cm depth, low Nitrogen, Phosphorus and Potassium values, low soil organic content in the range of 0.32 – 0.40 was recorded. Soil pH was in the range of 5.3 – 5.7 with occasional hot spots with values as low as 3.8. The top-soil layer consisted of a thin 2-3 cm organic layer above weathered clays with clasts of sandstone, coal shale and some coal in the top layers.

The plot was divided into a three-by-three Latin Square grid and after the initial soil sampling was carried out, Alder and Birch trees, along with a new experimental species Larch (*Larix decidua*) species were notch planted in November 2007.

Larch is a sturdy tree species, which is widely grown in the UK, initially for its timber. Later is has been identified as suitable for the rehabilitation, re-vegetation and reforestation of degraded lands (Gower and Richards, 1990; Ryu et. al., 2009; Kioke, 2009). It is considered as one of the most desirable tree species by the UK Forestry Commission (Bending, 1994). Plates 3.12 shows plot MD-07 before tree plantation in 2005 and Plate 3.13 shows young four year trees in 2011.

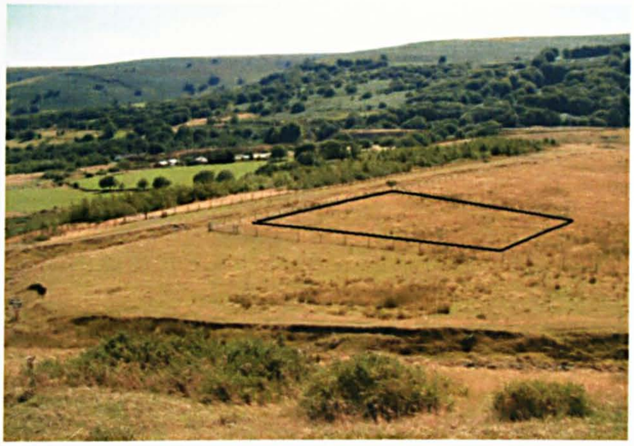


Plate 3.12: View of plot MD-07 from above the top field before planting in 2005

Plate 3.13: View of Plot MD-07 from above the top field after 4 years of planting in 2011.

3.5.6 MI-04

Plot MI-04 was planted in 2004 by the staff and students of Swansea Metropolitan University. Before planting, the plot was ploughed as per the recommended forestry guidelines using a normal tractor drawn agricultural plough (Moffat and McNeil, 1994). Although there is some research to show that ploughing the site before planting can improve plant establishment (Bending, 1993; Moffat et al., 1991), here this had an unfortunate effect. The plough blades made little impression on the compacted minespoils and penetrating just a few centimetres, did little more than peel away the grass turf that had colonised the original surface. Over the years, this ploughing has increased the problems of surface water runoff. There have also been large colonies of ants across the plot. Alder and Birch, the most widely used trees in land reclamation, were then notch planted into the newly exposed mine-spoils but their success has been limited.

Plate 3.14: MI-04 after ploughing and tree plantation in 2010.

Plate 3.15: Six year old tree above the ploughed trench.

Plot 14 shows the effects of ploughing before tree plantation. Notice the deep fissures in the soil profile and the presence of big rocks and shale at the top layer. Plot MI-04 was a failed reclamation experiment and has to be treated as a special case during sample analysis. Despite the challenges that this plot presented the results observed have been included when appropriate to the statistical test.

3.6 Summary

Located on top of Varteg Hill in the county borough of Torfaen in South Wales is an area characterised as a post-industrial mineral extraction landscape (Faber-Maunsell, 2006). The study site consists of approximately 5 Ha of degraded, compacted land, which has been created as the result of early land reclamation works following the closure of the Waun Hoscyn Extension (Varteg Hill) Opencast coal mine in 1962-1963. The site consists of mine spoils from these opencast workings, mainly coal measure shales and sandstones but with some admixture of coals and ironstone, some from deep mine spoil tips that covered part of the original mine site. After mining ended, the site was reclaimed by grass seeding as per National Coal Board Opencast Executive recommendations, although local people dispute that this was done properly and point to a nearby berm of topsoil, which was 'never redistributed'. These activities have left the site in an unreclaimed condition with poor vegetation cover, overgrazed lands, irregular landscapes with compacted soil and water logged marshy areas in depressions. Poor reclamation and management have allowed soil degradation, compaction, and gully erosion. In an attempt to reclaim the soil, a group of environmental volunteers have planted trees annually since 1991 as the Cradle for Nature (CfN) initiative. Different tree species and planting methods have been tested and tree vitality has been measured. Building on this project, this research has selected a plot of 30 x 30 m and planted 900 trees of three species in an attempt to evaluate the value of such tree plantings for the remediation of metal contamination and to gain a deeper understanding of mine-spoil chemistry and tree interactions.

Chapter 4 Materials and Methods details both the design of the planting and the analyses undertaken. This research looks at the chemical properties of the soil underlying the new study plot and the uptake of metals by leaves. It also looks at the soil and leaf chemistry of trees from older adjoining plots.

CHAPTER 4

MATERIALS AND METHODS

4 MATERIALS AND METHODS

Research Questions (RQ):

The review of literature helped identify some important questions which will be answered in this research using a variety of techniques, materials and methods. The main research questions identified at the Varteg Hill site in South Wales, UK are:

1) What are the concentration levels and spatial distribution of key metal contaminants: Zn, Cd, Mn, Cu and Pb in the reclaimed mine spoil at Varteg, South Wales?
2) What level of metal uptake is possible by Alder, Birch and Larch trees as represented by foliar analysis on the Varteg test plots?
3) How is foliar uptake of metal contaminants affected by the soil composition and age of tree plantation?
4) How is metal concentration in soil affected by the age of plantation?

The above mentioned research questions will be answered by various techniques which are outlined in the following section. Every heading will also identify the research question it aims at answering (RQ-).

4.1 Experimental Plot Selection

The current experimental study plot is situated on the reclaimed opencast coal spoils in Varteg, South Wales. A plot for the tree plantation was selected on the basis of an attempt to situate them over the degraded spoil, keeping in mind the topography of the adjoining plots studied. Care was taken to plant the trees on a similar landform as the adjoining reclaimed plots, which consisted of coal spoils and was in line with all the problems associated with coal spoils as outlined in section 1.6. The locations of the experimental plot and the adjoining reclaimed plots are shown in the aerial photos (plate 2 and 3) of the study sites in Chapter 3.

4.2 Species Selection

The species of interest used in the present study were *Alnus glutinosa* (Alder), *Betula pendula* Roth (Birch), *Larix europaea* (Larch), based on their ability to cope with the hostile conditions, such as soil compaction, problems of deep rooting, tolerance to drought conditions and metal resistance. *Betula* species are known to tolerate elevated levels of heavy metals and have deep rooting properties and hence they are considered as hardy pioneer species (Moffat and McNeill, 1994, Utriainen et al., 1997; Kozlov et al., 2000; Featherstone, 2004). *Alnus* species are a popular choice due to its nitrogen fixing properties and have been extensively used by the forestry commission for planting on urban, mined and brownfield sites (Fitter and Hay, 1987; Moffat and McNeill, 1992; Moffat and McNeill, 1994; French, 2004). *Alnus* species have also shown high survival rates under poor soil conditions (Moffat and McNeill, 1994; Roy, et al., 2007). *Larix* have been used in the past for the reclamation of former mine spoil sites (Bending and Moffat, 1999). *Larix*, when planted in a mixture with other trees such as *Betula* and *Alnus* species, have shown promising results as the latter provides the necessary nitrogen through symbiotic relationship with nitrogen fixing bacteria found in root nodules (Moffat and Roberta, 1988; Mofatt and McNeill, 1994). They are also widely used for its timber.

Selection of the tree species was based on:

- Detailed review of previous research into the role of the three selected species in heavy metal uptake and their role in restoration of former spoils (see section 2.11).
- Forestry commission guidance.
- However, in spite of their extensive use in urban, mined and brownfield sites, there is a lack of knowledge about the remediation capabilities of these trees. This research will explore the metal uptake and remediation potential of these trees.
- Similarity of the species planted on the experimental plot (MD-07) with the adjoining study plots, makes it possible to carry out a comparison of the uptake and storage of metals in leaves of trees of different age groups, although the earlier trials did not use the non-native *Larix* species.

The species selected and planted for the use of this study are tabulated in Table 4.1. All the trees planted were two year old saplings supplied by Elmcroft Growers of Newent in Gloucestershire.

Table 4.1: Tree species selected for the present study.

Species Planted	Abbreviation	Total No planted
Alnus glutinosa	AL	300
Betula pendula	BE	300
Larix europaea	LA	300

4.3 Plot preparation and design

The experimental plot was of a standard size of 30m x 30m which was sub-divided into 9 subplots. The tree species were planted in a classic Latin Square Design which is most widely used in agriculture (Lakic, 2002; Palaniswami, 2006; Haigh et al., 2013). Within each subplot, half of the trees were given a dose of natural compost (1kg) and the other half was a control (Figure 4.1).

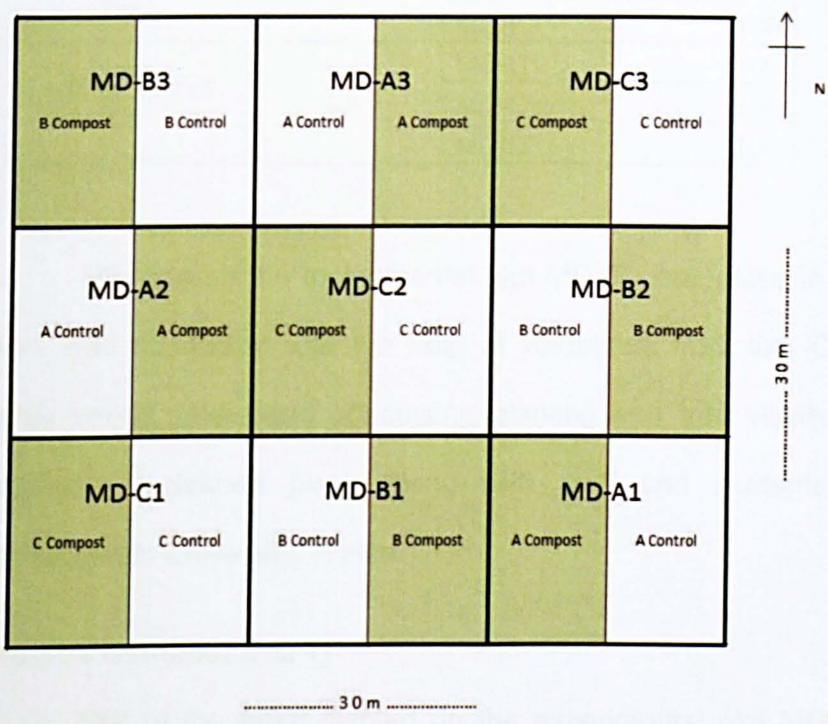


Figure 4.1: 9 sub-plots on the Experimental plot MD-07

On each of the sub-plots 100 saplings of the chosen variety were planted making a total of 900 trees of three different species. Keeping in mind best practice guidance for planting (Palaniswami, 2006) the trees were planted at 1m intervals between and within rows. This spacing was adopted for all experimental taxa.

Table 4.2: Number of trees planted on the sub-plots on plot MD-07

Species Planted	Species Abbreviation	Plot name	No planted
Alnus glutinosa (A)	AL	MD-A1	100
		MD-A2	100
		MD-A3	100
Betula pendula (B)	BE	MD-B1	100
		MD-B2	100
		MD-B3	100
Larix europaea (C)	LA	MD-C1	100
		MD-C3	100
		MD-C3	100

Planting on the experimental plot MD-07 took place in November 2007. This was conducted with the help of volunteers from the 'Cradle for Nature' team which have been conducting planting and tree vitality experiments on adjoining reclaimed plots, along with staff and students from Swansea Metropolitan University, Wales.

4.4 Soil Compost (RQ 1)

Half of the trees planted on the experimental plot MD07 were given a one-time dose of approx. 1kg of organic compost at the time of planting and half of the trees were planted without any compost. This was done to allow the new sapling to get acclimatised to the soil and soil environment. Compost was also added to test the possibility of a measurable change in the plant metal uptake.

4.5 Sample Collection

Soil samples were collected at a depth of 20-30cm from the top soil, with the help of a random number table to create co-ordinates for contamination mapping. Due to heavy soil compaction it was difficult to collect samples from deeper layers. The samples were tested for physical and chemical parameters. Standard tested were carried out for determining the soils physical composition. The standard tests carried out were Soil pH, Soil Moisture Content, Soil Organic Content, Soil Texture and Colour, Loss on Ignition and Carbonate Content (Heiri et al., 2001)

Choice of Metal selection: As this research looks at the contamination and remediation of degraded coal mine spoil, metals that naturally occur at such sites and that are specifically related to and pose a threat due to coal mining industry were considered. Previous metal, coal and iron ore mines which were widely spread in and around the site of investigation, added to the contamination of the site, as outlined in section 3.1. Cottenie's (Cottenie et al., 1982) chart for the distribution and uptake of metals by plants was the framework used for the selection of metals, as it represented the entire range of metal availability from highly available (Cd and Zn) to moderately available (Mn) to least available metals (Cu and Pb) for uptake. As outlined in table 2.4 and Section 2.6, Cd, Zn, Mn, Pb and Cu were selected due to the threat these metals pose to the site and the wider environment.

Most of these metals are essential nutrients to plants but when present in larger quantities can lead to phytotoxicity. Even at low quantities Cd, which is a non-essential element is readily available and found to be poisonous to plants, animals and humans alike (Johnson et al., 1978; Cooke et al., 1980; Alloway, 1995a). Cd is found to be present in close association with Zn which is present in the soil in colossal amounts and also highly available for uptake (Kiekens, 1995). On zinc-rich soils only a limited number of plants have a chance of survival (Reiman, 2002). Mn although an essential element, is found to cause metal toxicity at higher concentrations (Smith and Paterson, 1995; Kasraei et al., 1996). Manganese has been identified as a hazardous waste recommended for inclusion on the EPA National Priorities list (NPL) (HazDat, 2008; ATSDR, 2008) Cu and Pb although least available for uptake by plants, are found in abundance on such sites and cause toxicity at high levels (Alloway, 1990; Sieghardt, 1990; Alloway, 1995a; Baker and Sneft, 1995; French, 2004). All the metals under study are inversely proportional to soil pH, which means at low pH levels the availability of these metals increases (Bolan et al., 2003; Kirkham, 2004; Schulte and Kelling, 2004). Mine spoils generally have a pH ranging from 3.5 to 5.8 which can affect the presence and availability of these metals. Because of the presence, availability and toxicity of these metals at high levels, it is important to identify and quantify their presence on site.

In order to quantify the background concentrations of the potential heavy metals present in the soil, each soil sample was tested for the presence of the five metals: Cadmium (Cd), Zinc (Zn), Manganese (Mn), Copper (Cu) and Lead (Pb). Analysis for presence of Arsenic (As) was planned but could not be tested due to the lack of the analytical equipment availability.

Atomic absorption is the most widely used technique for determining elements in every conceivable matrix, especially the metallic ones. Various aspects must be considered to determine the technological potential of chemically characterising a sample (Skoog, 1985; Pyle et al., 1996). These may include: Sensitivity, cost, simplicity, range of concentration, precision, accuracy and waste generation. Of the different techniques available for analysis Atomic absorption spectroscopy (AAS) and inductively coupled plasma-atomic emission spectroscopy (ICP-AES) have been the preferred choice for elemental analysis of soil extracts and aqueous samples because of their reliability, utility, sensitivity and precision, analytical interferences and elimination and difficulties with contaminants (EPA, 1986; Robinson, 1995; Pyle et al., 1996). The other less commonly used techniques are Flame Photometry, Emission Spectroscopy and Potentiometric Stripping Analysis.

AAS is a relatively inexpensive and easy to use technique as compared to IEP-AES which is moderately easy to use but expensive technique as compared to AAS. Although ICP-AES is slightly quicker than the AAS and is applicable to elements for analysis, it does present a spectral interference problem which is well documented (Pyle et al., 1996; Thermo Elemental, 2001). This is due to line rich spectra produced by hot plasma source. The sample preparation time is fairly less for AAS as compared to ICP-AES. AAS also offers better sensitivity as compared to ICP and much better than flame photometry or emission spectroscopy (Robinson, 1995).

Based on the availability of the instruments, chemicals used in preparation of standard solutions used for detection, ease of use of instrument, sample preparation time and costs involved, all elements were analysed and tested using an Atomic Absorption Spectrophotometer (AAS). AAS analysis was conducted using an Agilent Technologies SpectrAA 220FS at Oxford Brookes University (Figure 4.9.1). The working of this instrument is discussed later in the chapter.

Random samples using random number table, were collected from the experimental plot MD-07. This was done to ensure maximum ground coverage for optimum number of samples. Care was taken to collect samples from areas close to the tree roots and also in-between two trees for maximum coverage. A total of 75 samples from the entire plot were collected which should be sufficient to detect the presence of heavy metals in soil on the MD-07 plot as well as the locations of any chemical hotspots. This sampling method was applied to all the study plots involved in this research.

In order to study the uptake capacity of trees on mine spoils, it was important to analyse the soil samples to establish the initial baseline metal content of these soils. To that end, soil samples were collected from the virgin soil in July 2007 prior to planting. These are referred to as Year 0 samples on MD-07 as they predate planting. Additional soil and foliar samples were collected in 2009 (Year 2) and 2010 (Year 3) from all of the study plots. This information is tabulated in table 4.3. For ease of study, 2007 samples are called Year 0 samples, 2009 samples are called Year 2 samples and 2010 samples are called Year 3 samples. Since the site was planted in November 2007, soil and leaf samples were not collected in July 2008 (year 1). The aim was to give enough time for the tree saplings to acclimatise to the soil environment and grow sufficient foliage to allow optimum sampling.

Soil samples were collected from the top soil layer at a depth of 20-30cm, although at some places, this was difficult due to the presence of large stones in the top layers of the soil profile. The soil samples were collected using gardening trowel or spade and were bagged in zip lock bags, labelled and brought to the laboratory. In the laboratory, these samples were prepared using standard sample preparation procedures (Bradshaw, 1980; Bending, 1999; Dingus, 1999; French, 2004; Head, 2006) in which the samples were air dried at room temperature, ground with the help of a mortar and pestle. Care was taken to prevent disintegration of smaller coal shales. The samples were then dry sieved through a <2mm sieve. This protocol was carefully followed throughout all the sampling during this research. These samples, which were later used for all of the physical and chemical analysis, were recorded and stored in dark, cool conditions in the laboratory at Oxford Brookes University.

Table 4.3: Sampling schedule at the different study plots.

Year	Plot	Soil	Alder Leaves	Birch Leaves	LarchNeedles
Year 0 2007	MD-A07	√			
	MD-B07	√			
	MD-C07	√			
Year 2 2009	PA93	√	√		
	TA94	√	√	√	
	SH97	√	√		
	CA03	√	√	√	
	MI04	√	√	√	
	MD-A07	√	√		
	MD-B07	√		√	
	MD-C07	√			√
Year 3 2010	PA93	√	√		
	TA94	√	√	√	
	SH97	√	√		
	CA03	√	√	√	
	MI04	√	√	√	
	MD-A07	√	√		
	MD-B07	√		√	
	MD-C07	√			√

Table 4.3 outlines the sampling schedule across the three years of sampling over the different study plots. Soil samples were collected from all the plots in year 0, 2 and 3. Leaf samples were collected post planting in years 2 and 3.

4.6 Soil Physical and Chemical Analysis (RQ 1)

4.6.1 Bulk Density

Soil bulk density is an indicator of soil compaction. As minespoils commonly suffer from soil compaction this is an important analysis. Soil bulk density was measured using the standard procedure outlined by Cresswell and Hamilton (2002) and has been widely used by many researchers (Phelps, 1983; Sheoran, 2010; Gudadhe, 2012). Core cylinders were used to collect soil samples for different depths in the soil. Samples were brought to the laboratory, oven dried (110^{0}C) and weighed. Bulk density is calculated as dry weight of soil divided by the volume.

4.6.2 Soil NPK (Nitrogen, Phosphorous and Potassium content)

One of the major problems associated with mine spoils is the lack of top soil material. Such sites suffer severely from NPK deficiency (Coppin and Bradshaw, 1982; Moffat and McNeil, 1994; Sheoran et al., 2008). For successful planting and site reclamation it is essential to know the NPK values of the soil. Soil samples were tested for NPK values using HANNA HI 3895 Soil NPK Kit in Year 0 and Year 3. The colour charts gives accurate measurements of the level of NPK in the soil.

4.6.3 Laboratory Analysis Methods

All the chemicals and acids used were of analytical grade. Glassware and plastic ware were prepared using standard laboratory guideline (Head, 2006). These were washed with 2% chemical decontamination solution before washing in 3% HNO_3 followed by acid wash and rinsed with deionised water and finally oven dried. Bucher funnels and flasks were used for heavy metal extraction from soil samples.

4.6.4 Soil pH

Soil acidity is one of the well-known problems associated with mine spoils (Bradshaw, 1982; Haigh, 1995; Lasat, 2000). Haigh (1995) pointed out that a characteristic feature of British mine spoils is a gradual reduction in pH over time due to soil weathering (for example from pH 5.5 to 3). Identification of soil pH is an essential measure for understanding soil quality. Soil pH was measured in the field using a digital pH meter with probe by BlueLab. Additionally pH (H_20) was also measured in the laboratory using the Rayment and Higginson (1992) method by mixing 10gm of air dried soil sample into 50ml distilled water and shaking on an orbital shaker for an hour before testing with a calibrated pH meter, fitted with a glass bulb electrode. To each sample 4ml of 0.125m $CaCl_2$ solution was added in order to make a 0.01m $CaCl_2$ solution. The pH ($CaCl_2$) was again recorded for the samples.

4.6.5 Loss on Ignition (Heiri et al., 1999)

Loss on Ignition (LOI) method proposed by Heiri et al. (1999) was used to calculate the soil organic matter (OM) and Carbonate Content (CO_3). This method has been widely accepted and used for determining the weight percentage organic matter and carbonate content in soil by means of LOI by sequential heating of soil samples in a muffle furnace. Heiri et al. (1999) also showed that the location of the samples in the muffle furnace can influence the results; hence the sample positions were randomised in the furnace and were placed in triplicates. In the first step organic matter is combusted to ash and carbon dioxide at a temperature between 500 and 550^{0}C and the OM is calculated using the following formula:

$LOI_{550} = ((DW_{105} - DW_{550})/ DW_{105}) * 100$ to give % organic matter (OM).

In the second step, carbon dioxide is evolved from carbonate, leaving behind oxide and the CO_3 is calculated as:

$LOI_{950} = ((DW_{550} - DW_{950})/ DW_{105}) * 100$ to give % carbonate content (CO_3) (Heiri et al., 1999)

4.6.7 Chemical Analysis of Soil Samples

Once collected, all the soil samples were digested by Ethylenediaminetetra-acetic acid disodium salt (EDTA-Na_2) solution and analysed using the Agilent Technologies SpectrAA 220FS, Atomic Absorption Spectrophotometer (AAS) as described in section 4.5. The protocol for metal extraction from soil samples is included in Appendix A.

Along with the AAS analysis, Year 0 soil samples were also used for different physicochemical analysis mentioned under section 4.6.1.

Year 2 and Year 3 soil samples were also analysed for the presence of heavy metals using AAS and for soil pH.

4.6.8 Foliar Sample collection and analysis

The samples were collected in July of 2009 (year 2) and 2010 (year 3). As with the soil samples, foliar samples were also collected using a random number table. Only the fully open larger leaves were collected from the top three quarters of the tree to ensure maximum storage of metals. Care was taken to collect an equal number of leaves from each tree sampled. All the foliar samples were collected in paper bags, labelled and immediately brought to the laboratory to avoid rotting. In the laboratory, the foliar samples were oven dried at (75-80^{0}C), allowed to cool and restored in the same paper bags to prevent cross contamination. Foliar samples were digested using the Milestone MLS-1200 MEGA microwave digester and then analysed using the AAS.

4.6.9 EDTA Extraction of Soil samples

Rationale

Soil is considered to be a multi-component complex system, acting as a reactor of the physical, chemical and biological behaviour of soils, making it difficult to assess the behaviour of pollutants in that soil (Manouchehri et al., 2006). Most research based on heavy metals focuses on the distribution and availability of these metals in the soil. The availability of metal concentrations for plant uptake differs considerably from the total amount of metal in the soil. This availability is heavily dependent on some of the soil characteristics such as organic matter, pH, adsorption etc. discussed in Chapter 1. In order to facilitate the extraction process, chelating agents such as EDTA, $CaCl_2$, NTA, DTPA etc. are used (Bermond et al., 1998; Barona et al., 2001; Fangueiro et al., 2002; Prokop et al., 2003; Cornu et al., 2007). There is much debate about the best possible chelating agent, but this is beyond the scope of this study, although this has been widely reviewed by many scientists (Chen et al., 1996; Ure, 1996;

Quevauviller et al., 1996; Dickinson et al., 2000; French, 2004; Manouchehri et al., 2005).

EDTA has been successfully used on a large scale in soil science for predicting the bioavailability of heavy metals and soil remediation processes (Feng et al., 2005; Dean, 2010; Dao et al., 2012). Like other chelating agents, EDTA might have some limitations with respect to the actual plant metal; however, it was only used for extraction of metals from soil samples. It was also a preferred choice because of its ease of use and the opportunity to make a contribution to the new data on its application and practical use on opencast mine spoils with a heterogeneous mixture of metal contaminants. Hence, for the purposes of this research EDTA was used to extract metals from soil samples.

Procedure

All soil samples from Year 0, 2 and 3 were digested in the laboratory and heavy metals were extracted by a powerful chelating agent ethylenediaminetetra-acetic acid disodium salt (EDTA-Na_2) as described by Quevauviller, et al. (1996). 10g of air dried soil samples were weighed into labelled centrifuge tubes to which 30 ml of EDTA solution was added. With every batch of 20 tubes containing soil samples, 1 tube with only the EDTA solution was prepared which served as a control. The samples were shaken on an orbital shaker SLM – OS – 300 for about an hour and then centrifuged at 3000 rpm for 5 minutes. From each tube, the supernatant was carefully filtered under vacuum into a Buchner flask taking care not to disturb the sediment pellet at the bottom of the centrifuge tube. Some more EDTA was added to the tubes and the process was repeated. The final filtrate was carefully decanted into labelled 100ml volumetric flasks with the help of Whatman 42 filter paper and made up to 100ml with deionised water prior to metal determination.

4.6.10 Microwave Digestion of Foliar samples

All the foliar samples were digested using the MLS-1200 MEGA Microwave Digestion System (MDS) with new MDR (Microwave Digestion Rotor) Technology. This method allows fast, simple, reliable, rapid and safe digestion of samples in a replicable and controlled environment where temperatures and pressures are maintained during each run (MLS Manual 1995). The MDS allows one step digestion of simultaneous samples at low and high boiling acids. The closed system minimises loss of sample and cross contamination along with ensuring regulated heating for all samples during all the runs. The digestion vessels used are specially designed and pressures sealed, and allow high temperatures to be reached which ensures complete digestion of the samples with no carbon residue. A blank reagent vessel was run with every batch of 10 sample vessels. Each vessel was filled with 1gm of dry crushed foliar sample. To this 5ml of HNO_3, followed by 2ml of H_2O_2, was added. Several dry organic materials often react violently when in contact with concentrated acids. To avoid any danger, 2-3ml of distilled water was added to each vessel to slow down the reaction. Once the vessels were filled and secured they were placed into the microwave cavity of the MDS unit and the programme was run for 20 minutes. Once the digestion programme was complete the MDS unit was allowed to cool for approximately 10-15 minutes and vessels were uncapped. The digested samples were transferred to 100ml volumetric flasks and made up to 100ml with deionised water prior to metal determination using the AAS. Extraction procedure for foliar samples was adapted from the MLS -1200 MEGA Operator Manual (1995)

4.7 Apparatus and Working of AAS

Analysis of EDTA extracted soil samples of years 0, 2 and 3 and HNO_3/ H_2O_2 extracted foliar samples were analysed by the Atomic Absorption Spectrophotometry (AAS). AAS analysis was conducted using an Agilent Technologies SpectrAA 220FS at Oxford Brookes University, Life Sciences Department (Plate 4.1).

Prior to using the AAS, the purity of the reagents was carefully checked to ensure accuracy of analysis. Reagents were also checked for the element of interest by running blank determinations. New bottles of standard stock solutions (1000 µg/mL) were used. Care was taken in soaking the apparatus in dilute nitric acid and rinsing thoroughly with distilled water prior to use. Only concentrated standards (1000 µg/mL) were stored. Any standard stock solutions prepared for analysis also known as 'working standards' were used immediately, as they have been found to deteriorate quickly (Koplik, 2010). Any leftover working standard solution was carefully disposed of.

4.7.1 Flame Spectroscopy

Flame spectroscopy is a useful analytical technique, which has widely been used for quantitatively and qualitatively analysing different elements in a given sample (Nichani, 2006). In this method the sample, in the form of a homogeneous liquid, is introduced into a flame where thermal and chemical reactions create "free" atoms capable of absorbing, emitting or fluorescing at characteristic wavelengths (Perkin-Elmer, 1996). Flame spectroscopy can be subdivided into the different processes such as Flame Emission Spectroscopy, Atomic Fluorescence Spectroscopy and Atomic Absorption Spectroscopy (Haswell, 1991).

Plate 4.1: Flame Atomic Absorption Spectrophotometer at Oxford Brookes University, Department of Life Sciences.

4.7.2 Atomic Absorption Spectroscopy

The technique of Atomic Absorption was first developed by Sir Alan Walsh of C.S.L.R.O. in the mid 1950's, and has now become one of the most preferred methods of elemental analysis. Walsh discovered that the majority of free atoms in the commonly used flames were present in the ground state, and that the flames did not have enough energy to excite these atoms (except for the Group I elements). Hence, a light source is used to excite these free atoms, which emit a narrow spectral line of the energy in the flame and the decrease in energy (absorption) was then measured (CMET, 2005).

The absorption measured is said to be proportional to the concentration of free atoms in the flame (CMET, 2005), and is calculated by the Lambert-Beer law.

$$\text{ABSORBANCE} \quad \log_{10} = \frac{I_o}{I_t} = K.\ C.\ L.$$

Where,

I_o = Intensity of incident radiation emitted by the light source

I_t = Intensity of transmitted radiation (amount not absorbed)

C = Concentration of sample (free atoms)

K = Constant (can be determined experimentally)

L = Path length

4.7.3 Components of AAS instrument

Hollow Cathode Lamp (HCL)

This is used as the radiation source to excite the free atoms in the flame. The hollow cathode lamp produces a narrow spectral line characteristic of the material of the cathode and the fill gas (neon or argon). Figure 4.2 shows the construction of a hollow cathode lamp. The hollow cathode (made of the element or its compound) and ring anode is enclosed in a glass envelope, filled with either argon or neon at a reduced pressure of 7.5 mBar (10 torr) (Agilent, 2000). The fill gas is chosen to reduce the spectral interference.

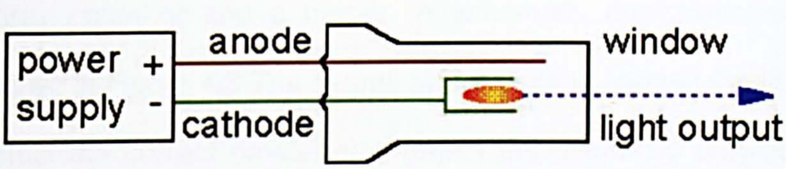


Figure 4.2: Schematic representation of HCL (Tissue, 1996, www.scimedia.com)

A potential of 150-400 volts is applied to the electrodes (anode positive and cathode negative), which causes the fill gas to ionize. The gas ions are accelerated by the applied potential, and collide with the cathode releasing a cloud of atoms from the cathode (this process is termed 'sputtering') (Haswell, 1991). These atoms are further excited by collisions with gas ions to a high-energy state. The atoms then emit characteristic wavelength as they fall to the ground state. Both the cathode and anode are designed to produce a stable contained discharge, and this yielded a very narrow line output (typically 0.001 nm) (Haswell 1991).

Atomization

For atomic absorption to occur, the atoms have to be introduced into the excitation beam as "free" atoms. Analysis is normally conducted from a solution in which the atoms are chemically bonded and solvated. To form free atoms, the solvent has to be removed and the chemical bonds broken to form free atoms (Issac and Kerber, 1971). In order to atomise the metal ions into free-state a hot air acetylene flame was used.

Flame atomization

The flame atomizer consists of three major components, a nebulizer, a spray chamber and a burner. A schematic representation of the system is shown in Figure 4.3 The overall system has to convert the liquid into an aerosol, select the correct droplet size (reject the oversized droplets) and transfer the sample to the burner, where atom formation occurs (Haswell, 1991). This is the heart of an AA system. If too large a droplet is introduced into the flame, the atoms may be lost before they are "freed" to absorb.

Examination of the process shows that the particles have to be de-solvated and converted to "free" atoms, before the flow of gases "wash" the particles out of the light beam (Ramirez-Munoz, 1968; Haswell, 1991). The operations of the basic components are:

Nebulizer

The nebulizer used in atomic absorption is pressure operated in which the flow of gases through the aperture creates a vacuum drawing the sample up into the capillary and the formed aerosol is introduces the sample into the flame, where aerosol is dissolved, evaporated and the analyte is atomised (Koplik, 2007). As the solution passes through the venturi an aerosol is formed. The nebulizer is designed to accept only specific sized droplets. For optimum sensitivity, particles of a consistent size (~ 5pm - 10pm) should reach the burner. Bigger droplets are prevented from entering into the flame and disposed of to the waste chamber (Issac and Kurber, 1971; Agilent, 2000). In an AAS two types of flame are commonly used, Air-acetylene flame and Nitrous oxide - acetylene flame. These flames are specific for the elements under examination. During the analysis of samples air-acetylene flame which is used for readily atomizable metals (Mn, Cd, Cu, Pb, Zn, Mg and Fe) (Koplik, 2007) was used.

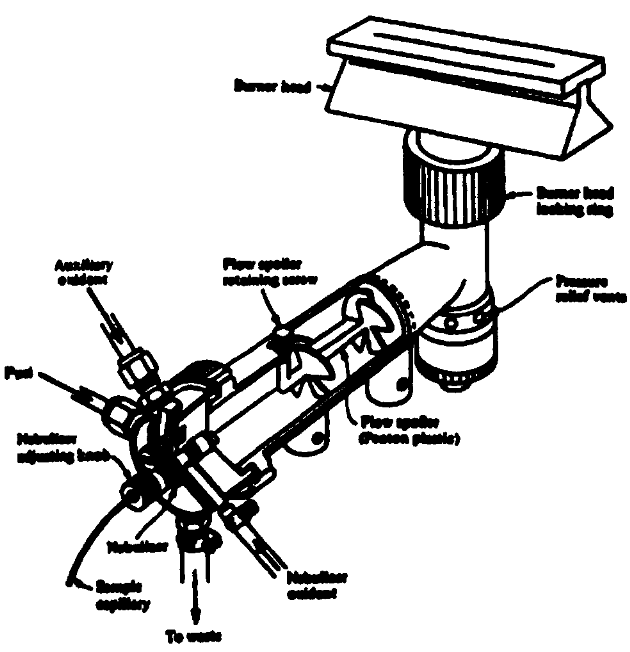


Figure 4.3: Schematic diagram of a Flame Atomizer (NMSU, 2006)

Monochromator

In atomic absorption spectroscopy a monochromator of high resolution is not essential. A medium resolution monochromator capable of 0.2 nm resolution which is adequate was used. The resolution of a monochromator (the minimum repeatable band-pass) depends on a variety of factors such as focal length, number of lines per millimetre on the grating and the width of the entrance and exit slits. For atomic absorption spectroscopy the most common slit sizes used are 0.2, 0.5 and 1.0 nm (Angilent manual, 2002). For the elements tested a slit size of 0.2 was used.

Detector

A photomultiplier tube (PMT) is almost exclusively used in atomic absorption as the detector. PMTs are very similar to phototubes in which a photocathode and a series of dynodes in evacuated glass enclosures are used. Figure 4.4 shows a schematic representation of an AAS showing the position of each of the components mentioned above.

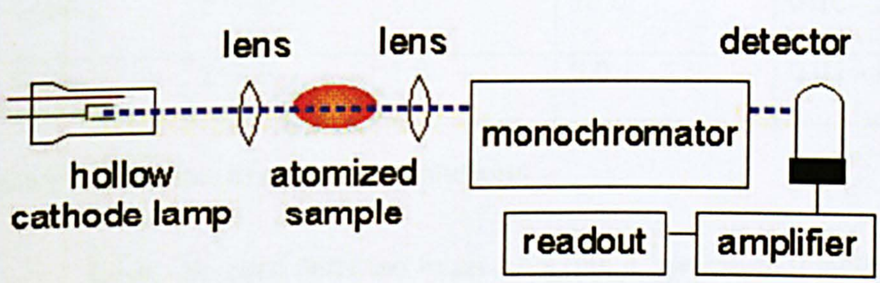


Figure 4.4: Schematic representation of an AAS (Tissue, 1996)

4.7.4 Calibration

Calibration curve is the most common method where interference effects are known to be absent. Usually at least three standards and a blank are used to cover the range 0.1 to 0.8 Abs. The calibration is performed by using the blank solution to zero the instrument. The standards are then analysed with the lowest concentration first, and the blank run between standards, to ensure the baseline (zero point) has not changed (Agilent, 2000). Standard calibration curves prepared for the five metals analysed were created using the settings depicted in Table 4.7.1 were adopted for the present analysis of samples.

Table 4.4: Instrument settings for analysis of the 5 metals studied.

Metal	Wavelength (nm)	Slit Width (nm)	Lamp Current mA	Optimum working Range µg/ml
Manganese	279.5	0.2	5.0	0.02 - 5
Cadmium	228.8	0.5	4.0	0.02 - 3
Copper	324.8	0.5	4.0	0.03 -10
Lead	217.0	1.0	10.0	0.10 - 30
Zinc	213.9	1.0	5.0	0.01 - 2

4.8 Preparation of standard solutions

Every element detected in an AAS has a minimum detection limit. Based on the minimum detection limit for the individual metals analysed working standards were prepared by adding the calculated amounts of individual standard solutions to 100ml volumetric flasks and finally making up the volume with deionized distilled water. These working standards were freshly prepared on the day of the analysis and the left over working standards were carefully disposed.

During the analysis of soil and foliar samples reagent blanks were analysed after every 15 samples, and standard solutions were analysed after every 10 samples to check the instrument drift.

In order to calibrate the results obtained from the AAS analysis, results from the soil analysis are checked against the soil analysis data collated by Swansea Metropolitan University (SMU) team on plot MI-04 (unpublished). Although SMU team used ICP-AES for analysing a small batch of samples, both results are in a calibrated range. In addition, results from the soil sample analysis are also checked against the data published in the interim report on Varteg Hill Reclamation and Coal Recovery Scheme by Faber Munsell (2006).

