The Effect of *in situ* spatial heterogeneity of Lead in soil on plant uptake

By Grace Oyiza Solomon-Wisdom

A thesis submitted for the Degree of Doctor of Philosophy of the University of Sussex.

Evolution, Behaviour and Environment Research Group

School of Life Sciences

University of Sussex.

Submitted May, 2015.

Declaration

I hereby declare that this thesis has not been and will not be, submitted in whole or in part to another University for the award of any other degree.

Signature…….. (Grace Oyiza Solomon-Wisdom)

Dedication

This thesis is dedicated first to God the Father, Son and the Holy Ghost for being my pilot in life and to the blessed memory of my dearly beloved and cherished parents, who made huge sacrifice for my upbringing and sound education. I lost mum and dad just about the start and conclusion of the PhD respectively. Their loving memories live on.

UNIVERSITY OF SUSSEX

Grace Oyiza Solomon-Wisdom

A thesis submitted for the degree of Doctor of Philosophy

The effect of *in situ* spatial heterogeneity of lead in soil on plant uptake

Abstract

The understanding of the spatial distribution of lead (Pb) in soil is important in the assessment of potential risks and development of remediation strategies for Pb contaminated land.

In situ heterogeneity of Pb was measured at two heavily contaminated sites in the United Kingdom using the Portable X-ray Fluorescence Spectrometer (P-XRF) over a range of spatial scales (0.02 to 50 m). The pattern of the distribution of Pb was very variable, and when expressed as heterogeneity factor (HF), it ranged from 1.2 to 3.2 (highly heterogeneous).

The effect of such Pb heterogeneity on plant uptake was investigated in greenhouse pot trials. Two earlier pot trials, which assessed the effect of Pb in a fixed concentration (1000 mg/kg) and in a range of concentration (100 to 10000 mg/kg) found a significant effect of the Pb added treatments, when compared to a control treatment (0 mg/kg Pb added). Biomass and uptake varied by 20 to 100% within and between 16 species/varieties. Results enhanced the selection of two species (*Brassica napus* and *Brassica juncea*) for further pot trials.

A third pot experiment with *Brassica napus* and *Brassica juncea* in simplistic binary model of heterogeneity found 20 to 60% lower uptake in the binary treatment, than homogeneous the treatment. Biomass was higher by 10 to 50% in *Brassica juncea* and 20 to 40% lower for *B. napus* in the bianary treatment, *when* compared to the homogeneous and control treatments.

The effect of a more realistic *in situ* heterogeneity on plant uptake was investigated in a further pot trial, which simulated low (LH), medium) (MH) and high (HH) heterogeneity treatments, compared to a homogeneous (HO) treatment. It detected a significant ($P < 0.05$) impact of heterogeneity on biomass and uptake between treatments and species. Four to five fold lower biomass were recorded in HH treatment, when compared to the HO treatment. Shoot and root uptake in (mg/kg) concentration increased with increasing heterogeneity with peak uptake (twice as high as HO treatment) in LH for *B*. *napus* and in HH and MH treatments for *B*. *juncea* respectively. Shoot and root Pb masses in (µg)

were maximum in HO and MH treatments respectively with 50 to 70% lower Pb mass in the HH treatment. Results showed that response to heterogeneity is species specific.

A sub-experiment explored the behaviour of plant roots in HH treatment and found 20 to 80% variation in root biomass between concentric patches with same nominal soil Pb concentrations. This provided insights into varied responses of these species to realistic Pb heterogeneity.

The research demonstrated that the presence and extent of *in situ* heterogeneity of Pb in soil plays an important role in Pb uptake by plants. It also showed that the homogeneous and simplistic binary model of heterogeneity do not give reliable estimates of plant growth and Pb uptake in realistic field conditions.

This work has implications for improving the efficiency of phytoremediation of Pb contaminated land, phytomining, reliability of risk assessment and models of human exposure to Pb.

Acknowledgements.

I am eternally grateful to the Most High God, Who is the source of my inspiration and all I have for His love, faithfulness and grace always.

I am profoundly thankful to my main Supervisor, Professor Mike Ramsey for unreservedly and dedicatedly supervising this work. I wholeheartedly thank Dr Libby John (University of Lincoln) for her helpful advice and suggestions especially on the plant aspect of this research. I am enormously thankful to Professor Jeremy Field for his co-supervisory role. My earnest and profound gratitude goes to Professor Elizabeth Hill for her help with the ball mill used for processing small core soil samples in this study.

My innumerable thanks goes to the Commonwealth Scholarship Commission in the United Kingdom (CSUK) for funding my PhD.

Thanks to the University of York and Dr Stefan Reindinger for the release and use of the Portable X-ray Fluorescence Spectrometer without which the field measurements would not have been possible. I acknowledge the help of Julia Gow, Mr Chris Coomb of Derbyshire Wildlife Trust and BlackRock respectively for granting access to the field sites (Gang Mine and BlackRock). Many thanks to Dr Libby John and Mrs Janet Ramsey for help in laying grids at the field sites.

Thank you to Dr David Dungworth of English Heritage Laboratory for the use of the X-ray microprobe without which the X-ray map of lead heterogeneity on some of my field soil samples would not have been carried out. I am also grateful to the United States Department of Agriculture (USDA), the North Central Regional Plant Introduction Station (NCRPIS)-Iowa State University, Iowa and the Royal Botanic garden (KEW) for supplying gratis some of the plant seeds used in this research. I am particularly grateful to Dr Laura Marek, Mr Mark Milliard and Janet Terry for processing the seed orders through.

I am extremely thankful to Mr Mick Henry, Mr Chris Dadswell and Mrs Frances Chick for use of the Chemistry teaching laboratory and technical support. Many thanks Chris, you have been tremendously helpful. I am immensely grateful to the Life Sciences Technical Services team (Dr Teresa Knapp, Dr Steve Pearce, Dr Martyn Stenning, Mr Peter Reed, Mr Andrew Black, Mr Andrew White and all members of the team), University of Sussex for technical support during the experimental work. I am sincerely thankful to all Staff at the School of Life Sciences, Research Enterprise Office (Dr Deeptima Massey) and the Doctoral School (Professor George Kemenes) for their various unique roles in supporting my PhD research. I cannot forget your outstanding contributions to research in Sussex.

I appreciate the University of Abuja for giving me the opportunity to pass through this programme by way of study leave. I humbly express my profound and deep gratitude to all members of staff of the Department of Biological Sciences, University of Abuja. I appreciate all, whose encouragement in various ways and degrees have brought me to this level in my academics. My sincere appreciation goes to, Professor A. A. Olatunde and family, Professor S. E. Kakulu, Professor N. R. Isu, Professor O.O. Olorode, Professor J.S.A. Adelabu, Mrs N.O. Adelabu and Dr.(Mrs). R W. Ndana for their kindness and thoughtfulness.

I am so indebted to my beloved parents for their passionate love, prayers, and unflinching support. Thank you, dad for your patience enduring my long absence from home, though you did not live to say 'welcome' as you promised. Dad and mum, you are no more here with us, but you are always fondly remembered. My heartfelt gratitude goes to my ever patient and beloved husband Reverend Wisdom Solomon, my lovely children Jeiel and Jehobed for their immeasurable love, patience, kindness and prayers. I appreciate your unwavering support through trying and challenging times and the sacrifice you made to come to the United Kingdom. Many thanks to my siblings for their prayers and support.

I am profoundly grateful to my spiritual fathers, Bishop David Oyedepo (PhD), Senior Prophet T.B Joshua, Reverend Chris Oyakhilome (PhD) and Pastor D.K Olukoya (PhD) for their prayers and gracious words of wisdom especially through very difficult times. Your Ministries have been great blessings. I appreciate all Ministers of the Holy Ghost revival in Nigeria. May you and the Ministries you represent be blessed and preserved.

Thank you to all family friends/well-wishers for their prayers and to my office mate (Dr Peter Rostron) for his kindness, especially at the beginning. Many thanks to Mrs Judith Field for her great sense of caring and the beautiful lessons for the kids.

The list is inexhaustible. Many thanks and God bless to everyone I have been unable to mention, but have been immensely helpful one way or the other throughout the entire PhD journey.

Table of Contents

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Tables

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 8

Abbreviations

AAS	Atomic Absorption Spectrometer
As	Arsenic
BJ 18	*Brassica juncea* (Indian mustard) PI 182921 variety
BJ 17	*Brassica juncea* (Indian mustard)PI 173874 variety
BJ 21	*Brassica juncea* (Indian mustard) PI 211000 variety
BJ 42	*Brassica juncea* (Indian mustard) PI 423604 variety.
BN SW	*Brassica napus* (Oil seed rape or rapeseed) PI 601261 Swedish variety.
Cd	Cadmium
DW	Dry weight
EA	Environment Agency
FW	Fresh weight
GI	Growth index
GSD_{samp} or GSD	Geometric standard deviation of sample
GM_{samp} or GM	Geometric mean of sample
HF	Heterogeneity factor
Hg	Mercury
HH	High heterogeneity
HO	Homogeneous
LH	Low heterogeneity
MH	Medium heterogeneity

MNDL	Mean number of dead leaves
MNTL	Mean number of true leaves
N/A	Not applicable
Ni	Nickel
Pb	Lead
PbO	Lead (II) oxide
P-XRF	Portable X-ray Fluorescence Spectrometer
RSDsamp	Relative standard deviation of sample
STDEV	Standard deviation
$\mathbf{s^2_{samp}}$	Sampling variance
$\mathbf{s^2_{anal}}$	Analytical variance
$\mathbf{s^2_{measure}}$	Measurement variance
TC HS	*Thlaspi caerulescens* (Alpine pennycress) supplied by Herbiseed
TC BR	*Thlaspi caerulescens* (Alpine pennycress) collected from Black rocks
TC GM	*Thlaspi caerulescens* (Alpine pennycress) collected from Gang Mine
USEPA	United States Environmental Protection
USDA	United States Department of Agriculture
XMP	X-ray Microprobe
Zn	Zinc
ZM B37	Zea mays (corn) B 37 variety
ZM OH43	Zea mays (corn) OH43 variety
ZM B73	Zea mays (corn) B 73 variety

ZM 64 Zea mays (corn) 644101 variety

Glossary

Analyte	The compound measured.
Bias	The difference between the expectation of the test result and an accepted reference value (CEN, 2005).
Bioavailability	A fraction of the chemical that can be absorbed by the body or any living tissue such as plant roots and shoots (Safae *et al*., 2008).
Biosynthesis	It is an enzyme- catalysed process in living organisms by which complex products are produced (Waynes and Ames, 2012).
Cell differentiation	It is the process by which a less specialised cell becomes more specialised (Wayne and Ames, 2012).
Cell elongation	The movement of actively dividing cells towards the stem.
Chlorosis	Absence of green colouration of the leaf.
Contaminant	A substance which has the potential to cause harm or pollution to water or land when present in it (Fahr *et al*., 2013).
Contaminated land	Any land which appears to the local authority in whose area it is situated to be in a condition of causing significant and/or potential harm (DEFRA, 2002).
Duplicate sample	One of the two samples obtained separately at the same time by the same sampling procedure (Ramsey and Ellison, 2007).

Excluder	A plant that is able to regulate the flow of potentially harmful metals into sensitive tissues of plants (Robinson, 1995).
Flowering time gene	Genes that regulate floral transition (Salehi *et al*., 2005).
Heavy metal	Metals of high atomic weight having densities greater than 5 mg/m^3 potentially toxic (Duffus, 2002).
Homogeneity/ Heterogeneity	The degree to which a property or a constituent is uniformly distributed throughout a quantity of material (Taylor *et al*., 2005).
Phytomining	Use of plants to extract trace elements (Robinson, 1995).
Phytoremediation	The use of plants to decontaminate polluted land, air and water (Reeves and Brooks, 1989).
Phytotoxic	Harmful or poisonous to plants (Kopottike *et al*., 2009).
Precision	The closeness of agreement between independent test results obtained under stipulated conditions such as repeatability and reproducibility (CEN, 2005).
Meristematic	Ability of cells or tissues to divide (Taku and Zheng-Hua, 2010).
Secondary cell wall	A structure located between the primary cell wall and the plasma membrane in plants (Deng *et al*., 1990).

Translocate	Transport of dissolved substance within a plant in phloem and across membrane (Lasat, 1996).

CHAPTER 1: INTRODUCTION

1.0 BACKGROUND OF STUDY.

Soil is a medium of interaction between the atmosphere, biosphere and the lithosphere. The presence of toxic elements in soils can be harmful to plants, animals and humans via this interaction (Kelerpteris *et al*., 2006). Soil plays a very complex and important roles as filter, buffer, storage and transformation systems, thus helping to protect the global ecosystem against the effects of pollution. However, the efficiency of these functions depends on the preservation of soil properties (Sharma and Dubey, 2005; Kabata-Pendias, 2010).

According to Jeana (2000), since the dawn of industrial revolution, mankind has been introducing numerous hazardous compounds into the environment at an exponential rate. These hazardous pollutants consist of variety of organic compounds and heavy metals, which can pose serious risks to human (Fahr *et al*., 2013). One of the most serious and long term outcomes of environmental pollution is heavy metal contamination of soils (Kabata-Pendias, 2010). Kitagishi and Yamane, (1981); Greener and Kochen, (1983); Strubelt *et al*., (1996); Huang *et al*., (1997); Johnson, (1998); Jeana, (2000); Bhuyian *et al*., (2010); Udeigwe *et al*., (2011) reported that heavy metals in the environment are sources of concern because of their potential reactivity, toxicity, mobility and non-biodegradable nature in the soil.