4.9 Contamination Factor (CF) and Pollution Load Index (PLI) (RQ – 1)

In order to identify whether or not the site was contaminated contamination factor and pollution load index tests were carried out.

Contamination Factor (CF)

In soil the contamination level of metals in calculated in terms of Contamination Factor (CF) which is calculated as follows:

$$CF = \frac{C_m \text{ Sample}}{(C_m \text{ Background})}$$

Where C_m Sample: is the mean concentration of a given metal

C_m Background: is the median concentration of the background soil sample

When the contamination factor (CF) refers to the following:

CF < 1 Low contamination

1 ≤ CF ≤ 3 Moderately high contamination

3 ≤ CF ≤6 Considerable high contamination

CF > 6 Very high contamination

Pollution Load Index (PLI)

Each site was assessed for the extent of metal pollution by employing the method based on the Pollution Load Index (PLI) developed by Thomlinson et al. (1980). The pollution load index provides a simple but comparative means for assessing a sites quality, where a value of PLI refers to:

PLI < 1denotes perfection (which means the site is not polluted)

PLI =1 baseline levels of pollutants are present

PLI > 1 deterioration of site quality

4.10 Contamination Contour Maps (RQ – 1)

SURFER v10 by MS Golden Software was used to create contour maps of contamination. Contaminant mapping was used to measure, identify and evaluate the presence of heavy metals in soil and provide a spatial distribution of metals under investigation on the experimental plot MD-07. This technique was also used to create a visual representation of the contaminants present at the site of study and also provides a baseline for site characterisation.

4.11 Tree Mortality (RQ – 2)

A population tally of tree mortality on the experimental site MD-07 was carried out after the final soil and foliar analysis in July 2010. The results are displayed graphically in Chapter 5.

4.12 Statistical and Computational analysis

After the soil and leaf samples were analysed they were statistically tested using the SPSS v19 software. The following statistical analyses were carried out:

a) Numerical and Graphical summaries of data (RQ – 1, 2, 3 and 4): Descriptive statistics and bar charts and cluster graphs were used to quantitatively describe the main features of the observed data set.

b) Paired sample t-test (RQ – 1, 2, 3 and 4): Paired sample t-test was used to determine whether or not there was significant difference between the means of two variables. The variables in this case were metals in soil at different times (age); metals in soil vs metals in leaves.

c) Pearson's correlation (RQ – 3 and 4): Pearson's correlation (r – value) provides the degree of strength and direction of a linear relationship between two or more variables (Weinberg & Abramowitz, 2008). This relationship can be positive (+1), neutral (0) or negative (-1). The Pearson's correlation was used to check if two or more variables were related.

d) Multiple regression analysis (RQ – 3 and 4): The main function of a multiple regression is to identify the relationship between several predictor or independent variables and a criterion or dependent variable (StatSoft, 2012). For this research the test was used to investigate the effect of age of planting on the amount of all the metals present in soil and leaves on all plots.

CHAPTER 5

RESULTS AND ANALYSIS

5 RESULTS AND ANALYSIS

The following chapter is mainly divided into three key sections. It looks at the presence and spatial distribution of metals: Cd, Zn, Mn, Pb and Cu in soil on plot MD-07 and the adjoining CfN plots. The second section will look at the uptake of these metals into Alder, Birch and Larch foliage and finally the third section will look at the relationships of metal contamination in soil and their subsequent uptake into leaves over time.

5.1 Contaminant mapping

Site investigation is essential for any *in situ* reclamation project. The aim of using the technique of contaminant mapping was to describe measure and evaluate the heavy metal contamination in soil and show its spatial distribution on the experimental study plot MD-07 in this research. This approach provides a visual representation of the contaminants present on the study site and also provides a baseline for site characterisation. Site MD-07 was selected as being the only plot which allowed sampling before planting and after planting. Contamination maps for MD-07 were produced for samples collected in 2007 before tree planting and in 2010, at the end of the second year of sampling.

5.1.1 Data Analysis and production of contaminant maps

Results from the soil sample analysis from plot MD-07 has been collated in tables 5.1 and 5.2. The GPS location of the individual sampling points and the data from soil sample analyses were combined to create interpolated contour contamination maps for each of the five metals studied; Mn, Cd, Cu, Pb and Zn.

Contamination maps were produced

- to identify the presence of individual elements;
- to establish whether or not chemical contamination exists on the site;
- to locate hotspots for each of the five metals studied and identify any pattern in the spread of these hotspots.
- to provide a baseline data for further investigations for current and future projects not only at the site of study but at different sites with similar history and characteristics.

Maps were created using the software programme SURFER 10, MS Golden Software v.2010 (Section 4.10). The data tables present the contamination results and compare them with the reference guideline figures (Section 1.5). Using a colour blocked scale, the four colours represent the chemical levels in normal soil; contamination threshold levels; critical soil levels, and contamination soil-levels that are so high that immediate action/ treatment is required.

These standard values for normal soil and contamination threshold levels have been collected from Davies & Roberts (1978); Kelly (1980); ICRCL (1987); Bending and Moffat (1999); DEFRA (2004); Environment Agency (2009). The values for action/treatment levels of contaminants are adapted from Kelly (1980). Although these values provide a useful database there is a gap in the literature for levels of contaminants which fall in between the contamination threshold and the action/ treatment level. To fill this gap, and for the purposes of this study, a 'Critical soil level' has been recognised to accommodate these intermediate chemical levels. Each map also shows the location of sampling points and concentration of metal at that point. The scale is provided at the right hand side of each map and the concentration is in mg/kg. (Fig 5.2.1 to 5.2.5)

5.2 MD-07 Contamination Maps

Since MD-07 is a new experimental plot, it was possible to collect soil samples in July 2007, before the planting of the 3 tree species followed by tree plantation in November 2007. A second batch of soil samples was collected in July 2009 and 2010. Contamination maps on MD-07 were created for 2007 and 2010. The 2007 planting shows background levels in these coal spoils and the 2010 sampling shows the effects of tree planation. The following sections compare the soil metal distributions at both times.

Site MD-07 before Planting

Table 5.1.1: Soil metal concentrations (mg/kg) at MD-07 from samples collected in 2007 (n=234).

MD-07 in 2007	Mn	Cd	Cu	Pb	Zn
Mean	1879	1.16	64	216	200
Minimum	1129	0.32	33	132	145
Maximum	2644	2.99	94	281	248
Standard Published Soil Values for UK					
Normal soil*	<500	<1	<40	<200	<100
Contamination threshold*	500-1500	1-2	80	320	250
Critical soil level	1500-2000	2-8	80-300	320-1000	250-500
Action/Treatment levels^	>2000	>8-10	>300	>1000	>500

*Standard values from Davies & Roberts (1978); Kelly (1980); ICRCL (1987); Bending and Moffat (1999); DEFRA (2004); Environment Agency (2009). ^ Action/Treatment Levels from Kelly (1980).

Before planting in 2007 all the test metals in site MD-07 were above normal soil levels. The site shows elevated levels for Mn, which requires action/treatment, and levels for Cd at the contamination threshold with some hotspots showing elevated critical soil levels. Cu, Zn and Pb are at levels above those of normal soils although below their contamination thresholds. Soil pH was fairly homogeneous across the plot (pH= 5.7, sd = 1.04), in spite of local hotspots (pH = 3.8).

Contamination maps show hotspots for all metals except Mn. If this site is not treated/ reclaimed the levels of contaminants could increase due to natural weathering and waterlogging and pose serious environmental concerns (McNeal and Bledsoe, 1992; Dang et al., 2002; Nwachukwu and Pulford, 2008)

Site MD-07 after planting – Maps based on soil data collected in 2010

Table 5.1.2: Soil metal concentrations (mg/kg) at MD-07 from samples collected in 2010 (n=234).

MD-07 in 2010	Mn	Cd	Cu	Pb	Zn
Mean	1532	1.07	52	204	173
Minimum	1003	0.28	31	120	105
Maximum	2156	2.45	79	269	237
Standard Published Soil Values for UK					
Normal soil*	500	<1	40	200	100
Contamination threshold*	1500	1-2	80	320	250
Critical soil level	1500-2000	2-8	80-300	320-1000	250-500
Action / Treatment levels^	2000	>8-10	300	1000	500

*Standard values from Davies & Roberts (1978); Kelly (1980); ICRCL (1987); Bending and Moffat (1999); DEFRA (2004); Environment Agency (2009). ^ Action/Treatment Levels from Kelly (1980).

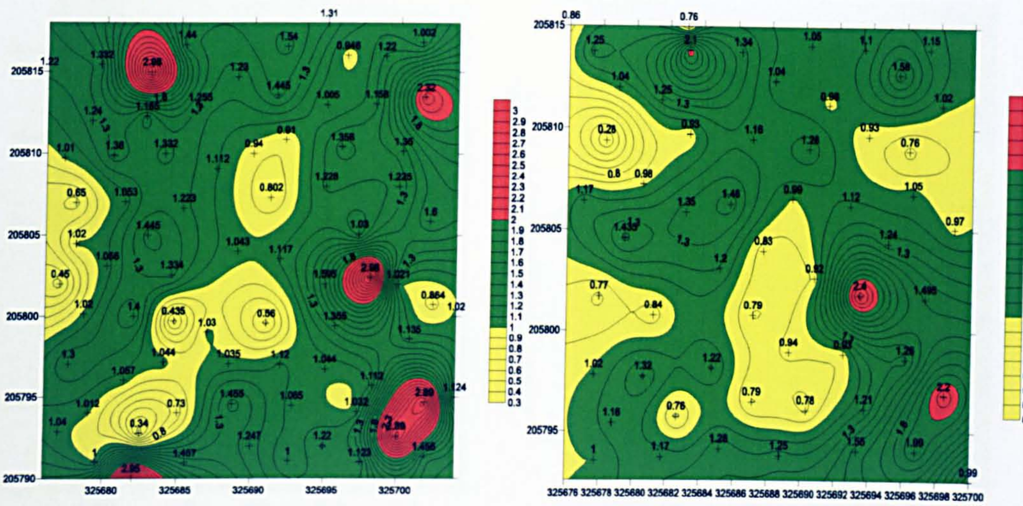
The difference in contaminant levels at the two time periods have been compared statistically using independent sample t-tests. Comparison of the data from before planting in 2007 and after planting in 2010 show a statistically significant initial decrease in the levels of contaminants ($p=0.002$). The levels of Mn, Cu and Zn have decreased by almost 14-18%. Cd shows a 6% decrease but this difference, although low, is also significant ($p=0.004$). These results show that the young tree leaves have started taking up metals thereby initiating the processes of remediation.

This change in metal loadings is represented in Tables 5.2.1 to 5.2.5. These show statistical test results, and the standard soil contamination values adapted from Davies & Roberts (1978); Kelly (1980); ICRCL (1987); Bending and Moffat (1999); DEFRA (2004); Environment Agency (2009).

Contamination Map

The following section compares the 2007 and 2010 contamination maps for each individual metal.

5.2.1 Cadmium (Cd) in Soil at MD-07 in 2007 and 2010



a) Cadmium (Cd) at MD-07 in 2007 *b) Cadmium (Cd) at MD-07 in 2010*

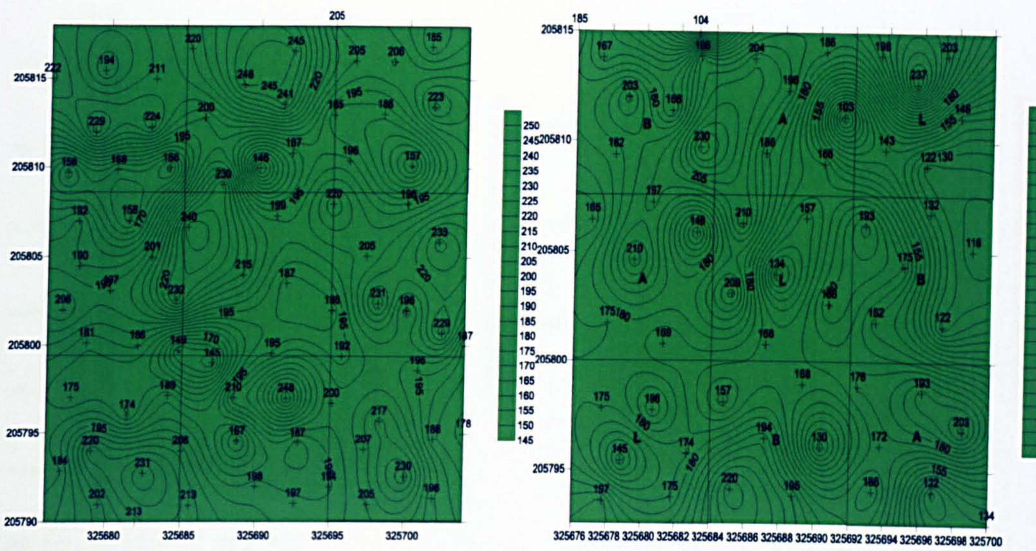
Figure 5.2.1: Contamination map for Cd in soil in 2007 and 2010

Table 5.2.1: Comparison of Cd in soil (mg/kg) at MD-07 from samples collected in 2007 and 2010.

Cd in Soil	Cd at MD-07 in 2007	Cd at MD-07 in 2010
Mean	1.16	1.07
Minimum	0.32	0.28
Maximum	2.99	2.45
Standard Published Soil Values for UK		
Normal soil*	<1	
Contamination threshold*	1-2	
Critical soil level	2-8	
Action / Treatment levels^	>8-10	

An independent sample t-test was conducted to compare the difference of Cd in soil in samples collected before planting in 2007 and after planting in 2010. The null hypotheses assumed that there was no difference between the amount of Cd in soil in 2007 and 2010. The mean amount of Cd in soil in 2007 was M= 1.16 (SD = 2.26) and in 2010, M= 1.07 (SD = 1.98). The mean difference of Cd in soil in 2007 and 2010 is 0.22. T-testing reveals that this is a significant ($p=0.004$) reduction in the concentration of Cd in soil.

5.2.2 Zinc (Zn) in Soil at MD-07 in 2007 and 2010



a) Zinc (Zn) at MD-07 in 2007 *b) Zinc (Zn) at MD-07 in 2010*

Figure 5.2.2: Contamination maps for Zn in soil in 2007 and 2010

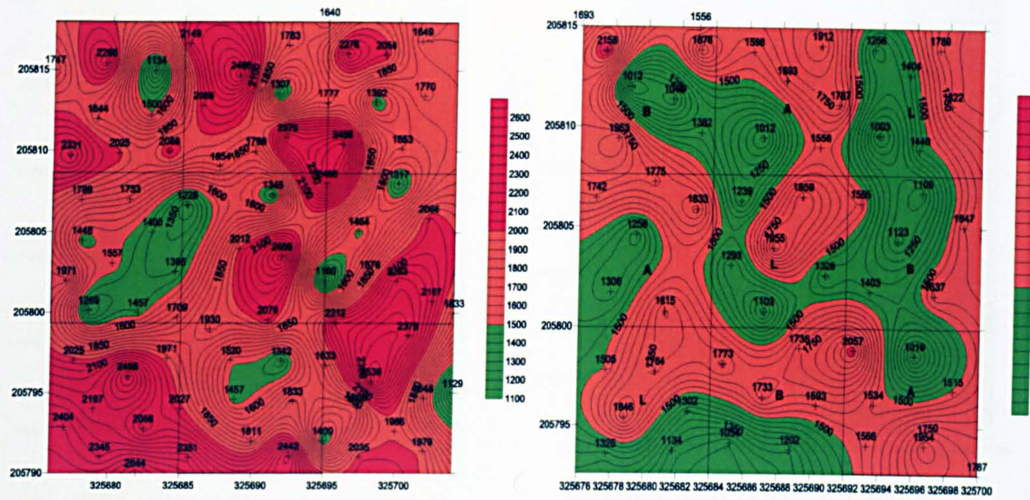
Table 5.2.2: Comparison of Zn in soil (mg/kg) at MD-07 from samples collected in 2007 and 2010.

Zn in Soil	Zn at MD-07 in 2007	Zn at MD-07 in 2010
Mean	200	173
Minimum	145	105
Maximum	248	237
Standard Published Soil Values for UK		
Normal soil*		<100
Contamination threshold*		100-250
Critical soil level		250-500
Action / Treatment levels^		>500

The independent sample t-test to compare the change of Zn in soil between 2007 (M= 192.78; SD = 29.21) and 2010 (M= 169.68; SD = 30.45) showed there was a significant difference ($p=0.005$) between the amount of

Zn in soil in 2007 and 2010. The mean difference of Zn in soil in 2007 and 2010 is 23.10 which showed a statistically significant reduction in soil Zn concentration.

5.2.3 Manganese in Soil at MD-07 in 2007 and 2010



a) Mn in MD-07 in 2007 *b) Mn at MD-07 in 2010*

Figure 5.2.3: Contamination map for Mn in soil in 2007 and 2010

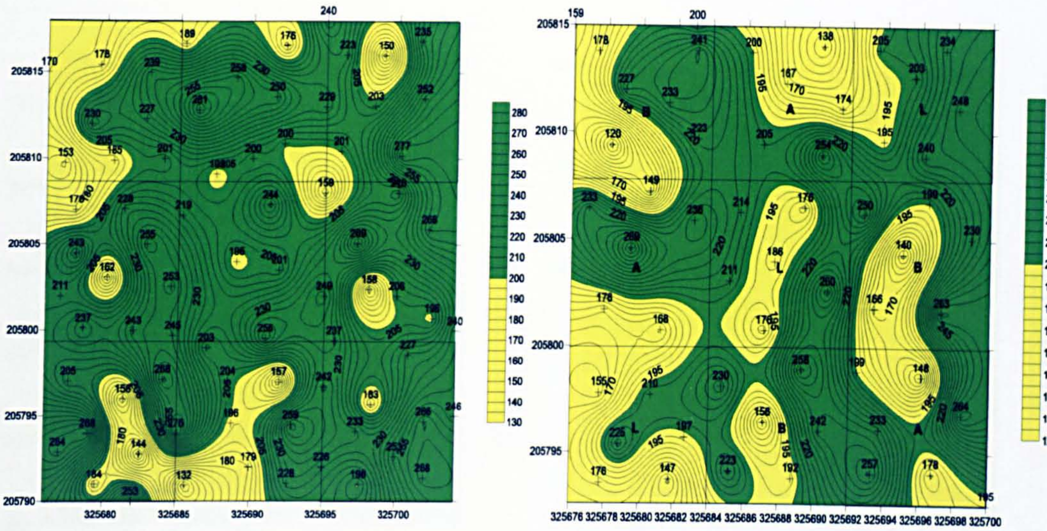
Table 5.2.3: Comparison of Mn in soil (mg/kg) at MD-07 from samples collected in 2007 and 2010.

Mn in Soil	Mn at MD-07 in 2007	Mn at MD-07 in 2010
Mean	1879	1528
Minimum	1129	1003
Maximum	2644	2156
Standard Published Soil Values for UK		
Normal soil*		<500
Contamination threshold*		500-1500
Critical Soil level		1500-2000
Action / Treatment levels^		>2000

The independent sample t-test to compare the change of Mn in soil between 2007 (M= 1879; sd = 383.4) and 2010 (M= 1528; sd = 244.4) showed there was a significant difference (p=0.002) between the amount of Mn in soil in 2007 and 2010. The mean difference of Mn in soil in 2007 and

2010 is 338.5 which showed a statistically significant reduction in soil Mn concentration.

5.2.4 Lead (Pb) in Soil at MD-07 in 2007 and 2010



a) Lead (Pb) at MD-07 in 2007 *b) Lead (Pb) at MD-07 in 2010*

Figure 5.2.4: Contamination map for Pb in soil in 2007 and 2010

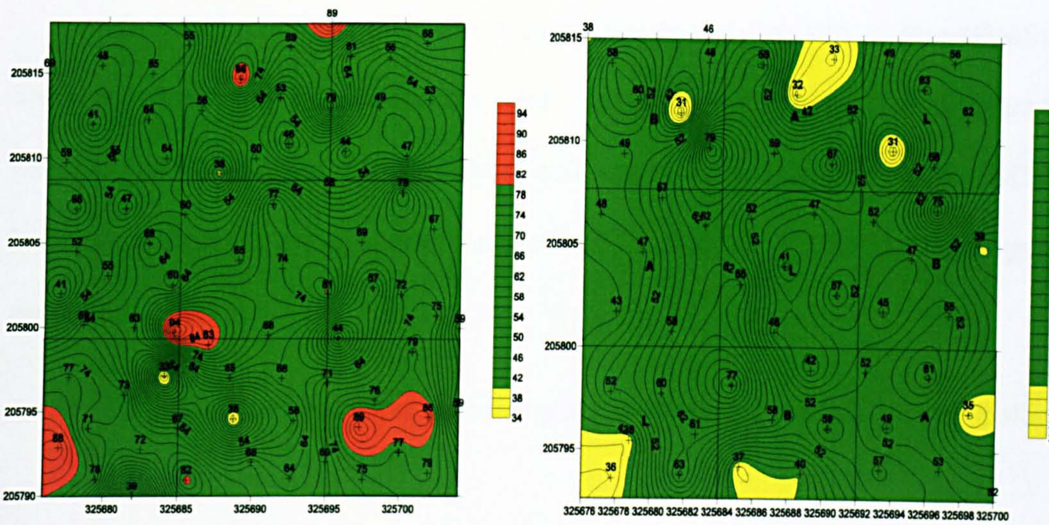
Table 5.2.4: Comparison of Pb in soil (mg/kg) at MD-07 from samples collected in 2007 and 2010.

Pb in Soil	Pb at MD-07 in 2007	Pb at MD-07 in 2010
Mean	216	204
Minimum	132	120
Maximum	281	269
Standard Published Soil Values for UK		
Normal soil*		<200
Contamination threshold*		200-320
Critical soil level		320-1000
Action / Treatment levels^		>1000

The independent sample t-test to compare the change of Pb in soil between 2007 (M= 221.82; SD = 34.20) and 2010 (M= 204.75; SD = 27.89) showed there was a significant difference (p=0.025) between the amount of Pb in soil in 2007 and 2010. The mean difference of Pb in soil in 2007 and

2010 is 17.07 which showed a statistically significant reduction in soil Pb concentration.

5.2.5 Copper (Cu) in Soil at MD-07 in 2007 and 2010



a) Copper (Cu) at MD-07 in 2007 *b) Copper (Cu) at MD-07 in 2010*

Figure 5.2.5: Contamination map for Cu in soil at 2007 and 2010.

Table 5.2.5: Comparison of Cu in soil (mg/kg) at MD-07 from samples collected in 2007 and 2010.

Cu in Soil	Cu at MD-07 in 2007	Cu at MD-07 in 2010
Mean	64	52
Minimum	33	31
Maximum	94	79
Standard Published Soil Values for UK		
Normal soil*	<40	
Contamination threshold*	40-80	
Critical soil level	80-300	
Action / Treatment levels^	>300	

The independent sample t-test to compare the change of Cu in soil between 2007 (M= 62.92; SD = 15.19) and 2010 (M= 50.97; SD = 7.10) showed there was a significant difference ($p=0.003$) between the amount of Cu in soil in 2007 and 2010. The mean difference of Cu in soil in 2007 and

2010 is 11.95 which showed a statistically significant reduction in soil Cu concentration.

5.3 Soil Metal Interaction – All plots

Knowledge of the different metals present at the site of investigation form the basis of this research. Soil samples were collected and each sample was analysed for the total soil metal concentration using EDTA extraction through the procedure described in chapter 4. The data analysed are used:

- To provide a baseline or background levels of metals in soil before tree plantation.
- Access the variability of metal concentration in the main site of investigation.
- Access the variability of metal concentration amongst different study plots over time
- To monitor and model the process of phytoremediation.

Plot MD-07 in 2007 was an undisturbed plot after the original reclamation done by the mining industry during the late 1960's. The main vegetation present on the plot was grasses, which were a part of the original reclamation programme. On the other CfN plots the only changes created over the years were due to tree plantation. Hence, soil samples from plot MD-07 in 2007 will be treated as background levels for all the sampling plots. These samples will be referred to as background levels (BL-07).

As described in Section 4.6.8, apart from site MD-07, which is the main site of investigation, soil and leaf samples were also collected from five adjoining plots in 2009 and 2010. The following sets of tables outline the descriptive statistics of soil metal concentration at all the plots studied in this research.

Table 5.3.1 outline the descriptive statistics of the background levels of metals (BL-07). Table 5.3.2 and 5.3.3 outline the descriptive statistics of metals on the different plots studied in 2009 and 2010 respectively. All the results are indicated in mg kg^{-1}.

Table 5.3.1: Descriptive statistics of metal contamination on MD-07 before planting in 2007. Results are indicated in mg kg^{-1} (n=213)

BL-07	Cadmium (Cd)	Zinc (Zn)	Manganese (Mn)	Lead (Pb)	Copper (Cu)
MEAN	1.21	192.79	1874.25	221.80	62.92
MIN	0.32	99.03	1044.54	133.64	20.32
MAX	2.99	245.32	2732.32	279.12	97.53
Std Dev	2.63	29.21	384.90	3.42	1.51

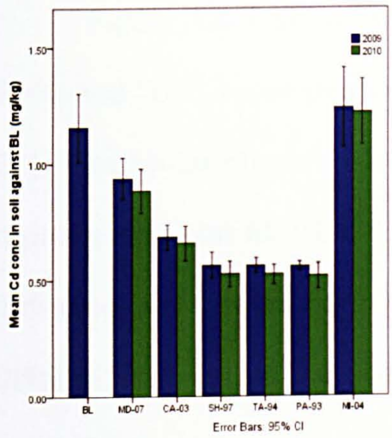
The descriptive statistics in tables 5.3.1 – 5.3.3 show a gradual decrease in soil metal concentration over the years when compared to the background levels of soil. In other words the older the plot the lower the concentration of metal in soil. The only exception is the concentration of metals on plot MI-04 which has values higher than the background soil values and all other plots. This is graphically represented by figure 5.3.1.

Table 5.3.2: Descriptive statistics of metal contamination on all plots in 2009. Results are indicated in mg/kg (n=210)

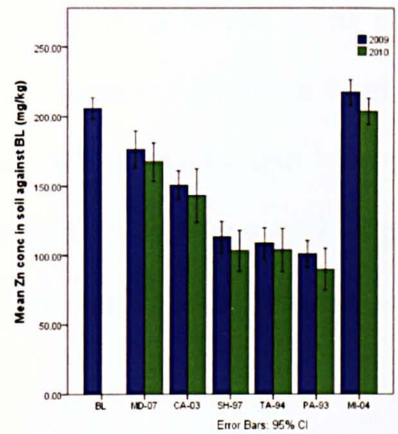
MD-07	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	1.12	176.75	1598.32	223.62	58.23
MIN	0.43	83.64	1233.50	134.11	32.39
MAX	2.85	232.03	2353.00	285.38	78.61
Std Dev	2.33	24.43	275.32	32.35	5.65
CA-03	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.70	155.45	2058.46	197.86	60.46
MIN	0.30	73.53	1332.13	122.74	32.53
MAX	1.27	203.33	2686.42	278.62	68.68
Std Dev	0.86	43.35	299.32	34.55	7.22
SH-97	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.64	124.56	1366.02	142.80	49.16
MIN	0.58	66.08	1034.75	104.96	32.32
MAX	0.73	176.05	1557.69	176.44	67.28
Std Dev	0.33	28.43	687.53	8.68	4.67
TA-94	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.61	100.03	1374.56	130.83	41.00
MIN	0.32	63.86	1033.96	100.25	22.05
MAX	0.82	155.75	1532.03	157.09	65.73
Std Dev	0.87	24.54	790.43	10.26	6.99
PA-93	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.57	94.32	1276.02	127.23	36.53
MIN	0.11	78.20	1132.32	99.40	22.56
MAX	0.68	174.11	1353.84	137.00	43.52
Std Dev	0.48	24.53	432.91	5.50	3.49
MI-04	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	1.42	214.20	2245.42	264.53	68.56
MIN	0.43	194.08	878.45	204.67	39.08
MAX	2.98	266.47	3059.32	298.73	99.73
Std Dev	1.53	21.66	654.67	19.45	1.43

Table 5.3.3: Descriptive statistics of metal contamination on all plots in 2010. Results are indicated in mg/kg (n=213)

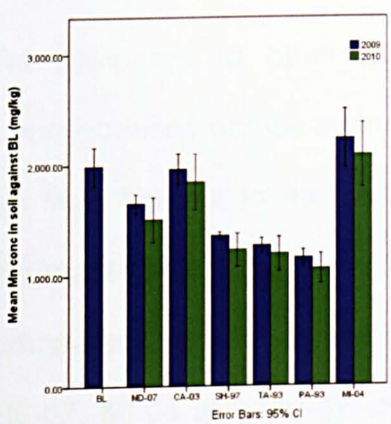
MD-07	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	1.07	169.26	1533.80	199.49	51.21
MIN	0.28	75.43	1003.00	131.16	30.42
MAX	2.45	221.82	2171.46	265.32	69.66
Std Dev	1.98	30.45	241.65	27.59	7.13
CA-03	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.63	146.94	2012.69	189.25	52.31
MIN	0.42	75.52	1292.06	128.74	31.58
MAX	1.25	193.17	2568.73	268.17	63.85
Std Dev	0.77	35.29	277.23	37.45	7.69
SH-97	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.60	111.74	1340.04	131.95	43.00
MIN	0.52	70.80	1048.92	117.03	31.16
MAX	0.69	165.00	1498.63	168.89	59.83
Std Dev	0.49	27.78	928.42	9.77	5.84
TA-94	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.59	97.30	1337.48	123.52	37.58
MIN	0.37	67.08	1048.82	104.96	25.93
MAX	0.78	147.55	1498.04	143.04	59.32
Std Dev	0.99	22.44	845.71	8.04	7.43
PA-93	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.56	89.43	1227.10	120.40	33.27
MIN	0.14	67.32	1116.48	101.24	27.37
MAX	0.64	163.79	1322.83	131.57	40.71
Std Dev	0.47	25.32	493.91	6.11	3.56
MI-04	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	1.36	206.53	2232.79	255.72	60.04
MIN	0.36	183.03	863.93	231.05	45.84
MAX	2.88	245.95	3056.36	288.48	94.73
Std Dev	1.34	17.33	677.42	14.42	1.36



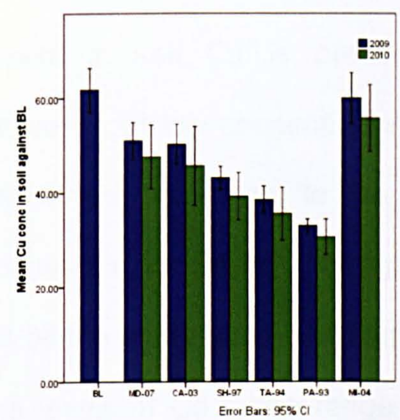
a) Mean Cd Conc in soil on all plots against BL



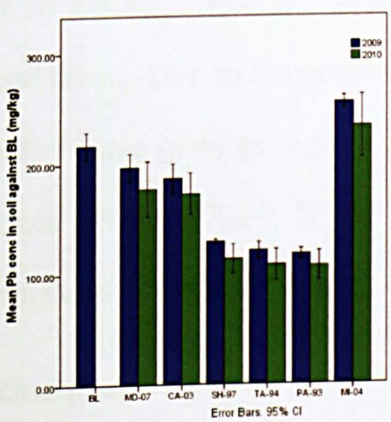
b) Mean Zn Conc in soil on all plots against BL



c) Mean Mn Conc in soil on all plots against BL



d) Mean Cu Conc in soil on all plots against BL



e) Mean Pb Conc in soil on all plots against BL

Figure 5.3.1: Mean metal concentrations in soil on all the plots in 2009 and 2010 along with background soil levels (BL). Error bars show 95% Confidence Intervals.

Figure 5.3.1 show concentration of metals in soil on all the plots in 2009 and 2010 in comparison with the background soil levels from 2007 (BL-07). Plot MI-04 showed elevated levels for all metals and hence the bars representing plot MI-04 are at the far end of each graph. The graphs are indicated with error bars showing 95% confidence interval - note the differences in the vertical scale.

Cadmium (Cd)

As compared to other metals present in soil Cd is present in low concentrations across all the plots. However, at low concentrations Cd can be very harmful to the soil and environment. According to the published standard values, the normal soil concentration for Cd is <1.0 mg/kg. When compared to the levels present across all the study plots, soil samples from BL-07, MI-04 and MD-07 showed high levels of Cd in the range of 1.07 – 1.36 mg/kg. These concentrations, however low are still above the normal soil levels. Due to the presence of hotspots these levels on BL-07, MI-04 and MD-7 have gone as high as 2.97 mg/kg which are at the critical soil level. On plots PA-93, TA-94, SH-97 and CA-03, the total concentrations averaged between 0.5 and 0.7 mg/kg, which is the range expected in normal soil.

Zinc (Zn)

Variability of Zn on all the plots is low. Only with the exception of PA-93 (97 mg/kg), the level of Zn was above the level of that in normal soil on all the plots with total concentrations averaging between 107 to 206 mg/kg. MI-04 showed a maximum concentration of 245 mg/kg.

Manganese (Mn)

Across all the study plots Mn shows high levels of contamination. When compared to the standard published values (Table 5.1), the concentrations of Mn in soil are mainly at critical soil levels. The background soil levels (BL-07) show heavy contamination of Mn in soil with the mean value of 1874 mg/kg. Only after four years of planting site MD-07 shows a reduction in the level of Mn present in soil. However, this level is still at the critical soil level when compared to the standard values. All other plots with the exception of MI-04 show a reduction in the amount of Mn over time.

Lead (Pb)

Low levels of Pb are present across all the plots. These levels are close to, and at some sites, within the normal soil levels. The background soil level is slightly above the normal soil level band with an average of 221 mg/kg with the exception of MI-04 which has an average Pb concentration of 255 mg/kg.

Copper (Cu)

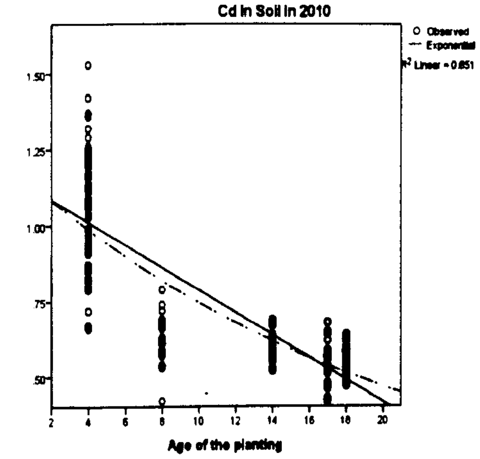
Across the plots there are low levels of Cu present ranging from 35 – 63 mg/kg which, when compared to the standard values, cover the normal to contamination threshold levels. The background soil levels show an average of 63 mg/kg of copper which is above the normal soil levels. Over time, the level of Cu at all the plots has gradually decreased. However only at site PA-93 and TA-94 which were planted in 1993 and 1994 respectively have the levels of Cu reduced to the normal soil levels. Despite being planted in 2004, plot MI-04 show levels similar to that of the background soil levels.

Plot MI-04

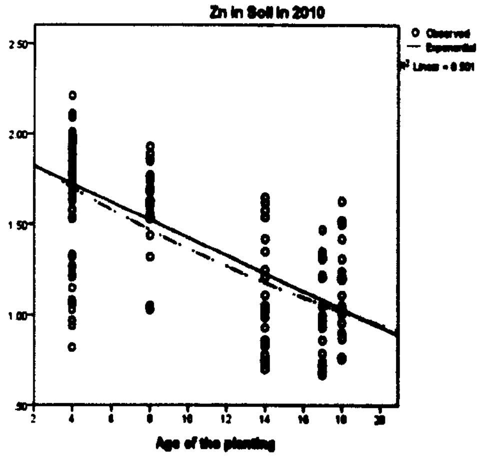
Plot MI-04 is a failed remediation experiment in which the plot was ploughed as per the recommended forestry guidelines, which not only scraped off the little organic layer present, but over the years increased the problem of metal contamination. This is evident from the results which show irregular spikes in the data. Despite the challenges that this plot presented, the results observed are odd and have been included where ever possible. Due to the anomalous results of this accidently created plot, acts as a proxy for the 50 year old reclaimed plot. Present site conditions and metal contaminants at this site are therefore treated as the closest representative of what the condition of the site would have been, if trees were directly planted into freshly mined soil before reclamation via grass seeding in 1963.

Statistical analysis:

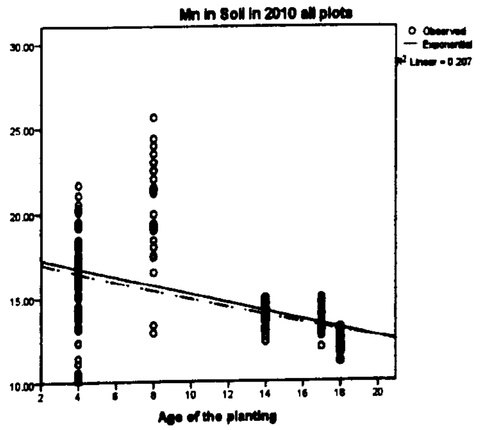
Multiple regression analysis was used to investigate the effect of age of planting on the amount of metals present in soil on five study plots namely, PA-93, TA-94, SH-97, CA-03 and MD-07. Results from 2009 and 2010 have been pooled together and the average has been used. Results from MI-04 were excluded from the analysis due to the high variability in metal concentrations. The scatter plots (Fig 5.3.2) showed a negative line of fit indicating that with an increase in age of planting there was a decrease in soil metal concentration. At 5% level, there is evidence that age of planting has a significant ($p=0.002$) effect on the concentration of metals in soil. The regression scatter plots are shown below with exponential and linear curves with R^2 values.



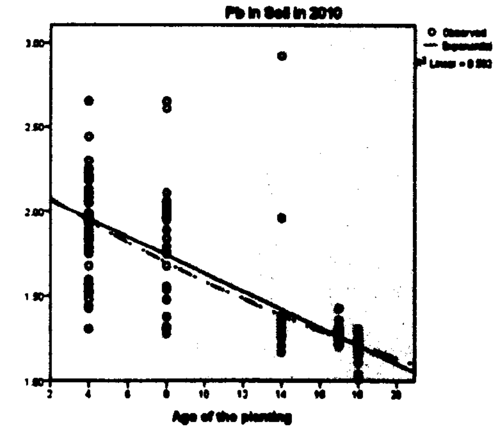
a) Scatter plot with regression curve for Cd in soil



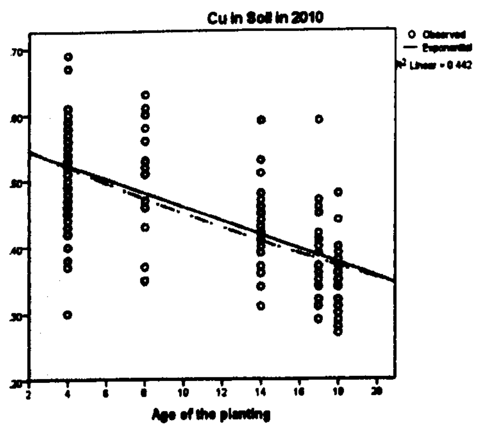
b) Scatter plot with regression curve for Zn in soil



c) Scatter plot with regression curve for Mn in soil



d) Scatter plot with regression curve for Pb in soil



e) Scatter plot with regression curve for Cu in soil

Figure 5.3.2: Scatter plots showing exponential and linear regression curves with R^2 values for metals in soil against age of planting on the five study plots. All metals showed a significance value p=0.00

It is evident that there are high levels of some metals present on all the test plots. However it is essential to determine the extent to which these levels could be regarded as signifying dangerous contamination. When the recording loadings are compared to the standard published value, it is clear that for most of the elements the level of concentrations is above that of the normal soil and, for the most part, above the contamination threshold and within the critical soil level band. In some cases, especially for Mn, concentration levels are above the highest action/treatment level, where contamination poses an immediate pollution threat to the environment. The tests for identifying site contamination by metal and in turn the pollution load developed by Thomlinson et al. (1980), which has been widely used, is employed here as follows.

5.4 Contamination Factor (CF) and Pollution Load Index (PLI)

Each site was assessed for the contamination level of metals in terms of Contamination Factor (CF) and the extent of metal pollution in terms of Pollution Load Index (PLI) developed by Thomlinson et. al (1980) (See section 4.9).

Contamination Factor (CF) which is calculated as follows:

$$CF = \frac{C_m \text{ Sample}}{(C_m \text{ Background})}$$

Contamination Factor (CF)

Table 5.4.1: Contamination factor for heavy metals on individual sites in 2009 and 2010

Site 09	PA93	TA94	SH97	CA03	MI04	MA07
Mn	1.23	1.34	1.34	2.02	2.22	1.52
Cd	1.05	1.05	1.10	1.20	2.03	1.94
Cu	0.70	0.86	0.97	1.24	1.37	1.15
Pb	1.00	1.05	1.11	1.60	2.15	1.72
Zn	1.03	0.93	1.07	1.42	1.97	1.65
Site10	**PA93**	**TA94**	**SH97**	**CA03**	**MI04**	**MA07**
Mn	1.22	1.33	1.34	2.00	2.22	1.52
Cd	1.01	1.07	1.09	1.16	2.05	1.94
Cu	0.73	0.82	0.95	1.15	1.33	1.13
Pb	1.00	1.03	1.09	1.57	2.12	1.66
Zn	1.00	0.92	1.05	1.37	1.94	1.61

Pollution Load Index (PLI)

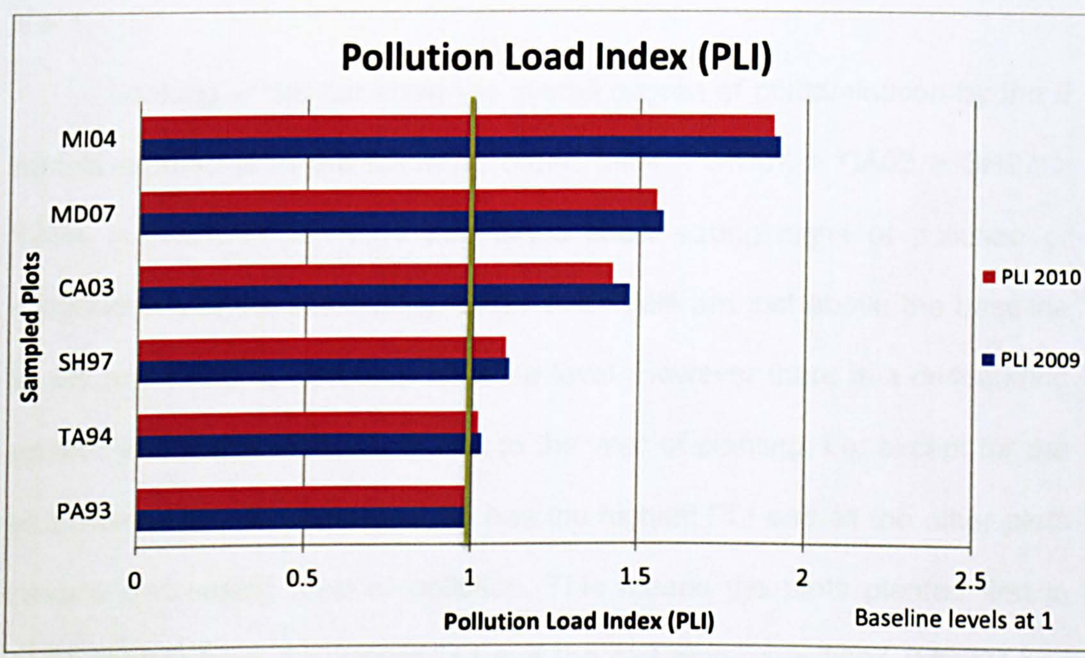


Figure 5.4.1: Pollution Load Index,(PLI) for the five metals studied at all the plots in 2009 and 2010.

Contamination Factor (CF): The contamination factor of the different metals analysed in 2009 and 2010 are presented in Table 5.4.1

Using the contamination factor categories described in section 4.9, all of the sites seem to suffer moderate contamination from heavy metals except for Cu on sites PA93, TA94 and SH97 in both the years. MI04 and CA03 have concentrations of Mn towards the higher end of moderate contamination. If not controlled, this contamination can cross the category and move into the high contamination category. MI04 also shows higher ends of moderate contamination for Cd and Pb.

Pollution Load Index (PLI): In order to determine whether the study sites suffer contamination or not, the pollution load index (PLI), was used. The PLI is a suitable analysis for providing a measure of the degree of overall contamination at a sample site. The results for the PLI are shown in Figure 5.4.1.

Looking at the bar chart the overall degree of contamination by the 5 metals studied is in the following order: MI04 > MD07 > CA03 > SH97 > TA94 > PA93. MI07, MI04 and CA03 show strong signs of pollution or deterioration of the site quality. SH97 and TA94 are just above the baseline levels and PA93 is almost at baseline level. However there is a descending pattern in the PLI when compared to the year of planting, i.e. except for the plot planted in 2004 (MI04) which has the highest PLI and all the other plots have a decreasing level of pollution. This means the plots planted first in 1993 (PA93) have the lowest PLI and the plot planted in 2007 (MD07) has the highest PLI with an exception of MI04.

Summary

Contamination maps in section 5.1 show the distribution of the five metals across the study plot MD-07, indicating the variation in the levels of individual contaminants on the same plot. Initial soil analysis and comparison of the contaminant levels with standard published values show that site MD-07 is heavily contaminated, especially with high levels of Manganese. Cadmium, although in lower concentration than the other metals still poses a toxic threat to the environment. The maximum level for Mn in 2007 was at the action/ treatment level. After planting young saplings in November 2007 the soil had shown initial improvement after only three years of planting in 2010. A 14 - 18% decrease in the level of Mn in soil in three years itself has brought down the chemical from action/treatment level to critical soil level on most of the MD-07 plot which is evident from the contamination maps. The results are statistically proven by independent sample t-test ($p= 0.002$). Similarly in many patches the levels of cadmium in soil have lowered from critical soil level to contamination threshold level. The site is not heavily contaminated with Cu, Pb and Zn although the levels are way above those of normal soil. However, if left untreated these levels could increase to critical levels due to inputs from the natural weathering of the mine spoils (Nwachukwu and Pulford, 2008; Dang, et al., 2002; Welch, 2013) as evident at plot MI-04. Meanwhile, results of the contamination mapping across the two time periods provide not only a good visual representation of the site condition but also a clear understanding of the problem areas such as hotspots.

In addition to the sampling and analysis carried out on MD-07, samples were also collected from 5 other adjoining plots (CfN plots) with similar topography and soil conditions as explained in Section 3.5. Soil samples collected from MD-07 in 2007 prior to planting are treated as background soil levels as the conditions and history of the site suggest that the soil conditions are similar to that after the initial so called 'reclamation' on site was carried out in the late 1960's. Initial soil analysis on the adjoining plots show varied levels of metals at the different plots and when compared to the background levels they showed a gradual decrease in soil metal concentrations over time. This is proven statistically by linear regression analysis. The only exception to this is site MI-04 which even after being re-reclaimed in 2004 show elevated levels of some metals especially Mn, Cd and Zn. These levels exceed even the background soil levels. One plausible explanation is that site MI-04 was ploughed using heavy machinery and trees were later planted in the notch planting style. The trenching has led to the loosening of the otherwise highly compacted mine spoil, exposing the chemicals from the deeper layers to weathering and making them available for plants and therefore potentially to animal intake, and entering into the food chain. Because of the highly irregularities of contaminants on this plot the results from MI-04 were excluded from the statistical analysis.

The regression analysis showed a strong statistical significant relation between age of planting and metals in soil. This means that the older the plantation the lesser is the amount of chemical in soil. Results show a gradual decrease in metal concentration for almost all the metals across all plots except MI-04. The level of pH across all the sites was averaged between 5 and 6 with no particular seasonal or topographic variation.

Although there is an almost steady decrease in metal concentrations across all the plots there is also on several plots, high standard deviations or confidence intervals, indicating variations or presence of hotspots. This reiterates the repeated arguments made on the variable nature of mine spoils. Mapping does help to better locate and treat these concentrations and hotspots, although there must be acknowledgement of the variable nature of mine spoils when working on such sites. The decrease in the amount of chemical on all the sites indicates the role played by the trees planted on these sites. This is confirmed at site MD-07 where, **within three years of planting, young trees are playing a key role in the uptake of chemicals from the soil.** The results from the foliar analysis in the further chapters should help confirm this.

Summary: Analysis of soil metal concentrations showed the following results:

- Site MD-07 is mainly contaminated with Cd and Mn which are at critical soil levels and with hotspots which require immediate action and moderately contaminated with Cu, Pb and Zn.
- After only 3 years of planting trees plot MD-07 shows a 14 - 18% decrease soil concentrations of Mn which brings down the loading level from action/ treatment level to critical soil level and a 6% decrease of Cd.
- PLI confirmed that of the six plots studied MI07, MI04 and CA03 show strong signs of pollution or deterioration of the site quality.
- The regression analysis showed a strong statistical significant relation between age of planting and metals in soil on all plots except MI-04.

5.5 Foliar metal uptake and interaction – MD-07

Soil results suggest that tree planting helps remove metals from the soils. This section demonstrates that trees themselves are involved in this process and different species are involved at different degrees. In order to examine the prospects for phytoremediation, it is essential to gather accurate data on metal uptake by plant species. Foliar samples were collected in year 2 and 3 (July 2009 and July 2010 respectively) from the experimental site as well as CfN plots. The following section examines metal uptake by *Alnus*, *Betula* and *Larix* species on the experimental plot MD-07; data from the CfN plots are discussed later. Tables 5.5.1 and 5.5.2 describes metal concentrations in leaves at site MD-07 in 2009 and 2010.

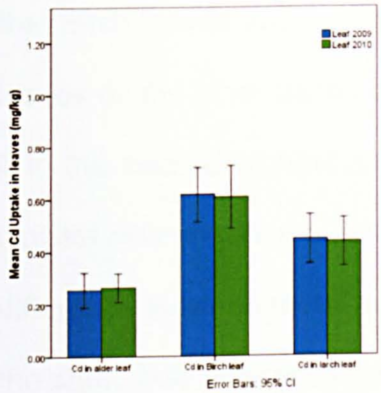
Table 5.5.1: Descriptive statistics of metal contamination in Alder, Birch and Larch foliage on MD-07 in 2009. Results are indicated in mg/kg (n=76)

MD-07 Alder	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.25	69.01	292.48	4.27	4.53
MIN	0.00	38.54	167.00	1.99	2.01
MAX	0.75	107.53	405.00	6.47	6.88
Std Dev	0.46	13.66	50.31	1.32	0.92
MD07 Birch	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.62	91.25	501.51	2.41	14.32
MIN	0.00	42.28	307.42	1.02	9.22
MAX	1.13	145.23	699.32	4.75	20.01
Std Dev	0.53	22.67	73.93	0.58	2.56
MD-07 Larch	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.56	38.88	599.32	12.90	3.80
MIN	0.00	12.24	322.04	4.31	1.05
MAX	0.79	62.55	789.32	17.85	10.45
Std Dev	0.46	12.34	113.01	2.69	1.56

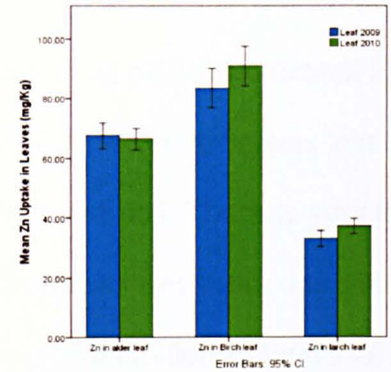
Table 5.5.2: Descriptive statistics of metal contamination in Alder, Birch and Larch foliage on MD-07 in 2010. Results are indicated in mg/kg (n=78)

MD-07 Alder	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.22	66.42	292.48	4.23	4.48
MIN	0.00	39.06	167.01	2.05	3.12
MAX	0.72	107.23	405.23	6.35	6.05
Std Dev	0.35	13.31	50.31	1.29	0.91
MD07 Birch	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.61	86.44	495.05	2.35	13.67
MIN	0.00	39.02	305.13	1.24	9.39
MAX	1.02	135.95	687.30	4.46	19.32
Std Dev	0.53	24.65	73.93	0.62	1.44
MD-07 Larch	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.53	35.23	591.43	12.35	3.35
MIN	0.00	17.12	302.34	3.97	0.92
MAX	0.76	56.34	789.59	16.79	9.55
Std Dev	0.43	9.75	113.01	3.03	1.42

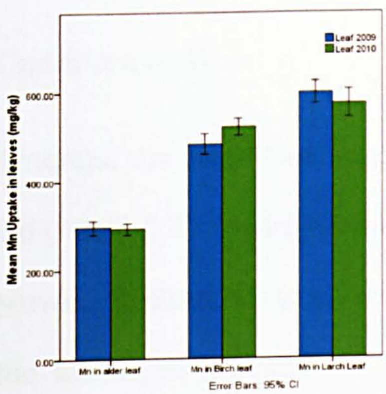
Tables 5.5.1 and 5.5.2 outline the descriptive statistics for foliar metal concentrations in Alder, Birch and Larch leaves in 2009 and 2010. There is not much difference in metal uptake between the two years and this is graphically represented in Figure 5.5.1. The figure compares the concentration of metals in the leaves of three trees on Site MD-07 in the two sampling seasons, July 2009 and July 2010. Graphs show error bars with 95% confidence interval - note the differences in the vertical scale. In contrast to the data from levels of metals in soil, there is no systematic difference between the two data sets.



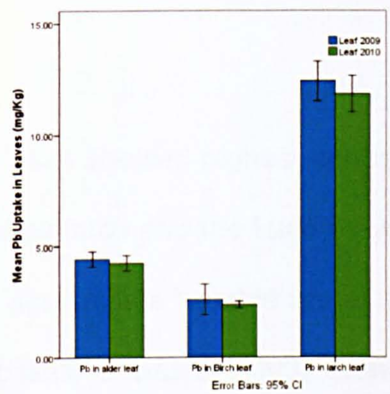
a) Mean Cd uptake in leaves in 2009 & 2010



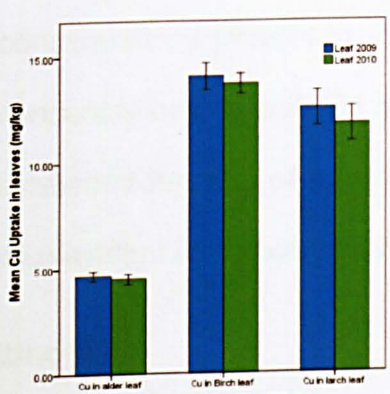
b) Mean Zn uptake in leaves in 2009 & 2010



c) Mean Mn uptake in leaves in 2009 & 2010



b) Mean Pb uptake in leaves in 2009 & 2010



e) Mean Cu uptake in leaves in 2009 & 2010

Figure 5.5.1: Concentration of metals in descending order in Alder, Birch and Larch leaves on Site MD-07 in 2009 and 2010. Error bars show 95% Confidence Intervals.

Figure 5.5.1 shows the metal concentration in leaves of the three trees over two years. From the graphs and descriptive statistics, it is evident that Birch leaves accumulate the maximum amount of Cd, Zn and Cu. Alder leaves on the other hand accumulates less of all metals except Zn, for which it is the second highest accumulator. Finally, larch leaves accumulate the highest concentration of Mn and Pb from the soil. There is very little obvious difference between metal accumulation across two years this could be due to the short time difference or due to the accumulation potential of annual tree leaves.

Cadmium (Cd)

Amongst the three tree species, birch trees showed highest concentration of Cd (mean 0.61 mg/kg) uptake followed by larch and the least by young alder leaves. Research has shown birch to accumulate notable amounts of Cd in the leaves at low soil pH values (Rosselli & Bashi, 2003; French, 2004). Although, the overall uptake of Cd was low, when compared to the overall concentrations present in soil the results were significant since, even at low concentrations in soil, Cd is a potential hazard. These leaf uptake results confirmed the role of trees in the reduction of soil metal contamination, as also evident on the contamination maps (Figure 5.5.1).

Zinc (Zn)

Significant quantities of Zn were accumulated by all three tree species, the highest after Mn in this study. Birch leaves accumulated the highest amounts of Zn with mean concentrations of 87 – 89 mg/ kg, followed by Larch needles with mean concentrations of 66 – 68 mg/ kg. Alder leaves show moderate levels of Zn accumulation. When compared to soil metal concentrations, foliar are promising and suggest that these species, especially Birch and

Larch may be effective for the phytoremediation of land contaminated with Zn (Borgegard & Hakan, 1989; Pulford, et al., 2001; Baltrenaites & Butkus 2007; Gallagher, et al., 2008; Moudouma, et al., 2012)

Manganese (Mn)

The availability of Mn in soil is greatly dependent on soil pH. At pH 5.5 – 6.0 Mn is soluble, available for uptake and can lead to Mn toxicity (Schulte & Kelling, 2004). Plants take up manganese as Mn^{2+} from soil, where it is highly mobile and can be leached, particularly from acidic soils. On site MD-07, which has an average pH of 5.7 Mn was present in quantities as high as 1800- 2000 ppm before tree plantation. After three years of planting, young Alder, Birch and Larch trees show a high uptake of Mn from soil, the highest for any element under the current study. Of the three tree species planted, young larch needles showed the highest accumulation of Mn in their leaves with a mean concentration ranging between 580 - 600 mg/kg, followed by birch and alders. Young trees leaves show great potential for Mn accumulation.

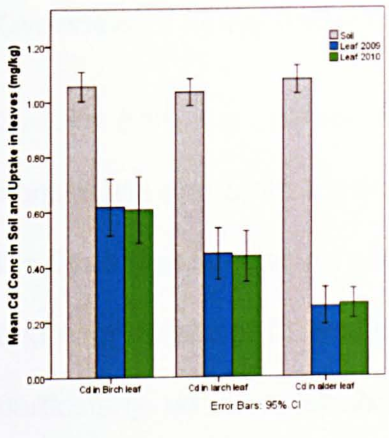
Lead (Pb)

Pb uptake into leaves was minimal in all three tree species. Very small quantities of lead were translocated into the leaves. Mean foliar values were between 2.4 – 4.0 mg/kg for alder and birch respectively, while Larch concentrations, although slightly higher, were only in the range of 12 – 14 mg/kg. The Pb concentrations in the site range from 199 – 223 mg/kg, which is just above the normal soil level. Very low measurable concentrations mean that phytoremediation of Pb by these tree species on this site is not a realistic option.

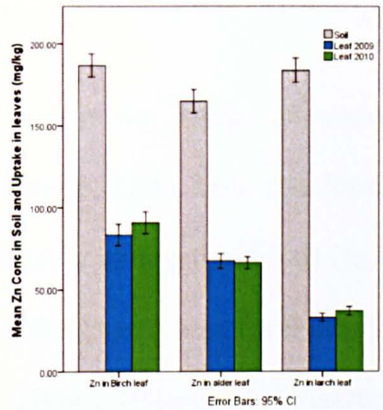
Copper (Cu)

Copper uptake in leaves was very low for all three species. Amongst the three trees, young birch leaves showed maximum concentrations with a mean uptake of 13 mg/ kg. When compared to the amounts present in the soil (Figure 5.5.2), the proportion taken into leaves was also very low. However, on site Cu concentrations in the soil range from 35 – 63 mg/kg, which are just above the standard values for normal soil and between the contamination threshold level. In other words, Cu does not pose an immediate threat to the site and the slow uptake by trees may be expected to reduce the soil levels towards normal condition in long term.

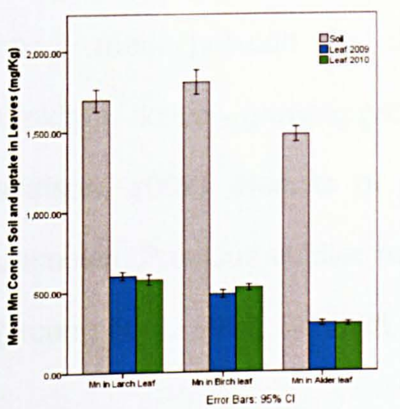
Foliar levels in three trees are compared with the soil metal concentrations and illustrated in the following graph. Figure 5.5.2 shows the concentration of metals in tree leaves over 2 years compared to the soil metal concentrations - note the differences in the vertical scales of each sub-graph. Once again results are represented in descending order of uptake. There is little variation between the metal concentration in leaves in 2009 and 2010, however, when compared to soil metal concentration Cd and Zn show the maximum uptake especially by Birch trees. Larch leaves show good uptake of Mn and Cd and a moderate uptake of Zn. Also Larch is the only species to show an appreciable uptake of Pb.



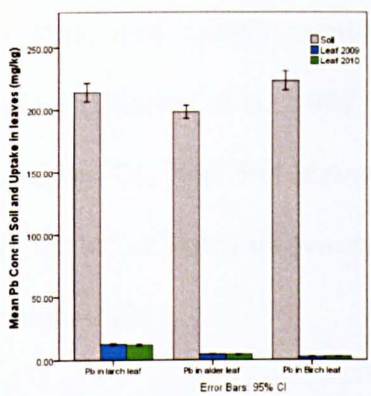
a) Mean Cd conc in soil and uptake by leaves



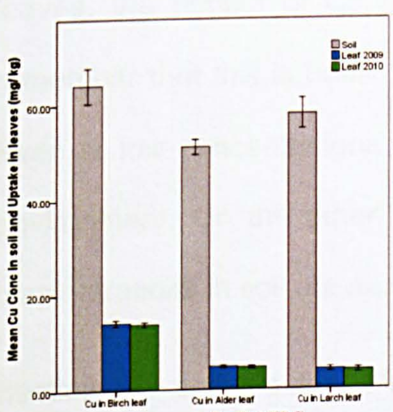
b) Mean Zn conc in soil and uptake by leaves



c) Mean Mn conc in soil and uptake in leaves



d) Mean Pb conc in soil and uptake in leaves



e) Mean Cu conc in soil and uptake by leaves

Figure 5.5.2: Concentration of metals (mg/kg) in Soil and its uptake by Alder, Birch and Larch leaves on Site MD-07 in 2009 and 2010. Error bars show 95% Confidence Intervals.

Overview of heavy metal uptake by trees

Results from the current research confirm the ability of young trees to translocate and store metals in their foliage. Data from the foliar uptake of the three tree species on site MD-07 show a strong uptake of Cd, Zn and Mn and poor uptake of Cu and Pb. These findings are new for these tree species particularly on mine spoils (French, 2004); Although Rosselli and Bashi (2003) have noted the uptake potential of Zn and Cd by *Betula* leaves at a heavy metal polluted site. Studies suggest that metals accumulate more rapidly in actively growing parts of the plant (Chaney et al., 1997; Pulford and Watson, 2002). Results of poor uptake of Cu and Pb however are not unknown. Previous studies have shown Pb to be highly unavailable to plants (Huang et al., 1997; Schmidt, 2003; French, 2004).

On comparing the amounts of different metals in soil and their uptake in leaves, the uptake of Cd by leaves may appear low, but one needs to remember that this is based on very low concentrations of the metal in soil. Even at low concentrations Cd is found to be very harmful to the soil and environment. On the other hand Pb uptake is negligible by trees but the concentrations in soil are much higher.

Section 5.1 showed that Mn contamination in soil at site MD-07 showed heavy contamination, with maximum level at the action/ treatment level. After only four years from planting tree saplings, the soil showed a 14 - 18% decrease in the level of Mn, which brought down the chemical level in soil from action/treatment to contaminated soil level as evident in the contamination maps (section 5.1.6). This coincides with the foliar uptake data which shows high concentrations of Mn in leaves, especially for Birch and Larch, but also in Alder trees too. Similarly, Birch leaves show a high uptake of Cd, followed by Larch, which has led to 6% decrease in the Cd level in soil.

Among the three tree species studied, Birch exhibited the highest concentrations for Cd and Zn. Larch needles showed the highest concentrations of Mn and Larch was the second highest accumulator of Cd. Alder trees were not the highest accumulator of any particular element but did accumulate considerable amounts of all elements except Cd, for which it was the lowest accumulator. Pb is known to be highly unavailable for metal uptake by plants and this is confirmed in this study by results from the three tree species planted on MD-07.

The following section explores the relationship between metal distributions in soil and leaves.

5.5.1 Metal distribution relationships: soil and leaves at MD-07

One of the factors in phytoremediation is the relationship between metal concentration in soil and uptake into plant tissues. In this case, the plant tissues explored are the leaves. If such relationships exist, it may be possible to predict the amount of metal that can be removed from the soil at specific concentrations. Initial relationships between foliar uptake and soil concentrations were conducted using Pearson's correlation (Table 5.5.3) using transformed data ($\log^{n \text{ or } 10}$) for foliar metals (year 3) and corresponding soil concentrations in year 3 for the three individual species. The Kolmogorov – Smirnov test for normality, which is appropriate for this sample size was conducted and all interactions except Pb and Cu in foliar samples were found to be log-normally distributed. Only significant results are shown in table 5.5.3 ($p<0.05$).

Table 5.5.3: Pearson's correlation matrix for metal in soil at MD-07 against metal in leaves of Alder, Birch and Larch trees.

	Mn Alder	Zn Alder	Mn Birch	Cd Birch	Zn Birch	Cu Birch	Mn Larch	Cd Larch	Zn Larch
Mn Soil	.248** 0.01		.288* 0.02				**.539** 0.05**		
Cd Soil				.371** 0.003				**.449** 0.002**	
Zn Soil		.286** 0.001			**.489** 0.002**				.276* 0.029
Cu Soil						.266** 0.035			

**- Significant at $p<0.05$; **- Significant at $p<0.01$. Values show Pearson's product correlation (r) and p values.*

Table 5.5.3 demonstrates that there are many significant positive correlations between the concentration of metals in the soil and the concentration in foliar samples. Pearson correlation suggests some strong relationships between metals in soil and subsequent uptake by leaves. Mn in alder, birch and larch trees is strongly correlated with soil concentration. Similarly, Zn in Birch, Alder and Larch leaves shows strong significant positive correlations with concentration of Zn in soil, while Cd has similar correlations with foliar concentrations in Birch and Larch but not Alder, and Cu only with Cu in Birch leaves. Despite its reputation as a metal excluder, Alders accumulate Mn and Zn in proportion to the amount in the soil, while Larch accumulates Mn, Cd, and Zn (but not Cu) and Birch accumulates four of the metals examined. Pb is not accumulated by any species and hence not included in the table above.

The next section explores the relationship between metal concentrations in the adjoining CfN plots and the uptake and accumulation of metals in trees on the individual plots. Data from all the plots has been pooled for individual species across all sites wherever essential for statistical analysis.

5.6 Foliar metal uptake and interaction – All plots reclaimed and experimental

The previous section established the patterns in the metal uptake by leaves of three tree species (Alder, Birch and Larch) and the soil on the experimental plot through the first three years after planting. In order to verify these results and to test the longer term potential of these trees to continue the process of metal and soil phytoremediation, further samples were collected from adjoining, older CfN plots, where planting dates range from 1993 – 2004.

Table 5.6.1 and 5.6.2 display the descriptive statistics of metal concentrations in Alder leaves across all of the study sites in 2009 and 2010. All results are recorded in mg/kg.

Table 5.6.1: Descriptive statistics of metal contamination in Alder leaves on the different CfN plots and the experimental plot MD07 in 2009 (n=59)

MD-07	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.21	69.01	240.82	4.27	4.53
MIN	0.00	38.54	167.00	1.99	2.01
MAX	0.75	107.53	405.00	6.47	6.88
Std Dev	0.46	13.66	50.31	1.32	0.92
CA03	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.32	82.69	307.73	4.65	4.91
MIN	0.01	54.75	189.00	2.15	3.02
MAX	0.52	98.56	433.00	6.90	7.31
Std Dev	0.36	12.35	58.51	1.33	1.08
SH97	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.23	63.56	243.53	3.86	4.76
MIN	0.04	31.24	201.24	2.53	2.21
MAX	0.42	89.43	357.66	6.49	7.94
Std Dev	0.49	18.56	41.55	1.49	1.46
TA94	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.25	64.24	268.63	3.41	4.99
MIN	0.05	33.61	199.33	1.53	2.04
MAX	0.31	78.42	375.03	6.78	8.43
Std Dev	0.55	9.54	26.35	1.26	1.34
PA93	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.27	62.66	292.55	3.91	5.50
MIN	0.00	32.45	136.22	1.94	1.02
MAX	0.30	70.43	389.50	5.77	10.30
Std Dev	0.42	15.32	68.53	1.42	1.95
MI04	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.49	87.34	318.21	5.31	5.42
MIN	0.00	32.35	201.32	3.22	4.22
MAX	0.86	109.35	437.77	8.96	7.84
Std Dev	0.53	19.38	68.43	1.43	0.93

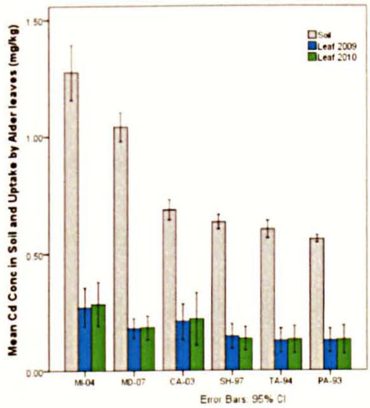
Table 5.6.2: Descriptive statistics of metal contamination in Alder leaves on the different CfN plots and the experimental plot MD07 in 2010 (n=52)

MD-07	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.19	66.42	238.48	4.23	4.48
MIN	0.00	39.06	167.01	2.05	3.12
MAX	0.72	107.23	356.23	6.35	6.05
Std Dev	0.35	13.31	50.31	1.29	0.91
CA03	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.32	71.56	307.73	4.65	4.91
MIN	0.10	52.12	189.17	2.20	3.17
MAX	0.52	96.35	433.21	6.92	7.64
Std Dev	0.36	11.48	58.51	1.31	1.08
SH97	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.20	60.04	243.82	3.78	4.69
MIN	0.09	32.04	201.19	2.66	2.01
MAX	0.37	87.19	354	6.37	7.66
Std Dev	0.47	17.29	37.88	1.27	1.15
TA94	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.21	55.85	264.85	3.76	4.61
MIN	0.08	32.19	195.02	2.01	2.24
MAX	0.29	71.52	371.42	6.23	7.39
Std Dev	0.41	9.52	34.46	1.26	1.48
PA93	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.23	54.32	289.50	3.58	5.16
MIN	0.00	32.66	137.32	1.99	0.00
MAX	0.30	70.41	398.05	5.30	9.01
Std Dev	0.39	15.80	60.19	1.01	2.07
MI04	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.41	77.80	311.40	5.25	5.35
MIN	0.00	33.26	221.62	3.21	4.01
MAX	0.80	108.03	434.00	8.32	6.94
Std Dev	0.41	18.60	62.52	1.37	0.93

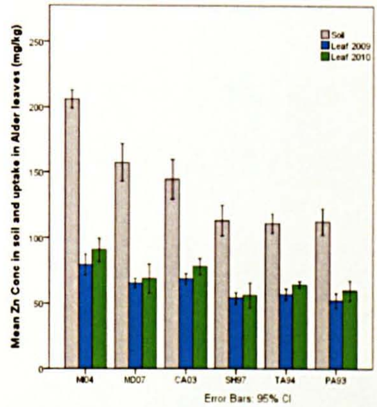
Tables 5.6.1 and 5.6.2 describe the metal concentrations in Alder leaves at the experimental site MD-07 and the CfN plots, planted between 1993 and 2004 as recorded in 2009 and 2010, two and three years after planting. The effect of time after planting on uptake by Alder leaves across the various plots and its subsequent impact on soil metal concentrations is explored below.

Metal uptake in Alder leaves:

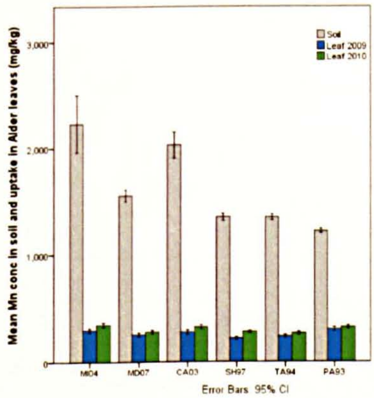
Across the whole range of plots examined, Alder leaves show a moderate uptake of Mn, Zn and to some level Cd. The levels of these metals in the leaves are almost consistent across all the plots with the exception of plot MI04 which show the highest uptake of Mn (M 311.40, SD 62.52) and Zn (M 77.80, SD 18.60). Comparison with the soil data (Fig 5.6.1) shows a gradual decrease in the amount of chemical in soil over the years. Alder leaves achieve little translocation of Pb and Cu from soil to the leaves. The graphs (Fig 5.6.1) below show the variation in metal uptake by Alder leaves across the different plots. Note the high amount of metals in soil and its subsequent uptake in leaves at MI04 (Fig 5.6.1 a – e).



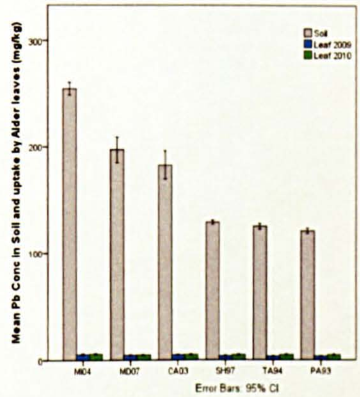
a) Mean Cd conc in soil and Alder leaves



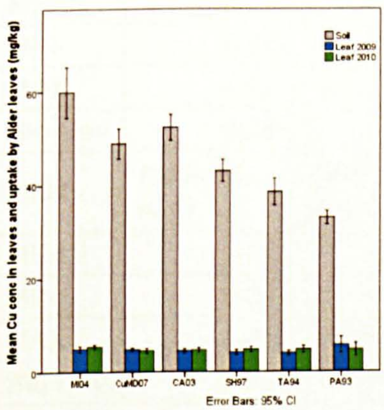
b) Mean Zn conc in soil and Alder leaves



c) Mean Mn conc in soil and Alder leaves



d) Mean Pb conc in soil and Alder leaves



e) Mean Cu conc in soil and Alder leaves

Figure 5.6.1: Concentration of metals in soil (mean 2009-10) and their uptake by Alder leaves at the various study sites in 2009 and 2010. Error bars show 95% Confidence Interval.

Metal uptake in Birch leaves

Tables 5.6.3 and 5.6.4 display the descriptive statistics of metal concentrations in Birch leaves across all of the study sites in 2009 and 2010; all results are indicated in mg/kg.

Table 5.6.3: Descriptive statistics of metal contamination in Birch leaves on the different CfN plots and the experimental plot MD07 in 2009 (n=63)

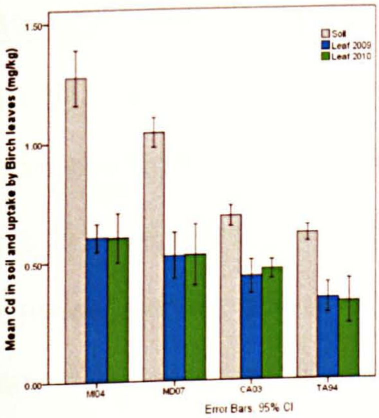
MD07	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.62	91.25	452.51	2.41	14.32
MIN	0.00	42.28	307.42	1.02	9.22
MAX	1.13	145.23	545.32	4.75	20.01
Std Dev	0.53	22.67	73.93	0.58	2.56
CA03	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.59	83.24	467.32	2.80	1.49
MIN	0.01	31.21	325.32	1.95	10.69
MAX	0.84	125.63	563.53	4.52	18.45
Std Dev	0.54	24.35	64.24	0.73	3.21
TA94	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.47	78.08	501.21	2.41	11.21
MIN	0.00	22.12	213.30	1.01	6.80
MAX	0.62	120.03	699.53	3.21	13.56
Std Dev	0.58	25.00	96.64	0.65	1.32
MI04	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.71	105.42	557.23	3.43	15.82
MIN	0.01	46.64	404.22	1.05	10.42
MAX	1.18	159.24	643.24	5.81	20.62
Std Dev	0.52	43.23	52.36	1.03	1.46

Table 5.6.4: Descriptive statistics of metal contamination in Birch leaves on the different CfN plots and the experimental plot MD07 in 2010 (n=68)

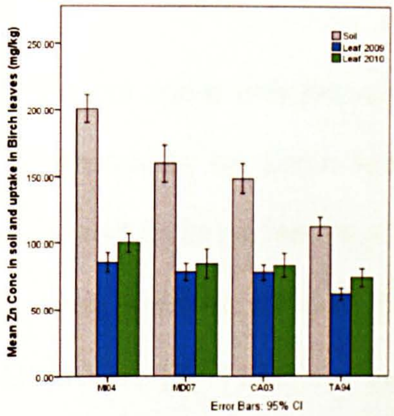
MD07	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.61	86.44	445.05	2.35	13.67
MIN	0.00	39.02	305.13	1.24	9.39
MAX	1.02	135.95	572.30	4.46	19.32
Std Dev	0.53	24.65	73.93	0.62	1.44
CA03	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.57	79.96	463.42	2.73	14.50
MIN	0.01	32.12	322.01	2.01	10.21
MAX	0.83	120.21	599.00	4.12	18.24
Std Dev	0.53	21.02	62.38	0.66	2.61
TA94	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.46	65.65	495.04	2.41	10.79
MIN	0.00	12.02	211.03	1.03	8.33
MAX	0.60	120.40	652.62	3.21	13.50
Std Dev	0.50	25.00	100.77	0.65	1.44
MI04	**Cadmium (Cd)**	**Zinc (Zn)**	**Manganese (Mn)**	**Lead (Pb)**	**Copper (Cu)**
MEAN	0.69	97.24	544.82	3.21	15.02
MIN	0.03	44.21	431.21	1.03	6.53
MAX	1.12	156.32	632.03	5.42	20.21
Std Dev	0.72	25.67	59.03	0.99	1.35

Birch trees were only planted on four of the seven plots studied in this research. Across the four plots studied Birch trees transferred the highest mean concentration of Cd (mean range between 0.46 and 0.69 mg kg^{-1}) and Zn (mean range between 78 to 100 mg kg^{-1}) to the leaves followed by Mn (mean range between 409 to 544 mg kg^{-1}). Birch leaves have shown a gradual decrease in metal concentration with time for most metals, with the exception of plot MI04 which showed high levels of Zn (M 97.24, SD 25.67), Cd (M 0.69, SD 0.72) and Mn (M 544.82, SD 59.03). Although this difference is not high, it does suggest two possible explanations, a) the amount of metal in soil has decreased over time hence leading to the low uptake by leaves and b) the older and mature the tree, the less is the uptake of chemicals, although there is no literature available to suggest this about the species under consideration. Birch also translocated some amount of Cu in the leaves (mean range between 10 – 15 mg kg^{-1}) as compared to Alder. Just like Alder leaves Birch do not show any uptake for Pb.