The term heavy metals has been widely used to refer to a group of metals and semi-metals that have been associated with contamination and potential toxicity (Duffus, 2002). High concentrations of heavy metals in some soils have been widely reported. Heavy metals such as lead (Pb), zinc (Zn), cadmium (Cd), nickel (Ni) and chromium (Cr) are released into the environment by many processes (Christina *et al*., 2000). For example, United States Environmental Protection Agency {USEPA} (1997) and USGS (2013), reported the presence of Cd, Ni, Pb, Zn, copper (Cu), chromium (Cr) and mercury (Hg) in soils at some hazardous waste sites previously used for mining and smelting activities in the United States. There is an estimate of over half a million heavy metal contaminated sites throughout the world (USEPA, 1997; USEPA, 2007).

The main threats to human health from heavy metals are associated with exposure to Pb, Cd, Hg and Arsenic (As) (Lars, 2003; ATSDR, 2007; Fahr *et al*., 2013). Lead (Pb) is one of the most widely distributed heavy metals. It is a bluish–grey metal, also known as plumbum or pigment metal, which occurs naturally within the earth crust (Environment Writer, 2000). Lead pollution of soil especially in mining areas is a widespread and

significant problem globally. Lead has been ranked second of all hazardous substances next to arsenic because of its toxicity (ATSDR, 2007). It exhibits extreme persistence and accumulation in soils, sediments and water (Traunfeld and Clement, 2001; Lee, 2013).

Lead has been made ubiquitous in the environment by anthropogenic activity (Griffith, 2002). It has been used by man for at least 5000 years and its early applications include its use as building materials, pigments, paints, ceramics and pipes for transporting water (Lars, 2003). Variety of industrial processes involve the use of Pb such as mining, smelting, manufacture of pesticides, dumping of municipal waste and burning of leaded fuels containing lead additives (Jeana, 2000; Seul-Ji *et al*., 2013). Other anthropogenic sources of Pb include the use of industrial emissions, landfill and sewage sludge (Jeana, 2000). An estimated 5.2 million tonnes of Pb are released into the environment annually from lead mining sites (USGS, 2013). Crustal abundance of Pb is much lower than the Pb produced by anthropogenic influences. Krauskopf, 1979; Jefferson, 2007; IST, 2007 estimate of Pb crustal abundance is between 10 and 14 mg/kg.

Lead contamination of soil can cause variety of environmental problems, including loss of vegetation, ground water contamination and toxicity to plants, animals and humans (Buchauer, 1993; Body *et al*., 1991; Huang and Cunningham, 1996; Yusuf *et al*., 2011). It has no known biological function in living organisms and is toxic at low concentrations (USEPA, 1997; Kabata-Pendias, 2010). Lead is toxic to humans and may be implicated in systemic poisons, building up in the body over an extended period of time and exposure (Bakerly, 1978; Hill *et al*., 1999). Purefoy (2010) reported that 30,000 people have been poisoned by Pb and estimated that 400 children have died due to Pb poisoning as a result of Pb contamination of residential soils in Zamfara, Northern Nigeria.

Due to anthropogenic use of Pb, most soils are likely to be enriched in Pb, especially within the top horizon (Kabata-Pendias, 2010).The steadily increasing amounts of Pb in surface soils in both arable and cultivated lands have been reported for various terrestrial ecosystems and anthropogenic Pb deposition extending back at least to Greek and Roman times has been traced in peat cores of European countries (Kabata-Pendias, 2010). Peat soils are regarded as a sink of Pb deposited by the atmosphere and might be a significant source of the metal to the fluvial system due to peat erosion processes (Rothwell *et al*., 2008). In Europe, areas around metal smelting complexes have been found to be heavily contaminated by Pb, Cd, Cu and Zn (Alloway, 1990; Fent, 2004; Panagos *et al*., 2013).

Soil Pb concentration values are different for every region. A similar value (100 mg/kg) was established in China for tea garden soils (Jin *et al*., 1987). However, there are no established values of Pb for soils in most developing nations. American Blacksmith Institute recorded 11000 mg/kg Pb in residential soils of Pb contaminated villages in Zamfara, Northern Nigeria (BI, 2011).This high Pb concentration resulted in a widespread Pb poisoning triggered by illegal artisan gold mining activities. Lead can be released into the environment through gold mining activities as a result of the association of the primary Pb mineral (galena or PbS) with the gold ore **nagyágite** {$Pb_5Au(Te, Sb)_4S_{5-8}$} (Effenberger *et al*., 1999). Galena may become associated with other secondary Pb minerals through weathering processes, oxidation and anthropogenic deposition (Richard *et al*., 2007). Lead contamination of soils and plants in gold mining areas of China and Nigeria are higher than in unmined areas (Zabowski *et al*., 2001; Salami *et al*., 2003). Lead concentrations of household dust of children sleeping areas in Zamfara was 2.5 times higher than the USEPA residential soil limit of 400 mg/kg (Taylor *et al*., 2013). The number of reported cases of Pb pollution in developing nations is an indication that Pb pollution is still an environmental issue to reckon with in developing parts of the world.

Davies (1977), stated that the upper limit for Pb content of an unpolluted soil in the United Kingdom should be established as 70 mg/kg. However, a recent survey (Ander *et al*., 2013) reported 180 mg/kg as the normal background concentrations (NBC) of Pb in English soils. That study (Ander *et al*., 2013) also reported Pb concentrations of 2400 and 820 mg/kg for non-ferrous metalliferous mineralised areas associated with mining activities and urbanised areas respectively. Previous studies by Argyraki (1997); Baker *et al*. (1994); Mcgrath and Loveland, (1992) in the United Kingdom have shown significant Pb contamination of some sites. One survey of soils in England and Wales reported Pb concentrations ranging from 30-1638 mg/kg with a median value of 40 mg/kg (McGrath and Loveland, 1992). Data supplied by the Geochemical Baseline Survey of the environment (G-Base) project run by the British Geological Survey, reported a top soil (0-150 mm depth) Pb concentrations in Derbyshire Dales of 996 mg/kg and the subsoil (300-450 mm/depth) Pb concentrations of 470 mg/kg (DEFRA, 2007).The highest recorded concentrations for some top and sub soils in Derbyshire were 35930 mg/kg and 24700 mg/kg respectively (DEFRA, 2007).

The high concentration of heavy metals in some soils is reflected in the higher concentrations in some plants and which can be biomagnified through the food chain ending up with animals and humans (Buszewski, 2000; Vamerali *et al*., 2010). The Pb levels of soils that are toxic to plants are not easy to evaluate, as it is not easy to predict

how much of soil Pb is bioavailable to plants (Davies, 1977). Although Pb is not an essential element, a small number of plants species proliferate in Pb contaminated areas and can potentially accumulate it in different parts of the plants depending on the species.

This ability of some plants to absorb heavy metals make them useful indicators of environmental pollution (Farago, 1994). Lead, like any other heavy metal, enters into plants cells and tissues through various uptake mechanisms. The roots are usually the first plant organ of contact with contaminated soil. One of the potential exposure routes of Pb into the human food chain is via the consumption of plants grown on contaminated soils (Argyraki, 2014). However, ingestion of Pb contaminated soil is a primary route of human exposure to Pb. The generic assessment criteria used to estimate the risk of contaminant to human from consumption of contaminated food crops as a concentration factor is based on the soil and plant contaminant concentrations.

The interaction of plants with heavy metals such as Pb in soil, and their heterogeneous distribution in soil can influence plant uptake of contaminant in soil. Reviews of AMC, (2009); Ramsey, (2010) show that new analytical techniques have become available (e.g. Portable X-ray Fluorescence Spectrometer) that enable the concentration of heavy metals in soil to be measured at a fine spatial scale whilst they are still in their original location (i.e. *in situ*). This new technology enables the quantification of this *in situ* heterogeneity of contaminants in soil at the scale that potentially affect plants, mainly via their roots. The Understanding of Pb spatial distribution within the soil is very important in the assessment of potential risks and the development of remediation strategies for contaminated sites (Thomas, 2010). This study has potential implications for risk assessment and phytoremediation of Pb contaminated land discussed in Chapter 7.

Increasing public concerns over the presence of certain chemical pollutants in the environment have led to a search for suitable technologies for clean-up of contaminated environments (Chaudhry *et al*., 2005; Lee *et al*., 2013). In recent decades, phytoremediation has emerged as a low cost, low–maintenance, environmentally friendly and renewable technology for *in situ* clean up, stabilisation and removal of organic and inorganic contaminants from the environment, which is considered more cost effective than *ex situ* decontamination methods (Chaudhry *et al*., 2005; Varemali *et al*., 2010; Thanh *et al*., 2013). Plant uptake of Pb poses a potential health risk to both animals and humans and at the same time may provide possible solutions for remediation of contaminated land.

Spatial heterogeneity of contaminants in soil refers to the pattern in which contaminants are distributed in the soil. A major factor that have been shown to impact significantly on plant uptake is the spatial heterogeneity of contaminants and scale of heterogeneity in relation to target receptors (Millis *et al*., 2004; Thomas, 2010). Sampling provides a useful estimate of contaminant concentration and spatial heterogeneity necessary to achieve reliable risk assessment and sustainable remediation strategies. Various sampling methodologies have been developed and used which are aimed at producing reliable measurements of contaminants (DoE, 1994; USEPA, 1996; Lyn *et al*., 2007a; Thomas *et al*., 2008). The development of *in situ* analytical techniques has enhanced the sampling of contaminated lands without disturbing the *in situ* structural heterogeneity. Taylor *et al*., (2005); Thomas *et al*., (2008) used the Portable X-ray Fluorescence (PXRF) to quantify *in situ* heterogeneity at scales across five orders of magnitude and over a range of scales respectively.

The understanding of spatial scales is relevant in the ecological studies of plants. Robinson, (1994); Hutchings and John, (2004) found that the impact of spatial heterogeneity of nutrient distributions relative to individual plant roots have significant effect on the performance of some plant species. Some studies (Millis *et al*., 2004; Manciulea and Ramsey, 2006; Menon *et al*., 2007; Moradi *et al*., 2009; Thomas, 2010) have shown that the pattern and scale of heavy metal heterogeneity can have a significant effect on plant performance and uptake.

Much of previous studies aimed at estimating plant uptake were based on pot experiments in hydroponics or homogeneously distributed trace metal medium (Huang and Cunningham, 1996; Ebbs *et al*., 1997; Huang *et al*., 1997; Quartacci *et al*., 2006; Turan and Bringu, 2007) or a field site where the soil-plant system is peculiar to that site (Clemente *et al*., 2005; Wang *et al*., 2012). A major drawback of previous works on plant uptake is that spatial heterogeneity in contaminants distribution is overlooked. Studies using Zn and Cd (Millis *et al*., 2004; Manciulea and Ramsey, 2006; Thomas, 2010) have shown significant differences between the simplistic models used and homogeneous media, but these models are not characteristic of the original spatial patterns of contaminant heterogeneity experienced by plants in realistic field conditions. Thomas, (2010) showed that Zn heterogeneity seen in the field can be simulated in pot trials to assess its effect on plant uptake. That study (Thomas, 2010) reported an extreme contrast between models used to map spatial distribution of trace metals in contaminated land investigations and the distribution of trace elements used in controlled studies to estimate plant uptake.

Research to date on heterogeneity of contaminants has tended to focus on Zn and Cd. Existing accounts only provided insights into the heterogeneities of these elements in soil. The study on Cd heterogeneity did not take into account realistic *in situ* heterogeneity. To the best of my knowledge, no controlled study assessing the effect of *in situ* heterogeneity of Pb in soil on plant uptake of Pb in pot experiments has been reported and no other research has attempted to simulate *in situ* heterogeneity of Pb based on field investigation and recreate the simulated heterogeneity in pot trial, although a similar but significantly different approach has been taken to understanding the effects of Zn heterogeneity (Thomas, 2010).

Work in this thesis assesses the impact of *in situ* heterogeneity of Pb in soil on plant uptake by quantifying *in situ* heterogeneity of Pb and simulating it in pot experiments to assess its effects on plant behaviour, shoot, root biomass and Pb uptake. It also evaluated a range of plant species in varied Pb concentrations which was important to select plants species that can thrive but respond to high levels of soil Pb. This study makes a unique contribution to important areas such as contaminated land investigation and remediation, environmental risk assessment of Pb and plant uptake research.

This thesis also describes the quantification of the *in situ* heterogeneity of Pb in soil over a wide range of scales (0.02- 50 m) at two field sites that were expected to be highly heterogeneous (Chapter 3). A series of pot trials then investigate (1) how a range of 13 plant species/varieties interact with a single homogeneous but quite high concentration of Pb in soil (1000 mg/kg of soil). The second pot trial examines how a narrower selection of 6 plant species interact with a range of homogeneous Pb concentrations varying from 100 to 10000 mg/kg. The third pot trial selects just two plant species to investigate how the two extremes of Pb heterogeneity (homogeneous and simplistic binary) affect their biomass, and uptake of Pb. The final pot trial uses the field measurements of Pb heterogeneity made in this study, combined with those from more homogeneous sites, to assess these same effects on the same plant species over a range of four realistic levels of Pb heterogeneities. The objectives of these experiments are to address the thesis aims (Section 1.1) and to test the hypotheses stated in the individual chapters.

1.1 RESEARCH OBJECTIVES

The major aim of this study is to quantify *in situ* spatial heterogeneity of Pb in selected Pb contaminated sites in the United Kingdom and simulate this *in situ* heterogeneity in pot trials to assess its effects on selected plant species, to work towards the following objectives:

1. Review existing knowledge on Pb contamination and effects on plants and human, plant uptake of Pb, potential Pb accumulators, plant behaviour in Pb contaminated growth medium or soil, heterogeneity and methods of quantifying *in situ* heterogeneity of Pb.
2. Determine *in situ* heterogeneity and concentration of Pb in soils at selected contaminated sites at a range of scales (e.g 0.02-50 m) using suitable measurement techniques (e.g Portable X-ray Fluorescence Spectrometer {P-XRF}).
3. Quantify the effect of Pb contamination on biomass and Pb uptake of a range of selected plant species in pot trials with a fixed Pb concentration of 1000 mg/kg and a range of Pb concentrations (100 to 10000 mg/kg).
4. Examine the response of selected plant species in greenhouse pot trials to forms of *in situ* heterogeneity of Pb in growth media that are (a) simple (i.e. in simplistic binary design) and (b) a simulation of a more realistic heterogeneity, based on the field observations, at three levels (low, medium and high)
5. Assess the significance and relevance of research findings and make recommendations for further work.

1.2 THESIS OUTLINE

This thesis is formed of eight Chapters. This first Chapter presents a general overview, justification and background to the research work, research objectives and thesis outline. Chapter 2 presents a review of literature on Pb contamination and effects on plants and human, potential Pb accumulator plants, plants behaviour in Pb contaminated soil and growth media, potential field sites, Pb speciation and bioavailability, heterogeneity and methods of quantifying heterogeneity.

Chapter 3 presents the results of the field work. *In situ* heterogeneity and concentration of Pb in two heavily Pb contaminated sites were investigated. A specific sampling design, in conjunction with appropriate *in situ* measurement techniques, were used to quantify Pb heterogeneity over a range of scales (0.02 m to 50 m). The design was easily adapted to different scales based on its systematic approach using the full balanced and the simplified balanced designs (Thomas *et al*., 2008). The measurement of *in situ* heterogeneity is expressed in the new form as a heterogeneity factor (HF) (already published in Ramsey *et al.,* 2013).

Chapter 4 presents the results of the seed germination experiment, the first and second pot trials. The seed germination experiment compared the suitability, viability and germination rates of 16 species/varieties to determine selection of plant species for the first pot trial. The first pot experiments compared within and between 13 species and varieties at a fixed added Pb concentration in the growth medium of 1000 mg/kg and control (0 mg/kg Pb added), which enhanced comparison of Pb uptake rates and further selection for the second pot trial. The second pot trial compared 4 selected species made up of 6 varieties in a range of Pb concentrations (100, 300, 3000, 10000 mg/kg). It was used to determine the most suitable plant species for further pot trials simulating *in situ* heterogeneity and a binary simplistic model.

Chapter 5 presents the results of the pot experiment which examined the effect of simplistic binary heterogeneity of Pb in the growth medium on the biomass and metal uptake of two plant species (*Brassica juncea* and *Brassica napus)*. Previous research using simplistic heterogeneity models found significant differences in plant growth and uptake of Zn and Cd (Manciulea and Ramsey, 2006; Thomas, 2010), but not for Pb. The main aim of this experiment was to assess whether the simplistic binary model would provide an insight into the effect of a simple Pb heterogeneity model on the selected plant species.

Chapter 6 presents the results of the pot trial simulating more realistic style of *in situ* heterogeneity, based upon the field measurements. It measures the effect on dry biomass of both root and shoot, the Pb concentrations of shoot and root, expressed in (mg/kg), (µg) and concentration factor (CF), for two plant species, grown in homogeneous, low, medium and high heterogeneity treatments. It also examined root response to different patch contrast (cells of varied Pb concentration) in the high heterogeneity treatment. Studies of some metallopytes have shown foraging of roots to different patch contrast in a contaminated growth medium e.g *Thlaspi caerulencens* showed root foraging toward high Zinc concentration (Schwartz, *et al*., 1999a; Whiting *et al*., 2000; Haines, 2002). This part of the experiment is to assess the impact of root placement with regards to Pb heterogeneity.

Chapter 7 discusses the key findings of this study and also assessed the wider significance and implications of these results, and whether a more realistic heterogeneity will be an important factor influencing the uptake of Pb and should therefore be taken into account in phytoremediation of Pb contaminated lands, and in the exposure assessment of humans to Pb.

Chapter 8 summarizes main findings from the thesis in relation to stated aims and objectives, strengths and limitations of this research, conclusions of thesis and makes suggestions for further research work that may be required.

CHAPTER 2: Review of sources and effects of lead, its forms and speciation, lead contaminated sites, plant uptake, accumulator plants and heterogeneity.

This chapter focuses on the review of literature on sources, effects, forms, and speciation of Pb and how it affects plant uptake of Pb from the soil, potential Pb contaminated sites, phytoremediation, Pb accumulator plants, heterogeneity and methods of quantifying *in situ* heterogeneity. The chapter also provides background information on the key themes of works discussed in the other chapters of this thesis.

2.0 SOIL LEAD CONTAMINATION.

2.1 Sources

This section examines soil Pb contamination with respect to possible anthropogenic sources of Pb and links to human exposure.

Soil, air and water are prone to contamination by Pb with the vast use of Pb in many human activities and materials. Metalliferous mining and dumping of wastes often produce Pb pollution (Wong, 2003; Freitas *et al*., 2004; Del Rio *et al*., 2006; Clemente *et al*., 2007). Mine tailings are known to have the most severe environmental effects due to high concentration of heavy metal implicated in many cases of soil Pb contamination (Safae *et al*., 2008). As stated in Chapter 1, an estimated 5.2 million tonnes of Pb is produced annually from mining sites (USGS, 2013).

Lead used in paints, petrol, explosives and disposal of municipal sewage sludge enriched in Pb also contribute to soil Pb contamination (Jackson and Watson, 1977; Levine *et al*., 1989). Before the withdrawal of Pb additives from petrol in the United Kingdom in 1988, an annual maximum Pb emission of 7500 tonnes from vehicles was reported by Thornton and Howarth, (1986) of which about 10% are deposited on and within 100 m of roads.

Many commercial products and materials contain lead including ceramic glazes, television glass, ammunition, batteries, medical and laboratory equipment such as X-ray shields, foetal monitors and electrical equipment (Environment Writer, 2000).

2.1.1 Health effects of lead.

This sub-section discusses the health effects of Pb to human and plants in relation to the implication of this study for estimation of human exposure to Pb in risk assessment and the assessment of the effects of Pb on plants in pot trials.

Effects of Pb on human health

This section describes the human health effects of exposure to Pb. One primary source of human exposure to Pb is by ingestion of Pb contaminated soil (pica) and inhalation of Pb solid phases in dust which have severe effect on children (ATSDR, 2012). However, recent studies (Pruvot *et al*., 2006; Duoay *et al*., 2008; Zhuang *et al*., 2009; Duoay *et al*., 2013; Argyraki, 2014) reported implications of human exposure via the consumption of Pb contaminated vegetables.

Several comprehensive reviews have examined the quantitative relationship between exposure of Pb contaminated soil and blood lead levels (BPb) in children (Dungan, 1980; Duggan and Inskip, 1985; ATSDR, 1992; Laidlaw and Taylor, 2011; Zahran *et al*., 2013). A dose-response relationship is often observed which reflects a change in BPb levels with a change in soil lead concentration (ATSDR, 1988; Reagan and Silverbird, 1989; Laidlaw *et al*., 2011; Zahran *et al*., 2013). Laidlaw *et al*. (2005) reported a relationship between blood lead levels of children living in urban areas of Syracuse and temporal variation of atmospheric Pb deposit.

Varied health effects are associated with Pb exposure. Studies on the effects of lead on children have demonstrated a relationship between exposure to Pb and a variety of adverse effects (ATSDR, 1988; Tristan-Montero, 2000; Laidlaw *et al*., 2011; Zahran *et al*., 2013). These effects include impaired mental and physical development, decreased haemo biosynthesis, and elevated hearing threshold, decreased serum levels (ATSDR, 1988; 1992), substance addiction and risk of infection with sexually transmitted disease among adults (Hu *et al*., 2014).

Low level exposure to Pb has been associated with deficits in early developmental years (Canfield *et al*., 2003). Canfield *et al*. (2003) studied the effect of lead poisoning on the cognitive functioning in children and infants and observed a decline in intelligence quotient (IQ) by 7.4 points between 1 µg/dL and 10µg/dL blood Pb and then a decline 4.6 points for every 10µg/dL increase after that. Needleman, (2004) reported that long term exposure to Pb interfered with bones and teeth metabolism and altered the permeability of blood vessels and collagen synthesis. Elliott *et al*. (1999) reported clinical

lead poisoning of children in England at blood Pb level >25 µg/dL. The symptoms of acute lead poisoning include headache, irritability, abdominal pain, nervous disorder, lead encephalopathy characterized by sleeplessness and restlessness (WHO, 1995). Lead has also been listed as a potential carcinogen in EPA Toxic Release Inventory (TRI) (Environment Writer, 2000; CDC, 2005a; IARC, 2006).

The actual number of children exposed to lead in dust and soil at concentrations adequate to elevate blood Pb levels cannot be estimated with the data now available, but the number of children potentially exposed to Pb in dust and soil can be estimated as a range of potential exposures to primary sources of Pb in dust and soil (ATSDR, 1988). An estimate of 5.9 to 11.7 million children are at risk worldwide (ATSDR, 1988; ATSDR, 1990; CDC, 1991; USEPA, 1998) reported in Charles, (1992).

Human and animal health threats posed by heavy metals is aggravated by their long–term persistence in the body and environment (Yoon *et al*., 2006). Barbosa *et al*. (2005), recorded a re-introduction of Pb into the bloodstream from bones of children and pregnant women undergoing remodelling. Clearance of Pb from the bloodstream is slow, partly due to the release of Pb from the bone (Hu *et al*., 2007). In a prospective analysis of the Normative Aging Study, higher patellar Pb levels, but not blood Pb, were associated with higher systolic blood pressure and abnormalities in electrocardiographic conduction (Cheng *et al*. 1998, 2001). Due to Pb persistence in the bone, studies by Gerr *et al*. (2002); CDC, (2005b); CDC, (2007) suggested bone Pb a metric of cumulative or long-term exposure to Pb as a better predictor of Pb-induced elevations in blood than blood Pb.

2.1.2 Effects of Pb on plant health

Elevated concentrations of trace metals in the soil can be potentially toxic to plants (Kopittke *et al*. 2009). When concentrations of Pb in plant cells accumulate above some threshold or maximum levels, it can cause direct toxicity by damaging cell structure (due to oxidative stress caused by reactive oxygen) and inhibit some cytoplasmic enzymes (Assche and Clijsters, 1990; Chhotu and Fulekar, 2009). However, threshold Pb concentration will vary considerably with different plant species (Brooks, 1994). Brooks, (1994) suggested a **threshold Pb concentration of 1000 mg/kg** for most plant species and classified plants, which accumulated > 1000 mg/kg Pb as hyperaccumulators. Some plant species have been reported to have accumulated > 1000 mg/kg without any symptom of Pb toxicity, while some have shown signs of Pb stress or toxicity at concentration less than this threshold (Baker, 1981; Brooks, 1989).Threshold concentration may also be dependent on the Pb concentration of the underlying

substrate (Brooks, 1994). There are currently no established threshold Pb concentration for most plant species. However, threshold CF has been established as as CF=1, CF<1, CF >10 for Pb **accumulators, excluders and hyperaccumulators** respectively.

Ashagre *et al*. (2013) defined phytotoxicity as the degree of toxic effect caused by a compound on plant growth and such damage that may be caused by a wide variety of compounds, including trace metals, pesticides, salinity, phytotoxins or allelopathy.

Peterson (1971); Foy *et al*., (1978); Bowen, (1979); Prasad and Strzalka, (1999) in Kabata-Pendias (2010) have reviewed basic reactions related to toxic effects of excess Pb as follows:

- Reactions of thiol groups with cations (i.e. Pb^{2+}).
- Damage to photosynthetic apparatus involved in several metabolic alterations is most significant.
- Occupation of sites for essential groups such as phosphate and nitrate.
- Affinity for reacting with phosphate groups of Adenosine diphosphate (ADP) or Adenosine triphosphate (ATP).
- Changes in permeability of cell.

Taiz and Ziegler (2002) also reported an indirect toxic effect of Pb caused by replacement of essential nutrients at cation exchange sites in plants. Choutu and Fulekar (2009), citing Boonyapookana *et al*. (2005), reported that Pb caused some phototoxic effects including chlorosis, necrosis, stunt growth of root/shoot and less biomass production in *Helianthus annus, Nicotiana tobacum and Vetiveria zizanioides*. Huang and Cunningham (1996) observed an inhibition of root growth due to Pb toxicity. Opeolu *et al*., 2010 and Deo and Nayak, 2011 reported decreased plant growth and chlorosis in plants as symptoms of Pb toxicity.

2.2 CHEMICAL FORMS AND SPECIATION OF LEAD.

This discussion gives background to the choice of Pb mineral used in pot trials reported in Chapters 4, 5 and 6. Lead can occur in soil in many different chemical forms, that affect its behaviour in general, and its uptake by plants in particular. Biochemical and toxicological evidence suggests that the chemical form and oxidation state (together expressing speciation) in which a metal is present in the environment can be a critical factor affecting the uptake of that metal (Kot and Namiesnik, 2000, Morgan, 2013, Roy and Macdonald, 2013).