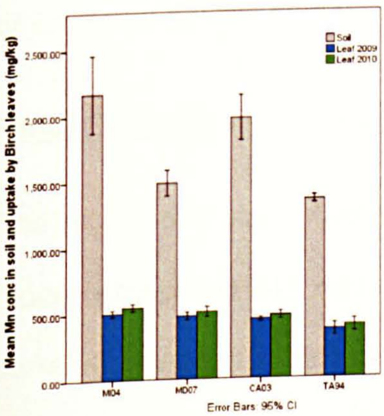
Figure 5.6.4 show the metal concentrations in soil at the various plots and their subsequent uptake by Birch leaves. Once again the results are in descending order of uptake and there is a gradual decrease in soil metal loadings with the age of planting; TA94 shows the least amount of chemicals in soil followed by CA03 and finally MD07 with the exception of plot MI04.



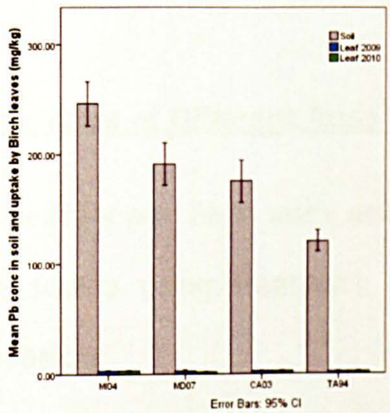
a) Mean Cd conc in soil and Birch leaves



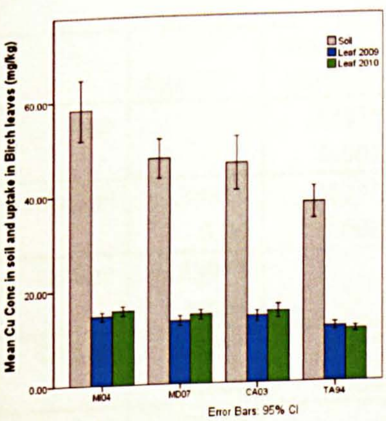
b) Mean Zn conc in soil and Birch leaves



c) Mean Mn conc in soil and Birch leaves



d) Mean Pb conc in soil and Birch leaves



e) Mean Cu conc in soil and Birch leaves

Figure 5.6.2: Concentration of metals in soil (mean 2009-10) and their uptake by Birch leaves at the various study sites in 2009 and 2010. Error bars show 95% Confidence Interval.

Metal uptake in Larch leaves

Larch was a new species introduced on the site and is only present at MD07. The descriptive statistics for uptake of chemicals by Larch leaves (table 5.5.2) show a good initial uptake of Mn, Cd and Zn in the leaves. Of the three species planted across all the plots Larch needles showed the highest accumulation of Mn in their leaves (M 599.32, SD 113.01). Young leaves also showed a good uptake of Cd (M 0.56, SD 0.46) and a moderate uptake of Zn (M 38.88; SD 12.34)

Metal Concentrations in Leaves in Plantings of Different Ages

The results of metal uptake by leaves for Alder and Birch trees across all the plots (except MI-04) was statistically tested using Pearson's coefficient correlation. And is outline in the section below.

Table 5.6.5: Pearson's correlation matrix showing soil metal concentrations against foliar metal concentrations for Alder and Birch trees across all the plots.

	Age	Mn Alder	Cd Alder	Zn Alder	Mn Birch	Cd Birch	Zn Birch
Age		.573** 0.002	.468** 0.001	.302** 0.001	.385** 0.001		.205** 0.004
Mn Soil	.388** 0.00	.453** 0.003			.348* 0.005		
Cd Soil	-.830** 0.001		.337* 0.008			.301* 0.007	
Zn Soil	-.672** 0.001	.366** 0.004		.489** 0.005	.393** 0.002		.589* 0.002
Cu Soil	- .651** 0.00						

**- Significant at p<0.05; **- Significant at p<0.01. Values show Pearson's correlation (r) and their significance (p) values. Bold figure boxes show high value.*

The concentrations of metal taken up by leaves for Alder and Birch trees across all the plots (except MI-04) was tested statistically using

Pearson's correlation (Table 5.6.5). Results show significant correlations ($p<0.001$) for Alder and Birch leaves. These results suggest some strong relationships between metals in soil and subsequent uptake by leaves. There are also strong relationships seen between metals in soil and leaves against the age of the plantation

<u>MI-04</u>: Due to the high variability of metal distribution and uptake the data was analysed twice once with MI-04 and once without MI-04 to reduce any discrepancies. Since a great variation was noticed the results for plot MI-04 were excluded from the statistical test. The correlations (r value and significance p value) and regression r^2 values are for soil and leaf samples from all plots except MI-04

<u>Correlation between metals in soil against age of planting:</u>

Results from the Pearson's correlation analysis show very strong negative, highly significant, correlations between Cd in soil against age of planting ($r= 0.830$, $p = 0.001$), Zn in soil against age of planting ($r = 0.672$, $p = 0.001$) and Cu in soil against age of planting ($r= .651$, $p = 0.00$).

<u>Correlation between metal in leaves against age of planting:</u>

Results also show a strong highly significant correlation between age of planting and Mn in Alder leaves ($r = 0.573$, $p = 0.002$), age against Cd in Alder leaves ($r= 0.468$, $p = 0.001$) and moderate but still highly significant, correlation between age and Mn in Birch ($r = 0.385$, $p = 0.001$) and moderate correlation between age against Zn in Birch ($r = 0.205$, $p = 0.004$).

Correlation between metals in soil against metal in leaves:

Foliar results show highly significant, correlations between Mn in soil and Mn in Alder ($r = 0.453$, $p = 0.003$); Zn in soil and Zn in Alder ($r = 0.489$, $p = 0.005$) and Zn in soil and Zn in Birch ($r = 0.589$, $p = 0.002$). There are also weaker but still highly significant correlations between Mn and soil and Mn in Birch.

Some other significant correlations between soil concentrations of Zn and foliar concentrations of Mn in Alder ($r = 0.366$, $p = 0.004$) and Birch ($r = 0.398$, $p = 0.002$) trees are harder to explain. They may suggest that the availability of Zn in the soil facilitates the uptake of Mn by the Alder and Birch leaves. Similar results have also been found for Larch trees (as shown in table 5.5.3) which were only planted on MD-07. The data for the correlations at individual plots also confirmed these links

5.7 Overview of heavy metal uptake by tree leaves on all plots

Data from the foliar uptake for Alder and Birch species on all the study plots have shown strong uptake for Mn, Zn and Cd and poor uptake with Cu and Pb. This is similar to the results obtained at the new site MD-07. The adjoining CfN plots were included to assay the potential of these trees for reducing the metal loading over a longer timer scale. The soil metal concentrations of Mn, Cd and Zn in the plots planted between 1993 and 2007 show the following rank order of MD-07>CA-03>SH-97>TA-94>PA-93 [Cd ($r = 0.830$, $p = 0.001$); Zn ($r = 0.672$, 0.001) and Mn ($r = 0.388$, $p = 0.00$)], which indicates that, as the age of the tree plantation increases, the level of soil metal contaminant loading decreases.

Foliar metal concentrations vary with metal and tree species. Pearson's correlation for Alder tree leaves show Significant strong positive correlation between age of planting and concentration of Mn and Cd in leaves, whereas Birch leaves show a, still significant, but more moderate correlation with age. This suggests that with an increase in age of Alder tree there is an increase in metal accumulation in leaves. The descriptive statistics may not suggest this but that may be due to the sampling size as only a handful of leaves (10g dried sample) were collected from each tree. If the uptake of metals in Alder at MD-07 is compared to the uptake of metals at the adjoining plots it is evident that with an increase in age of the planting there is an increase in the uptake of different metals by Alder leaves. Birch on the other hand shows strong uptake in young tree leaves as compared to trees planted 17 years ago on TA-94 plot.

When individual metals in soil are compared to their subsequent uptake into leaves Alder have shown a positive correlation between Mn in soil and their subsequent uptake in leaves. Zn is readily taken up by Alder and Birch with Birch leaves showing the maximum uptake. Similar results have been observed by Borgegard and Hakan (1989) in a study that examined the metal concentrations in Birch trees that had colonised over copper mine tailings. Zn, Pb and Cd concentrations in leaves of Birch trees were found to exceed values for leaf concentrations of trees grown on uncontaminated soil.

Previous studies by Huang et al., (1997); Schmidt (2003) and French (2004) have shown the inability of Alder leaves to translocate Pb and Cu in the leaves and this has been confirmed in the present study. As in plot MD-07, CfN trees show little uptake of Pb and Cu.

A new observation has been recorded between the correlation of Mn in leaves of Alder and Birch under the presence of Zn in soil. Nowhere in the literature has this been previously recorded. Similar results were also observed for Larch at MD-07 but additional long term field data for larch are required to verify these results. Mn and Zn are both essential elements in the soil, which are required in small amounts by the plants (Walter, 1988) and often coexist. However, correlation at such levels of concentration is new and not recorded previously.

<u>Summary:</u>

- On all the study plots Alder and Birch species have shown strong uptake for Mn and Zn with Birch leaves showing the maximum uptake for Zn and Cd. Larch showed maximum uptake of Mn in leaves and was the only tree to accumulate Pb from soil.
- With an increase in the age of the tree plantation, the level of soil metal contaminant loading decreases significantly.
- Alder tree leaves show significant strong positive correlation between age of planting and concentration of Mn and Cd in leaves.
- A new observation has been recorded between the correlation of Mn in leaves of Alder and Birch under the presence of Zn in soil.

5.8 Annual metal distribution in leaves

Some of the hyperaccumulator trees used for phytoremediation show annual differences in the uptake of different metals in leaves, which seems to be dependent on site and climatic site conditions (Windham et al., 2003). However, this is not always recorded, especially among non-hyperaccumulator species. Due to the elevated uptake of metals by leaves prior to leaf senescence, changes may occur within a single growing season (Windham et al., 2003; French, 2004). Here, data from the two field trial seasons is used to examine annual variations in metal uptake. These factors can make predicting the foliar uptake more difficult and, in the case of species that are harvested for phytoremediation, this may impact on predicting the optimum time of harvest.

Foliar metal concentration data from the second (2009) and third (2010) year were compared for each species and each chemical using paired t-test on ($\log x^{+\,n}$) transformed data in order to determine any significant differences (Table 5.7). The data from all the plots was pooled together. However, because of the unusual circumstances affecting MI-04 and its high variability, the data was analysed twice, once with MI-04 and once without MI-04, to reduce any discrepancies.

Table 5.7: T-test results for annual variability fluctuations of seasonal difference in metal uptake by leaves.

Metal	t-test result	species
Manganese	No significant difference between year two and three for any species	-NA-
Cadmium	Significant difference between year two and three	Larch (p = 0.003)
Copper	No significant difference between year two and three for any species	-NA-
Lead	No significant difference between year two and three for any species	
Zinc	Significant difference between year two and three	Birch (p= 0.026) Alder (p= 0.016)

Pooling the data across the different plots shows very little overall difference in metal uptake by leaves over the two years. Zinc is the only metal which shows significant annual difference in its uptake by Birch (p=0.026) and Alder (p=0.016) leaves. The results for Cd uptake by Larch (p= 0.003) are only from plot MD-07 where they were planted. Further research is required on Larch species to see if older trees would show a similar significant variation in annual metal uptake.

Results from the paired t-test at MI-04 show significant difference between annual metal uptake for Mn (p=0.004). This difference is however not observed for any other plot suggesting that the observation is a result of the high variability of the plot and not due to a sudden change in the environmental or seasonal conditions.

5.9 Soil metal concentration after last sampling season – compared against background levels (BL) for all plots

As explained in section 1.7 there are processes that take place within the soil especially in the rhizosphere, which facilitates or inhibit metal uptake by trees. Observations of the foliar metal concentrations have shown that trees accumulate a range of metals in their leaves and, presumably other plant tissues. The next questions is whether or not this metal uptake is sufficient to affect the overall soil metal loading and whether such changes can be detected just 3 years after planting.

The following section looks at the changes in the soil metal concentration at a) MD-07 after three years of tree planting, and b) difference in metal concentrations at the adjoining CfN plots after sampling older trees. The data from all the plots have been pooled together for graphical representation and does not show the variation within individual plots. However, the data will be statistically examined by paired t-tests. All these results will be compared against the background levels benchmark (BL) established in the previous section (5.2) of this chapter and the anomalous results from MI-04, where the topsoil protection of the minespoils had been stripped away. Figure 5.9.1 shows graphs for metal concentration in soil at all the study plots in 2010 against the background levels (BL). Results for all the metals show a decrease in soil metal concentration with age of planting except for plot MI-04 again outlining the Special nature of the plot. Statistical results from the paired sample t-test show significant decrease in soil metal concentrations for most of the metals on all sites. Data is represented in the Table 5.8

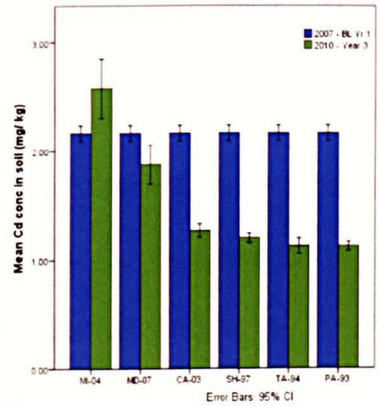
Table 5.8: Difference in soil metal concentration from 2007 to 2010: Significance (p) values from paired sample t-test.

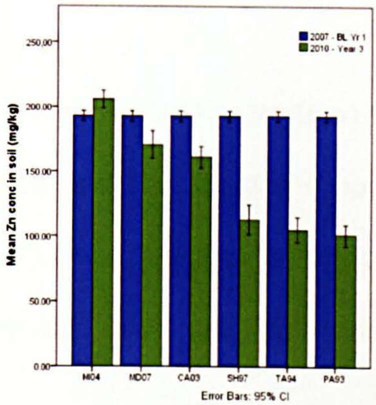
	PA-93	TA-94	SH-97	CA-03	MI-04	MD-07
Mn	0.02	0.03	0.021	--	--	0.03
Cd	0.00	0.03	0.00	0.02	-0.033	0.002
Cu	0.06				--	
Pb		.027	0.052	--	--	0.00
Zn	0.001	0.01	0.023	0.04	- 0.04	0.02

**- Significant at $p<0.05$; **- Significant at $p<0.01$; negative value means increase in soil metal concentration from 2007 to 2010.*

Table 5.8 shows a consistent pattern across some sites. For example the changes in Mn across PA-93, TA-94, SH-97 and MD-07 are statistically significant with the exception of CA-03 and MI-04. Similarly Cd and Zn show significant difference in metal concentrations across all the plots except MI-04 (Cd p= -0.033; Zn p = -0.04). This suggests that as the age of the plot increases, the concentration of metal in soil decreases which is also evident from the graphs in Fig 5.6.2. The role of plants in this phenomenon will be described in the next chapter.

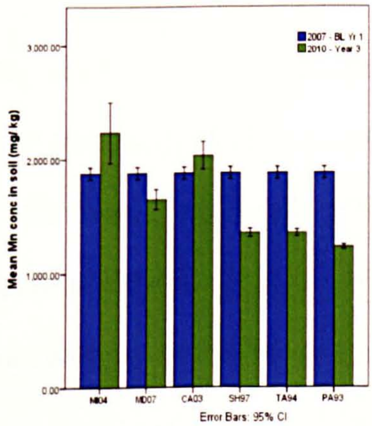
The negative p value at MI-04 means there is an increase in metal concentration (Zn and Cd) at the plot. The negative difference could be a result of a) an error due to low analytical recovery values or b) there is a genuine increase in the metal concentration due to various factors like exposed compacted minespoils due to ploughing, elevated soil metal concentrations due to weathering etc. which will be explained in the discussion chapter.

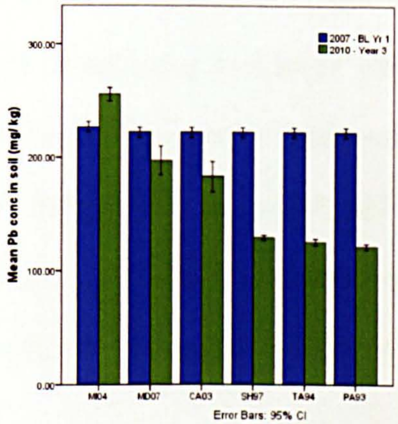




a) Mean Cd conc in soil in 2007 and 2010

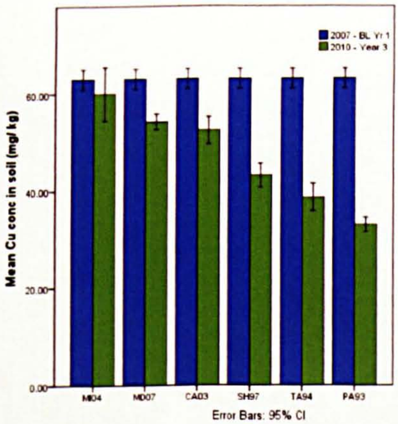
b) Mean Zn conc in soil in 2007 and 2010





c) Mean Mn conc in soil in 2007 and 2010

d) Mean Pb conc in soil in 2007 and 2010



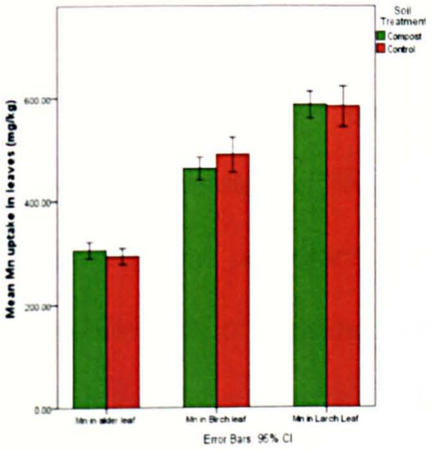
e) Mean Cu conc in soil in 2007 and 2010

Figure 5.7: Difference in soil metal concentration between Year 1 (BL-07) and Year 3 (2010) on all plots. Error bars show 95% Confidence Interval.

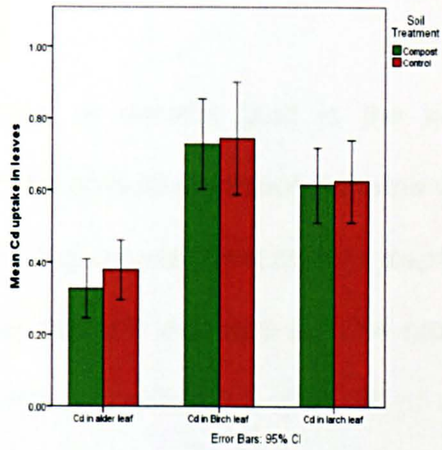
5.10 Addition of Compost

On the experimental plot a single dose of compost (1 Kg per tree) was added at the time of planting to half of the trees and the remaining half were left untreated. This was done to study the effect of compost on the growth and uptake of metals by tree leaves. Results from the foliar analysis are represented in figure 5.10.1

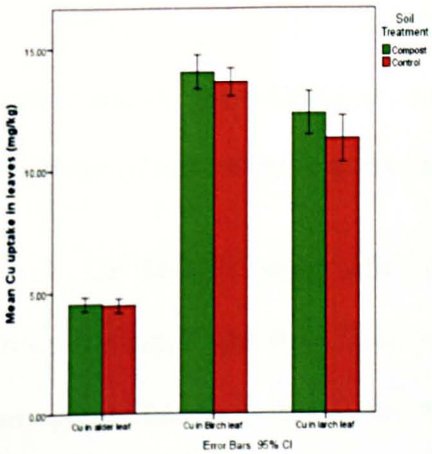
Results show no significant difference between the uptake of metals by trees which received an initial dose of compost and trees which had no soil treatment. An independent sample t-test was used to statistically test this difference. The t-test revealed that there was no significant difference between the amount of metal in leaves of trees which received a single dose of compost and trees which did not receive any compost treatment.



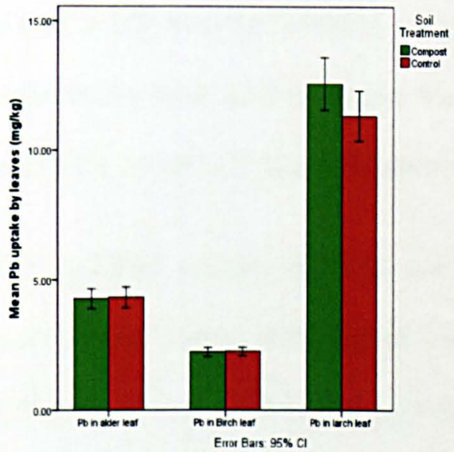
a) Mean Mn conc in compost & control soil



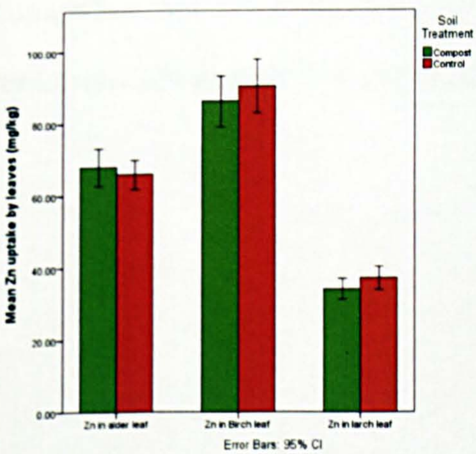
b) Mean Cd conc in compost & control soil



c) Mean Cu conc in compost & control soil



d) Mean Pb conc in compost & control soil



e) Mean Zn conc in compost & control soil

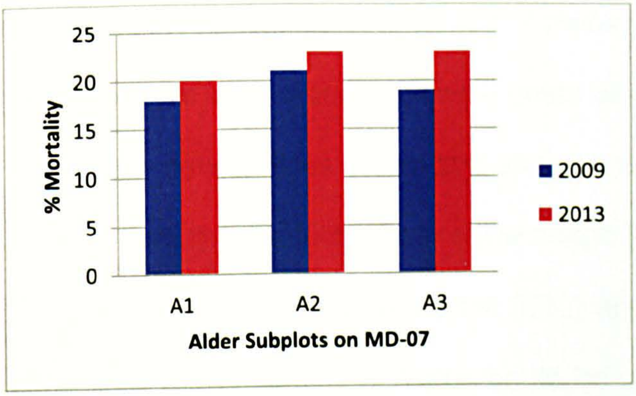
Figure 5.8: Concentration of metals in leaves from trees treated with compost and without compost on MD-07. Error bars show 95% Confidence Interval.

5.11 Tree mortality

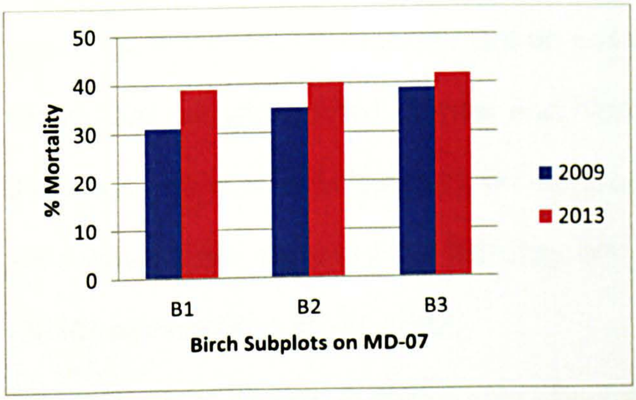
Survival of trees on contaminated or derelict land is the key to successful remediation. The success of any phytoremediation scheme would be related to the proportion of trees surviving on site, despite their capability for stabilisation or uptake. The planting scheme adapted for this study is suitable for phytoremediation, forestry and biomass production, all of which require establishment and survival and growth of plants.

Perry and Handley (2000) stated that in community forestry, excessive tree mortality may reinforce negative effects on land and or areas that are deprived of natural cycles. However, loss rates on MD-07 were relatively low

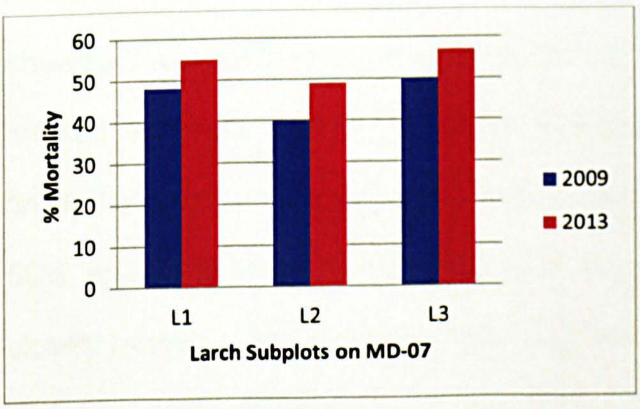
On MD-07, trees were planted in a Latin square of nine subplots, three for each species. Tree mortality was recorded at the end of the first sampling season in 2009 and in 2013. Figure 5.11.1 shows the percentage mortality of the three species at each subplot on MD-07. The average cumulative mortality in Alder was 19% and 22%, for Birch 35% and 40%, and for Larch 46% and 54%, in 2009 and 2013 respectively.



a) Percentage mortality for Alder at the three subplots on MD-07



b) Percentage mortality for Birch at the three subplots on MD-07



c) Percentage mortality for Larch at the three subplots on MD-07

Figure 5.9: Percentage mortality of Alder, Birch and Larch at the three subplots on MD-07 in 2009 and 2013.

Tree mortality during the first full growing season is often high because these young plantings may be more susceptible to environmental and soil conditions, especially on problematic or critical lands (Bending and

Moffat, 1999; Hutchings et al., 2001; Putwain et al., 2003). If mortality is very high then this may incur additional costs of replacement by the same or a completely new species depending on the end use of the site.

Results from the mortality study show a high loss in larch trees (mean= 46%) in 2009 followed by Birch (mean = 35%) and the lowest by Alder (19%) in 2009. The high mortality of Larch could be due a variety of factors such as poor stock quality, unsuitable planting method and seasonal variation on site, waterlogging or lack of establishment on soil with low fertility. However these results are not unexpected. Similar and higher results for Larch mortality in the initial years of establishment on derelict, contaminated and brownfield sites have been recorded by Bending and Moffat (1999), Webber et al. (2010) and Barbeito et al., (2012).

Haigh et al. (2013) studied the effect of planting method on growth of *Alnus* and *Quercus* trees on compacted coal spoils in Wales. Their results showed that growth rate and survival of both species depended on planting method and was highest for trench planting followed by pit planting and finally for notch planting. Their 3 and 5 year mortality rates for Alder were 55% and 44% respectively. They also found that the tree mortality was closely related to the degree of loose soil around the roots, which suggested that trees with lower density soils in the rooting zone showed greater survival.

Results have shown that the research questions set at the beginning of Chapter 4 have been answered. How these relate to the wider aims and objectives of this study is explained in the following chapter.

CHAPTER 6

DISCUSSION

6 DISCUSSION

This research started off with some questions that were identified after reviewing the literature on the reclamation and remediation of open cast mine spoils through forestation, especially in Wales. A test site was selected that displays many of the common problems of such lands namely metal contamination and soil compaction. This test site, at Varteg Hill, Torfaen, already had a tradition of NGO forestry experimentation and, as the focus of community forestry research for much of the period 1990-2004, had seen regular tree planting episodes (Haigh et al., 2013). In 2007, land obtained on this site was used for the creation of a new test plot for the experimental examination of the phytoremediation of some of the key tree species used in land reclamation in Wales, namely Alder, Birch and Larch. Two of these species had been planted widely previously by the CfN NGO project and the third, Larch, was one strongly commended for land reclamation sites by the UK's Forestry Commission (Forestry Commission 1991; Moffat and McNeil, 1994). The new test plot was established formally, as a Latin Square, to explore the role of these three species in the remediation of metals in the mine spoils. Tests also compared whether or not tree performance might be affected by some additional compost fertiliser on planting – in fact – this made no significant difference to the performance of the three tree species or their uptake of metals (Section 5.10).

The main aim of research measurement was to compare the uptake of five common metals contaminants of mine spoils. These were two metals regarded as readily mobilised by plants, namely Cadmium (Cd) and Zinc (Zn), two that were notable for being much less easy to mobilise, namely

Lead (Pb) and Copper (Cu), and one that was moderately mobilised, Mn (Cottenie et al., 1982).

To this end various soil test and leaf sample analyses were carried out to identify the extent of soil contamination at these sites and, through foliar analysis, to test the potential of tree species to remediate this damage. These findings were compared with benchmarks sourced from the literature regarding normal, critical and severe 'immediate action' levels of contamination (e.g Kelly, 1980; Alloway, 1990; Haigh, 1995 and Braithwaite, 1999) and with background level data from the site, prior to planting, and from an adjacent site where topsoil had been removed to expose the mine spoils to a fresh bout of weathering (MI-04) was additionally used.

Maps were recreated to show the distribution of each of these metal contaminants across the new and earlier test plots, which confirm the existence of contamination hotspots (Moffat, 2006). However, mappings for sequential years after planting on the new MD-07 test plot show that after only two or three years, metal levels in the soil were becoming significantly reduced (Section 5.1, Figure 5.2.1 – 5.2.5). Subsequent studies extended these tests to older plantings creating Chronosequence reaching back 17-18 years to 1993 and these conformed that the reductions in metal levels continued as each planting aged.

Foliar analysis, used to identify the amount of metal uptake by plant tissues, was carried out for Alder, Birch and Larch leaves. These demonstrate the metal accumulation potential of these trees and indicate both their role in taking up metals from the soil and the ways in which this changes through time.

The research questions are summarised in Table 6.1. This chapter revisits each one of these in turn and evaluates the project's findings with results reported elsewhere.

Table 6.1: List of research questions

1	What are the concentration levels and spatial distribution of key metal contaminants: Zn, Cd, Mn, Cu and Pb in the reclaimed mine spoil at Varteg, South Wales?
2	What level of metal uptake is possible by Alder, Birch and Larch trees as represented by foliar analysis on the Varteg test plots?
3	How is foliar uptake of metal contaminants affected by the soil composition and age of tree plantation?
4	How is metal concentration in soil affected by the age of plantation?

Q1: What are the concentration levels and spatial distribution of key metal contaminants: Zn, Cd, Mn, Cu and Pb in the reclaimed mine spoil at Varteg, South Wales?

This research question has been answered in two parts. Part one looks at the physical mine spoil conditions and part two looks at the mine spoil chemistry.

Mine spoil characteristics: Mining causes not only the disruption of the soil but of the entire natural ecosystem around it. Hence, the reclamation of mined land can predominantly be treated as an ecosystem reconstruction (Cooke and Johnson, 2002). Previous mining activities on the Varteg hills left not only a visual scar on the site but after sampling and analysis it was evident that the soil condition was deteriorating. The soil at Varteg suffers greatly from soil compaction, low organic matter and heavy metal contamination (Haigh and Sansom, 1999).

Soil compaction naturally develops on many reclaimed soils due to the weathering of mine spoils (Haigh, 1995). Results from the soil bulk density tests showed that site MD-07 shows a soil bulk density of 1.81 g/cm^3 at a depth of 50cm. Barnhisel (1988) described the term critical soil bulk density as the soil density at which root growth ceases. This however would vary based on the soil conditions, location, previous land use and plant species. According to Bending et al. (1992) the ideal minimum soil bulk density for forestry on disturbed land up to 50 cm depth should be <1.5 g/cm^3 and that for up to 100 cm depth should be <1.7 g/cm^3. On the study site with a bulk density of 1.81 g/cm^3 root penetration becomes difficult. This has been observed during plant vitality research on the adjoining CfN plots by Haigh (2000) and Haigh et al. (2011), where root penetration can be restricted to fissures and macro-pores.

Soil compaction directly affects the growth of the plant as the roots are unable to penetrate soils with high bulk density to absorb water and nutrients for growth and development. Sheoran et al. (2010) noted that severely compact mine spoils with less than 60cm of rooting depth and the presence of large bedrocks in the top layers, are unable to hold sufficient amounts of plant available water to sustain vegetation leading to drought like conditions. This phenomenon was visible across most of the plots in the current study.

Soil pH is another indicator of soil health and the possible success or failure of growing plants. At the study site, the pH on all the plots was in the range of 5.2 – 5.7. According to Moffat and Bending's report for the British Forestry Commission (1999) soils that are being reclaimed for forestry must have a pH in the range of 3.5 – 8.5.

Low soil moisture content was recorded at the site and soil texture analysis showed the minute occurrence of clay in the soil at the mine spoil site accounting for the lack of nutrients and soil moisture content recorded. As well as clay, organic matter also increases a soil's moisture holding capacity. According to the Forest service, reasonable topsoil has between 0.8% and 1.5% organic content, (Williams & Schuman, 1987). This site exhibited a thin organic top soil layer of approximately 5 cm above the dense compacted and weathered mine spoil layer. The mine spoils results also show low levels of organic content ranging from 0.3 % to 0.5 %. Loss on ignition (LOI) results, were slightly higher than expected but this could be a result of the presence of coal shale fragments combusted during the analysis. All of these observations point towards very poor soil conditions which are necessary to tackle in order to reclaim the soil.

Variation in climatic conditions such as temperature changes, wind and rainfall can affect the environment and soil system which in turn can affect the mine spoil, properties, processes, chemistry and the uptake of metals by plants. Soil is an integral part of nutrient cycles and two of the most important cycles affected by climate change are the carbon and nitrogen cycles as they play an important role in soils organic matter content (Brady and Weil, 2008). A healthy soil organic matter is essential for the development, growth and survival of soil ecosystem. An increase in temperature can lead to loss of organic matter, which in turn can increase CO_2 emission (SEPA, 1999). On the other hand an increase in atmospheric CO_2 due to climate change can augment biomass production and accumulation of metals in plants. This in turn can support and protect microbial populations against heavy metal stress (Rajkumar et al., 2013) and/or increase metal bioavailability. Warmer and wetter soils are likely to increase emission of N_2O from nitrogen-fertilised soils and this can result in the conversion of nitrate to nitrogen gas (SEPA, 1999).

Climatic variations affect the survival and performance of trees and their metal accumulation potential on such degraded coal spoils. This can be due to changes to various physical, chemical and biological properties of the environment and soil ecosystem (Scullion and Malinovszky, 1995; Rajkumar et al., 2013). Climatic changes leading to weathering of soil forming materials can also alter soil pH, heavy metal loads and their mobility and bioavailability within the soil system (SEPA, 1999). It is essential to plant trees like Birch and Larch which can translocate or mobilise these metals and prevent them from spreading in to the wider environment. Heavy rains on degraded mine spoils can result in surface runoffs and wetness, waterlogging due to soil compaction, leaching, rapidly changing soil conditions due to changes in weather and extreme susceptibility to drought due to shallow rooting (Scullion and Malinovszky, 1995; Marashi and Scullion, 2004). McVean (1953) suggested that Alders, due to their extensive root system, are able to survive on waterlogged soils. However, studies by Moffat and Roberts (1998); Moffat and McNeill (1994); Moffat et al. (1989) and Roy et al. (2007) have suggested that many trees including Alders perform poorly on prolonged waterlogging sites. The study area in Varteg does pose seasonal climatic challenges, mainly seasonal waterlogging, surface runoff due to lack of vegetation cover and drought conditions during summer. Hence, it is essential to establish a tree cover on such lands to help tackle and survive harsh climatic conditions.

<u>**Soil Metal Concentration and Contamination:**</u> Heavy metals in reclaimed mine spoils can have multiple origins. They can be by-products of coal, native to the spoil, additions due to depositions from mining activity, waste dumps during and after mining, pollution from the adjoining metal ore mines (or the former 'British' steel works a short distance down slope), as well as from transport vehicles, power generation effluents and emissions (Bradshaw, 1980; Hangyel and Benesoczky, 1992; Haigh, 1995; Faz Cano et al., 2002). Here, the former opencast coal spoil on Varteg Hill, was tested for the presence of five important heavy metals Cd, Zn, Mn, Cu and Pb. All of these metals are important plant nutrients at some concentrations (Lasat, 2000; Pulford and Watson, 2003). However, when present in quantities above the normal acceptable range of metals, these metals can become contaminants and toxic to soil, plants and in the wider environment.

On the new research plot, MD-07, contamination maps (Section 5.1), created using SURFER 10 MS Golden software, provided an opportunity to visually identify the presence and changes in the soil metal concentrations in three years of planting. When recorded metal loadings of the soil were compared to the published standard values, the soil at MD-07 showed extremely high levels of Mn, Cd and moderately high levels of Zn. Bolan et al. (2003) described soil pH as the key variable in controlling the metal partitioning in between the soil and solution- phase. High levels of Mn are found in soils with low organic matter and soils which are easily waterlogged (Schulte and Kelling, 2004), which is certainly the case at Varteg (cf Faber-Maunsel, 2006) and also in soils with low pH values (5.5 and lower) (Schulte and Kelling, 2004). Soil pH also affects the availability of Zn more than any other factor, with maximum Zn availability at low pH 5.4 (Schulte, 2004). Similarly, low pH facilitates the availability of Cd in soil (Kirkham, 2004). At the study site pH was generally found in the range of 5.2 – 5.7 which helps explains the availability of some these metals.

The initial soil sampling was carried out in Year 0 (2007), prior to tree planting, and this data is treated as Background level (BL-07). Plotting results on to the maps helps identify the presence and variation of metals across the experimental plot MD-07. Hotspots for different metals were scattered across the study plot. Comparing these maps with maps created after tree planting and establishment in Year 3 (2010), reveals a visible decrease in the soil metal concentration especially for Cd, Zn and Mn, so leading to the conclusion that planting trees has helped reduce the initial soil metal concentration. These observations reflect a 14 - 18% decrease in the level of Mn in soil and approximately 8% decrease in the level of Cd and Zn in three years. This was sufficient to reduce the chemical level from 'Action/Treatment' levels to 'Critical' soil levels on most of the MD-07 plot.

This exercise revealed a probable gap in the current published soil; contamination standard values. Although these provide a useful database, there is a gap between the Contamination Threshold and the Action / Treatment level, where, of course, a vast number of contaminated lands fall.