In natural environments, lead sulphide (galena) is the primary Pb mineral. It is stable over a wide pH range. Gee *et al*., (1995) reported that Pb is widespread in some contaminated sites, often as a relict blocky galena surrounded by Pb oxide/cerussite or hydrocerussite which has been interpreted as a weathering reaction of the Pb phases. Important lead bearing minerals identified in soils of mining and smelting sites include Pb hydroxide, cerrusite, hydrocerrusite ($Pb_3(CO_3)_2(OH)_2$, anglesite $PbSO_4$, galena PbS, lead oxide PbO which exist at different pH (Gee *et al*., 1995; Gee *et al*., 1997; Zyan and Ryan, 1999; USEPA, 2007). Richard *et al*. (2007) also reported chloropyromorphite ($Pb_5(PO_4)_3Cl$) as a Pb bearing mineral phase in phosphate-bearing systems, and in plumbojarosite Pb-contaminated soils.

Lead is often accumulated near the soil surface as a result of its sorption by soil organic matter (SOM). Studies conducted by Sipos *et al*. (2005) suggest that SOM plays an important role in the adsorption of Pb, but fixation by clay minerals is stronger. Pb distribution within the soil profile, whatever its source, is not uniform and it is usually associated with hydroxides and oxides of Fe and Mn in high concentration up to 20,000 mg/Kg (Kabata-Pendias and Pendias, 1999). Lead (Pb^{2+}) can be concentrated in calcium carbonate or phosphate particles at higher pH > 6 (Chardon *et al*., 2008). During weathering feldspar crystal loosened from rock below slowly changes into a clay mineral as it reacts with acidic water, and Pb cations in solution then adhere to the negatively charged clay mineral surface (Inez *et al*. 1998; Pires, 2004;). Some clay minerals e.g. kaolin have high specific surface area favouring adsorption by heavy metals such as Pb^{2+}, Cd^{2+}, Zn^{2+} and Cu^{2+}(Ma *et al*., 1995). Elliott *et al*. (1986) postulated that under acid conditions, the adsorption phenomenon dictates Pb bioavailability, while solubility, precipitation and complexation control bioavailability under neutral or alkaline conditions. In relation to clay mineralogy, Pires (2004); Pires *et al*. (2007), observed a strong relationship (r = 0.80) between kaolinite content of cambisol, lactosol, and organosol soils and Pb adsorption capacity.

In natural systems Pb is present in the (II) oxidation state over relevant conditions of pH and reduction-oxidation potential (Eh). The mineral plattnerite (PbO_2) occurs in some natural systems and is associated with other oxidation products such as cerussite and pyromorphite (USEPA, 2007). In general, the geochemical transport processes of Pb are not directly tied to redox conditions (Zyan and Ryan, 1999). However, Pb may form stable precipitates with redox-sensitive elements such as sulphur or iron (Richard *et al*., 2007). In sulphate-reducing systems, Pb is expected to form insoluble PbS precipitates and in moderately reducing systems, reductive dissolution of hydrous ferric oxides that contain adsorbed Pb could result in Pb mobilization (USEPA, 2007). Some important Pb bearing minerals in soil are summarised in Table 2.2.1.

Table 2.2.1: Some important Pb bearing minerals in soil.

Lead bearing minerals	Chemical formula
Anglesite	$PbSO_4$
Cerrusite	$PbCO_3$
Galena	PbS
Hydrocerrusite	$Pb_3(CO3)_2(OH)_2$
Pyromorphite	$Pb_5(PO_4)_3Cl$
Plattnerite	PbO_2
Plumbojarosite	$Pb_3Fe_2(OH)_6(SO_4)_2$
Lead II oxide	PbO

Ionic lead (Pb^{2+}), lead oxides and hydroxides, lead-metal oxy-anion complexes are the general forms of lead that are released into the soil, ground and surface waters (Jeana, 2000). Lead oxide particles can either be covered rapidly by a weathering crust of secondary minerals or can remain virtually unchanged over 18 months depending on the soil type (Birkefeld *et al*., 2007). Ionic Pb is the most common and reactive form of lead at pH 6, forming mononuclear and polynuclear oxides and hydroxides in the soil (GWRTAC, 1997; Birkefeld *et al*., 2007). The geogenic Pb content of residual soil is largely inherited from parent rocks. Hence the estimation that the geogenic concentration of Pb in soils that are derived from sandstones would typically be at their average concentration of ~ 10 mg/kg (Krausopf, 1979).

In most lead phytotoxicity studies, $Pb(NO_3)_2$ has been used as the Pb species in both soil and hydroponic cultures. Opeolu *et al*. (2010); Huang and Cunningham (1996); Lame *et al*. (2005); Kopittike *et al*. (2009) used $Pb(NO_3)_2$ in *ex situ* determination of lead concentrations of plants in growth media. Its widespread use is as a result of it readily dissolving in water to give clear colourless solution and Pb^{2+} as a product of dissociation (Mahjoubet *et al*., 2001).

However, Pb $(NO_3)_2$ can migrate between media and out of the pot (USDA, 1993). This Pb migration may affect the original heterogeneity within a growth medium, especially if it contains sand as a major component (Burkark and Kolpin, 1993; USDA, 1993).

2.2.1 Behaviour of Pb in Soil.

Lead is known to readily form a precipitate within the soil matrix, and has low aqueous solubility, in some cases it is not readily bioavailable to plants. Vega *et al*. (2007) reported that Pb sorption is lower than that of Zn and Cu, being the least mobile among other trace metals in the soil. Hyperaccumulation of Pb is rare in plants, due to the low solubility of most Pb compounds and ready precipitation of Pb by sulphate and phosphate in the root system (Baker *et al*., 2000).

About 0.005 to 0.13% of Pb in soil solution is available to plants (Davies, 1995, Wilson and Cline, 1996). Lead's behaviour in the soil is influenced by several factors. These include pH, soil organic matter (SOM), cation exchange capacity (CEC), redox potential (Eh) and granulometric composition (i.e. the measurement of grain sizes in sand, rock and other deposits). Granulometric composition is the content of granules of varying size in soil, expressed as a percentage of the bulk or of the quantity of granules of the examined sample (Grossgeim, 1979). These factors also affect the bioavailability and uptake of Pb by plants.

2.3 CONTAMINATED SITES IN THE UNITED KINGDOM.

This section gives an overview of Pb contamination of soil in England with the aim of helping to select suitable field sites for investigation of Pb heterogeneity in this research.

Various estimates have been made of the extent of the problem of Pb contamination in the United Kingdom. In the report of contaminated soils published in 1993, the Parliamentary Office of Science and Technology referred to an expert estimate of between 50,000 and 100,000 overall contaminated sites across the United Kingdom with an estimated value of 100,000 to 200,000 hectares (DEFRA, 2005). In more recent times, the United Kingdom Environment Agency (EA) has estimated about 300,000 hectares of contaminated lands in the United Kingdom due to both natural contamination and anthropogenic influence (DEFRA, 2007). DEFRA (2007) has identified 30,000 to 40,000 sites affected by heavy metal contamination with a total area of about 55,000 to 80,000 hectares.The advanced geochemical map of England and Wales (Figure 2.3.1) based upon earlier work (McGrath and Loveland, 1992) show the extent of Pb contamination in England.

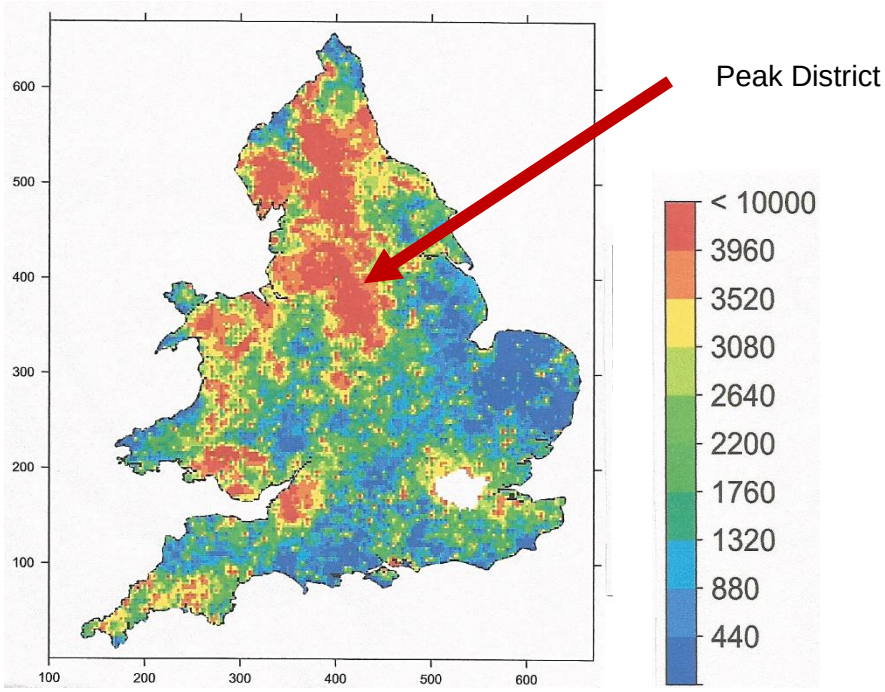


Figure 2.3.1: Advanced Soil Geochemical map of England and Wales showing Pb contaminated areas in the UK and extent of contamination. Lead concentrations in the scale above are mg/kg of soil. Arrow points to Derbyshire the Peak District where field sites are located (British Geological Survey, 2012 with permission from the British Geological Survey @NERC)

2.3.1 Potential Lead contaminated sites in Derbyshire

Derbyshire (Grid reference SK 372320) is in the East Midlands of England, the Northern part of the county overlays a portion of the Pennines, the famous chain of hills and mountains in England (DEFRA and EA, 2002a). The 254,615 hectares of land contains a large amount of thinly populated agricultural upland with 75% of population living in 25% of the area (DEFRA and EA, 2002b). The county had both rural and mining economy on both ends of the land and was very rich in natural resources like iron, limestone and lead and so is known for its historical mining activities especially of Pb (DEFRA and EA, 2002c).

Maskall and Thornton (1993), reported that the heavy metal contamination of Wirksworth Moor in Derbyshire is mainly due to its use for Pb smelting in medieval times (1300-1550 AD). McGrath and Loveland (1992) reported Pb concentrations ranging from 3 to 16338 mg/kg in Derbyshire Dales. The highest recorded concentrations for topsoil and sub soil were 35,930 mg/kg and 24,700 mg/kg respectively (DEFRA and EA, 2002a). In Derbyshire, Pb concentrations in soils are frequently elevated well above the national average primarily due to the natural underlying geology, with Pb mineral veins being enclosed within the carboniferous limestone that underlies it (DEFRA, 2005).

Anthropogenic contributions are also a major cause of elevated Pb concentration arising from historical Pb mining and smelting within the district (DEFRA and EA, 2002b). Lead mine stopped in the late 1800s, but most mines are now capped and covered with vegetation (DEFRA, 2007). Gee *et al*., (1997) studied the mineralogy and weathering processes in historical smelting slags and effects on metal mobilization, reported some lead contaminated sites in Derbyshire. The South Pennine ore field, the smelting places North–West Chesterfield, and smelting settlement East of Bolsover all in Derbyshire (Gee *et al*., 1997).

2.3.2 Phytoremediation of lead contaminated sites.

Interestingly, the same plant uptake mechanisms that pose potential risks to human from toxic heavy metals may also provide a possible solution to remediation of contaminated lands by phytoremediation. Phytoremediation is the name given to a set of technologies that use green plants for *in situ*, or in place, removal, degradation, or containment of contaminants in soils, sludge, sediments, surface and ground water (USEPA, 2000; Seul-Ji *et al*., 2013).

Various remediation technologies such as soil washing, soil flushing, electro kinetic process, stabilization and solidification have been used to reduce the effects of heavy metal contaminated lands (Babel and Kurniawan, 2003; Aziz *et al*., 2011; Sorvari *et al*., 2006). Faced with high rates of environmental pollution, especially by heavy metals, and the search for more environmentally friendly techniques to decontaminate highly polluted ecosystems, phytoremediation becomes very pertinent. Generally, the remediation of contaminated soils, ground and surface water requires the removal or containment of toxic metals from such contaminated areas. Phytoremediation methods in use, include phytomining, phytoextraction, rhizofiltration, phytostabilization and phytovolatilization (GWRTAC, 1997; Robinson *et al*., 2006; Antiochia *et al*., 2007; Machie *et al*., 2014; Cheng *et al*., 2015).

Phytoextraction is the uptake of contaminants by plant root and translocation within the plants and the contaminants generally removed by harvesting the plants (USEPA, 2000; Antiochia *et al*., 2007). This technology leaves a small mass to be disposed of than excavation of the soil or other media (Banuelos, 1997). It is the most often applied to metal-contaminated soil. Reeves and Brooks (1983); Robinson *et al*., (2006); Varameli *et al*., (2010) reported some disadvantages associated with phytoextraction as follows;

- Metal hyperaccumulators are generally slow-growing with a small biomass and shallow root system.
- Plant biomass must be harvested and removed, followed by metal reclamation or proper disposal of the biomass. Hyperaccumulators plants may accumulate significant metal concentrations above 1000 mg/kg.
- Metals may have a phytotoxic effect.

Phytostabilization is defined as the immobilization of a contaminant in soil through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants (Adler, 1996). Airst (1996) in USEPA (2000) defined phytostabilization as the use of plants and plant roots to prevent contaminant migration via wind and water erosion, leaching and soil dispersion.

Rhizofiltration is the adsorption or precipitation onto plant roots, or absorption of contaminants in solution into the roots due to abiotic or biotic processes (USEPA, 1997; Kabata-Pendias, 2010). It results in the containment of the contaminant, in which contaminants are immobilized or accumulated on or within the plant (USEPA, 2000).

Contaminants are then removed by physical removal of plants. Groundwater, surface water and waste water can also be treated using this technology (USEPA, 1992).

Phytovolatilization is defined by Thompson *et al*. (1998), as the uptake and transpiration of a contaminant by a plant, with the release of the contaminant or a modified form of it to the atmosphere. This technology is mainly applied to ground water and not applicable to soil.

Phytoming describes the exploitation of sub-economic ore bodies using plants. Metal hyperaccumulating plant species are grown, biomass harvested and burned up to produce bio-ores (Brooks *et al*., 1998; 1999). Phytomining trials have been carried out with elements such as nickel, gold and thalium. For example, the nickel (Ni) hyperaccumulator *Streptanthus polygaloides* (milk wort jewelflower) was found to yield 100 kg/ha of Sulphur-free Ni when used on some ore sites in United States (Brooks *et al*., 1998). *Brassica juncea* is also known to accumulate gold to concentrations of over 100 mg/kg gold per plant biomass on a dry matter basis (Robinson *et al*., 1997; 2003). An unusual hyperaccumulation (>500 mg/kg dry mass) of thallium has been determined in *Iberis intermedia* (candy tuft) and *Biscutella laevigata* (Buckler mustard) from southern France (Brooks *et al*., 1999; Anderson *et al*., 1999; Robinson *et al*., 1997; 2003). This is new and of great potential and can be applied to other elements such as Pb and Zn.

The concept of using plants to remediate heavy metal contaminated soils has been receiving increasing attention (Chaney, 1983; Baker *et al*., 1994; Raskin *et al*., 1994; Huang and Cunningham, 1996; Varameli *et al*., 2010; Romeh, 2010). However, the success of phytoremediation depends on several factors. Plants must produce sufficient biomass while accumulating high concentrations of metals (Blaylock and Huang, 2000), and should also be able to accumulate environmentally important toxic metals (e.g Cd, Pb, etc.). Some plants have the potential to absorb a wide variety of metals from soils (Chen and Cutright, 2001). However, only a small group of plants have the capacity to take up and sequester one or more of the following metals in their shoots: > 10,000 mg/kg for Zn and Mn, > 1,000 mg/kg for Ni, Co, Cu and Pb, and > 100 mg/kg of Cd (Mei *et al*., 2002; Pollard *et al*., 2002); these plants are referred to as ‘hyperaccumulators’. Brooks *et al*., (1998) reported about 400 hyperaccumulators, but a few Cd or Pb hyperaccumulators have been discovered (See discussion in Section 2.5.3).

Jeana, (2000) and USEPA, (2000) reported some limitations of phytoremediation as follows: (i) it is restricted to the rooting depth of remediative plants, (ii) remediation with plants is a lengthy process, and thus it may take several years to remediate a

contaminated soil, and it may still not be fully remediated. Several concerns have been raised about phytoremediation, such as the use of invasive and exotic species which can affect biodiversity and consumption of contaminated plants by wildlife (Jeana, 2000; USEPA, 2007). According to USEPA (2000; 2007), harvested plant biomass produced from the process of phytoextraction may be classified as hazardous waste, therefore subject to proper handling and disposal, and unfavourable climatic conditions may limit plant growth and phytomass production, thus decreasing efficiency of phytoremediative process.

Several methods have been described for disposal of heavy metal contaminated biomass. The commonly used methods are biomass desiccation, pre-treatment by compaction, composting, pyrolysis and final disposal by incineration or direct disposal (McGrath, 1998; Blaylock and Huang, 2000; Sas-Nowosielska, 2004; Gosh and Singh, 2005). United States Environmental Protection Agency {USEPA} (1997), reported some economic problems associated with phytoremediation. As an emerging technology, standard cost (an estimated and predetermined cost of phytoremediation under normal conditions) such as the estimate of $1 to $2 million or more depending on the size of land is not readily available. Subsequently, the ability to develop cost comparisons and to estimate project costs will require determination on a site-specific basis, and this is often influenced by the potential application and the cost comparisons to other methods (USEPA, 1997).

New studies (Lievens *et al*., 2008; 2009; Michal *et al*., 2012) showed that heavy metal contaminated biomass can be converted to biofuel and other useful products. These studies reported the conversion by fast pyrolysis at high temperature (623-873 K) of heavy metal contaminated willow resulting from phytoremediation with high concentrations of Pb, Cd, Cu and Zn to heavy metal free biofuels and wide range of useful products which include aliphatic and aromatic hydrocarbons, esters, ethers, acids, aldehydes/ketones, N-compounds and S-compounds. However, the charcoal/ash fraction contained heavy metals at low concentrations ranging from 60-400 mg/kg (Michal *et al*., 2012). Leviens *et al*., 2009 suggested the future use of this method to reduce pollution and production of other valuable products.

2.4 TRANSPORT AND UPTAKE OF LEAD BY PLANTS.

A root-shoot interaction is important in determining the overall response of plants to uptake and acquisition of nutrient and contaminant from the soil (Bassisirad, 2000). Under moderately acidic conditions (pH < 5.5), Pb cations are more mobile, while the anions tend to sorb to mineral surfaces (GWARTC, 1997). The reverse is the case under basic conditions (pH > 7) within the soil matrix. Pb uptake varies significantly over its concentration range and is dependent upon the forms and species of Pb that occur in soils (Kabata-Pendias, 2010).

Plants have evolved highly specific mechanisms to take up, translocate and store nutrients and this has implication for how plants deal with toxins such as Pb (Lasat *et al*., 1998). Metal movement across biological membranes is mediated by proteins with transport functions (Lasat *et al*., 1996). The uptake of Pb involves specific membrane transporters binding with specific ions which may be monovalent or divalent cations (Lasat *et al*., 1996; 1998). However, Pb may mimic certain divalent cations such as calcium (Ca) which is of important metabolic significance (Millstone, 1997). This could enhance Pb transport in plant tissues by binding to specific Ca transporters (Lasat *et al*., 1998). Kalavrouziotis *et al*., (2009) have reported antagonistic, synergistic and biphasic interactions between some essential macro elements and Pb in *Brassica oleracea* (Brussel sprout) grown on soil irrigated with treated municipal waste water. Lead translocation from roots to shoot is however greatly limited (Zimdahl, 1975).

A number of genes responsible for metal transport in plants have been identified to play crucial role in tolerance and uptake of heavy metals (Lasat *et al*., 1998; Kumar *et al*., 2012). For example the ZIP1-4, ZNT1, IRT1, 2COPT1-5, AtNramp1/3/4, LCT1 and CNGC metal transporters present in the plasma membrane cytosol interface in plants (Hall and Williams, 2003). Some of these transporters are also present in the Golgi apparatus and Endoplasmic recticulum (ER), which are plant transport systems involved in the acquisition, distribution and homeostasis of toxic metals (Kumar *et al*., 2012). These gene families corresponding to these transporter are large and diverse, thus helping plants to cope with various types of heavy metal stress and movement of metal ions at both cellular and sub-cellular levels (Kumar *et al*., 2012). These gene families also provide high affinity for metal ions and their transport in plants (Kumar *et al*., 2012).

Malone *et al*. (1974) identified Pb deposits in cell walls outside the plasma lemma or plasma membrane (cortical cytoplasmic region) as it precipitates and crystals. However,

Meyers *et al*. (2008) reported that root tissue uptake of Pb is mainly intracellular and it is aggregated in cell vacuoles. This deposition of Pb is important for its subsequent accumulation in root tissue. The mechanism of metal uptake by hyperaccumulator plants and the basis of their metal specificity are poorly understood; the phytochemistry involved varies considerably depending on the plant species (Brooks, 1998). Several studies have suggested that metals are detoxified in hyperaccumulators by binding with low molecular weight ligands such as histidine (Kramer *et al*. 1996). A study of two species of *Thlaspi*, (*T*. *caerulescens* and *T. rotundifolium*) showed that Pb is more concentrated in roots than shoots, thus indicating low mobility of Pb from the roots to the shoots and immobilization of the heavy metal in the roots (Safae *et al*., 2008). Uptake of Pb occurs passively with mass flow of water through the root by active transport across plasma membrane of root epidermal cells (Yoon *et al*., 2006). Schwartz *et al*., (1999); Pierret *et al*., (2005), reported that the uptake of contaminant from the soil by plants may depend on root structure and functional architecture. The diagram of a typical root showing the xylem, phloem and other sieve structures involve in transport and translocation of water and mineral elements is shown in Figure 2.4.1

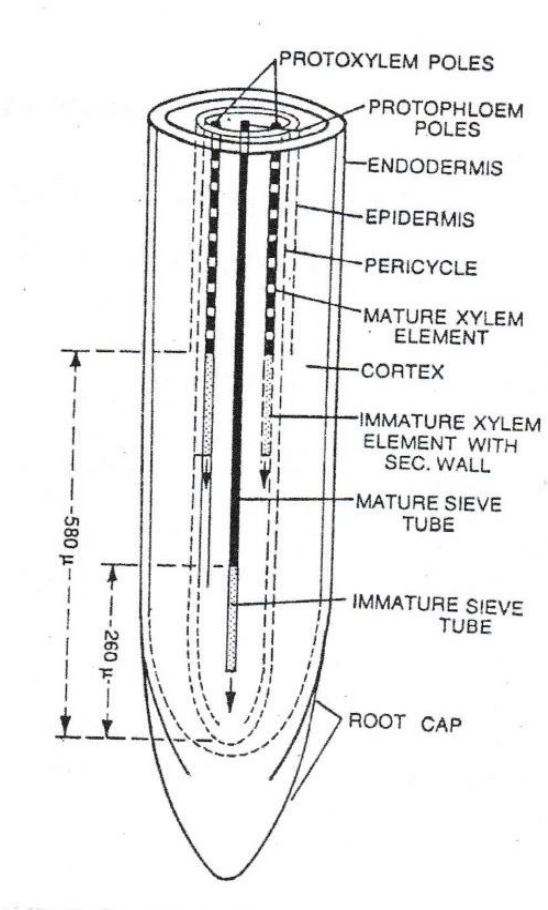


Figure 2.4.1: Longitudinal section (L.S) of a typical root. (Source: Pandey, 2005 with permission from the S. Chand Publishing, April, 2015).

All plants take up metals in varying degrees from the substrates in which they are rooted (Rotkittikhun *et al*., 2006). Diehl et *al*. (1983) observed that Pb–tetra alkyls from petrol additives in soils are quickly converted to water-soluble Pb compounds that are easily available to plants. As a result of this, vegetation grown in soils polluted with tetra alkyls show relatively large Pb enrichment in both vegetative and generative organs.

Baker (1981), proposed two basic strategies by which higher plants can tolerate large amounts of metals in their environment: (i) bioaccumulation in the upper plant parts at both high and low soil concentration, (ii) Exclusion related to restricted metal transport, thus allowing accumulation in roots over a wide range of soil concentration. Mishra *et al*., 2006 reported that exclusion is the first defence strategy of plants to metal contamination.

However, more recent studies suggest that plants have developed a wide range of tolerance mechanisms that are activated in response to Pb exposure. Fahr *et al.*, (2013) reported that Pb affects plants primarily through their root systems and plant roots rapidly respond either (i) by the synthesis and deposition of the plant polysaccharide callose, creating a barrier that stops Pb entering (ii) through the uptake of large amounts of Pb and its sequestration in the vacuole accompanied by changes in root growth and branching pattern or (iii) by its translocation to the aboveground parts of plant in the case of hyperaccumulator plants. The synthesis of mechanical barriers such as callose as a defence against Pb stress in plants has been reported (Bacic *et al*., 2009; Krzeslowska, 2009; 2011; Samardakiewicz *et al*., 2012).

2.4.1 Factors affecting plant uptake of lead.

The chemistry of metal, anions, minerals, soil organic matter (SOM) and colloids interaction within the soil matrix is central to the phytoremediation concept (Lasat, 1998). Several factors affect the bioavailability and uptake of lead by plants. These include the following:

pH

Soil pH is a significant parameter controlling the uptake of metal contaminants. It is one of the major soil factors controlling metal bioavailability. A soil pH > 7 may precipitate Pb as hydroxides, phosphates, carbonates as well as promote formation of Pb-organic complexes that are rather insoluble (Kabata-Pendias, 2010). Decreasing pH will often increase Pb solubility and mobility. At lower pH (i.e < 6), lead in the soil has greater potential to translocate from roots to shoots. Chardon *et al*. (2008), observed Pb take up by clay mineral surface at higher pH (>7) forming a discreet phase of hydrocerussite. It is well known that acidity affects plant growth and uptake of nutrients directly and

indirectly, but normal plant growth can be achieved at a pH range of 5-7 (Shu *et al*., 2001).