In 2005 the Environment Agency (under part 2A of the EPA), estimated about 300,000 ha of contaminated land existed in England and Wales alone, and of 781 contaminated sites, just 35 were designated as 'special sites' (Action / Treatment) that need immediate attention. The remainder of these sites are left untreated and these include the majority of the lands counted 'derelict' in the UK, according to the National Land use Database (2001) which equates to approximately 66,000 ha. More recent figures are not available. However, it remains important to deal with these contaminated sites that fall into this treatment gap and so, for the purpose of this study, a 'critical soil' level was recognised, which would accommodate these chemical levels (Table 6.2).

Table 6.2: Standard published values for soil metal concentration

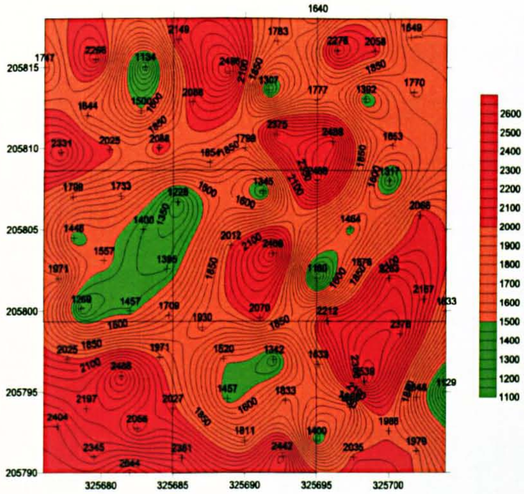
Standard values (mg/kg)	Mn	Cd	Cu	Pb	Zn
Normal soil*	<500	<1	<40	<200	<100
Contamination threshold*	500-1500	1-2	80	320	250
Critical soil	1500-2000	2-8	80-300	320-1000	250-500
Action / Treatment levels^	>2000	>8-10	>300	>1000	>500

*Standard values from Davies & Roberts (1978); Kelly (1980); ICRCL (1987); Bending and Moffat (1999); DEFRA (2004); Environment Agency (2009). ^ Action/Treatment Levels from Kelly (1980).

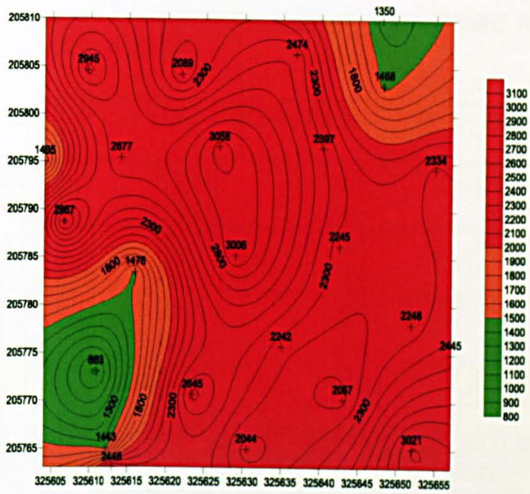
The measure of the degree of the overall contamination of the site was explored with the aid of the Pollution Load Index (Section 5.4). Tests provided substantial evidence of soil contamination at all sites. The levels of pollution discovered were greatest for MI-04 followed by BL-07 (which after tree planting is MD-07) and CA-03. Statistical analysis of soil metal concentrations for all the plots showed a mean decrease in metal concentration over time. Although, these tests are highly significant, there are also high standard deviations on several plots, indicating variations or presence of hotspots. This reinforces assertions about the variable nature of mine spoils (Bradshaw, 1980; French, 2004; Mmolawa et al., 2011). Mapping does help to locate and treat these hotspots but there must be acknowledgement of the variable nature of mine spoils when working on such sites.

However, it is recommended that such mapping exercises be included in all reclamation planning, especially for sites that fall into new 'critical soil' category, especially if the aim is to remediate such sites for public access like parks, open lands and forestry. For abandoned, poorly reclaimed sites like the one in the current research, where the mining authorities provide a one-time reclamation programme with limited funds, community involvement could be sought for an effective and sustainable regeneration of sites by linking a 'green approach' for land reclamation. Community forestry could be adapted for sites open to public access with areas planted with trees or other flourishing ecosystems (National Urban Forestry Unit, 2001). For areas with dangerous hotspots, restricted access may be necessary, while effecting remediation by planting trees.

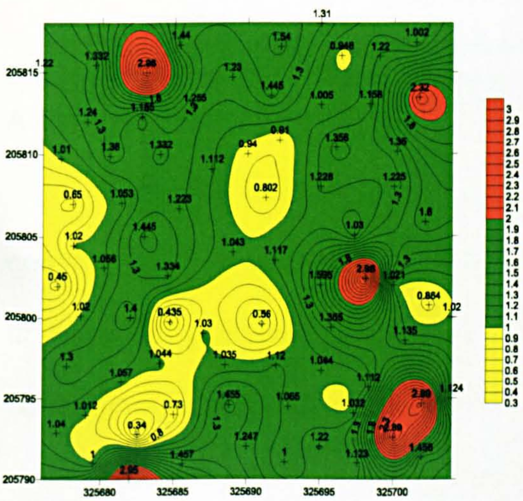
Site MI-04: Special Case- As outlined in Section 3.5.6, plot MI-04 was unusual on this site. This site was prepared for planting by ploughing, as per the recommended forestry guidelines, using a normal tractor drawn agricultural plough. This had unfortunate consequences, which may stand as a warning to others. In this failed remediation experiment, ploughing not only scraped off the little organic layer that existed but also exposed the opencast mine spoils buried beneath. These were now exposed to the full force of weathering and this seems to have sparked a new cycle of metal release and exacerbated the problem of metal contamination. This is evident from the results, which show irregular spikes in the data. Despite the challenges that this plot presents, the results observed have been included wherever possible and may stand, in this project, as an approximation of the conditions that would have held after the site was originally reshaped and prior to it being recovered with topsoil. The site is, thus, a special case that highlights the risks involved in ground preparation for forest planting. Contamination maps created for plot MI-04 show negative results. Maps for Cd and Mn against background levels showed visible negative results, meaning that the concentrations of these contaminants is higher than on neighbouring undisturbed surfaces. Independent sample t-test comparisons of the data of background soil levels (BL-07) and metal concentrations on plot MI-04 (planted in 2004) showed statistically significant higher levels of contaminants for Mn, Cd, Pb and Zn ($p < 0.05$) in the plough disturbed plot.



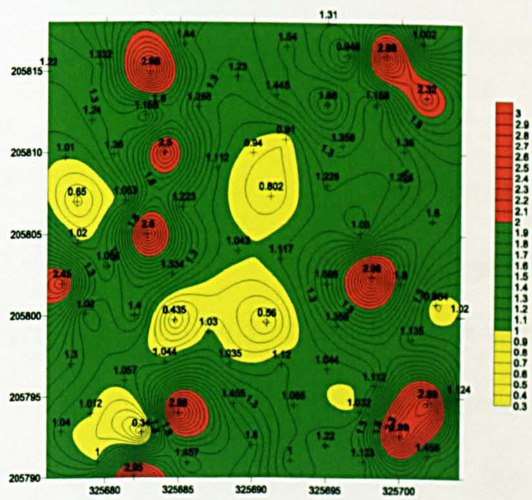
6.1 a) Manganese (Mn) at BL-07 in 2007



6.1. b) Manganese (Mn) at MI-04 in 2010



6.1 c) Cadmium (Cd) at BL-07 in 2007



6.1 b) Cadmium (Cd) at MI-04 in 2010

Figure 6.1: Comparison of Mn and Cd in soil (mg/kg) between BL-07 and MI-04.

Table 6.3: Soil metal concentrations (mg/kg) at BL-07 from samples collected in 2007 and MI-04 from samples collected in 2010 (n=150).

Zn in Soil	Mn in Soil	Cd in Soil	Pb in Soil	Cu in Soil	Zn in Soil
Mean BL-07 Mean MI-04	1867 2232	1.21 1.36	216 255	64 60	200 206
Minimum BL-07 Minimum MI-04	1129 863	0.32 0.36	132 231	33 45	145 183
Maximum BL-07 Maximum MI-04	2644 3056	2.69 2.88	281 288	94 94	248 245
Normal soil*	<500	<1	<200	<40	<100
Contamination threshold*	500-1500	1-2	200-320	40-80	100-250
Critical soil level	1500-2000	2-8	320-1000	80-300	250-500
Action / Treatment levels^	>2000	>8-10	>1000	>300	>500

*Standard values from Davies & Roberts (1978); Kelly (1980); ICRCL (1987); Bending and Moffat (1999); DEFRA (2004); Environment Agency (2009). ^ Action/Treatment Levels from Kelly (1980).

Table 6.3 records the increase in metal concentration at MI-04. These confirm that because the soil layer was scrapped off on plot MI-04 as a part of its initial pre-planting soil preparation, the site ended up being more contaminated, possibly achieving a state which is not unlike that of the non-reclaimed plot when mining ended in 1963. This project conceives this accident as a proxy for the original land surface.

There is some debate over the best method of planting. Across site MD-07 planting was carried out by simple notch planting. Trees establishment and growth has been successful after planting. Elsewhere, the adjoining CfN plots have explored different planting methods. Haigh et al. (2013) studied the effects of planting on the growth of *Alnus* and *Quercus* species on the adjoining CA-03 plot. The study involved testing three types of planting strategies: notch, pit and trench planting on mine spoils. Results showed that growth and survival of both species was better for trench planted trees followed by 'park and garden' style pit planting followed by 'forestry style' notch planting.

However, trenching the soil before planting also disrupted the thin top soil layer and exposed the already degraded and contaminated surface soil as evident on MI-04. Results from the soil chemical analysis showed high levels of metal in soil, most of them exceeding the background soil levels, while those for Mn were at action/treatment levels due to weathering and water logging. Consequently foliar metal concentrations were observed to be higher than on other plots. This technique of trenching might be useful when the aim is phytoextraction of metals from the soil. There is also some research to show that ploughing the site before planting can improve plant establishment (Bending, 1993; Moffat et al., 1991). However, both approaches may have an unfortunate effect on soil metal contamination. Hence, as in normal forestry practice, based notch planting is still the preferred style of planting.

Q2: What level of metal uptake is possible by Alder, Birch and Larch trees as represented by foliar analysis on the Varteg test plots?

The findings here suggest that tree planting provides physical and biological balance to the environment. Researchers have noted that the tree planting provides a canopy layer, a healthy root system and a biologically active forest floor, together forming a 'green cap' which stabilises the soil (Glimmerveen, 1996; Pulford and Watson, 2003; Mertens, 2006).

Use of trees as a vegetation cover over metal contaminated sites has gained popularity over the last two decades (Moffat and Houston, 1991;

Dobson and Moffat, 1993; Bending, 1993; Forestry Commission, 1994; Dobson and Mofatt, 1995; Hodge, 1995; Arduini et al., 1996; Glimerveen, 1996; Kozlov et. al., 1996; Moffat, 1997; Pisano and Rockwood, 1997; Aronsson and Perttu, 2001; Prasad and Freitas, 2003; Pulford and Watson, 2003; Rosselli and Bashi, 2003; Brunner et al., 2008). Trees are considered a sustainable, low-cost option, especially when there is no time pressure on the land being reused (Riddell-Black, 1993). Depending on the type and concentration of contaminants, specialist trees can be selected to stabilise the soil and waste material or, trees with hyperaccumulation properties, to help clean-up metal contaminated soils. However, with hyperaccumulator trees, the additional time and costs of management and harvesting may need to be considered (Rascio and Navari-Izzo, 2011).

Foliar metal concentrations measured over the two years show that different tree species take up varied amounts of different metals (Table 6.4). These results were compared with the normal range of metals in plants assembled from various references found in the literature (Table 6.5). The concentrations of metals in 3 year old foliage at MD-07 were within normal range when compared to all the ranges presented by different researchers.

Table 6.4: Mean Foliar metal concentration on Site MD-07

Species	Mn	Cd	Cu	Pb	Zn
Alder	293	0.7	4.50	4.23	66.4
Birch	495	1.87	13.7	2.3	86
Larch	599	1.72	3.3	12.3	35

Table 6.5: Foliar metal concentrations reference data (mg / kg)

Species	Mn	Cd	Cu	Pb	Zn
Normal concentration in leaves: Kabata – Pendias and Pendias (1992)	NA	0.05 – 0.20	5 – 30	5- 10	27 -150
Normal concentration in leaves : Alloway (1995a)	NA	0.10 – 2.00	5 – 20	0.2 – 20	1 – 400
Normal concentration in plant: Ross (1994)	NA	0.20 – 0.80	4 – 15	0.1 – 10	8 – 400
Normal concentration in Alder: Mertens (2006) (B): Wang and Jia (2010) (Larch)	30-100 (B)	0.05 – 0.23	5.8 ± 9	5.0 ± 0.5	65 ± 12

The variation of metal concentration in plant species grown in the same soil has been well documented (Page et al., 1981; Pulford and Watson, 2003; Hamon et al., 2004; Hermle et al., 2006). Results from foliar metal uptake on MD-07 show trees having an affinity towards the uptake of different metals. Of the three tree species planted, young larch needles showed the highest accumulation of Mn in their leaves (mean 580- 600 mg/kg), followed by birch and alders. Birch on the other hand showed maximum concentration for Cd (1.5 – 1.8 mg/kg) followed by larch and the least by young alder leaves and it also accumulated the highest amounts of Zn (87 – 89 mg/ kg), followed by alder and larch leaves. None of the trees showed any useful uptake for Cu although they were in range of the standard published values. Pb is known to be highly unavailable for uptake in soil (Huang et al., 1997; Schmidt, 2003; French, 2004) and this was evident in these three tree species although, Larch was the only tree to accumulate some Pb in the needles (10.1 - 12.3 mg/kg).

Cottenie et al. (1982) illustrated the levels at which different metals were translocated in the aerial parts of the plant. The concept is described in Section 2.9 and the results from this study have been highlighted on the metal distribution diagram created by Cottenie et al. (1982). The distribution of metal uptake by leaves of the three trees studied: Alder, Birch and Larch are superimposed on the original diagram and is illustrated in Figure 6.2

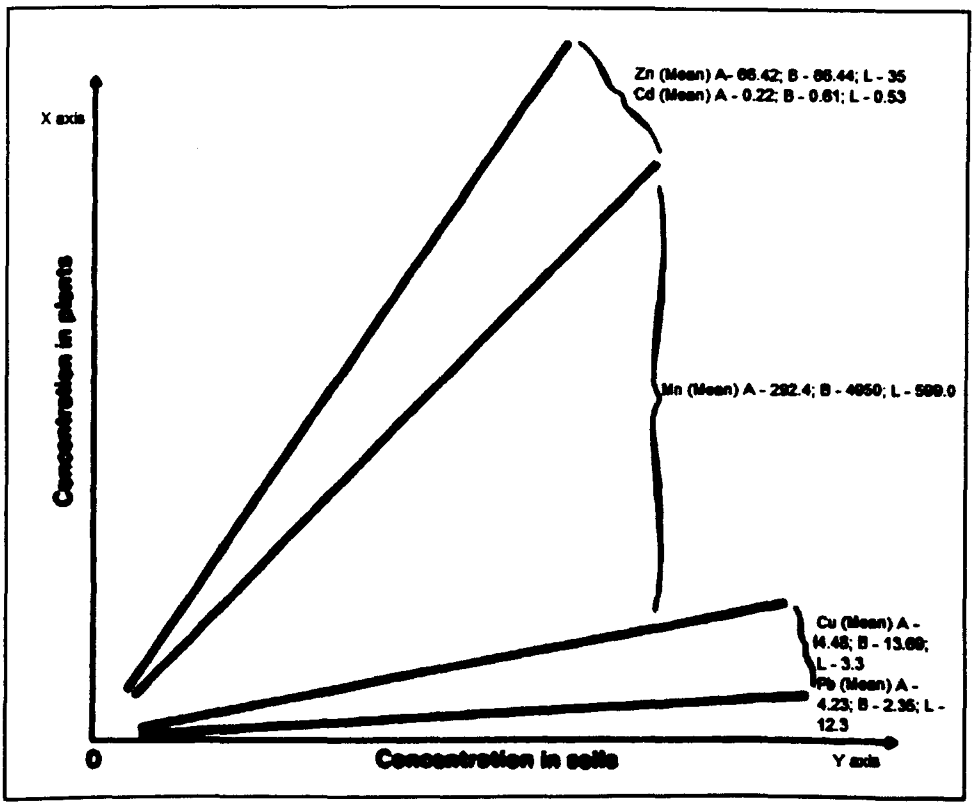


Figure 6.2: Distribution of metals into leaves of Alder (A); Birch (B) and Larch (L) against the metal concentration in soil (Adapted from Cottenie et al., 1982)

The results, as anticipated, align with Cottenie et al. (1982)'s concept where Cd and Zn, which are two of the most mobile toxic elements, show the greatest uptake when compared to the concentrations in soil, followed by Mn and the least for Cu and Pb. The following graphs rank the uptake of these metals by the three trees. Error bars show 95% Confidence Intervals - Note differences in the vertical scales.

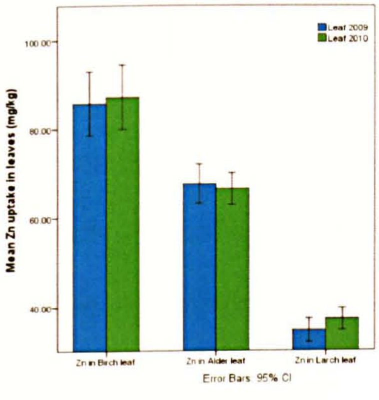


Fig. 6.3 a) Zn uptake ranks at MD-07 B>A>L

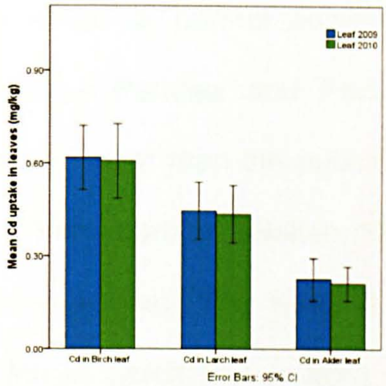


Fig. 6.3 b) Cd uptake ranks at MD-07 B>L>A

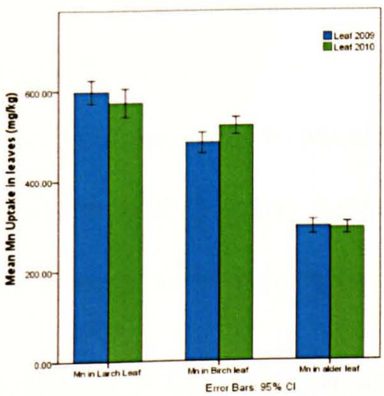


Fig. 6.3 c) Mn uptake ranks at MD-07 L>B>A

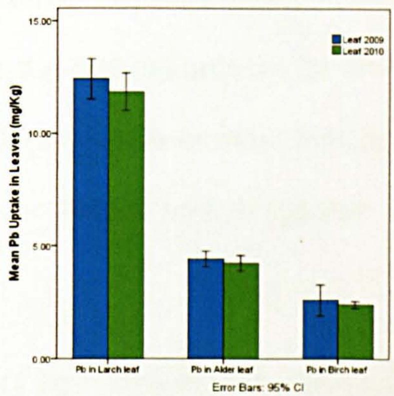


Fig. 6.3. d) Pb uptake ranks at MD-07 L>A>B

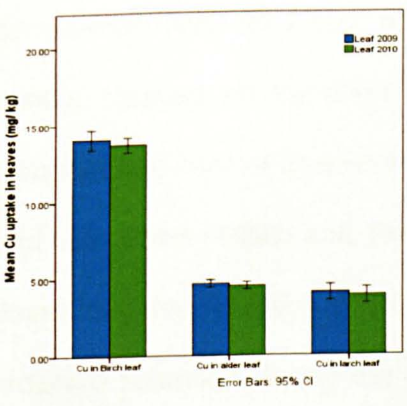


Fig. 6.3 e) Cu uptake ranks at MD-07 B>A>L

Figure 6.3: Ranks of metal accumulation in trees at MD-07 in 2009 and 2010. A – Alder, B – Birch, L- Larch

Figure 6.3 shows the ranks of metal uptake by Alder, Birch and Larch trees. Birch trees show the highest accumulation of Zn, Cd and Cu. The results observed for Zn were in the range of normal published values (Freedman & Hutchinson 1981; Kabata – Pendias and Pendias 1992) whereas the observed values for Cd were higher than the published values for Birch (Freedman & Hutchinson 1981; Borgegard and Hakan, 1989). Larch showed the maximum concentrations for Mn and Pb. Kula et al. (2012) observed elevated concentrations of Mn in Larch leaves from a Mn Ore contaminated site which has soil Mn levels of 9000 mg/kg. This shows that in highly Mn contaminated soils Larch needles are able to accumulate greater levels of Mn. Alder did not accumulate the highest amount for any particular metal but was able to accumulate moderate levels of most metals in leaves. Mertens (2006) found similar results for Alders and suggested that Alder were more suited for phytostabilisation.

Addition of Compost: A single dose (1 kg / tree) of compost was added to half of the trees planted on the experimental plot (MD-07) to check if composting had an effect on tree establishment and metal uptake. The results showed no variation between metal uptake by trees without any compost and that of trees with added compost. Previous studies by Bardos and Van Veen (1996) and Pinamonti et al. (1997) showed that at very high doses of compost (50 – 300t/ ha) plants showed improved yield and greater moisture retention during the summer. There were no reports of increased metal accumulation due to composting in the present study.

Q3: How is foliar uptake of metal contaminants affected by the soil composition and age of plantation?

When data was collected from the adjoining study CfN plots, planted between 1993 and 2004, for the foliar uptake, Alder and Birch species showed strong uptake for Mn, Zn and Cd. This is similar to the results obtained at the experimental plot MD-07. However, Pearson's correlation for Alder trees shows strong positive correlation between age of planting and the concentration of Mn and Cd in leaves, whereas Birch leaves show a moderate to weak correlation with age (Cottenie et al.,1982). This suggests that with an increase in age of Alder tree there is an increase in metal concentration, which is additional to any increase in total uptake resulting from the increasing biomass of the plantation.

A new, apparently unrecorded observation correlates Mn concentrations in leaves of Alder and Birch with concentrations of Zn in soil. This suggests that Zn facilitates the uptake of Mn in these tree leaves. Similar results were also observed for Larch at MD-07 but additional long term field data are required to verify these results. Mn and Zn are both essential elements in the soil which are required in small amounts by the plants (Alloway, 1995) and do coexist but this correlation at such high levels of concentration are not recorded previously.

Results from Pearson's correlation show a strong highly significant correlation between age of planting and Mn in Alder leaves ($r = 0.573$, $p = 0.002$), age against Cd in Alder leaves ($r= 0.468$, $p = 0.001$). This suggests that with an increase in age the leaves accumulate more metal. Although this is not obvious from the descriptive statistics outlined in section 5.6, Pearson's correlation and regression analysis, however, show a strong, highly significant increase in metal concentration. Similarly Birch show a moderate but still significant, correlation between age and Mn in Birch ($r = 0.385$, $p = 0.001$). This suggests that as the age of the tree increases the metal accumulation in leaves also increases.

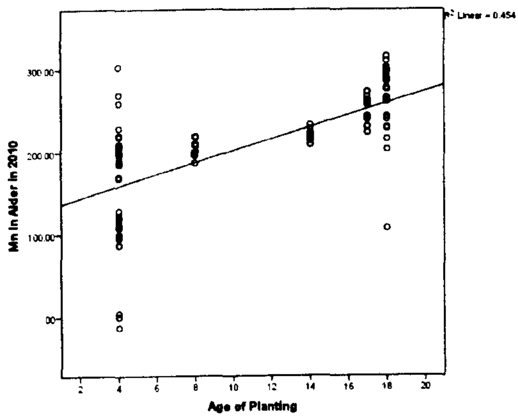


Fig 6.4 a) Conc of Mn in Alder over time

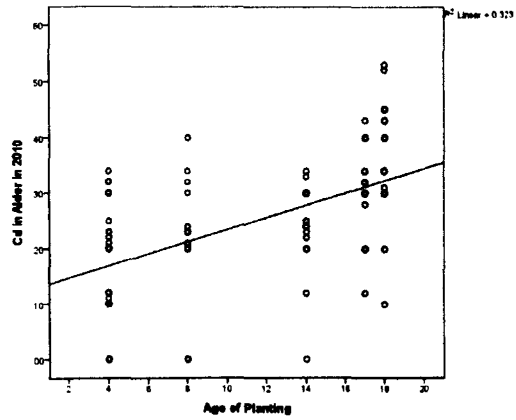


Fig 6.4 b) Conc of Cd in Alder over time

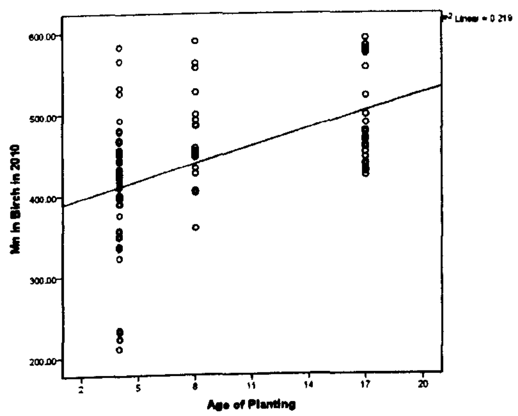


Fig. 6.4 c) Conc of Mn in Birch over time

Figure 6.4: Scatter graphs showing the Concentration of Mn (Alder and Birch)and Cd (Alder) in foliar over time.

Q4: How is metal concentration in soil affected by the age of plantation?

The metal concentration of Cd, Zn, Mn, Cu and Pb in mine spoils at Varteg Hill site shows a decrease through the 18 years of forest plantation.

This change is detectable even during the relative short initial period observed at site MD-07 (Section 5.1). Adding the data from the CfN plots shows soil metal concentrations decreasing gradually over time. The only exception is the disturbed plot MI-04, where the protective layer of grass and topsoil was removed exposing the mine spoils to new weathering (Department of the Environment, 1989; Bransden, 1991; Moffat and McNeil, 1994; Bending and Moffat, 1999) and where, hence the results can be treated as a proxy for the site prior to reclamation.

Results in the present study show that in 18 years of tree planting the soil metal concentrations have decreased by 52% for Cd, 47% for Zn, 35% for Mn, 48% for Cu and 44% for Pb. Based on the reduction in soil metal concentrations over time it is possible to project 40-45 years for concentration of Mn to reach a normal soil level and about 20 – 25 years for Cd levels to reach normal soil levels, provided the soil is not further disrupted by physical activities (e.g. plot MI-04) or a sudden increase in plant mortality and decay of plant material, which will return metals that are stabilised or accumulated back to the soil. In soil the concentrations of Cu, Pb and Zn have reduced to normal soil levels after about 15 years of planting. The decrease in the soil metal concentration can be due to 2 factors:

a) First is the uptake of metals by leaves which has been observed in this research, as well as by other plant tissues and/or by the trees fungal associates. Of course, there is the issue of these metals returning to the soil organic layer after leaf fall and decay and leaching back into the ground. Although Sinha et al., (2006) outline that plants that take up the metals into their tissues also bio-transform the metals into inert forms in their tissues. This means that, when these metals return back to the soil, they are not as toxic. However, this is an area of debate.

b) Second are the processes within the soil – *root substrate* and organisms of the rhizosphere, which absorb the metals and accumulate them in tissues or adsorb onto the roots. Controversially, these may also preventing the migration of metals into the soil by immobilisation the metals and preventing their further transfer by erosion and deflation (Vangronsveld et al., 1995; Mertens, 2006; Brunner et al., 2008).

Depending on the amount of chemical present in soil and the characteristics of the tree species planted, trees may be appropriate for phytostabilisation or phytoextraction (French, 2004; Mertens, 2004; Cunningham and Berti, 2012). Reducing the risk of the dispersal of pollutants into the environment must be the focal point irrespective of the method of remediation selected (Mertens, 2006). Ross (1994) stated that the entry of metals into the food chain is more dangerous than the leaching of metals into the ground.

Phytoextraction or Phytostabilisation: Alder, Birch and Larch all show a considerable uptake of specific heavy metals in the soil. However, these are not above standard published values for metal accumulation in trees with the exception of Mn in all species (Lobersli and Steinnes, 1988; Migeon et al., 2009; Millaleo et al., 2010; Kula et al., 2012) and Cd and Zn in Birch (Eltrop et al., 1991; Borgegard & Hakan, 1998; Pulford et al., 2001; Baltrenaites & Butkus 2007; Gallagher et al., 2008) and Larch species (Wang and Jia, 2010; Nevel et al., 2011; Fabien et al., 2012). Mn is an essential nutrient and readily available for uptake. In a study conducted by Page et al. (2006), it was observed that Mn from the soil was rapidly released in to roots and immediately into the plant xylem and through transpiration reached the photosythentically active leaves, within seven days of exposure. Cd too is a highly mobile metal in soil (Cottenie et al., 1982; Naidu et al., 1997) and readily available, for uptake by plants (Roberts and Johnson, 1978; Alloway, 1995; Cooke and Johnson, 2002). Based on the results observed, Birch and Larch can be used as phytoextractors for Cd and Zn contaminated sites. However these species do not accumulate all the metals present in site. Hence soil metal concentrations must be checked before initiating any remediation programme which involves the two tree species. Mn is present in large quantities in the soil and due to its bioavailability, is readily accumulated in trees. However, sites which are severely contaminated with Mn, Birch and Larch would be a good addition to remediate the soil (Wang and Jia, 2010; Kula et al., 2012).

Across the plots Alders have accumulated varied amounts of most metals except Pb, which has been reported to be highly unavailable to plants (Steinnes, 1990; Vedagiri and Ehrenfeld, 1991; Pulford et al., 2001; Morgan and Connolly, 2013). However these levels are in the range of normal soil levels (Kabata – Pendias and Pendias 1992; Alloway, 1995; Mertens, 2006) hence, eliminating the potential of Alders as phytoextractors.

Over time, Alder have shown to accumulate higher levels of Mn and Cd from soil as compared to Birch or Larch. This shows that the older the plant the greater the accumulation potential of the tree. Studies have been carried out to test the uptake of nutrients in Alders from soil over time (Zavitkovski & Newton 1971; Zavitkovski & Stevens 1972; Turner et al., 1976). Results showed the older trees to accumulate larger amounts of nutrients as compared to younger trees. This is in line with the Mn and Cd uptake by mature Alder trees on CfN plots as compared to MD-07. The irregular results of Mn and Cd accumulation at MI-04 are due to the irregular nature of the plot, which has higher metal loads which leads to higher metal accumulation of these bioavailable metals in leaves (Mertens et al., 2004).

On the site, soil metal concentration levels for all the metals under consideration have shown a gradual decrease, which suggests: a) the metals are mobilised by these trees, especially Alder, this could be due to its nitrogen fixation (Fitter and Hay 1987; Moffat and McNeill, 1992; Moffat and McNeill 1994; French 2004) or that older trees accumulate more amounts of Mn and Cd which have shown the highest reduction in soil metal load b) Birch and Larch show high accumulations for Mn, Cd and Zn from soil, c) these metals are stored in the other growing tissues of these trees, like roots, bark, stem, etc. (Kahle, 1993; Clemens et al., 2002; Pulford et al., 2001; French, 2004).

The most popular trees used for phytoextraction are Salix and hybrid Populus, which are known for their metal hyperaccumulation (Landberg and Greger, 1994; Pulford et al., 2001; Nathanail and Bardos, 2004; French, 2006). These trees are fast-growing and genetically diverse hence provide an opportunity to select particular genotypes with traits for high metal uptake at higher metal concentrations (Pulford & Dickinson, 2003).

The validity of the species under study, as phytoextractors is estimated from the amount of metal accumulated in leaves. In order to consider a species for phytoextraction metal accumulation in roots, stem branches and leaves need to be calculated as well. (French, 2004; Mertens et al., 2004; Mertens, 2006, Vamerali et al., 2009). For metal extraction by harvesting, trees that can tolerate excessive pollution, coppice easily and extract high amounts of metals from soil are needed (Mertens, 2004; Mertens, 2006), such as Birch and Larch for Mn, Cd and Zn specific sites. In addition to this phytoextraction requires regular monitoring and cost for harvesting and processing of the plant material (Cunningham and Lee, 1995; Flathman and Lanza, 1998). It has been widely acknowledged that phytoextraction by tree plantation is not a feasible option as problems related to crop disposal, labour costs and uncertain timescales create challenges. Furthermore, to date, no species is known to accumulate all of the toxic metals present in the environment at the same time (Glimmerveen 1996; Finzi et al., 1998; Salt et al., 1998; Pulford and Watson, 2003; Robinson et al., 2005). However, phytoextraction is a viable option for site specific remediation where trees may be tailored – i.e. selected specifically to deal with the metals present. Through this study, it is possible to suggest that, Birch can be used for phytoextraction of Mn, Cd and Zn and Larch for the extraction of Mn and Cd. Alder, however with its low metal uptake may be more suited for phytostabilisation on such sites. The high amounts of Zn, Mn and Cd accumulation in Birch, Larch and to a certain extent Alder trees in combination provide compelling results, which can be fed into the literature for metal accumulation by these trees and may be used in such tree selection calculations.

For successful phytostabilisation of mine spoils, it is essential to consider the challenges presented by such lands for reclamation as a whole. These commonly include compaction, infertility due to lack of nitrogen, as well as other macronutrients such as P, K. and Mg, salinity, soil acidity, high bulk density and water logging. These problems interact with the impoverished soil ecological system on such sites, which restrict its capacities to process and recycle organic compounds. This is why under forest such sites quickly develop a surface layer of partially decomposed humus, such as that typical for most entisols.

For such reasons, it is essential to select plant species which are best suited to the site conditions. Haigh (1995) pointed out that planting trees that benefit the quality of the soil by processes of nitrogen fixation, encouraging microbial growth and/or confining metal toxicity to some level may be useful. The tree species planted on the mine spoil site in this research target these soil conditions. These trees are pioneer species and Alder and Birch are especially tolerant of the harsh on-site conditions such as dense soils and waterlogging (Pulford et al., 2001; Forestry Commission, 2002; Moffat, 2006). Haigh (1995) pointed out that successful reclamation of mine spoils relies mainly on the formation of a self-sustaining rooting medium, in other words, a living soil system. This is why the CfN team is currently working on monitoring the growth and development of earthworm communities on the site. Preliminary results already suggest that earthworm biomass is strongly correlated with forest density (Haigh, 2013 personal communication).

Tree performance: Alder trees provide nitrogen fixation by root nodules through symbiotic relations with micro-organisms. They have been widely used in forestry based restoration. On the current site, Alder trees have shown successful growth with the lowest mortality and have accumulated useful quantities of metals in their leaves. Regression analysis also shows that the concentration of metals in Alder leaves decreases with the age of plant and differences in metal uptake and deposition over two soil sampling years do not shown any significant increment in soil metal concentrations. This suggests that the release of these metals back into the environment due to leaf litter decay does not appreciably affect the soil concentration. These values do not increase from Year 1 to Year 2 and so dispersal into the environment is not of major concern. Alder trees however do not regenerate on site; there are very few volunteer Alder seedlings. However, they serve as a valuable 'nurse' species, which improves the organic matter of the mine soil and improves the nitrogen capacity of the soil.

Birch trees are pioneering species, which easily colonise open areas (Moffat, 2006). Birch trees are reputed in forestry as a tree species that improves soil quality (Priha, 1999). Soil pH, organic matter, nutrient content and microorganism populations have been found to increase under birch covers and the C:N ratio is found to decrease (Gardiner, 1968, Miles and Young, 1980; Nohrstedt, 1988; Mikola, 1995). All of these factors are known to facilitate metal availability and uptake by trees. Researches have also found the densities of microorganism Frankia, root nodule symbiont of Alder, to be higher under Birch trees rather than the host (Smolander, 1990; Priha, 1999). This mychorrhizal association also facilitates metal uptake by the plant, which is evident from the results obtained. Birch species accumulated the highest amounts of Cd and Zn in the leaves. Birch is a fast growing tree with deep rooting system which enables it to penetrate into the deeper layers of soil and accumulate higher amounts of metals. This could be another reason why young trees show high levels of metal accumulation. Borgegard & Hakan (1998) studied biomass, root penetration and heavy metal uptake in Birch in a soil cover over Cu tailings and found that the leaves stored levels of Zn, Pb and Cd that were higher than the published values for uncontaminated soil. Similar results have been found for Mn, Cd and Zn on the study site. Birch leaves have shown promising results and can be used for metal specific sites remediation, especially for Mn, Cd and Zn.

However, in 2010 there were visible signs of yellowing of leaves probably resulting from: 1) High Mn toxicity in soil which leads to leaf chlorosis (Moroni et al., 2003; Rosas et al., 2007; Mora et al., 2009). 2) Lack of water in the compacted mine spoil. 3) Lack of potassium or phosphorus in the soil, which is characteristic of mine spoils (Bradshaw, 1997). On the mine spoil, Birch trees showed low to moderate mortality, again reiterating its capability to survive under metal stress and uptake large amounts of metals. The maximum mortality was experienced in the first year, while the plant was getting acclimatised to the soil conditions, once acclimatised the tree shows great potential for metal accumulation and tolerance to harsh site conditions.

Larch trees, which are a favoured tree species for forest-based reclamation, were a new introduction to this site. The tree was mainly introduced due to its popularity with the Forestry Commission for woodland and reclamation and for its success on degraded mine spoils (Bending, 1993; Moffat and McNeil, 1994). The plant showed the highest mortality rates in the first year of planting. Similar results, in its initial years of establishment on derelict, contaminated and brownfield sites have been recorded by Bending and Moffat (1999), Webber et al. (2010) and Barbeito et al. (2012). From the foliar chemical analysis, Larch needles showed the highest accumulation of Mn in their leaves and a high uptake of Cd. Similar results have been found by Myre and Camire (1994); French et al. (2006) and Leonelli et al. (2011). Findings from the analysis of Japanese larch needles showed improvement in nitrogen nutrient content on planting in mixtures with Alder and Birch trees (Moffat, 1994; Mofatt and McNeill, 1994; Bending and Moffat, 1999; Mofatt, 2000).

Mine spoils in different parts of the world face many problems that can prevent adequate re-vegetation and in turn restoration of these lands, many of which are due to the poor solid physical and chemical conditions and an impoverished soil biological system. Bradshaw (1983) provides an overview of a short and long term remediation for mined lands, but most of this concerns short term (immediate) treatments for the first time remediation of newly mined lands and include high costs. They also often involved major ground engineering like ripping out compacted layers, deep tillage, the construction of drains, applications of large amounts of top soil and ground covers, etc. Table 6.6 details some of the long term treatments recommended. The table also displays where the tree species studied in this research were considered appropriate.

Table 6.6: Major problems of mined land, their long-term treatments (Bradshaw, 1983) and tree species from this research which meet the criteria.