Soil Organic Matter (SOM)

Soil organic matter (SOM) is a component of the soil,which is made up of plant and animal residues at the their different stages of decomposition (Brady and Weil, 1999; Kabata-Pendias, 2010). It influences the physical and chemical properties of the soil. It is involved in the improvement of soil structure and aggregation, water retention, absorption and retention of contaminants in soil, provision of buffering capacity, cycling and storage of plant nutrient (Brady and Weil, 1999;Troeh and Louis, 2005). SOM increases soil fertility and can enhance uptake of nutrient and contaminants by providing cation exchange sites for macronutrients, micronutrients and heavy metals (Troeh and Louis, 2005). It also act as plant nutrient reserve and trace elements released via the process of SOM mineralisation (Troeh and Louis, 2005; Kabata-Pendias, 2010).

SOM is a major sink of soil carbon (Jain *et al*., 1997). It is composed of humic and non–humic substances. Soil organic matter has a great sorption capacity, which is beneficial in reducing the activities of of an excess trace metals (Kabata-Pendias, 2010). The adsorption of some metals such as Ni, Cu, Pb and Cd is significantly enhanced by humic substances in the soil (Laxen, 1985). Humic substances are easily adsorbed by clay and oxides in soil. Kabata-Pendias, 2010 reported that SOM plays an important role in the adsorption/ co-precipitation of most trace elements in soil. SOM is also known to incease the cation exchange capacity of the soil (Sholkovitz and Copland, 1981). Juma, (1999); Brady and Weil, (1999); Troeh and Louis, (2005); Kabata-Pendias classified humic substances into three viz:(i) fulvic acid (fulvate), which have low polymerization capacity, high acidity and mobility, (ii) humic acids (humates) with medium acidity and mobility, spherocolloidal polymerization ability and soluble in alkali and (iii) humins (a generic name for materials with highest molecular weight that are darkest in colour, insoluble in acids or alkali and most resistant to microbial attack). Humins are aging products of humates and fluvates with high degree of polymerization and low acidity (Kabata-Pendias, 2010). Metal-fulvic acid complexes with lower stability constants usually are more readily soluble and bioavailable to plants (Kabata-Pendias, 2010). Kabata-Pendias, (2010) reported that the Interactions between humic substances and metals such as Pb have been described as ion exchange, surface sorption, chelation, coagulation and peptization.

Basta *et al*. (2005), reported that the atomic properties of Pb has strong affinity for SOM and formation of inner-sphere metal surface complexes. Soil organic matter is one of the

most important factor increasing Pb bioavailability (Jin *et al*., 2005). Stevenson (1983) reported that organic substances play important role in biochemical weathering and geochemical cycling of nutrients. It has been observed by Cheng (1977) that organic matter (OM) content of soil has a complex influence on the behaviour of trace elements.

Cation Exchange Capacity (CEC)

Cation exchange capacity is the number of exchangeable cations per dry weight that a soil is capable of holding at a given pH value and available for exchange at the soil-water solution (Robertson *et al*., 1999). It is a measure of soil fertility, nutrient retention capacity and buffering capacity (Mengel, 2011). Organic matter (OM) increases soil CEC by increasing the number available negative charges. Cation exchange capacity increases with increasing pH and this influences metal bioavailability, speciation and uptake (Mengel, 2011).

Metal speciation in general is influenced by their cation exchange capacity. The rate of trace elements downward migration is affected by CEC (Kabata-Pendias, 2010). The affinity of trace elements for soil constituents is strongly influenced by their electrochemical properties and closely related to the specific surface area and CEC of minerals (Kabata-Pendias, 2010). Similarly, it was observed by Tan (1998), that variable charge of both clays and organic particles enhances the formation of different organo-mineral complexes which greatly influence mobility in the soil.

Soil Microorganisms.

Root growth affects properties of the rhizospheric soil and stimulates the growth of microbial consortium (Lasat, 1998). According to Crowley *et al*. (1991), some microorganisms excrete organic compounds which increase bioavailability and facilitate root absorption of metals. Microorganisms are very important in the production, consumption and transportation processes in the ecosystem. Jaisi *et al*. (2008) reviewed that microbe-clay interactions are responsible for biological reduction of Fe^{3+} to Fe^{2+} which has great affinity for the surface–complexation sites of Pb thus increasing mobility in natural environment. A low rate of decomposition of vegetation having a high concentration of Pb and Zn is apparently due to reduction by microbial activities (Williams *et al*., 1977). A strain of *Pseudomonas maltophilia* was shown to reduce the mobility of toxic Cr^{6+} to non-toxic Cr^{3+} and also minimize environmental mobility of other toxic ions such as Hg, Pb, and Cd (Blake *et al.,* 1993; Park *et al.,* 1999).

Fahr *et al*., (2013) reported that Pb uptake is greatly affected by rhizospheric processes. Lin *et al*., (2004) explained that the ability of *Oryza sativa* L.(rice) to absorb high levels of Pb from the soil by a decrease in soil pH was due to root exudates, solubilisation of Pb by rhizosphere microorganisms and complexation of Pb with organic matter at the soil–root interface. Larger amounts of NH_4OAc extractable Pb are found in the rhizosphere than in bulk soil, pointing to the involvement of root activities in changing Pb availability (Lin *et al*., 2004).

Mycorrhiza is the non-pathogenic, mutualistic symbiotic association of obligate soil-borne fungi with the roots of higher plants. Pawlowska and Charvat, (2004); Ratti and Upadhyay, (2013) reported some functions of mycorrhizae in heavy metal uptake from the soil. This includes their help in increasing plant tolerance to heavy metal and the provision of an attractive system to advance plant-based environmental clean-up. The hyphal network of mycorrhizae functionally extends the root system of their plant hosts, thus, increasing the potential to take up heavy metal from an enlarged soil volume by enhancing root absorption area of plants (Ratti and Upadhyay, 2013). Mycorrhizae facilitate the establishment and survival of vegetation under heavy metal stress conditions and heavy metal chelation using compounds produced by the extra-radical mycelium (Ratti and Upadhyay, 2013). Metal contaminant in soil can be bound to mycorrhizae using free amino, hydroxyl, carboxyl and other groups present in its fungal cell wall which suggest that microbial biomass may affect the mobility of metals in the soil system (Pawlowska and Charvat, 2004; Ratti and Upadhyay, 2013).

Root Exudates.

Root exudates play an important role in the uptake of several essential elements and contaminants by plants (Lasat, 1998). Some grass species have been reported to exude from their roots a class of organic acids called siderophores (mugenic and avenic acids), which caused significant enhancement of bioavailability of soil-bound iron and zinc (Kanazawa *et al*., 1994; Cakmak, 1996a; 1996b). Studies by Pellet *et al*. (1995) and Larsen *et al*. (1998) demonstrated that some plant exudates are involved in plants tolerance through exudation of citric and malic acids in tolerance to aluminum in the soil. Rotkittikhun *et al*., (2006) suggested an influence of exudates on speciation of Pb in Pb-accumulating species like *Ageratum conyzoides* (goatweed)*, Sonchus arvensis* (corn sow thistle) and *Euphorbia hirta* (asthma plant). The uptake of Pb is based mainly on the plant species (e.g *Oryza sativa* {rice}) interaction between roots structures, synthesized exudates and the rhizosphere biochemical properties (Brown, 1995; Shadid *et al*., 2012; Fahr *et al*., 2013).

Root/foliar uptake.

The root zone is the primary area affecting the mobilization of Pb (USEPA, 2000). Remediation of Pb with plants requires contaminants contact with the root zone of the plants. The rate of trace element uptake will positively correlate with its available pool at the root surface (Kabata-Pendias, 2010). Foliar uptake is governed by surface properties of leaves and aerial deposition though concentrations found in shoots may be partly translocated from the root (Kabata-Pendias, 2010).

Dalenberg and Van Driel (1990), have calculated 73-95% of total Pb content in leaves of some field crops arose from aerial deposition of trace metals on the leaf surfaces. A fraction of the trace elements absorbed by the leaves may be leached from plant foliage by rain water. For example, Pb can be partly washed off from the leaves suggesting some superficial deposition on leaf surface (Kabata- Pendias, 2010). Kabata-Pendias (1969) and Little and Martin (1972) in Kabata-Pendias, (2010), observed a greater leaf penetration for Cu ,Zn and Cd than for Pb. Translocation of metals can be metabolic and non-metabolic, usually primarily controlled by root pressure and leaf transpiration (Lasat, 1998). Lin *et al*., (2004) reported the ability of roots to modify the mobility and the bioavailability of Pb by changing rhizospheric conditions which can significantly enhance the success of phytoremediation programme.

2.4.2 Quantification of plant uptake.

A plant's capacity to accumulate metals from the soils can be expressed by a concentration factor (CF) (Safae *et al*., 2008). It is defined as the concentration of a particular chemical in a biological tissue per concentration of that chemical in the tissue surroundings (Abdul and Bivin, 2009). Several terms has been used in different studies. In certain studies, concentration factor is also known as phytoextraction or bioaccumulation factor (Baker, 1981; Safae *et al*., 2008; Akinci *et al*., 2010). It is estimated for Pb as the ratio of Pb concentration in the aerial + below-ground part of plants and soil Pb concentration (both expressed on a dry weight (DW) basis), and expressed mathematically as (Rotkittkhun *et al*., 2006).

$$CF_{total} = \frac{\text{Concentration of Pb in shoots and roots} \frac{\text{mg}}{\text{kg}} \text{DW}}{\text{Concentration of Pb in soil} \frac{\text{mg}}{\text{kg}} \text{DW}}$$

$$CF_{total} = \frac{\text{Cshoot and root}}{\text{Csoil}} \quad (2.4.1)$$

Where

$C_{shoot\ and\ roots}$ = concentration of Pb in shoots and roots (mg/kg) (DW)

C_{soil} = concentration of Pb in soil (mg/kg) (DW).

$$CF_{shoot} = \frac{\text{Concentration of Pb in shoots} \frac{mg}{kg}\ \text{DW}}{\text{Concentration of Pb in soil} \frac{mg}{kg}\ \text{DW}} \quad (2.4.2)$$

$$CF_{root} = \frac{\text{Concentration of Pb in the roots} \frac{mg}{kg}\ \text{DW}}{\text{Concentration of Pb in the soil} \frac{mg}{kg}\ \text{DW}} \quad (2.4.3)$$

Lead translocation in plants from root to shoot can be measured using translocation factor (TF) which is given below:

$$TF = Cs/Cr \quad (2.4.4)$$

Where, C_s and C_r are metal concentrations (mg/kg) in the shoot and root, respectively.

According to Rotkittkhun *et al*. (2006), the standard definition for CF and TF of accumulators and hyper accumulators have not been identified. However, CF have been categorized further as hyper-accumulators, accumulator and excluder to those samples with CF >10, >1 and <1, respectively (Reeves and Baker, 2000). At present, four criteria are used: (i) Concentrations of heavy metals in plants shoots (lead > 1000 mg/kg; Baker *et al*., 1994), (ii) If the concentration of heavy metal in above ground part is 50-500 times more than in usual plants (i.e Pb >5 mg/kg; Shen and Liu, 1998), (iii) The metal concentration in shoots are invariably greater than that in roots, or shoots/root quotient >1 (Baker and Whiting, 2002), (iv). Phytoextraction coefficient >1(Chen *et al*., 2004). Phytoextration coefficient is the logarithmic function ($Y=\log_e C_{shoot+root}/C_{soil}$) of the CF equation {Equation 2.4.1 above} (Safae *et al*., 2008; Abdul and Bivin, 2009). Where Y= Phytoextraction coefficient. Wherein, TF > 1 indicates that the plant translocate metals effectively from root to the shoot and are considered a hyperaccumulator (Baker and Brooks, 1989).

Ulrich (2003) further identified metal precipitators, indicators and tolerant species. **Excluders** can regulate the flow of toxic metals to some parts of the plants using certain

physiological and biochemical mechanism of the plants (Lasat, 1996 and Baker, 1981). **Indicators** are tolerant to elevated concentrations until a threshold is reached or exceeded resulting in chlorosis in some plants while **tolerant** species are able to take up metal above threshold with mild to no observable effect of the contaminant (Ulrich, 2003; Kareen *et al*., 2013).

2.4.3 Lead Accumulating Plants.

Plants which accumulate heavy metals are known as metallophytes. Metallophytes can differ largely in their heavy metal contents (Bothe *et al*., 2010). Several plants show potential for Pb accumulation from the soil (Baker and Brooks, 1989). All plants have the ability to accumulate “essential” metals (e.g. Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Se, V and Zn) from the soil, although different concentrations are required for growth and development (Chhotu and Fulekar, 2009). This ability also allows them to accumulate some other “non-essential” metals (Al, As, Au, Cd, Hg, Pb, Pt, Sb, Te, Ti and U), which have no known biological function (Djingova and Kullef, 2000). Some have evolved tolerance to large amounts of metals in their environment through exclusion, inclusion and bioaccumulation (Baker, 1981).