Limiting Factor	Variable	Problem	Long- term Treatment	Trees from this research
Physical	Structure	Too Compact	Vegetation	Birch and Larch
		Too Open	Vegetation	Alder, Birch and Larch
	Stability	Unstable	Regrade or vegetation	Alder, Birch and Larch
	Mositure	Too dry	tolerant species	Larch
Nutrition	Macronutrients	Nitrogen deficiency	Legume or N-fixer	Alder
		Other deficiency	Fertiliser or tolerant species	Alder and Birch
	Micronutrients	Deficient	-	-
Toxicity	pH	High or Low	Lime or tolerant species	Alder and Birch
	Heavy Metal	Too high	Inert covering of tolerant cultivar	Birch, Larch and Alder

Mine spoils present challenging physical and chemical problems that must be taken into account for successful reclamation. In South Wales, soil auto compaction is a common consequence of mining and care needs to be taken to avoid further compaction during restoration. Chemical contamination is another damaging attribute of many mine spoils. Contamination maps are a good starting point for portraying and communicating the presence and variability of metal loadings in mine spoils. The use of contamination maps in small or big scale reclamation programmes is highly recommended because it highlights that while average conditions may appear relatively benign, most spoils include hotspots that rise into levels of serious contamination and potential toxicity.

Of the three tree species planted on the site, Larch shows maximum accumulation of Mn in leaves followed by Birch and then Alder. Birch leaves, on the other hand, contain high concentrations of Cd, followed by larch, and maximum concentration of Zn, followed by Alder. All three tree species are well suited for the remediation of mine spoils. There is a low risk of recycling of Mn, Cd, Cu, Zn and Pb into the environment as these species do not accumulate critical amounts of metal, except for Cd in Birch and Mn and Cd in Larch. Also results from 2 years of leaf sampling do not show any increment in metal concentrations in the soil, which suggests that the re-release of metals due to leaf litter decay is too small to alter the soil metal concentration or even off-set the amount of metal sequestered elsewhere in the plant, for example woody materials, roots, etc (Kahle, 1993; Clemens et al., 2002). These trees not only improve the soil quality and visual appearance but also stabilise the spoil waste (Fitter and Hay, 1987; Moffat and McNeill, 1992; Pulford et al., 2001; Mertens, 2006). All three species prove their worth by being capable of thriving in this challenging environment. However, none of the three species are suited for phytoextraction for all of the metals present in the soil (Pulford et al., 2001; French, 2006; Mertens et al., 2006). Although, Birch and Larch have shown potential for extracting Mn, Zn and Cd from soil. It is possible to estimate a time-line of 35-40 years for Mn, approximately 25-30 years for Cd and about 2 decades for Zn, to bring the soil contamination to normal background levels. That is, provided the soil is not further disrupted by physical or biological activities, which may recontaminate the spoil, as the experience of MI-04 shows (Stockfisch et al., 1999; Koch and Stockfisch, 2006).

Statistical analysis of metal concentrations in soil and leaves has shown that uptake of Mn in Alder and Birch leaves on all plots and Larch on new experimental plot is positively correlated to the presence of Zn in the soil. This has not been previously recorded.

Forestry for Land reclamation: The use of forestry for reclaiming contaminated and derelict land has been greatly advocated and is now being put into use. Planting trees provides many social, environmental and economic benefits. Forestry is a beneficial tool for reclaiming large areas of derelict landscapes that have been degraded by destructive mining and industrial activities (Perry and Handley, 2000). There are many reasons to recognise forestry as an after use for reclaimed land, which include site stabilisation, prevention from harsh climatic conditions, timber production, landscape enhancement (Moffat and McNeil, 1994), improved soil stability and fertility and development of an ecosystem for establishment of natural habitats. It has been widely established that trees have a potential for phytoremediation and provide solutions to remove or stabilise the contaminants along with reducing wind erosion, surface runoff and leaching (Dobson and Moffat, 1993; Bending, 1993; Forestry Commission, 1994; Dobson and Mofatt, 1995; Hodge, 1995; Arduini et al., 1996; Kozlov et al, 1996; Moffat, 1997; Pisano and Rockwood, 1997; Prasad and Freitas, 2003; Pulford and Watson, 2003; Rosselli and Bashi, 2003).

French (2004) studied the potential of woody trees for remediating brownfield industrial contaminated sites. French found *Salix* and *Populus* to accumulate maximum amount of Cd and Zn in stem and foliage. Contrary to the findings of this research, Birch and Larch showed no substantial accumulation of metals and were hence not included in developing a model for uptake of metals. The time line model generated by French for testing the metal accumulation potential of *Salix* and *Populus* spp was only based on three years data, rather than a long time field trail study. Mertens (2006) conducted a similar study to test the phytoextraction and phytostabilisation potential of trees (Alder, Poplar, Ash and Maple) for afforestation of degraded sediments. Mertens suggested alder was more suited for phytostabilisation and poplar was most suited for phytoextraction. Phytoextraction and phytostabilisation are cost-effective, in-situ techniques although, extraction and harvesting of biomass for metal mining involves some cost (Lasat, 2002; French, 2004; Mertens et al., 2004; Mertens, 2006, Vamerali et al., 2009).

This research has tested the potential of three of the most commonly used trees in land reclamation work in Wales – Alder, Birch and Larch to remediate land contaminated by coal mining. All three tree species have shown potential, some greater than others, to reclaim these mine spoils, either immobilising the metals or by translocating metals into its tissues. Hutchings (2002) reported that the use of trees to improve metal contamination on sites is possible for moderate levels of contaminants, but rare for high levels of contaminants. This is substantiated by this project. The study site is contaminated with elevated levels of metal contaminants, which forestation is reducing to safer levels. It has been observed that different trees have accumulated varied levels of specific metals. Based on the current research, the following recommendations are made for remediating metal loadings on land damaged by open cast coal mining and which poses problems like soil compaction, soil contamination, high bulk density, low fertility and nutrient deficiency.

1) Careful site investigation, and the creation of contamination maps, allow visual identifications of the geographical variability in soil metal concentrations over time and help identify and treat metal hotspots. Such mapping exercises might be included in all reclamation plans, especially for sites where metal loadings are above the threshold of contamination in whole or part and especially if the aim is to reclaim such degraded sites for public access like parks, open lands and forestry.
2) Trees planting with selected species able to remediate the metal contaminants of the mine spoils would reduce levels of contamination and also permit naturalistic geo-ecological succession and foster self-sustainability. Forestation also provides an opportunity for cost effective community engagement in land regeneration.
3) Alders act as 'Ayurveda' for degraded mine spoils by providing nitrogen fixation and results suggest that planting these trees mixed with Birch species, which readily colonises infertile soil and high density soils and that also accumulate high levels of heavy metals, does much to restore fertility to the soil system.
4) Especially for sites contaminated with Cd, Zn and Mn, Birch trees are the recommended tree choice, especially when planted with alders.
5) Larch trees, show promising results in the first 4 years after planting, especially for Mn and is the only tree species to accumulate Pb.

Future investigations: Soil-root-metal interactions are the basis for remediation (Dickinson, 2000; Laureysens et al., 2005) and, while the metal interaction with roots could not be addressed in this research, it would be a valuable avenue for future work. Larch shows promising initial metal uptake results but a longer time frame is necessary to prove the value of this tree as a phytoextractor of Mn and Pb.

CHAPTER 7

CONCLUSION

7 CONCLUSION

Degradation of reclaimed former opencast coal mine sites poses a serious and international environmental problem. Reclaiming such lands to a condition where their qualities become self-sustaining is becoming imperative. A considerable portion of the land which counted 'reclaimed' after a single treatment using top soil and grass is becoming degraded due to long term problems such as auto-compaction and soil contamination caused by the weathering of newly exposed mine spoils (Phelps and Holland, 1987; Bending and Moffat, 1999; Haigh, 2000). Although, these problems cannot be cured completely, they can be managed and controlled. Here, it is suggested that soil development is key to reclaiming mine spoils sustainably and that phytoremediation of such mine spoils by the establishment of trees encourages improvements to soil qualities that include the reduction of their loading with metal contaminants. A healthy soil system, as defined by Doran and Zeiss (2000, p4), includes

"the capacity of a soil to function, within ecosystem and land use boundaries, to sustain biological productivity, maintain environmental quality, and promote plant and animal health".

This study has shown that planting trees on derelict lands can improve the soil by systematically reducing toxic metal concentrations.

In this research, set on a former reclaimed open-cast coal mine spoil on the Varteg Hills in South Wales, soil metal contamination and the role of selected trees in phytoremediation is studied. The study has involved multi-year investigations of new test plots established specifically for this investigation (MD-07) and the comparison of these findings with earlier plantations on the same site (CfN). Collectively, the test plots data covers a period of 18 years after plantation.

These mine spoils in South Wales present many challenges for land reclamation. Physical soil conditions are problematic. On mine spoils the soil bulk density is considered to be the best test for measuring land success because high densities restrict plant root development (Ramsey, 1986). The critical limiting density for root development in soils is often recognised as 1.6-1.8 g/m^3 (Haigh, 2000). On the Varteg Hill former opencast site, the soil bulk density is often close to 1.6 g/cm^3 at 30 cm depth and about 1.8 g/m^3 at 50 cm depth. Soil acidity is also commonly a major problem for land reclamation and soil pH should not exceed the range pH 3.0 to 9.0 (Moffat and Bending, 1992; Wong, 2003). However at Varteg, soil pH is recorded in the range pH 5.3 – 5.7 across all the plots. So, while these soils suffer from serious compaction, acidity is not a problem. By contrast, metal contamination is a major issue on this as well as many other former industrial sites (Bradshaw, 1980; Maiti & Saxena, 1998; Maiti, 2006; Nwachukwu and Pulford, 2008).

Phytoremediation has been extensively used for the remediation of contaminated spoils (Vangronsveld et al., 1995; Banuelos et al., 1997; Chaney et al., 1997; Burken and Schnoor, 1997 & 1999; Blaylock, 2000; Raskin and Ensley, 2000, Pulford and Dickinson, 2005). Trees have often been used in these contexts because of the depth of their root penetration and their ability to generate biomass in amounts far greater than grasses of forbs (Moffat and McNeil, 1994). However, there have been few systematic investigations into the short and medium term impacts of forestation on the levels of metal contaminants in opencast coal-mine spoils or of the potential of some tree species on the remediation of specific metal contaminants. This project addresses some of these gaps in the research literature by examining the role in metal remediation of three of the key tree species used in the reclamation of former opencast coal-mine lands in Wales: *Alnus glutinosa* (also known as Common; European or Black alder), *Betula pendula* (Silver Birch or European White Birch) and *Larix decidua* (also known as *Larix europaea*) Common or European Larch. The role of these three species in the uptake of metals is explored through examining five metals. Cottenie et al. (1982) proposed a concept of metal uptake in plants and outlined the rank of uptake of these metals in to trees. Two of the metals studied commonly show high levels of uptake from contaminated soils, Cd and Zn, two commonly show low uptake, CU and Pb and one, Mn, is intermediate (Cotenie et al., 1982). Although, the results are in line with Cottenie's results the metal distribution pattern proposed by Cottenie does not consider metal uptake over time, which in the case of this research decreases with the age of planting, with the exception of Alder.

This project began by exploring the soil loadings of the five metals selected for study. First metals loadings were analysed in soils that had not been treated by forestation and on a site where the topsoil had been artificially removed, so exposing the mine spoils to weathering. These two data sets provided benchmarks against which the impacts of forestation could be compared. Next, the distribution of these metals was mapped across the test plots. Here, the considerable variation of contaminants within plots, and localised hotspots was displayed with the aid of contamination maps. The identification of hotspots enables a targeted assessment of soil contamination and remediation. Thereafter the effects of planting Alder, Birch and Larch trees in the mine spoil were explored and it was found that, within the first three years of planting there were significant reductions in the levels of metal contamination, as evident from the contamination maps.

The guidelines provided by different agencies including ICRCL, CLEA, Defra and those provided by a range of researchers (Davies & Roberts, 1978; Kelly 1980; Kabata Pendias and Pendias, 1992; Gupta, 1996; Kumar, et al., 2007) give values for normal, contamination threshold and action/ treatment level soils. However, a large proportion of metal contaminants on such mine spoils fall between the contamination threshold and action treatment level. These soils are not included in the official 'contaminated land' category but are harmful to the soil and environment and need attention (Hutchings, 2002). To understand these metal levels, and treat them, a new 'critical soil' level has been created. Trees are unlikely to increase the risks on land contaminated by heavy metals and hence can be used to break the contaminant pathway and receptor contaminant linkage. This is achieved through establishment over soils, to either reduce soil metal by uptake into trees or by immobilising them. In the present study trees within 3 years of establishment reduced the soil metal levels for Mn by 14-18%, which was sufficient to reduce the chemical level from 'Action/Treatment' to 'Critical' soil levels; and the level of Cd and Zn was reduced by 8%. These results provide a useful database and identify a gap, which includes a vast majority of contaminated and degraded lands that fall between the contamination threshold and action/ treatment levels.

The uptake or immobilisation of metals by trees formed the basis of this reclamation programme. Alder trees used in the present study were used for their nitrogen fixation properties and acted as a nurse tree and facilitated paedogenesis. Of the five metals *selected* for this study, two are known to be highly mobile (CD, Zn), two relatively immobile (Pb, Cu) and one is intermediate (Mn). Across all plots alder leaves accumulated moderate amounts of most metals and showed the lowest mortality. Birch trees on the other hand showed the highest accumulation of Cd, Zn uptake, which were significantly correlating with soil metal concentrations and second highest accumulation for Mn. Pb was highly unavailable to both Alder and Birch trees. However, Larch a new species introduced on the site showed the maximum concentration of Pb uptake, in its needles. Larch needles also showed the highest accumulation of Mn amongst all three tree species. This shows that planting trees in combination is beneficial as, different trees selectively remove different metal contaminants while some species in addition also benefit the soil and ecosystem in other ways. For example, Alder provides nitrogen fixation. Birch is found to add value for wildlife, by attracting birds (Moffat and McNeil, 1994). Also, when reclamation is done through forestry, as recommended through this research, planting native tree species is advised, as this makes it easier for the tree to acclimatise to the environment and also promotes community engagement (Moffat, 2006).

In addition to high uptake of metals in leaves, strong highly significant correlation between age of planting and Mn in Alder leaves ($r = 0.573$, $p =$

0.002), and age of planting against Cd in Alder leaves (r= 0.468, p = 0.001) was recorded which suggests that increasing age, allows Alder leaves to accumulate more metal. Similarly Birch show a moderate but still significant, correlation between age and Mn (r = 0.385, p = 0.001). This suggests that as the age of the tree increases the metal accumulation in leaves also increases.

A new observation, not previously recorded in the literature, suggests a correlation of Mn in leaves of Alder and Birch in the presence of Zn in soil. Larch showed a similar correlation between the concentrations of Mn in needles and levels of Zn in soil. As Larch trees were only planted on four year old MD-07 plot, additional long term field data for larch are required to verify these results.

The uptake of the metals: Zn, Cd, Mn, Cu and Pb into the leaves of Alder, Birch and Larch trees collected over two growing seasons over a 4 to 18 year old period on a degraded opencast mine spoil have shown a great reduction in soil metal concentrations over time. Based on the reduction of metal concentration in soil and due to uptake in leaves it is possible to project 40 – 45 years for concentration of Mn to reach a normal soil level and about 20 – 25 years for Cd and Zn levels. This assumes that the soil is not further disrupted by physical activities like natural disasters, further deforestation, disruption of soil profile by ploughing, erosion etc. or biological activities like tree death and decay. These activities would potentially return the chemicals back to the soil, root decay may release the immobilised metals back in to the soil profile etc. which may recontaminate the site.

In the present study Alder, Birch and Larch have all shown considerable uptake of specific heavy metals in the soil. However, these uptake levels are not above the standard published values for metal accumulation in trees with the exception of Mn in all species and Cd and Zn in Birch and Larch species. However on contaminated spoils, phytoextraction is a viable option for site specific remediation where, trees may be tailored to deal with specific metal types present in the soil. This can be effectively done by: a) identifying the amount and spatial distribution of soil contaminants; followed by b) selecting tree species in combination to target the specific metals; and finally c) modelling the length of time needed for effective sequestration. Through this study, it is possible to suggest that on such metal contaminated spoils, Birch can be used for phytoextraction of Cd, Zn and Mn and Larch for the extraction of Mn and Cd, which would take about 40-45 years for Mn and about 20 – 25 years for Cd and Zn levels to reach a normal soil level. Alder, however with its low metal uptake may be more suited for phytostabilisation on such sites as they serve as a valuable 'nurse' species, which improves the organic matter of the mine spoil and also improves the nitrogen capacity of the soil. The high amounts of Zn, Mn and Cd accumulation in Birch, Larch and to a certain extent Alder trees in combination provide compelling results, which can be fed into the literature for metal accumulation by these trees and may be used in such tree selection calculations.

The establishment of woodland on contaminated lands either immobilises and stabilise the contaminants in soil or facilitates the removal of these contaminants from soil by their uptake within the plant. The success of removal or stabilisation depends on the location of the site and metals present in the site, status and level of contamination and soil properties (Hutchings, 2002). Site preparation before planting is another important step towards successful reclamation. It has been evident that removing the soil top layer before planting, as advocated by some researchers (Forestry Commission, 1991; Bransden, 1991; Moffat and McNeil, 1994) and which was adopted on MI-04, is not advisable especially on such mine spoil, as it exposes the mine spoils to a fresh bout of weathering, taking the soil back to its original unreclaimed state. Some of the major problems associated with the formation of soil structure and in turn establishment of woodland are structural decline, acidity and heavy metal contamination built up over time (Haigh, 1995). Through this research a long-term data set is provided which looks into establishment of three native pioneering trees mainly used in land reclamation. In order to improve the soil quality and soil health, the use of nitrogen- fixing plants like Alders is suggested, which is an alternative to the costly and harmful provision of fertiliser. This study has also established the role of Alder in metal uptake and accumulation in leaves and its short and long-term effect. Coal spoil often pose the issues of soil compaction and metal contamination, and Birch, with its deep rooting systems can help tackle the issues of compacted soil. Also through this research Birch has shown great potential in the uptake of Cd and Zn, two of the most critical, toxic metals in mine spoils along with Larch which shows high accumulation potential for Mn and Cd in foliage. All of this demonstrates the ability of the three trees to be planted as top candidates for reclaiming contaminated soils and in turn providing stability to the soil.

Wider prospects: The problem of contamination of degraded coal spoils is wide spread. Before the 1970's, when no laws were in place to reclaim lands after industrial and mining activities, the sites were mainly abandoned or poorly reclaimed with grass seeding and to below acceptable levels. The EA (2006) has reported that the remediation and reuse of contaminated and derelict lands is supported by public funds in the UK. They also identified that priority needs to be given to the coal and metal mines of Wales and southwest and northern England which continue to cause pollution, despite being closed for many years. Although the report clearly outlines that the reclamation of these lands does not fall under the responsibility of a single body, they have accepted that currently they do not have a national strategy to tackle the problem. According to the EU statistics presented in the EEA report (2002), the UK alone has approximately 10,000 sites officially classed as contaminated and over 100,000 potentially contaminated sites. With such high numbers of abandoned, degraded and contaminated sites and hundreds of more sites yet to be identified and characterised, more awareness and progress into reclamation of these sites is needed. According to Nkonya et al. (2011) report for IFPRI, more than 24% of global land area has been affected by land degradation. While these sites pose a safety and environmental threat on the land quality and establishment, biodiversity and the overall human and local environment, these lands also present an opportunity for new, ecological and economical assets. The current rate at which deforestation occurs, suggests that the world's rain forests could completely disappear in a hundred years. Also the recent increased frequency and magnitude of storm events mean landscapes are more vulnerable to erosion and potential reactivation of metal contaminants unless sites like these are protected by forestry. Reclaiming these lands to woodland not only provides a safe, pollution free, aesthetically-pleasing green space, it also provides opportunities for low-cost community forestry projects, thereby increasing the global green cap and reducing the impacts of global warming.

Research has shown that creating green spaces on derelict and contaminated waste lands have a positive psychological effect on people's behaviour. It also enhances the biodiversity of the region, the property value of the area and averts accidents like the one in Aberfan (1966) by stabilising the slope. Reclamation via forestry can be done as a part of a County or NGO led community project or can be included in the curriculum at schools and university level as a sustainability project. The litter created from these woodlands can be used for controlled wood chip burning at power stations like the 'Drax power station'. This solves the problem of disposal of harvested tree materials that have accumulated high levels of metals and reduce the burning of coal to generate power.

In 2002, the Forestry Commission developed a systemic approach for assessing the potential of contaminated land to supporting woodland. The approach comprised of six stages: stage 1 and 2 site investigation; assessment of heavy metals and effect on trees; consideration of addition of amendments; determination of tree establishment on contaminated land; role of trees in mobilising and/ or stabilising metals; testing the viability of woodland establishment. Since its inception in 2002 there have been no published reports on the development of the project. The current research involved all of the above six stages and results obtained can contribute towards the development of the Forestry Commission project and the data can add to the bank of knowledge for reclamation agencies and site developers.

This research, establishes the prospects of Alder, Birch and Larch trees, in not only remediating metal contaminated and derelict soil, but also in creating a woodland ecosystem that can self-sustain, prosper and provide a range of end uses, including community forestry, and help target the wider global problems of deforestation, contamination and global warming. It also conclusively, demonstrates that forestation is an effective means of metal remediation on the moderately contaminated lands produced by opencast coal-mining in Wales and similar mine spoils across UK.

REFERENCES

Abbott, L. K. and Murphy, D. V. (2003a). *Soil Biological Fertility: A Key to Sustainable Land Use in Agriculture.* Kluwer Academic Publishers. Dordrecht, Boston, pp 121-129.

Abbott, L. K. and Murphy, D. V. (2003b). *Soil biological fertility: A key to sustainable land use in agriculture.* Dordrecht, Boston, Kluwer Academic Publishers, pp 151-167.

Agilent Technologies (2000). *Practical Multi-Element Hollow Cathode Lamps for Atomic Absorption Spectrometry: Application Note*. New York, Agilent Technology, pp. 1-50.

Akoto, O, Ephraim, J. H. and Darko, G. (2008). Heavy Metals Pollution in Surface Soils in the Vicinity of Abundant Railway Servicing Workshop in Kumasi, Ghana. *International Journal of Environmental Research, 2(4), pp. 359-364.*

Alloway, B. J. (1990) Soil Processes and the behaviour of metals. *In* Alloway, B. J. (Ed) *Heavy Metals in Soil*, Springer, London, pp. 7 – 28.

Alloway, B. J. and Jackson, A. P. (1991). The Behaviour of Heavy Metals in Sewage Sludge-Amended Soils. *The Science of the Total Environment.* 100, pp. 151-176.

Alloway, B. J. (1995a). *Heavy metals in soils.* London, Blackie Academic and Professional, pp. 613

Alloway, B. J. (1995b). *Cadmium. In: Heavy Metals in Soil.* Alloway, B. J. (Ed.) London, Blackie Academic and Professional, pp. 283-311.

Alriksson, A. and Eriksson, H. M. (2001). Distribution of Cd, Ck, Pb and Zn in Soil and Vegetation Compartments in Stands of Five Boreal Tree Species in N. E. Sweden. *Water, Air and Soil Pollution: Focus* 1, pp. 461-475.

Ang, L. H, Tang, L, Ho, W, Hui, T. F. and Theseira, G. (2010). Phytoremediation of Cd and Pb by four tropical timber species grown on an Ex-tin mine in peninsular Malaysia. *World Academy of Science, Engineering and Technology.* 38. Available at: http://waset.org/journals/waset/v38/v38-43.pdf
Accessed 7 February 2010

Angelone, M. and Bini, C. (1992). Trace elements concentrations in soils and plants of Western Europe. *In* Adriano, D. C. (Ed.), *Biogeochemistry of trace metals*, Lewis Publishers, Boca Raton , pp. 19–60.

Armstrong, W. (2009). *Technical report on coal properties of energy build group plc. in South Wales, United Kingdom for western Canadian coal corporation.* CA10196/TR01-R. Edinburgh Forestry Commission, pp. 12

Arduini, I, Godbold, D. L, and Onnis, A. (1996) Cadmium and copper uptake and distribution Mediterranean tree seedlings. *Physiology Plantarum,* 97, pp. 111–117.

Argonne National Laboratory (2006). *Environmental Science Division. Advancing informed environmental decision making* [online]
Available at:
http://www.evs.anl.gov/project/dsp_fsdetail.cfm?PrintVersion=trueandid=88
[Accessed 12 June 2008]

Aronsson, P. and Perttu, K. (2001). Willow vegetation filters for wastewater treatment and soil remediation combined with biomass production. *Forestry Chronicle*, 77 (2), pp. 293–299.

ATSDR (2005). *Toxicological profile.* U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, pp 1-25.

ATSDR, (2008). *Toxicological Profile for Cadmium.* US Department of Health and Human Services. Agency for Toxic Substances and Disease Registry, pp. 1-31.

Audet, P. and Charest, C. (2007). Heavy metal phytoremediation from a meta-analytical perspective. Environmental Pollution, 147, pp. 231–237.

Baath, E. (1989). Effects of heavy metals in soil on microbial processes and populations (a review). *Water, Air and Soil Pollution*, 47(1-3), pp. 335-379.

Bachman, G. R. and Miller, W. B. (1995). Iron chelate inducible iron/manganese toxicity in *Zonal geranium. Journal of Plant Nutrition*, 18, pp.1917–1929.

Baker, A. J. M, McGrath, S. P, Sidoli, C. M. D, and Reeves, R.D. (1998). The possibility of in situ heavy metal decontamination of polluted soils using crops of metalaccumulating plants. *Resource in Conservation Recycling*. 11, pp. 41–9.

Baker, D. E. and Senft, J. P. (1995) Copper *In* Alloway, B. J. (ed.) *Heavy Metals in Soil*. Blackie Academic and Professional, London, pp. 179-202.

Baker, A.J. M, McGrath, S. P, Reeves, R. D. and Smith, J. A. C. (2000). Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils. *In* Terry, N, Bañuelos, G. S, and Ajwa, H. A. (Ed.) *Phytoremediation of contaminated soil and water*. CRC Press, Boca Raton, FL. pp. 85–107.

Bakonyi, J, Nagy, Z. A. and Ersek, T. (2003). Standard and Swedish variant types of the hybrid alder *Phytophthora* attacking alder in Hungary. *Pest Management Science*, 59, pp. 484–492.

Ballard, T. M. (2000). Impacts of forest management on northern forest soils. *Forest Ecology and Management*, 133, pp. 37–42.

Baltrënaitë, E, and Butkus, D. (2007) Modelling of Cu, Ni, Zn, Mn and Pb transport from soil to seedlings of coniferous and leafy trees, *Journal of Environmental Engineering and Landscape Management*, 15(4), pp. 200-207.

Bañuelos, G. S, Ajwa, H. A. Mackey, L. L, Wu, C, Cook, S. and Akohoue, S. (1997). Evaluation of different plant species used for phytoremediation of high soil selenium. *Journal of Environmental Quality*, 26, pp. 639–646.

Bañuelos, G. S, and Ajwa, H. A. (1999).Trace elements in soils and plants: an overview. *Journal of Environment Science Health*, Part A. 34(4), pp. 951–74.

Barbeito I, Dawes MA, Rixen C, Senn J, and Bebi P. (2012). Factors driving mortality and growth at treeline: a 30-year experiment of 92,000 conifers. *Ecology.* 93(2), pp. 389-401.

Barbiroli G, Casalicchio, G and Raggi, A. (2004). A new approach to elaborate a multifunctional soil quality index. *JSS – J Soils and Sediments* 4 (3), pp. 201 – 204.

Barbiroli G, Casalicchio G and Raggi A. (2006). Developing a multifunctional tree-structured quality index for soil management. *Management of Environmental Quality: An International Journal* 17(3), pp. 339 – 370.

Barcelo, J, and Poschenrieder, C. (2003). Phytoremediation: principles and perspectives. *Contribution to Science,* 2(3), pp. 333-344.

Barceloux, D. G. (1999). Manganese. *Journal of Clinical Toxicology,* 37, pp. 293–307.

Bardos, R. P. and Van Veen, H. J. (1996). Review of longer term or extensive treatment technologies. *Land Contamination and Reclamation,* 4, pp. 19-36.

Barnhisel, R. I. (1988). Correction of physical limitations to reclamation. In: L. R.Hossner, editor. *Reclamation of surface-mined lands, vol. 1.* CRC Press, Boca Raton , Florida, pp. 191–211.

Barona, A, Aranguiz, I. and Elias, A. (2001). Metal associations in soils before and after EDTA extractive decontamination: implications for the effectiveness of further clean-up procedures. *Environmental Pollution*, 113, pp. 79–85.

Baum, C, Hrynkiewicz, P, Leinweber, P. and Meibner, R. (2006). Heavy-metal mobilization and uptake by mycorrhizal and nonmycorrhizal willows (*Salix × dasyclados*). *Journal of Plant Nutrition and Soil Science*, 169, pp. 516–522.

BBC (2011). Varteg Hill opencast mine bid rejected by councilors. Press Release, 18 January 2011.

Bending, N. A. D. (1993). *Site factors affecting tree response on restored opencast ground in the South Wales Coalfield*. PhD Thesis. University of East London, London, UK.

Bending, N. A. D, Moffat, A. J. and Roberts, C. J. (1992). Site factors affecting tree response on restored opencast ground in the South Wales coalfield. *Land Reclamation*, 91. Amsterdam: Elsevier., pp. 18-39.

Bending, N. A. D. and Moffat, A. J. (1993). Site factors affecting tree response on restored opencast ground in the South Wales coalfield. (ed. M.C.R. Davies). *Land Reclamation, an End to Dereliction?* Elsevier Applied Science, London, pp 469.

Bending, N. A. D. and Moffat, A. J. (1999). Tree performance on mine spoils in South Wales coalfields. *Journal of Applied Ecology* 36 (5), pp. 784-797.

Bengough, A. G. (1997). Modelling rooting depth and soil strength in a drying soil profile. *Journal of Theoretical Biology,*186, pp. 327-338.

Bell, R. M. (1992). Higher plant accumulation of organic pollutants from soils. *USEPA, Risk Reduction Engineering Lab.* EPA/600, pp. 138.

Bermond, A, Yousfi, I. and Ghestem, J. P. (1998). Kinetic approach to the chemical speciation of trace metals in soils. *Analyst*, 123, pp. 785–789.

Bernard, L, Maron, P. A, Mougel, C, Nowak, V, Leveque, J, Marol, C, Balesdent, J, Gibiat, F. and Ranjard, L. (2009). Contamination of Soil by Copper Affects the Dynamics, Diversity, and Activity of Soil Bacterial Communities Involved in Wheat Decomposition and Carbon Storage. *Applied and Environmental Microbiology*, 75, pp. 7565-7569.

BGS (2009). Opencast Coal Statistics 2008-2009. [online]. Available at: http://www.bgs.ac.uk/mineralsuk/mines/coal/occ/archive.html
Accessed 4 January 2009

Bhuiyan, M. A. H, Parvez, L, Islam, M. A. Dampare, S. B. and Suzuki, S. (2010). Heavy metal pollution of coal mine-affected agricultural soils in the northern part of Bangladesh Journal of Hazardous Materials, 173(1-3), pp. 384-392.

Biswas, A. K. (2010). *Removal of Cadmium from aqueous phase by adsorptive column test using fly ash as the adsorbent.* Masters Thesis, Civil engineering department, Jadavpur University, India.

Black, H. (1995). Absorbing possibilities: Phytoremediation. *Environmental*

Health Perspectives. 103(12), pp. 1106-1108.

Blake, L. and Goulding, K. W. T. (2002). Effects of atmospheric deposition, soil pH and acidification on heavy metal contents in soils and vegetation of semi-natural ecosystems at Rothamsted Experimental Station, UK. *Plant and Soil*, 240(2), pp. 235-251.

Blaylock, M. J. (2000). Field demonstrations of phytoremediation of lead-contaminated soils *In* Terry, N and Banuelos, G. (Ed.), *Phytoremediation of Contaminated Soil and Water*, CRC Press, Boca Raton FL, pp. 1–12

Blaylock, M. J, Salt, D. E, Dushenkov, S, Zakhrova, O, Gussman, C, Kapulnik, Y, Ensley, B. D. and Raskin, I. (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environment Science and Technology*, 31, 860–865.

Bolan, N. S, Adriano, D. C, Natesa, R. and Koo, B. J. (2003). Effects of organic amendments on the reduction and phytoavailability of chromate in mineral soil. *Journal of Environmental Quality*, 32(1), pp. 120–128.

Borgegard, S. O. and Hakan, R. (1989). Biomass, root penetration and heavy metal uptake in birch, in a soil cover over copper tailings. *Journal of Applied Ecology*. 26, pp. 585.

Borovik, A. S. (1989). Characterisation of metals in biological systems. In: Shaw, A. J. (Ed.) *Heavy Metal tolerance in plants: an evolutionary approach*. CRC Press. Florida, pp. 3-5.

Bradshaw, A. D. and Chadwick, M. J. (1980). *The Restoration of Land.* London: Blackwell Scientific Publication. 6. pp. 69 and 141.

Brady, N. C. and Weil, R. R. (2008). *The Nature and Properties of Soils, 14th ed.* Pearson Prentice Hall: NJ, USA.

Braithwaite, R. (1999). *Twenty two soil contaminant profiles. Properties, source, abundance and importance in contaminated land investigation*. Zero Environmental Publication, Warwick, UK.

Bransden, B. E. (1991). Soil protection as a component of gravel raising. Soil Use and Management, 7(3), pp. 139-144.

Brennan, M. A. and Shelley, M. L. (1999). A model of the uptake, translocation, and accumulation of lead (Pb) by maize for the purpose of phytoextraction. Ecological Engineering, 12, pp. 271–297.

Brevik, E. C. (2011). Soil health and productivity. *Soils, Plant Growth and Crop Production,* 1, pp. 2.

Brevik, E. C. (2013). The Potential Impact of Climate Change on Soil Properties and Processes and Corresponding Influence on Food Security. *Agriculture*, 3, pp. 398-417.

British Land Reclamation Society. (2006). *Sustainable and Beneficial reuse of land* [online] (updated 10 Jan 2007). Available at: www.blrs.org Accessed 3 September 2007

Broad, K. F. (1979). *Tree Planting On Man-Made Sites in Wales.* Forestry Commission Occasional Paper 3.Forestry Commission, Edinburgh, UK.

Brooks, R. R. (1987). *Serpentine and its vegetation.* Croom Helm, London.

Brooks, R. R, Morrison, R. S, Reeves, R. D, Dudley, T. R. and Akman, Y. (1979). Hyperaccumulation of nickel by Alyssum Linnaeus (Cruciferae). *Proceeding of Royal Society London*, 203, pp. 387– 403.

Brooks, R. R, (1998). *Plants that hyperaccumulate heavy metals.* Wallingford, CAB International, pp. 380.

Brown, S. L, Chaney, R. L, Angle, J. S. and Baker, A. J. M. (1994). Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc- and cadmium contaminated soil. *Journal of Environmental Quality*, 23, pp. 1151–7.

Brownridge, J. D. and Shafroth, S. M. (2003). Pressure dependence of energetic (<160 keV) focused electron beams arising from heating or cooling (LiNbO3) pyroelectric crystals. *Applied Physical Letters*, 83, pp.1477–1479

Brunner, I, Lustera, J, Günthardt-Goerga, M. S. and Frey, B. (2008). Heavy metal accumulation and phytostabilisation potential of tree fine roots in a contaminated soil. Environmental Pollution, 152(3), pp. 559–568.

Buckland, S. T, Campbell, C. D, Mackie-Dawson, L. A, Horgan, G. W. and Duff, E. I. (1995). A method for counting roots observed in minirhizotrons and their theoretical conversion to root length density. *Plant and Soil,* 153, pp. 1–9.

Burken, J. G. and Schnoor, J. L. (1997). Uptake and metabolism of atrazine by poplar trees. *Environment Science and Technology.* 31, pp. 1399–406.

Burken, J. G and Schnoor, J. L. (1999) Distribution and volatilisation of organic compounds following uptake by hybrid poplar trees. *International Journal of Phytoremediation.* 1, pp. 139–151.

Campaign to Protect Rural England. (2008). *Eco-towns and the Planning System* (London: CPRE) [online]
Available at:
http://www.cpre.org.uk/?option=com_finder&view=search&q=mining
Accessed on 26 November 2012.

Capuana, M. (2011). Heavy metals and woody plants- biotechnologies for phytoremediation. *Forest Biogeosciences and Forestry,* 4(1), pp. 7-15.

Carrel, S. (2009). Opencast coalmine surge 'weakens UK's authority at climate change talks, Guardian, 14 August 2009.
Retrieved from: http://www.guardian.co.uk

Casado, M, Anawar, H. M, Garcia-Sanchez, A. and Santa Regina, I. (2008). Cadmium and zinc in polluted mining soils and uptake by plants (El Losar mine, Spain). *International Journal of Environmental Pollution,* 33, pp. 146–159.

Cataldo, D. A. Wildung, R. E. and Garland, T. R. (1987). Speciation of trace inorganic contaminants in plants and bioavailability to animals: An overview. *Journal of Environmental Quality*, 16(4), pp. 289-295.

Chadderton, C, Elliott E. and Williams, G. (2011). A guide to assessing the health and wellbeing impacts of opencast mining. Wales HIA Support Unit, Opencase guidance, 522.

Chaney, R.L. (1988). Metal speciation and interactions among elements affect trace element transfer in agricultural and environmental food-chains. *In* J.R. Kramer and H.E. Allen (ed.). *Metal speciation: theory, analysis and applications*. Lewis Publishers Chelsea, MI. pp. 218–260.

Chaney, R. L, Malik, K. M, Li, Y. M, Brown, S. L, Brewer, E. P, Angle, J. S, (1997). Phytoremediation of soil metals. *Current Opinion in Biotechnology*; 8, pp. 279 – 284.

Chang, F. H. (1992). Influence of trace metals on some soil nitrogen transformations. *Journal of Environmental Quality*, 11, pp. 1–4.

Charvat, I. and Pawlowska, T. E. (2004). Heavy metal stress and developmental patterns in arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology*, 70, pp. 6643–6649.

Chen, B, Shan, X. Q. and Qian, J. (1996). Bioavailability index for quantitative evaluation of plant availability of extractable soil trace elements. *Plant and Soil* 186, pp. 275-283.

Cheng, S. (2003). Heavy metal pollution in China: origin, pattern and control. *Environmental Science and Pollution Research,* 10, pp. 192–198.

Chernykh, N.A. (1991). Alternations of the concentrations of certain elements in plants by heavy metals in the soil. *Soviet Soil Science*, 23(6), pp. 45-53.

Clemens, S, Palmgren, M. G and Kramer, U. (2002). A long way ahead: understanding and engineering plant metal accumulation. Trends in Plant Science, 7(7), pp. 309–315.