Safae *et al*. (2008) reported that Pb is accumulated in roots of two ecotypes of *Thlaspi caerulescens* in West Morocco. Potential hyperaccumulator species such as *Armeria maritime* (sea pink)*, Arabidopsis halleri* (rockcress)*, Ambrosia artemisiifolia* (ragweed)*, Brassica napus* (oil seed rape)*, Brassica juncea* (Indian mustard)*, Brassica oleracea* (including common cultivars such as cauliflower, broccoli, cabbage, kale, Brussel sprout)*, Festuca ovina* (sheep fescue)*, Helianthus annus* (sunflower)*, Thlaspi rotundifolium* (round leaved pennycress), *Triticum aestivum* (bread wheat) and *Zea mays* (maize or corn) have been reported (Baker *et al*., 1994; Deram and Pettit, 1997; Reeves and Brooks, 1983; Bert *et al*., 2000). The most frequently cited Pb hyperaccumulator is the cultivar *Thlaspi rotundifolium* (L). Gaud–Beaup (round leaved penny-cress) which can accumulate a shoot Pb concentration of 8500mg/kg (Reeves and Brooks, 1983). However, *Thlaspi rotundifolium* has a small biomass and slow growth rate. *Brassica juncea* (L) Czern also demonstrated an ability to accumulate Pb to a higher degree when grown in a nutrient solution that had high concentration of soluble Pb as Pb $(NO_3)_2$ as much as 1.5% (m/v) of Pb (Kumar *et al*., 1995). It showed little ability to translocate Pb to its shoots when grown on soils where Pb^{2+} bioavailability was limited.

Baker and Walker (1989), reported a Pb accumulation of 130-8200 mg/kg shoot dry weight of *Thlaspi rotundifolium*. Barry and Clark (1978), recorded shoot lead values of

13 to 11,750 mg/kg in *Festuca ovina*. Shinwell and Laurie (1972) also recorded a value of 2740 mg/Kg in the roots of *Thlaspi caerulescens* colonizing a lead mine district in the Pennines, England. Tanhan *et al*. (2007) reported Pb concentration of over 1000 mg/kg in the shoot and 30453 mg/kg in the roots in Siam weed (*Chromolaena odorata* (L.) (Siam weed) growing in an ore dressing plant in Bo Ngam, Thailand. Thanh *et al*., 2013 reported 898 to 2,850 mg/kg in shoots compared to 65 to 90 mg/kg in the roots of *Biden pilosa* {L} (Spanish needle) and *Ludwigia adscendens* {L} (water primrose) respectively growing on contaminated soils in Vietnam. Tables 2.4.1 and 2 .4.2 show some Pb accumulator plants, concentration of Pb in roots and shoots, concentration factor, translocation factor and land use.

Table 2.4.1: Lead concentrations (mg/kg DW) in shoots and roots of selected plant species grown in nutrient solution (hydroponics) 20µm/l Pb and soil contaminated with 2500 mg/kg lead in the form of lead nitrate. (Huang and Cunningham, 1996).

Plant species	Shoots Solution (mg/l)	Roots Experiment	CF_{total}	Shoots Soil experiment (mg/kg)	Roots	CF_{total}
Zea mays cv. Fiesta	375	2,280	0.16	225	1250	0.59
Brassica juncea 531268	241	19500	0.02	97	3460	1.42
Brassica juncea Czern	65	9580	0.01	45	3580	1.45
Brassica juncea 184290	32	5260	0.01	30	2310	0.9
Thlaspi rotundifolium	226	28700	0.01	79	6350	2.57
Thlaspi caerulescens	64	26200	0.002	58	5010	2.03
Ambrosia artemisifolia	95	4670	0.02	75	2050	0.85

Key**: CF_{total} varied between species and growth media type (Pb contaminated soil medium and hydroponic solution) with generally higher CF_{total} observed in the Pb contaminated soil medium than the Pb hydroponic solution.**

Table 2.4.2: Average lead concentrations in soil, shoot and root (mg/kg dry weight) from sites 1-4 in Thailand. 1-open pit area, 2-Stockpile area, 3-Ore dressing plant, 4---Tailing pond. (Adapted from Rotkttikhum *et al*., 2006)

Family	*Scientific name*	Land use	Type	Pb in soil	Pb in shoot	Pb in root	CF_{total} & TF
Asteraaceae	*Ageratum conyzoides*	4	Herb	38776	3183	4446	0.2 0.7
	Chromolaena odoratum	1,2,3	Shrub	118967	3520	9870	0.2 0.3
	Crassopcephalum crepidiodes	2,4	Herb	40622	1906	7903	0.2 0.2
Cyperaceae	*Cyperus difformis*	2	Herb	95500	1310	6000	0.08 0.1
Equisetaceae	*Equisetum debile*	1,2	Herb	78890	1505	21025	0.64 0.1
Euphorbiaceae	*Euphorbia heterophylla*	2	Herb	31830	6700	15130	0.30 0.4
Poaceae	*Imperata cylindrical*	1,2,3	Grass	106640	1430	10923	0.12 0.1
	Microstegnum cilliatum	3	Grass	175170	12200	128830	0.81 0.1
	Pennisetum polystachyon	2,3,4	Grass	104860	6205	24705	0.15 0.3
	Phragmites vallatoria	1	Grass	111670	403	17170	0.14 0.02
Fabaceae	*Vigna umbellata*	1,2	Herb	64750	2857	10330	0.20 0.3
Malvaceae	*Sida rhombifolia*	3	Undershrub	175500	9070	99670	0.62 0.1
Polygalaceae	*Polygala umbonata*	3	Climber	175500	21670	14580	0.21 1.5
Rubiaceae	*Spermacoce mauritiana*	3	Herb	172500	28370	78830	0.62 0.4

Key: CF_{total} and TF varied between plant species/families and land use. Land use shows high soil Pb concentration with generally higher soil Pb in predominantly used areas such as (1) –open pit area, (2) stockpile area and (3) ore dressing. The plant shoot and root Pb concentrations is also dependent on the soil Pb concentrations of these areas.

2.5 REVIEW OF POTENTIAL LEAD ACCUMULATING PLANT SPECIES.

This section reviews potential Pb accumulating plants, as part of the literature review (continuing from section 2.4.3) but also with the more specific aim of enabling the selection of suitable plant species for the first pot experiment (Chapter 4). Plants discussed in this review have shown potential to accumulate Pb in their tissues at varying concentrations. The amount of Pb taken up by plant species is partially dependent on the concentration of Pb in the polluted soil (Bothe *et al*., 2010). In most Pb accumulating plants, Pb is predominantly accumulated in the roots. However, in some species, the concentration of Pb in shoots can be particularly high and the partitioning of Pb between shoots and roots differs from one metallophyte to another (Kramer, 2010). Increased interest has been shown in plants with unusual potential for accumulation of more than 10,000 mg/kg Zn and Mn, 1000 mg/kg Al, As, Se, Ni, Co, Cu, Cr, Cu and Pb and 100mg/kg Cd in their above ground biomass known as hyperaccumulators (Reeves and Brooks, 1983). As mentioned in Section 2.4.3, there are some species that have been reported to hyperaccumulate Pb, such as *Thlaspi rotundifolium* ssp.*cepaeifolium* which can accumulate 8500 mg/kg Pb in its shoots (Reeves and Brooks, 1983) and *Thlaspi caerulescens* which was investigated in this study as a representative of this genus. *Thlaspi rotundifolium* is typically found growing in Zn and Pb mining regions (Likar and Pongrac, 2010). This species (*Thlaspi rotundifolium*) will not be discussed further here due to its very small biomass (<1g/plant), which makes experiments on the effects of heterogeneity technically impractical, and to a lesser extent due to the very poor availability of its seeds.

Enhancement of biomass production in hyperaccumulator plant species such as the genus *Thlaspi* is currently a subject of current debate and potential area of future research. For example Lasat *et al*., (1998); Kumar *et al*., (2012) have suggested the potential enhancement of biomass in *Thlaspi caerulenscens* (*Noccaea caerulescens*) and *T*. *rotundifolium* through genetic manipulation. Kumar *et al*., (2012); Ahmed *et al*., (2012) reported that the genes responsible for rapid growth, well developed root system and luxuriant aboveground biomass in *Brassica* species (e.g *Brassica juncea*, *Brassica rapa* and *Brassica napus*) could be genetically modified to enhance biomass production in potential heavy metal hyperaccumulator species with low biomass. Such genetic manipulation has been achieved for improving heavy metal tolerance in some plant species (Anjum *et al*., 2012) Reisinger *et al*., (2008) have reported the transfer of the gamma-glutamylcysteine {*y*-ECS} and gluththione synthase {GS} (a heavy metal tolerant genes) from a transgenic bacteria (*Escherichia coli*) to *Brassica juncea*. This *y*-

ECS and GS gene produce Y-Glu-Cys synthase responsible for improving tolerance against Pb, As, Cd and Zn in *B. juncea*. However, the potential limitation of the genetic manipulation of plants to improve heavy metal tolerance and biomass for efficient phytoremediation is the unknown specificity of such genes {e.g potential pathogenicity of bacteria genes apart from enhancement of heavy metal tolerance and biomass production} (Reisinger *et al*., 2008; Kumar *et al*., 2012).

The selection of plant species for this review were based on the following criteria:

1. Ability to accumulate Pb in their shoots and roots with specific reference to their concentration factor (CF) expressed as the ratio of Pb concentration of the shoot and roots to that in the soil, and translocation factor (TF) as the ratio of Pb in the shoot to that in the roots (see definitions in Section 2.4.2).
2. Root mass, lateral size, depth and morphology in comparison with scales of heterogeneity to be investigated.
3. Whether species is native to field areas where heterogeneity can be quantified. However, some non-native plant species may be useful in pot trials.
4. Practicability of obtaining seed and growing these species or varieties in pot trials. The key characteristics of each of plant species are compared (Table 2.5.1) and the two species selected for the third and fourth pot trials are discussed in Chapter 5: Sections 5.5.1 to 5.5.2.

Table 2.5.1: Lead accumulator species reviewed with those selected for the first pot trial in red

Plant species	Growth period/height	Pb concentration mg/kg DW	CF_{total}/TF	Root mass	Root depth	Growth conditions	Growth medium	Seed availability	References
***Brassica juncea* (Indian mustard)**	40-60 days/1-2m [5]	9,580 mg/kg-roots, 3,580 mg/kg-shoots [4]	1.7/0.4 [4]	Denseroot mass[1, 5]	90-120 cm [1]	Biennial, pH4-8, moderately tolerant to soil acidity, 500-4200 mm rainfall, temperature 6-27^{O}C [4]	Soil [4]	Available [61]	1-Woods *et al*., 1991; 2-Huang & Cunningham 1996; 3-Hemmingway, 1995; 4-Meyers *et al*., 2008; 5-Duke, 1982
***Brassica napus* (Oil seed rape or rapeseed)**	40-60 days/ 1-1.5m [9, 6]	984 mg/kg-roots, 354 mg/kg –shoots [11]	0.8/0.4 [11]	Branched tap root may be present depending on how it is grown, root diameter is 15-17 mm [8, 9]	60-90 cm [10,]	Requires full to partial shade, pH 5-7, grown in sandy loam to clay loam soil [7]	Soil [11]	Available [61]	6-Potts *et al*., 1999; 7-Chardin *et al*., 2001; 8- Chimbira and Moyo, 2009; 9-Crook and Ennos, 1993; 10- Ennos *et al*., 2001, 11-Carlson and Bassaz, 1997
***Thlaspi caerulescens* (Alpine pennycress)**	Winter or summer annual plant/ 10-20cm [12]	5,010 mg/kg-root, 58 mg/kg – shoots [17]	2.0/0.01 [17]	Tap root present with normal trivalent branching structures in the soil. Root morphology differs with different growth materials used depending on contamination gradient [15, 16]	5-15 cm [13]	Grow on contaminated soils and disturbed areas[14, 16]	Hydroponic [17]	Available [61]	12-Brown *et al*., 1995a, 13-Haines, 2002;14- Schwartz *et al*., 1999a; 15-Chaney *et al*., 1997; 16-Cleal, 1994; 17-Huang and Cunningham., 1996
***Rumex acetosa* (sorrel)**	Annual herbaceous plant native to the British isles/20-55 cm [21]	345 mg/kg—root, 115 mg/kg—shoot [20]	0.1/0.3 [20]	Large yellow, forking and long tap root, forms basal rosette at the shoot [19]	40-60 cm19	Small requirement for growth, found in forests, meadows, parks and wastelands [18]	Soil [20]	Available [61]	18-Atila and Mathe-Gaspus, 2005; 19- Davidson, 2006; 20-Gaweda, 2009; 21-Allen and Hafield, 2004,

***Helianthus annus* (sun flower)**	Warm season annual plant native to North America/1.5-2m[25, 22]	844 mg/kg—roots, 358 mg/kg—shoots [24]	0.1/0.4 [24]	Tap root [23]	25 cm [23]	Survive periods of drought and perish after frost. Does not tolerate partial shade. Prefers neutral to slightly alkaline soil. 25, 22	Hydroponically [24]	Available [61]	22-Jean, 1994; 23-Shella *et al.* , 1974; 24-Dushenkov *et al.* , 1995;1997; 25-Motloch, 2000
***Zea mays* (maize or corn)**	Annual. 60-90days/2-3m [26]	398 mg/kg-root, 176 mg/kg—shoot [27]	1/0.4 [27]	Fibrous roots, substantial root biomass with lateral root and root hairs. 27	10-12 cm [30]	Facultative long night pant, greater than 10 ^{o}C of temperature, plant matures usually during the summer months, shoot biomass 3710kg/ha[28, 27]	Soil [27]	Zea mays japonica (sweet corn) available in the UK [61]	26-Wilkes, 2004; 27-Kalogerakis et al., 2005; 28-Brennan and Shelley, 2005; 29-Erwin *et al*., 2005; 30-Janice *et al*., 2010
***Bidens alba* (Shepherd needles, beggarticks, Spanish or butterfly needle)**	Annual, biennial and perennial shrub or tree/15-20cm [31]	214 mg/kg-root, 569 mg/kg-shoot 35	1.1/0.4 [35]	Tap root sometimes fibrous [33]	60 cm [33]	Grow on agricultural areas, coastland and disturbed areas [32]	Soil [34, 35]	Available [61]	31-Crowe and Parker, 1981; 32-USDA, 2008; 33-Weedon, 1973; 34-Wang *et al*., 2007; 35-Yoon *et al*., 2006
***Gentiana penneliana* (wiregrass or gentian)**	Annual, biennial and perennial shrub or tree, distributed in North-west Africa, Asia, East Australia and Europe/8cm [36]	2200 mg/kg-roots, 4100 mg/kg-shoots [37]	1/1.9 [37]	Fibrous primary roots with secondary rootlets, tout fleshy or woody taproot with several linear cylindrical roots forming a collar. [36]	80-120 cm [36]	Grow on contaminated soils. [36]	Soil [37]	Available and it takes about 4 weeks to germinate. [61]	36-Ting-nung *et al*., 1988; 37-Yoon *et al*., 2006;