CMET (2005). Atomic Absorption Spectrophotometry. [online]
Available at: http://www.cmetindia.org/aas.html
(Accessed on 3 June 2012)

Colbourn, P., & Thornton, I. (1978). Lead pollution in agricultural soils. *Journal of Soil Science, 29*, 513–526.

Conesa, H. M, Garcia, G, Faz, A. and Arnaldos, R. (2007). Dynamics of metal tolerant plant communities' development in mine tailings from the Cartagena-La Union Mining District (SE Spain) and their interest for further revegetation purposes. *Chemosphere*, 68, pp. 1180-1185.

Cooke, J. A. and Johnson, M. S. (2002). Ecological restoration of land with particular reference to the mining of metals and industrial minerals: a review of theory and practice. *Environmental Reviews*, 10(1), pp. 41–71.

Cooper, E. M, Sims, J. T., Cunningham, S. D, Huang, J. W, Berti, W. R. (1999). Chelate assisted phytoextraction of lead from contaminated soils. *Journal of Environmental Quality,* 28, pp. 1709–1719.

Coppin N J and Bradshaw A D (1982). *Quarry reclamation.* Mining Journal Books, London.

Cornu, J. Y, Staunton, S. and Hinsinger, P. (2007). Copper concentration in plants and in the rhizosphere as influenced by the iron status of tomato (*Lycopersicum esculentum* L.). *Plant Soil*, 292, pp. 63–77.

Cottenie, A, Velghe, G, Verloo, M. and Kiekens, L. (1982). *Biological and Analytical Aspects of Soil Pollution*. Laboratory of Analytical and Agrochemistry, Town or Uni? Belgium.

Countryside Commission (1993). *Opencast coal mining* - CCP 434, London, pp.15.

Cresswell, H. P. and Hamilton, G. J. (2002). Bulk density and pore space relations. *In* N.J. Mckenzie, K.J. Coughlan, H.P. Cresswell (Eds.), *Soil Physical Measurement and Interpretation for Land Evaluation.* Chapter 3. CSIRO Publishing, Collingwood, Australia, pp. 35–59.

Cunningham, S. D. and Lee, C. R. (1995). Phytoremediation: Plant-Based Remediation of Contaminated Soils and Sediments. *Bioremediation, Science and Applications*,43, pp. 145-156.

Cunningham, S. and Berti, W. (2012). Remediation of contaminated soils with green plants: an overview. *In Vitro Cellular and Developmental Biology. Plant.* 29, pp. 207-212.

DAFF (Department of Agriculture, Forestry and Fisheries) 2011. National Policy on Organic Production. 7th Draft. From
http://www.nda.agric.za/doaDev/sideMenu/plantProduction/doc/NationalPolicyOrganicFarmingDraft7.pdf
Accessed on 6 May 2012)

Dang, Z, Liu, C. and Haigh, M. J. (2002). Mobility of heavy metals associated with natural weathering of coal mine spoils. *Environmental Pollution* 118 (3), pp. 419-426.

Dao, L, Morrison, L, Kiely, G. and Zhang, C. (2013). Spatial distribution of potentially bioavailable metals in surface soils of a contaminated sports ground in Galway, Ireland. *Environmental Geochemistry and Health,* 35, pp. 227–238.

Das, M, and Maiti, S. K. (2005). Metal mine waste and phytoremediation. *Asian Journal of Water, Environment and Pollution,* 4(1), pp. 169-176.

Davey, C. B. (1999). Tree growth and essential nutrient elements. *Soil Sci. Soc. Am. J.* 17, pp. 59–60

Davies, B. E. and Roberts, L. J. (1978). The distribution of heavy metal contaminated soils in northeast Clwyd, Wales. *Water, Air and Soil Pollution* 9 (4), pp. 507-518.

Davies, B. E. (1995) Lead. *In* Alloway, B. J. (ed.) *Heavy Metals in Soil.* Blackie Academic and Professional, London, pp. 206-220.

Davies, M. C. R. (1991). Contaminated Land. *In* Davies, M. C. R. (Ed.). *Land Reclamation: An End to Deriliction?* , Elsevier Sciences Publishers, pp. 315-325.

Davies, R, Hodgkinson, R, Younger, A. and Chapman, R. (1995). Nitrogen loss from a soil restored after surface mining. *Journal of Environmental Quality*. 24(6).

Dawson, J. O, Christenson, T. W. and Timmons, R. G. (1984) Nodulation by *Frankia* of *Alnus glutinosa* seeded in soil from different topographical positions on an Illinois spoil bank. *The Actinomycetes*, 17, pp. 50–60.

Dean, J. R (2010). Heavy metal bioavailability and bioaccessibility in soil. *Methods in Molecular Biology,* 599, pp. 15–36.

Dec J. and Bollag, J. M. (1994) Dehalogenation of chlorinated phenols during oxidative coupling. Environment Science and Technology, 28, pp. 484–490.

DEFRA (2002). Note on the withdrawal of ICRCL trigger values. *Contaminated Land Advice Note (CLAN)*. 3/02, pp. 15.

DEFRA (2004). *Soil Action Plan for England and Wales 2004-2006.* Defra Publications, London, pp. 45.

DEFRA (2008) Guidance on the Legal Definition of Contaminated Land. [online] Available at:

http://archive.defra.gov.uk/environment/quality/land/contaminated/documents/legal-definition.pdf
(Accessed 14 October 2012)

DEFRA and Environment Agency (2002). *Contaminants in soil: collation of toxicological data and intake values for humans: Cadmium.* Bristol, Environment Agency.

Densmore, R. V. (2005). Succession on subalpine glacier mine spoil: effects of revegetation with *Alnus viridis*. *Arctic Antarctic Alpine Research*, 37, pp. 297–303.

Department of Energy and Climate Change (2013). Historical coal data: coal production, availability and consumption1853 to 2012 [online]. Available at:
https://www.gov.uk/government/statistical-data-sets/historical-coal-data-coal-production-availability-and-consumption-1853-to-2011
Accessed 3 September 2013

Department of the Environment (1989). *Environmental Impact assessment: A guide to the procedures.* London. Thomas Telford Publishing, pp. 65.

Department of Agriculture, Fisheries and Forestry (2011). Soil Health Knowledge Bank [online] Available at:
http://soilhealthknowledge.com.au/index.php?view=article&catid=8%3Asoil-biological-attributes&id=48%3Asoil-biology-beneficials&tmpl=component&print=1&page=&option=com_content&Itemid=12

(Accessed 1 September 2013)

Dickinson, N. M. (2000). Strategies for sustainable woodland on contaminated soils. *Chemosphere* 41, pp. 259 – 263.

Dickinson, N. M, Mackay, J, Goodman, A and Putwain, P. (2000). Planting trees on contaminated soils: Issues and guidelines. *Land Contamination and Reclamation* 8(2) pp. 87 – 101.

Dix, M. E, Klopfenstein, N. B, Zhang, J-W, Workman, S. W. and Kim, M. S. (1997). Potential use of*Populus* for phytoremediation of environmental pollution in riparian zones. *In:* Klopfenstein, N. B, Chun, Y. W, Kim, M. S. and Ahuja, M. R. (Eds.), *Micropropagation, Genetic Engineering, and Molecular Biology of Populus.* US Department of Agriculture, USA, pp. 206–211.

Dobson, M. C. and Moffat, A. J. (1993). *The potential for woodland establishment on landfill sites.* HMSO.

Dobson, M. C. and Moffat, A. J. (1995) A re-evaluation of objections to tree planting on containment landfills. *Waste Management and Research,*13, pp. 579-600.

DoE (1997). Mineral Planning Guidance Note 7: *Relcamation of mineral works.* Department of Environment, London, pp. 76.

DoE (1999). Mineral Planning Guidance Note 3: *Coal mining and colliery spoil disposal.* Department of Environment, London, pp. 60.

Donahue, R. L, Miller, R. W, and Shickluna, J. C. (1990). *Soils: An introduction to soils and plant growth (5th ed.).* Prentice-Hall, pp. 234.

Doran, J. W. and Zeiss, M. R. (2000). Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology,* 15, pp. 3–11.

Douben, P. E. T. (1994). The ecotoxicological significance of cadmium intake and residues in terrestrial small mammals. *Ecotoxicology and Environmental Safety,* 29, pp. 101–112.

Downing, T. E, Moles, J, McIntosh, I. and Garcia-Downing, C. (1998). Indigenous Peoples and Mining: Strategies and Tactics for Encounters. London: International Institute for Environment and Development, MMSD Project, 57, pp.3-44.

Draper, N. R. and Smith, H. (1981). *Applied regression analysis 2^{nd} ed.* John Wiley, New York, pp. 736.

Dudka, S. (1992). Factor analysis of total element concentrations in surface soils of Poland. *Science of the Total Environment, 121*, pp. 39–52.

Dushenkov, V, Kumar, P. B. A. N, Raskin M. (1995). I. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environment Science and Technology*. 29, pp. 1239–1245.

Dutta, R. K. and Agarwal, M. (2003). Restoration of opencast coal mine spoil by planting exotic tree species: a case study in dry tropical region. Ecological Engineering, 21(2-3), pp. 143-151

EAA (2009). *3.6 Soil Degradation.* European Environment Agency. [online] Available at: http://www.eea.europa.eu/publications/92-9157-202-0/page306.html Accessed on 12 January 2013

Ebbs, S. D and Kochian, L. V. (1997). Toxicity of zinc and copper to Brassica species: implications for phytoremediation. *J Environ Qual.* 26, pp. 776–81.

Eckel, W. P. and Langley, W. D. (1988). A background-based ranking technique for assessment of elemental enrichment in soils at hazardous waste sites. *In* Super fund '88: Proceedings of the 9th National Conference, Nov 28–30, Washington, DC, The Hazardous Materials Control Research Institute, Silver Spring, pp. 286–288.

Eltrop, L, Brown, G, Joachim, O. and Brinkmann, K. (1991). Lead tolerance of *Betula* and *Salix* in the mining area of Mechernich, Germany. *Plant Soil.* 131, pp. 275.

Environment Agency (2005). Evaluation of models for predicting plant uptake of chemicals from soil. Report SC050021/SR. Bristol: Environment Agency. pp. 17-23. Available at: http://environment-agency.gov.uk/commondata/acrobat/sc050021_2029764.pdf

[Accessed 17 October 2008]

Environment Agency (2006). *Ecological Risk Assessment: Public Consultation*. Bristol, Environment Agency.

Environment Agency, 2009. *Compilation of organic chemical data for the derivation of Soil Guideline Values*. Report SC050021/SR7. Environment Agency. Bristol.

EPA (1999). *Phytoremediation resource guide*. Washington: U.S. Environmental Protection Agency, EPA 542-B-99-003.

EPA (2000). *Introduction to phytoremediation*. Washington: U.S. Environmental Protection Agency, EPA/600/R-99/107.

Ernst, W. H. O. (2000). Revolution of metal hyperaccumulation and phytoremediation hype. *New Phytologists*, 146, pp. 357-358.

EU, (2003). *Technical guidance document on risk assessment*, EUR 20418 EN/1. Brussels: European Commission Joint Research Center, pp. 1-39.

Europa (2011). Risk assessment of Cadmium in Swedish environment. [online]. Available at: http://ec.europa.eu/enterprise/sectors/chemicals/files/reports/sweden_risk_ass_cadmium_jan2011_en.pdf

Accessed 26 July 2012

Evangelou, M. W, Robinson, B. H, Günthardt-Goerg, M. S. and Schulin. R.

(2013). Metal uptake and allocation in trees grown on contaminated land: implications for biomass production. *International Journal of Phytoremediation*, 15(1), pp. 77-90.

Faber-Maunsell (2006). *Varteg Coal Recovery and Reclamation Scheme, Blaenavon, South Wales, Hydrogeology and Contaminated Land Assessment. Final Interpretive Report*, 2 Volumes. Faber-Maunsell AECOM: Birmingham and Bargoed, Glamorgan Power Company Ltd. [online]
Available at:
http://www.planapps.torfaen.gov.uk/varteg%20hill_blaenavon/04.P.09210%20Varteg%20Hill%20May%2009/Faber%20Maunsell%20-%20Interpretative%20report%20-%20vol%201%20text.pdf
[Accessed on 23rd March 2012]

Faeth, P. (1994). *Evaluating the Carbon Sequestration Benefits of Forestry Projects in Developing Countries*, World Resources Institute, Washington, D.C.

Fangueiro, D, Bermond, A, Santos, E, Carapuca, H. and Duarte, A. (2002). Heavy metal mobility assessment in sediments based on a kinetic approach of the EDTA extraction: search for optimal experimental conditions. *Analytica Chimica Acta*, 459, pp. 245-256.

Faz Cano, A, Mermut, A. R, Ortiz, R, Benke, M. B. and Chatson, B. (2002). ^{13}C CP/MAS-NMR spectra of organic matter as influenced by vegetation, climate, and soil characteristics in soils from Murcia, Spain. *Canadian Journal of Soil Science*, 82(4), pp. 403–411.

Featherstone, A. W. (2004). *Rewilding in the north-central Highlands: an update.* ECOS 25, pp. 4–10.

Feng, M. H, Shan, X. Q, Zhang, S. and Wen, B. (2005). A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl2, and NaNO3 extraction methods for prediction of bioavailability of metals in soil to barley. *Environmental Pollution*, 137(2), pp. 231–240.

Fernåndez, I, Cabaneiro, A. and Carballas, T. (1997). Organic matter changes inmediately after a wildfire in an Atlantic forest soil and comparison with laboratory soil heating. *Soil Biology and Biochemistry*, 29, pp. 1–11.

Finzi, A. C, Canham, C. D. and van Breemen, N. (1998). Canopy tree–soil interactions within temperate forests: species effect on pH and cations. *Ecological Application*, 8, pp. 447–454.

Fitter, A. H. and Hay, R. K. M. (1981) *Environmental Physiology of Plants* 2nd ed. Academic, San Diego, California, pp. 357.

Fitter, A. H. and Hay, R. K. M. (1987). *Environmental physiology of plants.* 2nd ed. Academic, London.

Flathman, P. E. and Lanza, G. R. (1998). Phytoremediation: Current Views on an Emerging Green Technology. *Journal of Soil Contamination*, 7(4), pp. 415-432.

Fontes, M. P. F, Matos, A. T, Costa, L. M. and Neves, J. C. L. (2000). Competitive adsorption of zinc, cadmium, copper and lead in three highly weathered Brazilian soils. *Communications in Soil Science and Plant Analysis*, 31, pp. 2939–2958.

Forestry Commission (1991). *Forestry Practice.* London. UK. HMSO. Chapter 9 (76), pp. 1-70

Forestry Commission (1994). Creating new native woodlands. Forestry Commumication Bulletin, 112, pp. 1–74

Forestry Commission (1999). *England forestry strategy: a new focus for England's woodlands.* Forestry Commission, Cambridge.

Forestry Commission (2003). *Roots - The software for sustainable growth.* LRU IN FULL News. 2.

Foy. C. D, Weil, R. R. and Coradetti, C. A. (1995). Differential manganese tolerances of cotton genotypes in nutrient solution. J Plant Nutrition, 18, pp. 685–706.

Franchin, C, Fossati, T, Pasquini, E, Lingua, G, Castiglione, S. and Torrigiani, P. (2007). High concentrations of Zn and Cu induce differential polyamine responses in micropropagated white poplar (*Populus alba* L. 'Villafranca'). Physiologia Plantarum, 130, pp. 77–90.

Freedman, B. and Hutchinson, T. C. (1981). Sources of metal and elemental contamination of terrestrial environment: Effect of Heavy Metal pollution on Plants. *Pollution Monitoring Series*, 2, pp. 35-94.

French, C. J. (2004). *Tree planting for phytoremediation: The fate of soil contaminants on brownfield sites*. Ph. D. Thesis. School of Biological and Earth Sciences. Liverpool, University of Liverpool.

French, C. J, Dickinson, N. M, Putwain, P. D. (2006). Woody biomass phytoremediation of contaminated brownfield land. Environmental Pollution 141, pp. 387-395.

Gallagher, F, Pechmaan, I, Bogden, J, Grabosky, J. and Weis, P. (2008). Soil Metal concentrations and productivity of *Betula* as measured by field spectrometry and incremental annual growth in an abandoned urban Brownfield in New Jersey. *Environmental Pollution*. 156, pp. 699-706.

Gardiner, A. S. (1968). *The reputation of birch for soil improvement*. Forestry Commission Research and Development Paper 67, pp.9.

GGAT (2008). The Glamorgan Gwent Archaeological Trust ltd. [online]. Available at

http://www.ggat.org.uk/cadw/historic_landscape/blaenavon/english/Blaenavon_019.htm.

Accessed 12 March 2009.

GATT (2009). Historic Landscape CharacterisationL Blaenavon HLCA 019 Mynydd Varteg Opencast. [online] Available at:

http://www.ggat.org.uk/cadw/historic_landscape/blaenavon/english/Blaenavon_019.htm

(Accessed 10 March 2009)

Ghose, M. K. and Majee S. R. (2000). Assessment of the impact on the air environment due to opencast coal mining – an Indian case study. Atmospheric Environment, 34, pp. 2791–2796.

Ghose, M. K. (2005). Soil conservation for rehabilitation and revegetation of mine-degraded land. *TIDEE – TERI Information Digest on Energy and Environment,* 4(2), pp. 137-150.

Ghosh, A. B, Bajaj, J. C, Hassan, R. and Singh, D. (1983). Laboratory manual for oil and water testing. Division of Soil Science and Agricultural Chemistry. IARI, New Delhi, India, pp. 11-22

Ghosh, M. and Singh, S. P. (2005a). A Review on Phytoremediation of Heavy Metals and Utilization of It's by Products. *Asian Journal on Energy and Environment,* 6(4), pp. 214-231.

Ghosh, M. and Singh, S. P. (2005b). A comparative study of cadmium phytoextraction by accumulator and weed species. *Environment Pollution,* 133, pp. 365-371.

Glass, D. J. (2000). Economic potential of phytoremediation. *In* Raskin, I. and Ensley, B. D. (eds) *Phytoremediation of toxic metals: using plants to clean-up the environment*. New York, John Wiley and Sons, pp. 15-32.

Glimmerveen, I. (1996) *Heavy metals and trees.* Edinburgh: Institute of

Chartered Foresters, pp. 206.

Göhre, V. and Paszkowski, U. (2006). Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta*, 223, pp. 1115-1122.

Good, J. E.G, Williams, T. G, Moss, D. (1985).Survival and growth of selected clones of birch and willow on restored opencast coal sites. *Journal of Applied Ecology*. 22, pp. 995– 1008.

Göransson, A. and Philippot, S. (1994). The use of fast growing trees as 'Metal-collectors'. *In*: *Willow Vegetation Filters for Municipal Wastewaters and Sludge, Proceedings of a Study Tour, Conference and Workshop in Uppsala*, Sweden, pp. 129-131.

Gould, A. B, Hendrix, J. W, and Ferriss, R. S. (1996). Relationship of mycorrhizal activity to time following reclamation of surface mine land in western Kentucky. I Propagule and spore population densities. *Canadian Journal of Botany,* 74, pp. 247-261.

Gower, S. T. and Richards, J. T. (1990). Larches: deciduous conifers in an evergreen world. *BioScience*, 40, pp. 818-826.

Graystone S. J, Wang, S, Campbell, G. D. and Edwards, A. C. (1998) Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biology and Biochemistry,* 30, pp. 369–378.

Green, S. (2005). *Birch dieback in Scotland*. Forestry Commission: Information

Note 72, pp. 1-6.

Griffin, G. J, Hale, M. G. and Shay, F. J. (1976) Nature and quantity of sloughed organic matter produced by roots of axenic peanut plants. *Soil Biology and Biochemistry*, 8, pp. 29–32.

Griffiths, D. G. (1992). Land reclamation in Wales: aims and strategies of the Welsh development Agency. *Land Degradation and Rehabilitation*, 3, pp. 157-159.

Gudadhe, S. K. and Ramteke, D. S. (2012). Impact of planting on coal mine spoil characteristic. *International Journal of Life Science Biotechnology and Pharma Research*, 1(3), pp. 84-92.

Haigh, M.J. (1993). Newsletter of the British Association for Better Land Husbandry (1), Oxford, UK.

Haigh, M. J. (1995). Soil Quality standards for reclaimed coal-mine disturbed lands: A discussion paper. *International Journal for Surface Mining. Reclamation and Environment* 9, pp. 187-202.

Haigh, M.J. and Sansom, B. (1999). Soil compaction, runoff and erosion on reclaimed coal-lands (UK). *International Journal of Surface Mining, Reclamation and Environment* 13(4). pp. 135-146.

Haigh, M.J. (2000a). *Reclaimed Land: Erosion Control, Soils and Ecology.* Rotterdam: A.A Balkema, pp. 421.

Haigh, M.J. (2000b). The aims of land reclamation. *Land Reconstruction and Management* 1 pp. 1-20.

Haigh, M.J. (2002). Land reclamation and deep ecology: in search of a more meaningful Physical Geography. *Area 34* (3). pp. 242-252.

Haigh, M.J. (2003). Soil conservation and the husbandry of the soil's organic system. *Ecology and Future: Bulgarian Journal of Ecological Science* 2 (3/4), 7-10.

Haigh, M. J, Plaming, K, Cullis, M. and Jenkins, R. (2008). Evaluation of cambial electrical resistance for the appraisal of tree vitality on reclaimed coal lands. International Journal of Mining Reclamation and Environment, 23(1), pp. 21-32.

Haigh, M. J, Reed, H, Flege, A, D'Aucourt, M, Plaming, K, Cullis, M, Woodruffe, P, Sawyer, S, Panhuis, W, Wilding, G, Farrugia, F, Powell, S. (2013) Effect of planting method on the growth of *Alnus glutinosa and Quercus petraea* in compacted opencast coal-mine spoils, South Wales. *Land Degradation and Development.* http://onlinelibrary.wiley.com/doi/10.1002/ldr.2201/full (Accessed 9 May 2013).

Halcrow, Sir W. and Partners (1986). *Assessment of Landslip Potential: South Wales (Rhondda Valleys Landslip Potential Assessment)*, Report for Department of the Environment and Welsh Office.

Hamon, R. E, McLaughlin, M. J, Gilkes, R. J, Rate, A. W, Zarcinas, B, Robertson, A, Cozens, G, Radford, N. and Bettenay, L. (2004). Geochemical indices allow estimation of heavy metal background concentrations in soils. *Global Biogeochemical Cycles*, 18, pp. 1014-1018.

Handley, J. F. and Perry, D. (1998) Woodland expansion on damaged land: reviewing the potential. *Quarterly Journal of Forest*, 92, pp. 297-306.

Hangyel, L. and Benesoczky, J. (1992) Research on the effects of municipal effluents on the reclamation of spoil heaps at opencast coal mines. United Nations Economic Commission for Europe, Committee on Energy, Working Party on Coal, *Symposium on Opencast Coal Mining and the Environment*, ENERGY/WP.1/ SEM.2/R.17: pp. 8.

Harmens, H, Koevoets, P. L. M, Verkleij, J. A. C. and WHO, Ernst (1994) The role of low molecular weight organic acids in mechanisms of increased zinc tolerance in *Silene vulgaris* (Moench) Garcke. *New Phytologist*, 126, pp. 615–621

Haswell, S. J. (1991). *Atomic Absorption Spectrometry; Theory, Design and Applications*. Elsevier, Amsterdam.

Hatcher, J. (1993). *The History of the British Coal Industry*. Volume I. Oxford: Clarendon Press.

Hawes, M. C, Gunawardena, U, Miyasaka, S. and Zhao, X. (2000). The role of root border cells in plant defence. *Trends in Plant Science*, 5, pp. 128–133.

HazDat. (2008). *HazDat Database: ATSDR's Hazardous Substance Release and Health Effects Database*. Agency for Toxic Substances and Disease Registry.

Head, K. H. (2006). Manual of Soil Laboratory Testing: Soil classification and compaction tests. 3rd Edition. Taylor and Francis, Boca Raton, United States.

Henry, J. R. (2000). *An Overview of the Phytoremediation of Lead and Mercury*. National Network of Environmental Management Studies (NNEMS), pp. 1–31.

Heinrichs, H, Schultz-Dobrick, B. and Wedepohl, K. J. (1980). Geochim Cosmochim. Acta, 44, pp. 1519-1532.

Heiri, O, Lotter, A. F. and Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of Paleolimnology* 25, pp. 101–110.

Hermle, S, Günthardt-Goerg, M. S, and Schulin, R. (2006). Effects of metal-contaminated soil on the performance of young trees growing in model ecosystems under field conditions. *Environmental Pollution*, 144(2), pp. 703-14.

Hester, R. E. and Harrison, R. M. (2001). Assessment and reclamation of contaminated land. *Issues in Environmental Science and Technology*.16 http://www.rsc.org/images/CHISampleRecords_tcm18-40125.pdf

Hodge, S.J. (1995). *Creating and managing woodlands around towns*. Forestry Commission Handbook No. 11, HMSO, London, pp. 25.

Hovmand, M. F, Nielsen, S. P. and Johnsen, I. (2009). Root uptake of lead by Norway spruce grown on ^{210}Pb spiked soils. *Environmental Pollution*, 157, pp. 404–409.

Howe, P. D, Malcom, H. M. and Dobson, S. (2004). *Manganese and its Compounds: Environmental Aspects.* World Health Organization, Geneva, Switzerland, pp. 1-26.

Huang, J. W, Chen, J, Berti, W. R. and Cunningham, S. D. (1997). Phytoremediation of lead contaminated soils: role of synthetic chelates in lead phytoextraction. *Environment Science and Technology,* 31, pp. 800– 805.

Hung, H, Mackay, D, (1997). A novel and simple model of the uptake of organic chemicals by vegetation from air and soil. *Chemosphere*, 35, pp. 959-977.

Hutchings, T. R. (2002). *The opportunities for woodland on contaminated land: Information Note 44.* Edinburgh, UK, Forestry Commission., pp. 1-3

Hutchings, T. R, Moffat, A. J. and Kemp, R. A. (2001). Effects of rooting and tree growth of selected woodland species on cap integrity in a mineral capped landfill site. *Waste Management and Research. The Journal of The International Solid Wastes And Public Cleansing Association, ISWA* 19 pp. 194-200.

Hutton, M, Roberts, R. D, Johnson, M. S. (1978). Lead contamination of small mammals from abandoned metalliferous mines. Environmental Pollution, 15(1), pp. 61-69.

ICRCL (1987). *Inter Departmental Committee on the Redevelopment of Contaminated Land: Guidance Notes* (59/83) London, HMSO, pp. 21.

International Energy Outlook (2009). Energy production. [online] Available at: http://arsiv.setav.org/ups/dosya/25025.pdf

Accessed 8 June 2010

Issac, R. A. and Kerber, J. D. (1971). Atomic absorption and flame photometry: Techniques and uses in soil, plant and water analysis. In L. M. Walsh ed. *Instrumental Methods for Analysis of Soils and Plant Tissue* Soil Society of America, Madison, Wisconsin.pp. 17-38.

Jarup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulliten,* 68 (1), pp. 167-182.

Jensen, J. K, Holm, P. E, Nejrup, J, Larsen, M. B and Borggaard, O. K. (2009). The potential of willow for remediation of heavy metal polluted calcareous urban soils. Environmental Pollution, 157, pp. 931–937.

Jing Y-D, He, Z-L, Yang, X-E. (2007). Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. *Journal of Zhejiang University B*, 8(3), pp. 192–207.

Johnston, G. G. and Kipphut, G. W. (1988). Microbially mediated Mn(II) oxidation in an oligotrophic Arctic lake. *Applied and Environmental Microbiology*, 54, pp. 1440-1451.

Jung, M. C. (2008). Heavy Metal concentrations in soils and factors affecting metal uptake by plants in the vicinity of a korean Cu-W mine. *Sensors* 8, pp. 2413-2423.

Kabata-Pendias, A. and Pendias, H. (1992). *Trace Elements in Soil and Plants* 2nd edn. Boca Raton, Florida, CRC Press.

Kabata-Pendias, A. (2001). *Trace Elements in Soils and Plants* 3rd edn. Boca Raton, Florida, CRC Press.

Khan, A. G, Kuek, C, Chaudhry, T. M, Khoo, C. S. and Hayes, W. J. (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, 41, pp. 197–207.

Kahle, H. (1993). Response of roots of trees to heavy metals. *Environmental and Experimental Botany* 33: 99-119.

Karpati, A. S, Handel, S. N, Dighton, J. and Horton, T. R. (2011) *Quercus rubra*-associated ectomycorrhizal fungal communities of disturbed urban sites and mature forests. *Mycorrhiza*, 21, pp. 537–547.

Kasraei, R, Rodriquez- Barrueco, C, and Igual, M. (1996). The effect of Al and Mn on growth and mineral composition of *Casuarina equisetifolia* Forst. *Fertilizers and Environment* pp. 75-81.

Kavamura, V. N. and Esposito, E. (2010). Biotechnological strategies applied to the decontamination of soil polluted with heavy metals. *Biotechnology*

Advances, 28, pp. 61-69.

Kerai, M. (2013). Coal in 2012. [online]
Available at:
https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/170721/et_article_coal_in_2012.pdf
Accessed 10 May 2013

Kelly, R. T. (1980). Site investigation and materials problems. In: *Proc. Conf, Reclamation of Contaminated Land*, Eastbourne, SCI, London, B2/1–B2/14.

Key R.S. (1994). Invertebrate conservation in quarries, mines, sand, clay and gravel pits. *English Nature Species Conservation Handbook 6.3*, English Nature, Peterborough.

Kieffer, F. (1991) Metals as essential trace elements for plants, animals, and humans. In: Merian E (ed) *Metals and their compounds in the environment.* VCH, Weinheim, pp 481–489.

Kiekens, L. (1995) 'Zinc' *In:* Alloway, B. J. (ed.) *Heavy Metals in Soil.* Blackie Academic and Professional, London, pp. 284-303.

Klang-Westin, E. and Perttu, K. (2002). Effects of nutrient supply and soil cadmium concentration on cadmium removal by willow, *Biomass Bioenergy*, 23, pp. 415–426.

Koch, H. J. and Stockfisch, N. (2006). Loss of soil organic matter upon ploughing under a loess soil after several years of conservation tillage. *Soil Tillage Research*, 86, pp. 73–83.

Koeppe, D. E. (1981). The uptake, distribution, and effect of cadmium and lead in plants. *Stevens Report, Stevens Institute of Technology, 7*(3), pp. 197–206.

Koplik, R. (2010). Atomic Spectrometry: Advanced strategies in food analysis. *Journal of Analytical Atomic Spectrometry*. 18, pp. 241-245.

Kopponen, P, Utriainen, M, Lukkari, K, Suntioinen, S, Karenlampi, L, and Karenlampi, S, Clonal, (2001). Difference in copper and zinc tolerance of birch in metal-supplemented soils. *Environmental Pollution*. 112(1), pp. 89 – 97.

Kozlov, M. V, Wilsey, B. T. Koricheva, J. and Haukioja, E. (1996). Fluctuating asymmetry of birch leaves increases under pollution impact. *Journal of Applied Ecology*. 33(6), pp. 1489-1495.

Kozlov, M. V, Haukioja, E, Bakhtiarov, A. V, Stroganov, D. and Zimina, S. N. (2000). Root versus canopy uptake of heavy metals by birch in an industrially polluted area: contrasting behaviour of nickel and copper. Environmental Pollution, 107, pp. 413–420.

Kula, E, Hrdlička, P, Hedbávný, J, and Švec, P. (2012). Various content of manganese in selected forest tree species and plants in the undergrowth. *Časopis Beskydy*, 5 (1), pp. 19–26.

Kumar, P. B, Dushenkov, V, Motto, H. and Rasakin, I. (1995). Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental Science and Technology*, 29, pp.1232– 1238.

Kundu, N. K. and Ghose, M. K. (1997) Shelf life of stock-piled topsoil of an opencast coal mine. *Environmental Conservation*, 24 (1), pp. 24–30.

Kuzovkina, Y. A, Keen, M, and Quigley, M. (2004). Cadmium and Copper Uptake and Translocation in Five Willow (*Salix* L.) Species. *International Journal of Phytoremediation*. 6(3), pp. 269 – 287.

Kuznetsova, T, Rosenvald, K, Ostonen, I, Helmisaari, H. S, Mandre, M. and Lõhmus, K. (2009). Survival of black alder (*Alnus glutinosa* L.), silver birch (*Betula pendula* Roth.) and Scots pine (*Pinus sylvestris* L.) seedlings in a reclaimed oil shale mining area. *Ecological Engineering*, 36, pp. 495–502.

Labrecque, M, Teodorescu, T. I. and Daigle, S. (1995). Effect of wastewater sludge on growth and heavy metal bioaccumulation of two *Salix* species. Plant Soil, 171, pp. 303–316.

Larcher, W. (1995). *Physiological plant ecology*. Springer Verlag, New York, pp. 585.

Lagerwerff, J. V, Brown, D. L. and Biersdorf, G. T. (1993). *In Trace Substances in Environmental Health*, (ed) Hemphill, D. D. University of Missouri, Columbia, pp. 71-78.

Lakic, N. (2002). Classification of latin squares. *Journal of Agricultural Sciences*, 47(1), pp. 105-112.

Lamoreaux, R. J. and Chaney, W. R. (1978). The Effect of Cadmium on Net Photosynthesis, Transpiration, and Dark Respiration of Excised Silver Maple Leaves. Physiologia Plantarum, 43(3), pp. 173-320.

Landberg, T. and Greger, M. (1996). Differences in uptake and tolerance to heavy metals in Salix from unpolluted and polluted areas. *Applied Geochemistry,* 11, pp. 175–180.

Lasat, M. M. (2002). Phytoextraction of toxic metals: A review of biological mechanisms. *Journal of Environmental Quality.* 31, pp. 109-120.

Lasat, M. M. (2011). *The use of plants for the removal of toxic metals from contaminated soil.* Environmental Protection Agency. United States.

Laureysens, I, Blust, R, De Temmerman, L, Lemmens, C, and Ceulemans, R. (2004). Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture: I. Seasonal variation in leaf, wood and bark concentrations. *Environmental Pollution*, 131(3), pp. 485-494.

Laureysens, R, Blust, L, Temmerman, C, Lemmens, R. and Ceulemans, C. (2004). Clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture: I. Seasonal variation in leaf, wood and bark concentrations. Environmental Pollution, 131, pp. 485–494.

Le Bot J, Kirkby, E. A. and Beusichem, M. L. (1995). Manganese toxicity in tomato plants: effects on cation uptake and distribution. *Journal of Plant Nutrition*, 13, pp. 513–525.

Lehn, H. and Bopp, M. (1987). Prediction of heavy metal concentrations in mature plants by chemical analysis of seedlings. *Plant Soil*, 101, pp. 9–14.

Lee, C. G, Chon, H. and Jung, M. C. (2001). Heavy metal contamination in the vicinity of the Daduk Au–Ag–Pb–Zn mine in Korea. Applied Geochemistry, 16, pp. 1377–1386.

Leonelli, G, Battipaglia, G, Cherubini, P, Morra di Cella, U. and Pelfini, M. (2011). Chemical elements and heavy metals in European larch tree rings from remote and polluted sites in the European Alps. *Geografia Fisica e Dinamica Quaternaria*, 34, pp. 195–206.

Lepp, N. W. (1981). *Effects of Heavy Metal Pollution on Plants* Vol 1. Applied Science Publishers, London. 111-143.

Liang, C. N. and Tabatabai, M. A. (1978). Effects of trace elements on nitrification in soils. *Journal of Environmental Quality*, 7, pp. 291–293.

Lingua, G, Castiglione, S, Todeschini, V, Franchin, C, Fossati, T, Peterson, E. A, Biondi, S. and Berta, G. (2005). Selection of elite poplar clones for phytoremediation of soil contaminated by heavy metals in field and glass-house experiments. *Proceedings of the XVII International Botanical Congress*, Vienna, pp. 17–23.

Løbersli, E. M. and Steinnes, E. (1988). Metal uptake in plants from a birch forest area near a copper smelter in Norway. Water, Air and Soil Pollution, 37, pp. 25–39.

Lone, M. I, He, Z. H, Stoffella, J. and Yang, X. (2008). Phytoremediation of heavy metal polluted soils and water: progresses and perspectives. *Journal of Zhejiang University Science*, 9(3), pp. 210–220.

Loveland, P. J. and Thompson, T. R. (2002). Identification and Development of a national set of soil quality indicators, *RandD Project Record P5-053/PR/02*. Environment Agency, Bristol, UK.

Lucas, R. E. and Davis, J. F. (1991). Relationships between pH values of organic soils and availabilities of 12 plant nutrients. *Soil Science*, 92, pp. 177–182.

Lynch, J. M. and Moffat, A. J. (2005). Bioremediation – prospects for the future application of innovative applied biological research. *Annals of Applied Biology*. 146, pp. 217-221.

Máchová, P, Malá, J, Cvrčková, H. and Vaněk, T. (2007). Heavy metals uptake by the hybrid aspen and rowan-tree. *Journal of Forest Science*, 53(11), pp. 491–497.

Maier, R. M, Pepper, I. L. and Gerba, C. P. (2009) Environmental Biology. 2nd ed. London, Elsevier.

Maiti, S. K. and Saxena, N. C. (1998). Biological reclamation of coalmine spoils without topsoil: An amendment study with domestic raw sewage and grass-legumes mixture. *International Journal of Surface Mining, Reclamation and Environment*, 12, pp. 87–90.

Maiti, S. K. (2007). Bioreclamation of coalmine overburden dumps—with special empasis on micronutrients and heavy metals accumulation in tree species. Environmental Monitoring and Assessment, 125 (1-3), pp. 111-122.

Maiti, S. K. and Mukhopadhyay, S. (2010). Phytoremediation of metal mine waste. *Applied Ecology and Environmental Research*. 8(3), pp. 207 – 222.

Malcolm, D. C. and Worrell, R. (2001). Potential for the improvement of silver birch (*Betula pendula* Roth.) in Scotland. *Forestry*, 74, pp. 439–453.

Manouchehri, N, Besancon, S. and Bermond, A. (2006). Major and trace metal extraction from soil by EDTA: equilibrium and kinetic studies. *Analytica Chimica Acta*, 559, pp. 105-112.

Marashi, A. R. A. and Scullion, J. (2004). Porosity and hydrological changes in surface mine soils. *Proceedings ISCO 2004 Conference. "Conserving Soil and Water for Society"*. Brisbane, Australia, pp.5

Marmiroli, N. and McCutcheon, S. C. (2003). Phytoremediation: Transformation and Control of Contaminants. Wiley-Interscience, John Wiley & Sons, Inc., Hoboken, New Jersey.

Marschner, P. and Baumann, K. (2003). Changes in bacterial community structure induced by mycorrhizal colonization in split-root maize. Plant Soil, 251, pp. 279–289.

McGinness, S. (1999). *Treatment of Acid Mine Drainage*. Research Paper 99/10. House of Commons, London

McGrath, S. P. and Loveland, P. J. (1992). *The Soil Geochemical Atlas of England and Wales*. Blackie, Glasgow, pp.101.

McGregor, S.D, Pulford, I. D, Duncan, H.J. and Wheeler, C.T. (1995). Uptake of heavy metals from contaminated soil by trees. *In* Glimmerveen, I, (ed) *Heavy metals and trees*. Proceedings of a Discussion Meeting, Glasgow. Edinburgh: Institute of Chartered Foresters, pp. 171– 176.

McLean, J. E. and Bledsoe, B. E. (1992). *Behavior of metals in soils*. EPA/540/S-92/018, pp. 15.

McNear Jr., D. H. (2013). The Rhizosphere - Roots, soil and everything in between. *Nature Education Knowledge*, 4(3), pp. 1

McVean, D. N. (1953). Biological flora of British Isle. *Alnus glutinosa* (L) Gaertn'. *Journal of Ecology*, 41, pp. 447-466.

MEGA operator manual. (1995). *Milestone microwave laboratory systems MLS 1200 microwave digester operation manual*, London, Milestone, pp. 70.

Mehra, A. and Farago, M. E. (1994). Metal ions and plant nutrition. *In* Farago,

M. E. (Ed.), *Plants and the Chemical Elements. Biochemistry, uptake, tolerance and toxicity* VCH, Weinheim, Germany, pp. 31–66.

Menon, S, Hansen, J, Nazarenko, L. and Luo, Y. (2002). Climate effects of black carbon in China and India, *Science*, 297, pp. 2250–2253.

Merrington, G. and Alloway, B. J. (1994). The transfer and fate of Cd, Cu, Pb and Zn from two historic metalliferous mine sites in the U.K. Applied Geochemistry. 9(6), pp. 677-687.

Mertens, J. (2006). *Afforestation of degraded material: fate of metals under different tree species.* Ph. D. Thesis. Ghent University, Belgium.

Mertens, J, Vervaeke, P, Schrijver, A. D. and Luyssaert, S. (2004). 'Metal uptake by young trees from dredged brackish sediment: limitations and possibilities for phytoextraction and phytostabilisation'. *Science of the Total Environment*. 326, (1-3), pp. 209-215.

Mertens, J, Van Nevel, L, Staelens, J, De Schrijver, A, Tack, F, De Neve, S, Meers, E. and Verheyen, K. (2011). Elevated Cd and Zn uptake by aspen limits the phytostabilization potential compared to five other tree species. *Ecological Engineering*, 37, pp. 1072-1080.

Migeon, A, Richaud, P, Guinet, F, Chalot, M, and Blaudez, D. (2009). Metal accumulation by woody species on contaminated sites in the north of France. *Water, Air, and Soil Pollution*, 204(1-4), 89-101.

Mikola, P. (1985). *The effect of tree species on the biological properties of forest soil.* National Swedish Environmental Protection Board, pp 1-27.

Miles, J. and Young, W. F. (1980). The effects on heathland and moorland soils in Scotland and northern England following colonization by birch (*Betula* spp.). *Bulletin d'Ecologie (France)*, 11, pp. 233-242.

Millaleo, R. et al. (2010). Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition*, 10(4), pp. 470-481.

Mitchell, J. S. (2006). *Patterns of and controls over N inputs by green alder (Alnus viridis ssp. fruticosa) to a secondary successional chronosequence in interior Alaska.* M.S. Thesis, Universityof Alaska Fairbanks.

Milton, A, Cooke, J. A. and Johnson, M. S. (2003). Accumulation of Lead, Zinc, and Cadmium in a Wild Population of*Clethrionomys glareolus* from an Abandoned Lead Mine. *Archives of Environmental Contamination and Toxicology*, 44(3), pp. 405-411.

Minnich, M. M, McBride, M. B. and Chaney, R. L. (1987) Copper activity in soil solution: II. Relation to copper accumulation in young snapbeans. *Soil Science Society of America Journal*, 51, pp. 573–578.

Mitchell, R. L. (1998). *Chemistry of the Soil*, 2nd Eds, Reinhold, New York, pp. 320.

Mmolawa, K. B, Likuku, A. S. and Gaboutloeloe, G. K. (2011). Assessment of heavy metal pollution in soils along major roadside areas in Botswana African. *Journal of Environmental science and technology*, 5(3), pp. 186-196.

Moffat, A. J. and Roberts, C. J. (1988). The use of large-scale ridge and furrow landforms in forest reclamation. *Research Information Note 137*, Forestry Commission Research Division, Farnham, pp. 12.

Moffat, A. J, Roberts, C. J. and McNeill, J. D. (1989). The use of nitrogen-fixing plants in forest reclamation. *Research Information Note 158*, Forestry Commission Research Division, Farnham, pp 18.

Moffat, A. J. and Houston, T. J. (1991). Tree establishment and growth at Pitsea landfill site, Essex, U.K. *Waste Management and Research*, 9(1), pp. 35-46.

Moffat, A. J. and McNeill, J. D. (1992). Alders - valuable components of woodland on reclaimed land. *Mineral Planning* 53, pp. 23-24.

Moffat, A. J. and McNeill, J. D. (1994). Reclaiming Disturbed Land for Forestry. *Forestry Commission. Bulletin* 110, pp. 2 HMSO: London.

Moffatt, A. J. (1999). Minimum soil depths for the establishment of woodland on disturbed ground. *Arboricultural Journal* 19, pp.19-27.

Moffat, A. J, Foot, K, Kennedy, K, Dobson, M and Morgan. G (2008). Experimental Tree Planting on UK Containment Landfill Sites: Results of 10 Years' Monitoring. *Arboriculture and Urban Forestry*, 34(3), pp. 163.

Morgan, J. B. and Connolly, E. L. (2013). Plant-Soil Interactions: Nutrient Uptake. *Nature Education Knowledge,* 4(8), pp. 21-32.

Moudouma, C. F. Riou, C, Gloaguen, V. and Saladin. G. (2013). Hybrid larch (*Larix eurolepis* Henry): a good candidate for cadmium phytoremediation? *Environmental Science and Pollution Research*, 20(3), pp 1889-1894.

Mora, M, Rosas, A, Ribera, A, and Rengel, R. (2009). Differential tolerance to Mn toxicity in perennial ryegrass genotypes: involvement of antioxidative enzymes and root exudation of carboxylates. *Plant Soil,* 320, pp. 79–89.

Morin, M. (1981). Heavy metal concentrations in three year old trees grown on sludge-amended surface mine spoil. *Symposium on Surface Mining Hydrology, Sedimentology and Reclamation*, University of Kentucky, Lexington, pp. 297–306.

Moroni, J, Scott, B, and Wratten, N. (2003). Differential tolerance of high manganese among rapseed genotypes. *Plant Soil,* 253, pp. 507-519.

Mortada, W. I, Sobh, M. A, El-Defrawy, M. M. and Farahat, S. E. (2001). Study of lead exposure from automobile exhaust as a risk for nephrotoxicity among traffic policemen. *American Journal of Nephrology,* 21, pp. 274–279.

Myre, R. and Camiré, C. (1996). The effect of crown position and date of sampling on biomass, nutrient concentrations and contents of needles and shoots in European larch. *Trees* 10, pp. 339–350.

Naidu, R, Kookana, R. S, Sumner, M. E, Harter, R. D. and Tiller, K. G. (1997).

Cadmium sorption and transport in variable charge soils: a review. *Journal of Environmental Quality,* 26, pp. 602–617.

Nathanail, C. P. and Bardos, R. P (2004). Chemistry for contaminated land. In *Reclaimation of Contaminated Land.* John Wiley and Sons Ltd, Chichester, 217-231.

National Coal Board Opencast Executive (1982). *Coal Mining and Quarry: Records.* Opencast Executive Meeting, COAL 81/10.

National Land Use Database. (2001) Survey. [online]
Available at: www.environment-agency.gov.uk
Accessed 25 March 2013

National Swedish Environmental Protection Board, Rapport 3017, pp.27.

National Urban Forestry Unit. (2001). *Urban forestry in practice: Community involvement in land reclamation.* CS/25.

Nevel, L. V, Mertens, J, Staelens, J, Schrijver, A. D, Tack, F. M. G, Neve, S. D, Meers, E. (2011). Elevated Cd and Zn uptake by aspen limits the phytostabilization potential compared to five other tree species. *Ecological Engineering*, 37, pp. 1072–1080.

Nichani, V, Li, W. I, Smith, M. A, Noonan, G, Kulkarni, M. and Kodavor, M. (2006). Blood lead levels in children after phase-out of leaded gasoline in Bombay, India. *Science of the Total Environment,* 363, pp. 95–106.

Nkonya, E, Gerber, N, von Braun, J. and De Pinto, A. (2011). *Economics of Land Degradation – The Costs of Action versus Inaction.* International Food Policy Research Institute, Washington, D.C. IFPRI 68.

NMSU (2006). AAS Nebulisation Chamber, New Mexico State University [Online]
Available at: http://web.nmsu.edu/~kburke/Instrumentation/AAS_Nebulizer.html
Accessed 5 December 2012

Nohrstedt, H-Ö. (1988). Nitrogen fixation (C2H2-reduction) in birch litter. *Scandinavian Journal of Forest Research,* 3, pp. 17-23.

Nriagu, J. O. and Pacyna, J. M. (1988). Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature*, 333, pp. 134 – 139.

Nwachukwu, O. I, and Pulford, I. D. (2008) Comparative effectiveness of selected adsorbant materials as potential amendments for the remediation of lead-, copper- and zinc-contaminated soil. *Soil Use and Management*, 24, pp. 199–207.

Osmond, J. and Upton, S. (2012). *Growing Our Woodlands in Wales - the 100,000 Hectare Challenge: The 100,000 hectare challenge.* Forestry Commission. Wales, pp. 9-11.

Page, A. L, Bingham, F. T. and Chang, A. C. (1981). Effects of Heavy Metal Pollution on Plants. *Applied Science*, 1, pp. 72 -109.

Page, V, Weisskopf, L, Feller, U. (2006). Heavy metals in white lupin: uptake, root-to-shoot transfer and redistribution within the plant. *New Phytologist*, 171(2), pp. 329-341.

Pahlsson, A. M. B. (1989). Toxicity of heavy metals (Zn, Cu, Cd, Pb). to vascular plants: A literature review. *Water, Air and Soil Polution* 47, pp. 287-319.

Palaniswami, U. (2006). *Handbook of statistics for teaching and research I plant and crop science*. Harworth Reference Press, New York.

Paterson, D, Mackay, D, Tam, W. and Shiu, Y. (1990). Uptake of organic chemicals by plants: a review of processes, correlations and models. *Chemosphere*, 21, pp. 297–331.

Patterson, W. A. and Olson, J. J. (1983). Effects of heavy metals on radicle growth of selected woody species germinated on filter paper, mineral and organic soil substrates. , *Canadian Journal of Forest Research*, 13(2), pp. 233-238.

Perkin-Elmer (1996). Analytical methods for atomic absorption spectrometry. Perkin Elmer Inc. United States. (online) Available at: http://eecelabs.seas.wustl.edu/files/Flame%20AA%20Operating%20Manual.pdf Accessed on 10 August 2007.

Perry, D. and Handley, J. (2000). Forestry Commission Technical Paper 29: *The potential for woodland on urban and industrial wasteland in England and Wales.* Edinburgh, Forestry Commission, pp. 8.

Perttu, K. (1993). Biomass production and nutrient removal from municipal wastes using willow vegetation filters. *Journal of Sustainable Forestry,* 1(3), pp. 57–70.

Phelps, L. B. (1983). Characterization of mine spoil properties as tool for premining planning. In: *Proceedings of the 1983 Symposium on Surface Mining Hydrology, Sedimentology and Reclamation*, Lexington, KY, pp. 47-51.

Phelps, L. B. and Holland, L. (1987). Soil compaction in topsoil replacement during mining reclamation. *Environmental Geochemistry and Health*, 9, pp. 8–11.

Phipps, D. A. (1981). *Effects of Heavy Metal Pollution in Plants.* Applied Science Publishers, London, pp. 154.

Pichtel, J. and Salt, C. A. (1998). Vegetative growth and trace metal accumulation on metalliferous wastes. Journal of Environmental Quality, 27, pp. 618–624.

Pinamonti, F, Stringari, G, Gasperi, F. and Zorzi, G. (1997). The use of compost: its effects on heavy metal levels in soil and plants. Resources, Conservation and Recycling, 21, pp. 129-143.

Pisano, S. M. and Rockwood, D. L. (1997). Stormwater phytoremediation potential of Eucalyptus. *In* Proceedings 5th. Biennial Stormwater Research, pp. 4-9.

Plass, W. T. (1977). Growth and survival of hardwood and pine interplanted with European alder. USDA Forest Service Research Paper. NE-376, pp. 1-10

Prasad, M. N. V. and Freitas, H. M, (2003). Metal hyperaccumulation in plants - Biodiversity prospecting for phytoremediation technology. Electronic Journal of Biotechnology, 6(3), pp. 89-96.

Priha, O. (1999). *Microbial activities in soils under Scots pine, Norway spruce and Silver birch.* Finnish Forestry Research Institute, Reserch paper 731, pp. 50.

Prokop, Z, Cupr, P, Zlevorova-Zlamalikova, V, Komakek, J, Dusek, L. and Holoubek, I. (2003). Mobility, bioavailability, and toxic effects of cadmium in soils samples. *Environmental Research*, 91, pp. 119-126.

Pulford, I. D, Watson, C. and Mcgregor, S. D. (2001). Uptake of Chromium by trees: Prospects for phytoremediation. *Environmental Geochemistry and Health.* 23(3), pp. 301-311.

Pulford, I. D. and Watson, C. (2003). Phytoremediation of Heavy Metal-Contaminated Land by Trees - A Review. *Environment International.* 29(4), pp. 529-540.

Pulford, I. D and Dickinson, N. M. (2003). Phytoremedation technologies using trees. *Trace Elements in the Envronment.* Elsevier, pp. 375-395.

Pyle, S. M, Nocerino, J. M, Deming, S. N, Palasota, J. A, Palasota, J. M, Miller, E. L, Hillman, D. C, Kuharic, C. A, Cole, W. H, Fitzpatrick, P. M, Watson, M. A. and Nichols, K. D. (1996). Comparison of AAS, ICP-AES, PSA, and XRF in determining lead and cadmium in soil. *Environmental Science and Technology*, 30(1), pp. 204–213.

Queirolo F, Valenta P, Stegen S, Breckle SW (1990) Heavy metal concentrations in oak wood growth rings from the Taunus (Federal Republic of Germany) and the Valdivia (Chile) regions. Trees 4: 81–87.

Quevauviller, P, Lachica, M, Barahona, E, Rauret, G, Ure, A, Gomez, A. and Muntau, H. (1996). Interlaboratory comparison of EDTA and DTPA procedures prior to certification of extractable trace elements in calcareous soil. *The Science of The Total Environment,* 178, pp. 127-132.

Rajkumar, M, Prasad, M. N. V, Swaminathan, S. and Freitas, H. (2013). Climate change driven plant–metal–microbe interactions. *Environment International*, 53, pp. 74–86.

Ramanathan, A. L, Anandan, P, Chidambaram, S, Ganesh, N, Srinivasanmorthy, K. and Kathiresan, R. M. (2002). Environmental impact assessment in opencast mining region. Case study from the lignite mining area, Neyveli, Tamilnadu. Environnmental Hazards in South Asia. Capital publishing company, 3, pp. 296-301.

Ramani, R. V, Sweigard, R. J, Clar, M. L. (1990). Reclamation (Reclamation Planning). In: Kennedy B.A. (ed.) *Surface Mining*. 2nd Edition. Littleton, Colorado: Society for Mining, Metallurgy, and Exploration, Inc. pp. 750-769.

Ramirez-Munoz, J. (1968). *Atomic Absorption Spectroscopy*, Elsevier Publishing Co, New York, pp. 438.

Ramsey, W. J. H. (1986). Bulk soil handling for quarry restoration. *Soil Use and Management,* 2, pp. 30-39.

Raskin, I. and Ensley, B. D. (2000) (eds). *Phytoremediation of toxic metals using plants to clean up the environment.* New York: Wiley, pp. 316.

Rawlinson, H, Putwain, P. D. and Dickinson, N. M. (2000). Constraints to the establishment of community woodlands on closed landfill sites. *Aspects of Applied Biology* 58, pp. 123-128.

Rayment, G. E. and Higginson, F. R. (1992). *Australian Laboratory Handbook of Soil and Water Chemical Methods.* Inkata Press, Melbourne, Australia, pp. 67.

Rees, S, Pulford, I. D. and Duncan, H. J. (1998). Utilising contaminated and disturbed industrial land for short rotation coppice. *Contaminated soil.* London: Thomas Telford, pp. 1225– 1226.

Reichman, S. M. (2002). *The Responses of Plants to Metal Toxicity: A review focusing on Copper, Manganese and Zinc.* Australian Minerals and Energy Environment Foundation, Melbourne, Australia, pp. 1-28.

Reimann, C. and de Caritat, P. (2000). Intrinsic flaws of element enrichment factors (EFs) in environmental geochemistry. *Environment Science and Technology*, 34, pp. 5084–5091.

Ren, F. C, Liu, T. C, Liu, H. Q. and Hu, B. Y. (1993) Influence of zinc on the growth, distribution of elements. and metabolism of one-year old American ginseng plants. Journal of Plant Nutrition, 16, pp. 393–405.

Riddell-Black, D. A. (1993). *Review of the potential for the use of trees in the rehabilitation of contaminated land.* Report CO 3467. Water Research Centre, Medmenham, pp. 25.

Rieuwerts, J. S. (2007). The mobility and bioavailability of trace metals in tropical soils: a review. *Chemical Speciation and Bioavailability.* 19(2), pp. 75-85.

Rietveld, W. J, Schlesinger, R. C. and Kessler, K. J. (1983). Allelopathic effects of black walnut on European black alder coplanted as a nurse species. *Journal of Chemistry and Ecology,* 9, pp. 1119–1133.

Roberts, R. D. and Johnson, M. S. (1978). Dispersal of heavy metals from abandoned mine workings and their transference through terrestrial food chains. *Environmental Pollution*, 16(4), pp. 293–310.

Roberts, P. (2007). Coal is Toxic. (Online) Available at:
http://nonewcoal.greens.org.au/coal/toxicity/dust/effects-on-health/coal-is-toxic/
Accessed on [2 April 2008]

Robinson, J. W. (1995). *Instrumental Analysis, 5th ed.* New York, Marcel Dekker.

Robinson, B. H, Mills, T. M, Green, S, Chancerel, B, Clothier, B, and Fung, L. E. (2005). Trace element accumulation by poplars and willows used for stock fodder. *New Zealand Journal of Agriculture Research*, 48, pp. 489–497.

Rockwood, D. L, Ma, L. Q, Alker, G. R, Tu, C. and Cardellino, R. W. (2001). Phytoremediation of contaminated sites using woody biomass. Final Report to the Florida Center for Solid and Hazardous Waste Management.

Rope, S. K., Arthur, W. J, Craig, T. H. and Craig, E. H. (1988). Nutrient and trace elements in soil and desert vegetation of Southern Idaho. *Environmental Monitoring and Assessment*, 10, pp. 1-24.

Rosas, A, Rengel, Z, Mora, M. (2007). Manganese supply and pH influence growth, carboxylate exudation and peroxidase activity of ryegrass and white clover. *Journal of Plant Nutrition,* 30, pp. 253-270.

Ross, S. M. (1994).Toxic metals: fate and distribution in contaminated ecosystems. In: Ross, S. M, editor. *Toxic metals in soil – plant systems.* Chichester: Wiley, pp. 189–243.

Rosselli, W. and Bashi, K (2003). 'Phytoextraction capacity of trees growing on metal contaminated soil.' *Plant and Soil.* 256(2), pp. 265- 272.

Roy, S, Khasa, D. P. and Greer, C. W. (2007). Combining alders, frankiae and mycorrhizae for the revegetation and remediation of contaminated ecosystems.

Canadian Journal of Botany, 85(3), pp. 237-251.

Ryu, K, Watanabe, M, Shibata, H, Takagi, K, Nomura, K. and Koike, T. (2009). Ecophysiological responses of the larch species in northern Japan to environmental changes as a basis for afforestation. *Landscape Ecological Engineering,* 5, pp. 99–106.

Salt DE, Smith RD, Raskin I. (1998). Phytoremediation. *Annual Rev Plant Physiology.* 49, pp. 643–68.

Schnoor, J. L, Lich, L. A, Mccutcheo, T. S. C., Wolfe N. N. and Carreira, L. (1995). Phytoremediation of organic and nutrient contaminants. *Environmental Science and Technology.* 29(7), pp. 318A-323A.

Schnoor, J. L. (1996). *Environmental modelling: fate and transport of pollutants in water, air, and soil.* Wiley Interscience Publication, New York.

Schnoor, J. L. (1997). *Phytoremediation - Technology Evaluation Report.* Groundwater Remediation Technologies Analysis Centre. Pittsburgh, USA.

Schroder, J. (1987). Plant hormones in plant-microbe interactions. *In* Kosuge, T. and Nester, E. W. (Ed.) *Plant-microbe interactions: molecular and genetic perspectives.* Macmillan, New York, pp. 40-63.

Schulte, E. E. (2004). Soil and applied iron. Understanding plant nutrients. A3554. [online] Available at:

http://corn.agronomy.wisc.edu/Management/pdfs/a3554.pdf

Accessed 18 May 2013

Schulte, E. E. and Kelling, K. A. (2004). Soil and applied Manganese. Understanding plant nutrients. A2526. [online] Available at: http://corn.agronomy.wisc.edu/Management/pdfs/a2526.pdf

Accessed 25 May 2013

Schmidt, U. (2003). Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. *Journal of Environmental Quality*, 32 , pp. 1939–1954.

Scottish Environment Protection Agency (1999). Threats to soil quality. [online] available at: http://www.sepa.org.uk/land/soil/threats_to_soil_quality.aspx

Accessed 21 February 2014

Scullion, J. (2006). Remediating polluted soils. *Naturwissenschaften*, 93(2), pp. 51-65.

Scullion, J. and Malinovszky, K. M. (1995). Soil factors affecting tree growth on former opencast coal land. *Land Degradation and Rehabilitation,* 6, pp. 239-249.

Sengupta, M. (1993). *Environmental impacts of mining: Monitoring, restoration and control.* Lewis Publisher, Boka Raton, FL, pp. 368.

Sharma, R. K, Agrawal, M. and Marshall, F. (2007). Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotoxicology and*

Environment Safety, 66(2), pp. 258-266.

Sheoran, V, Sheoran, A. S. and Poonia, P. (2009). Phytomining: A review. *Minerals Engineering*, 22(12), 1007–1019.

Sheoran, V, Sheoran, A. S. and Poonia, P. (2010). Soil reclamation of abandoned mine land by vegetation: A review. *International Journal of Soil, Sediment and Water*, 3(2), pp.1-22.

Shi, S, Richardson, A. E, O'Callaghan, M, Deangelis, K. M, Jones, E. E, Stewart, A, Firestone, M. K. and Condron, L. M. (2012). Effects of selected root exudate components on soil bacterial communities. *FEMS Microbiology and Ecology*, 77(3), pp. 600–610.

Sinha, R. K, Herat, S. and Tandon, P. K. (2004). 14. Phytoremediation: role of plants in contaminated site management. *Book of Environmental Bioremediation Technologies*, Springer, Berlin, Germany, pp. 315–330.

Skoog, D. A. (1985). Principles of instrumental analysis, 3rd ed, New York, Saunders, pp. 479.

Slattery, W. J. and Ronnfeldt, G. R. (1992). Seasonal variation of pH, aluminium and manganese in acid soils from north-eastern Victoria. Australian Journal of Experimental Agriculture, 32, pp. 1105–1112.

Smilde, K. W, van Luit, B. and van Driel, W. (1992). *Plant and Soil*. 143, pp. 233-238.

Smith, K. A. and Paterson, J. E. (1995). Manganese and cobalt. *In* Alloway, B. J. (Ed.), *Heavy Metals in Soil*, John Wiley & Sons, New York, pp. 224–244.

Smith, R. H. A. and Bradshaw, A. D. (1972). Stabilization of toxic mine wastes by the use of tolerant plant populations. *Transactions of the Institution of Mining and Metallurgy: Sect. A*, 81, pp. 230–237.

Smith, K. M, Abrahams, P. W, Dagleish, M. P, Steigmajer, J. (2009). The intake of lead and associated metals by sheep grazing mining-contaminated floodplain pastures in mid-Wales, UK: I. Soil ingestion, soil–metal partitioning and potential availability to pasture herbage and livestock. *Science of the Total Environment*, 407(12), pp. 3731-3739.

Smolders, E. & Degryse, F. (2002). Fate and effect of zinc from tire debris in soil. *Environmental Science and Technology*, 36, pp. 3706–3710.

Smolander, A. (1990). Frankia populations in soils under different tree species – with special emphasis on soils under *Betula pendula*. *Plant and Soil*, 121, pp. 1-10.

Sparks, D. (2003). *Environmental Soil Chemistry*. Elsevier Science. USA, pp. 634.

Steinnes, E. (1990) Copper *In* Alloway, B. J. (ed.) *Heavy Metals in Soil*. Blackie Academic and Professional, London, pp. 395-409.

Stockfisch, N, Forstreuter, T. and Ehlers, W. (1999). Ploughing effects on soil organic matter after twenty years of conservation tillage in Lower Saxony, Germany. *Soil and Tillage Research,* 52(1-2), pp. 91-101.

Su, Y. Z, Zhao, H. L, Zhang, T. H. and Zhao, X. Y. (2004). Soil properties following cultivation and non-grazing of a semiarid sandy grassland in northern China. *Soil Tillage Research*, 75, pp. 27–36.

Suciu, I, Cosma, C, Todica, M, Bolboaca, S. D. and Jantschi, L. (2008). Analysis of Soil Heavy Metal Pollution and Pattern in Central Transylvania. *International Journal of Molecular Science, 9*(4), pp. 434-453.

Susarla, S, Medina, V. F. and McCutcheon, S. C. (2002). Phytoremediation, an ecological solution to organic contamination. *Ecological Engineering*, 18, pp. 647–658.

Suthersan, S. (1997). *Remediation Engineering Design Concepts.* New York: Lewis Publishers, pp. 498.

Sweigard, R, Burger, J, Zipper, C, Skousen, J, Barton, C. and Angel, P. (2007). Low compaction grading to enhance reforestation success on coal surface mines. *Forest Reclamation Advisory No. 3. USDOI Office of Surface Mining*, pp. 1-6.

Tangahu, B. V, Abdullah, S. R. S, Basri, H, Idris, M. and Anuar, N. (2011). A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation. *International Journal of Chemical Engineering*, (2011), pp. 31.

Tao, S, Chen, Y. J, Xu, F. L, Cao, J. and Li, B. G. (2003). Changes of copper speciation in maize rhizosphere soil. *Environmental Pollution* 122: 447-454.

Tao, S, Liu, W. X, Chen, Y. J, Xu, F. L, Dawson, R. W, Li, B. G, Cao, J, Wang, X. J, Hu, J. Y. and Fang, J. Y. (2004). Evaluation of factors influencing root-induced changes of copper fractionation in rhizosphere of a calcareous soil. *Environmental Pollution*, 129, pp. 5–12

Taylor, G. J. and Foy, C. D. (1985). Differential uptake and toxicity of ionic and chelated copper in *Triticum aestivum*. *Canadian Journal of Botany*, 63, pp. 1271–1275.

Terry, N. and Banuelos, G. (eds.). (2000). *Phytoremediation of Contaminated Soil and Water*. London, Lewis Publishers, pp. 674.

Thermo Elemental (2001). *AAS, GFAAS, ICP or ICP-MS? Which technique should I use? An elementary overview of elemental analysis*. U.S.A. S002B Rev 02/01.

Thornton, I. (1996). Heavy metal contamination of soils and plants in the vicinity of a lead–zinc mine Korea. *Applied Geochemistry*, 11, pp. 53–59.

Tissue, B. M. (1996) Integrating Hypermedia Tutorials in Instrumental Analysis, *Division of Analytical Chemistry,* 110th National ACS Meeting, Chicago, IL.

Tomlinson, P. (1982). The environmental impact of opencast coal mining. *The Town Planning Review.* 53(1), pp. 5-28.

Tomlinson, D. C, Wilson, J. G, Harris, C. R. and Jeffrey, D. W. (1980). Problems in Assessment of Heavy Metals in the Estuaries and the Formation of Pollution Index. *Helgoland Marine Research,* 33, pp. 566-575.

Trigg, A. B. and Dubourg, W. R. (1993). Valuing the environmental impacts of opencast coal mining: the case of the Trent Valley in North Staffordshire. CSERGE Working Paper GEC 93-19. [online]
Available at: http://cserge.ac.uk/sites/default/files/gec_1993_19.pdf
Accessed on 4 august 2010

Turner, D. W, Cole, J, and Gessel, S. P. (1976). Mineral nutrient accumulation and cycling in a stand of red alder (*Alnus Rubra*). Journal of Ecology, 64(3), pp. 965-974.

Uren, N. C. (1991). Manganese and Cobalt. In Alloway, B. J. (Ed) Heavy Metal in Soil, Blackie Academic and Professional, London, pp. 224-244.

Uri, V, Tullus, H. and Lohmus, K. (2002). Biomass production and nutrient accumulation in short-rotation grey alder (Alnus incana (L.) Moench). plantation on abandoned agricultural land. *Forest Ecology and Management.* 161, pp. 169-179.

USDA (United States Department of Agriculture) (2000). *Natural Resources Conservation Service. National Soil Survey Handbook*, 430-VI. [Online] Available at: http://soils.usda.gov/technical/handbook/ Accessed on 7 May 2008

USEPA (2000). Introduction to Phytoremediation. EPA/600/R-99/107 [Online] Available at : http://www.clu-in.org/download/remed/introphyto.pdf. Accessed [18 May 2012]

USEPA. (2006). In Situ Treatment Technologies for Contaminated Soil: Engineering Forum Issue Paper. EPA 542-F-06-013.

Utriainen, M. A, Karenlampi, L. V, Karenlampi, S. 0. and Schat, H. (1997). Differential tolerance to copper and zinc of micro-propagated birches tested in hydroponics. *New Phytologist* 137, pp. 543-549.

Vamerali, T, Bandiera, M, Coletto, L, Zanetti, F, Dickinson, N. M, and Mosca, G. (2009). Phytoremediation trials on metal-and arsenic-contaminated pyrite wastes (Torviscosa, Italy). *Environmental Pollution*, 157(3), pp. 887-894.

Van Assche, F. and Clijsters, H. (1990). Effects of metals on enzyme activity in plants. *Plant, Cell and Environment*, 13, pp. 195-206.

Vance, G. F. (2009). Environmental Protection, Practices, and Standards *In* Karmis, M. E, Ramani, R. V, Gluskoter, H. J, Luttrell, G. H. and Vance, G. F. (Ed.) *Meeting Projected Coal Production Demands in the U.S.A. - Upstream*

Issues, Challenges and Strategies. The Virginia Center for Coal and Energy Research, Virginia Polytechnic Institute and State University. pp. 166-208.

Vangronsveld, J, Van Assche, F. and Clijsters, H. (1995). Reclamation of a bare industrial area contaminated by non-ferrous metals: *in situ* metal immobilization and revegetation. *Environment Pollution,* 87, pp. 51 – 59.

Vangronsveld, J, Herzig, R, Weyens, N, Boulet, J, Adriaensen, K, Ruttens, A, and Mench, M. (2009). Phytoremediation of contaminated soils and groundwater: lessons from the field. *Environmental Science and Pollution Research*, 16(7), pp. 765-794.

Vazquez, M. D, Barcelo, J, Poschenrieder, C. H, Madico, J, Hatton, P., Baker, A. J. M. and Cope, G. H. (1992). Localization of Zinc and Cadmium in *Thlaspi caerulescens*(Brassicaceae), a Metallophyte that can Hyperaccumulate both Metals. *Journal of Plant Physiology*, 140(3), pp. 350-355.

Vedagiri, U. and J. Ehrenfeld. (1991). Effects of*Sphagnum* moss and urban runoff on bioavailability of lead and zinc from acidic wetlands of the New Jersey Pinelands. *Environmental Pollution*, 72, pp. 317–330.

Vidal, J (2008). Coal's return raises pollution threat. The Guardian [online]

Available at: http://www.theguardian.com/environment/2008/nov/23/fossil-fuels-pollution

Accessed on 10 March 2010

Walker, T. S, Bais, H. P, Grotewold, E. and Vivanco, J. M. (2003). Root exudation and rhizosphere biology. *Plant Physiology*, 132, pp. 44–51.

Walley, C. (1994). Carving out a future? *Rural Wales,* Summer, pp. 22-24.

Walter, K. H. (1988). Manganese fertilizers. *In* R. D. Graham, R. J. Hannam, & N. C. Uren (Eds.), *Manganese in soils and plants* Dordrecht: Kluwer Academic, pp. 225–241.

Wan Ngah, W. S. and Fatinathan, S. (2010). Adsorption characterization of Pb(II) and Cu(II) ions onto chitosan-tripolyphosphate beads: kinetic, equilibrium and thermodynamic studies. *Journal of Environmental Management,* 91, pp. 958–969.

Wang, Z. L, Cai, C. L, Li, X. Y, Shi, X. R. and Tao, H. (2004). Nonlinear extension multi-factorial evaluation method of soil heavy metal pollution. *Soils*, 36 (2), pp. 151–156.

Wang, X. and Jia, Y. F. (2010) Study on adsorption and remediation of heavy metals by poplar and larch in contaminated soil. *Environmental Science and Pollution Research*, 17(7), pp. 1331–1338.

Ward, N. (1996). The place of trees in the reclamation of disturbed land. In: Moffat, A. J. (Eds.). *Recycling land for forestry. Proceedings of the Forestry Commission and BLRS conference.* Forestry Commission Technical Paper 22: Forestry Commission. Edinburgh, pp. 1-3.

Watmough, S. A. and Dickinson, N. M. (1995). Dispersal and mobility of heavy metals in relation to tree survival in an aerially contaminated woodland soil. *Environmental Pollution*. 90, pp. 135- 142.

Watmough, S. A. and Hutchinson, T. C. (2004). The quantification and distribution of pollution Pb at a woodland in rural south central Ontario, Canada. Environmental Pollution 128, pp. 419-428.

WDA (2009). Mining Waste Directive [online] Available at: http://wales.gov.uk/topics/environmentcountryside/epq/waste_recycling/legislation/miningwaste/?lang=en
Accessed on 10 April 2011

Weaver, J. E. (1982). Root development of field crops. 2nd ed. London, McGraw Hill Book Company, pp. 378.

Webber, J. F, Mullett, M. and Brasier, C. M. (2010) Dieback and mortality of plantation Japanese larch (*Larix kaempferi*) associated with infection by *Phytophthora ramorum*. New Disease Reports 22(19). http://www.ndrs.org.uk/pdfs/022/NDR_022019.pdf

Welch, A. H, Westjohn, D. B, Helsel, D. R. and Wanty, R. B. (2000). Arsenic in ground water of the United States: occurrence and geochemistry. *Ground Water*, 38, pp. 589–604.

Westphal, L. M. and Isebrands, J. G. (2001). *Phytoremediation of Chicago's brownfields - consideration of ecological approaches and social issues.*

Proceedings Brownfields 2001 Conference, Chicago, IL, USA, BB-11-02.

WHO (1981). *Environment Health Services in Europe 5: Guidelines for evaluation of environmental health service.* WHO Regional Publications, European series, No. 90.

Williams, R. D. and Schuman, G.E. (1987). *Reclaiming mine soils and overburden in the western United States.* Soil Conservation Society of America, Ankeny, Iowa. USA, pp. 322.

Windham, L, Weis, J. S. and Weis, P. (2003). Uptake and distribution of metals in two dominant salt marsh macrophytes, *Spartina alterniflora* (cordgrass) and *Phragmites australis* (common reed). *Estuarine Coastal and Shelf Science*, 56, pp. 63–72.

Wong, M. H. (2003). Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere,* 50 (6), pp. 775–780.

Wu, J, Hsu, F. C. and Cunningham, S. D. (1994) Chelate-assisted Pb phytoextraction: Pb availability, uptake, and translocation constraints. *Environment Science and Technology, 33*, 1898-1904.

Zavitkovski, J. and Newton, M. (1971). Litter fall and litter accumulation in red alder stands in western Oregon. *Plant and Soil*, 35(2), pp. 257-268.

Zavitkovski, J. and Stevens, R. D. (1972). Primary productivity of red Alder ecosystem. *Ecology*, 53, pp. 235-242.

APPENDIX 1

Extraction and Analysis of Heavy Metals from Soil

Reagent

0.05 M neutral EDTA solution (recipe for 5L)

Dissolve 93 g ethylenediaminetetra-acetic acid disodium salt (EDTA-Na_2) in about 2 L deionised water, using a magnetic stirrer to effect dissolution. Adjust to neutral pH with ammonia solution, decant into a 5 L flask and make up to volume with deionised water.

Equipment requirements

Spatula

50 mL centrifuge tubes (new or acid-washed)

100 mL volumetric flasks

100 mL or 250 mL Buchner flasks

Buchner funnels (to take 55 mm or 42.5 mm filters)

Tube rack

Retort stand

Top pan balance

Orbital shaker (fitted with wire basket to contain centrifuge tubes)

Centifuges (equipped with turret to hold 50 mL tubes)

Whatman 55 mm and 42.5 mm GF/F filter papers, or similar (e.g., Whatman 42)

Tweezers for handling filters

Glass Pasteur pipettes

Sample Preparation

In the laboratory, spread out samples in labelled plastic trays and leave for a few days to dry out. Once dry, use a pestle and mortar to break up any large lumps, and then sieve each sample through a 2 mm sieve. Store sieved samples in labelled plastic bottles.

EDTA Extraction

1. For each sample in triplicate, weigh accurately about 10 g soil into a labelled centrifuge tube. Add about 30 mL EDTA to each tube (use the scale on the side of the tube) and to a further empty (labelled) tube as a control*. Screw on the caps tightly and shake on an orbital shaker for 60 minutes.
2. Centrifuge at 3000 rpm for 5 minutes, ensuring that the turret on the centrifuge is balanced.
3. For each tube, carefully filter the supernatant under vacuum into a Buchner flask, taking care not to disturb the sediment pellet at the bottom of the centrifuge tube. Add a little more EDTA to the filter funnel to ensure that all the extracted metals are washed from the filter paper.
4. Carefully decant the filtered supernatant into a labelled 100 mL volumetric flask. Rinse the Buchner flask with a little more EDTA and add it to the volumetric flask.
5. Add a further 30 mL EDTA to the centrifuge tube, shake for a further 15 minutes, and repeat steps 2 – 4. Finally, make the volumetric flask up to volume with EDTA.
6. Thoroughly clean out each emptied centrifuge tube (soil residue should be emptied into the bin, not down the sink), sequentially rinse with tap water,

distilled water and then some of the filtered sample, and then fill each with sample. Discard any remaining solution.

* prepare 1 control after every 10 samples per day.