***Oryza sativa* (rice)**	Tropical, subtropical and warm climate annual grass/1-2m [38, 39, 40]	6284 mg/kg-shoot, 6373 mg/kg—root [43]	1/0.9 [43]	Root system possesses aerenchyma tissues, consist of seminal adventitious and lateral roots, and possess trichoblasts extending to form roots hairs. [38, 40, 45, 44]	30-60 cm [45]	Grows best at summer temperatures of 24-25 ^{O}C, mostly cultivated in humid coastal lowlands and deltas. Arrested at growth temperatures < 10 ^{O}C, 42-49dm rainfall, pH 4-8 [41, 42]	Soil [43]	Available but imported from China and India and expensive. [61]	38-Duke, 1978; 39-Duke, 1982; 40-Cherepanov, 1995; 41-Duke and Ayensu, 1985; 42-Zhukovsky, 1971; 43-Rotkittikhun *et al*., 2006; 44-Chao-wen *et al*., 2006; 45-Duke, 1981: 61-Seeds online, 2012
***Vetiveria zizaniodes* (vetiver)**	Perennial grass native to India/1.5m, growth period 12-24 months [46, 47]	359 mg/kg-shoot, 4940 mg/kg [52]	1.5/0.1 [52]	Root system spread horizontally; possess vertical, growing tufts on the roots. Presence of secondary and tertiary fibrous roots. 48, 49	3-4 m [49]	Prefers fertile soils with a pH 4-7, temperature range of 15-20 ^{O}C, roots ready for harvest at 12-24 months, requires hot humid climate and highly drought tolerant. [50, 48]	Soil [52]	Available and imported from India between October and November. [61]	46-Andras *et al*., 2006;2011; 47-Andras et al., 2010a; 2010b; 48-Jackson, 2001; 49-Andras *et al*., 2009;50-Lavania, 2003; 51-Lavania *et al*., 1998; 52-Huang and Cunningham, 1996
***Chromolaena odorata* (Siam weed)**	Widely distributed Neotropical shrub native to tropical America/2.5m, 10m when climbing vegetation and 0.8m at 40 day. Growth period of 12 months. [53, 54, 59]	3,520 mg/kg-shoot, 9870 mg/kg-root [57]	1.9/0.4 [57]	Dense extensive root mass, fibrous shoot system is slightly toothed. [55, 58]	30 cm [60]	Invasive weed. Growth pattern varies in different ecosystem. [58]	Soil [57]	Not available in the UK. [61]	53-Schmidt and Schilling, 2000; 54-Ezeibekwe et al., 2010; 55-Cruttwell and Skarrat, 1996; 56-Kushwaha *et al*., 1981; 57-Rotikittikhun et al., 2006; 58-Chandrasekaran and Swamy, 2010, 59-Weed management guide, 2012; 60-Rouw, 1991:

2.5.1 Summary of comparison of plant species reviewed.

Thlaspi caerulescens had the highest CF_{total} value (Table 2.5.1; Figure 2.5.1), followed by *Chromolaena odorata*, *Brassica juncea*, *Vetiveria zizaniodes*, *Bidens alba*, *Gentiana penneliana*, *Zea mays*, *Oryza sativa*, *Brassica napus*, *Rumex acetosa* and *Helianthus annus* in order of decreasing CF (Figures 2.5.2). All plant species showed low TF (<0.5) except *G*. *penneliana* and *O*. *sativa.* *Chromolaena odorata* and *T*. *caerulescens* had the highest CF_{total} of all species (Figure 2.5.1 and Table 2.5.1). In terms of CF_{total} nearly all plant species except *Rumex acetosa* and *Helianthus annus* might be fit for selection for the first pot trial in this research.

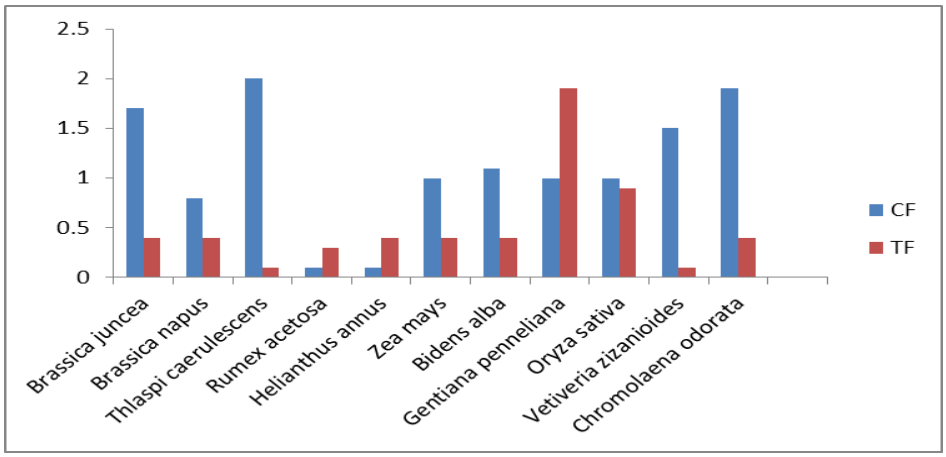


Figure 2.5.1: Comparison of total Concentration Factor (CF_{total}), Translocation Factor (TF) of Pb accumulating plant species in this review.

However, some of the species were not fit for selection considering growth conditions that can be achievable in the available facilities and time. They all possess substantial root masses and depths to enhance metal uptake, but *V*. *zizaniodes* and *C*. *odorata* have long (500 to 800 days) growth periods (Figure 2.5.2 and Table 2.5.1). The long period of growth rendered *V*. *zizaniodes* unfit for selection. *Chromolaena odorata* could be harvested at 40 days with a height of approximately 0.8 m, but the seeds were not readily available in the United Kingdom and seed viability was not certain. This precluded the selection of this species for the first pot trial, despite its high CF_{total}.

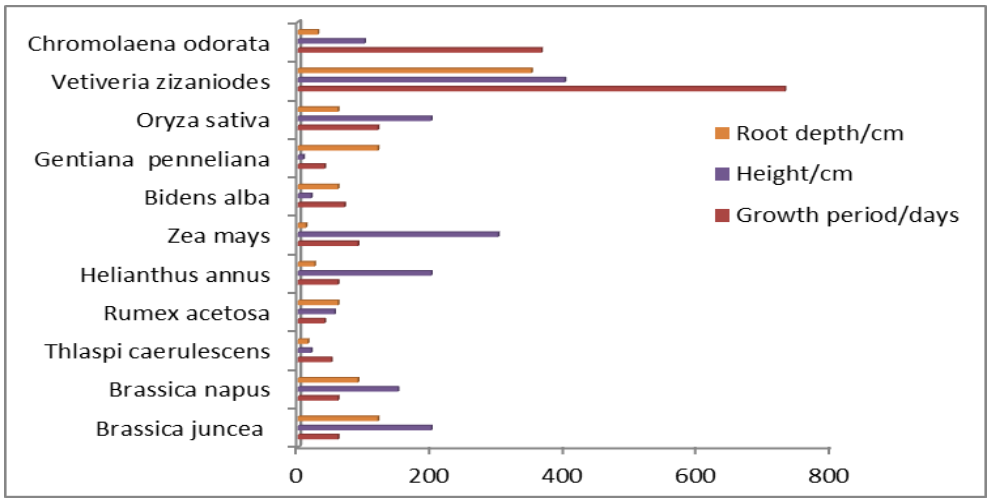


Figure 2.5.2: Comparison of Concentration root depth, height and growth periods of potential Pb accumulating plant species.

Two varieties of Oryza sativa seeds bred for metal uptake (Short duration variety- Pusa Jaldi Dhan-1 and medium duration variety- Pusa Basmati-1) were usually available, imported from China and India and sold at a high cost of €99 per pack of 20 seeds. Viability of the seeds in pot trial was not known. *Rumex acetosa* and *Helianthus annus* were not suitable for selection based on their CF_{total} 0.1 and TF values of 0.3 and 0.4 respectively. All plant species except *Thlaspi caerulescens* and *G. penneliana* have heights which suggest substantial biomass (Table 2.5.1). *Brassica napus* is a Pb accumulator plant considered fit for selection in the first pot trial because its similar root depth and growth period to that of *Brassica juncea* also fit for selection for the first pot trial. Six of these species viz *Brassica juncea, Brassica napus*, Gentianna *penneliana*, *Thlaspi caerulescens*, *Zea mays* and *Biden alba* were selected for the first pot trial.

2.6 HETEROGENEITY

This section gives a background literature on heterogeneity and how it has been previously expressed and quantified which is relevant to thesis aim to assess the effect of *in situ* heterogeneity of Pb on plant uptake.

Materials in the terrestrial environment are rarely homogenously distributed, either spatially or temporally and one consequence of this *in situ* heterogeneity is usually uncertainty in measurements made on that material (Taylor *et al*. 2005). Horwitz (1990) defined heterogeneity as a degree to which a property or constituent is uniformly distributed throughout a quantity of materials. Thompson (1999) stated that almost everything that is worth analysing is actually or potentially heterogeneous, and that any sample is likely to have a composition that is different from the mean composition of the target and therefore no two samples will have the same composition.

Ecologically, soil heterogeneity is described as the patchiness (the degree to which one patch differs from another) of soil components in relation to the size of the patch or scale (Hutchings and John, 2004). Myers (1997), described heterogeneity in relation to soils from a pile of soil. The pile may appear homogenous from a distance, but an inspection at a higher resolution reveals a range of colours, sizes, shapes, opacities and composition. This analogy relates to the *ex situ* study of soils, it is also applicable to the study of soils in an undisturbed *in situ* conditions. Spatial heterogeneity is ubiquitous in nature (Albert, 2000). Measurements of heterogeneous land surface processes are greatly influenced by measurement techniques and also by scales of sample, size, density and a real coverage of the domain (Albert, 2000).

In situ heterogeneity arises because each analyte is usually distributed heterogeneously in space within the sampling target. This has been subdivided into that between different types of mineral holding the analyte, which has been called ‘constitutional’ heterogeneity, and that caused by non-random spatial distribution of each mineral phase, called ‘distributional’ heterogeneity (Gy 1992). *In situ* heterogeneity, of either type, is often different at each spatial scale (e.g., ranging from µm to km). Gy (1992) described further these two types of heterogeneity: constitution heterogeneity and distribution heterogeneity.

i. **Constitution heterogeneity**: This is defined as the intrinsic property of materials that consist of different types of particles and can be estimated theoretically from the material properties if they are known (Gy, 1992).
ii. **Distribution heterogeneity**: It refers to the concentration of the determinand within a lot varying systematically along time or distance i.e. an expectance value

of determinand is a function of time and/or the location where the sample was taken (Gy, 1992). This type of heterogeneity and the sampling variance it generates can be estimated experimentally (Gy, 1992).

Ex situ heterogeneity usually arises from chemical preparation of test samples. The analytical uncertainty (U_{anal}) is usefully defined to include any chemical preparation of the test sample, and therefore includes the contribution from the *ex situ* heterogeneity of laboratory samples.

2.6.1 Consequences of heterogeneity.

Large uncertainties generated in most contaminated land investigation is one of the consequences of soil heterogeneity. Uncertainty has been defined as an estimate attached to a test result which characterizes the range of values within which the true value is asserted to lie (ISO, 1993). Although sampling and analytical errors may cause variability in measurements, Ramsey and Argyraki (1997) observed heterogeneity as the most often and main contributor to uncertainty in measurement of contaminants. Measurement uncertainty estimated using standard deviation includes both field sampling and chemical analysis. Measurement uncertainty also refers to all the variance that arises from both random and systematic errors from both sampling and analytical methods in geochemical soil surveys excluding geochemical variance (Ramsey, 2010). This can be estimated using the equation 2.6.5 below.

$$U=s_{meas}=\sqrt{(s^2_{samp} + s^2_{anal})} \qquad (2.6.5)$$

The variance arising from sampling (s^2_{samp}) which is primarily caused by heterogeneity is often the dominant factor in the estimation of measurement uncertainty (Argyraki *et al*., 1997, Taylor *et al*., 2005). The difference between individual sample means ($\bar{x}$) for a particular site investigation can be estimated using the variance (s^2) (Taylor *et al*., 2005). By taking duplicate samples from sampling locations, the variance of sampling in s^2_{samp} equation 2.6.5 can be estimated thus isolating the variance that arises from heterogeneity. This is represented in equation 2.6.6

$$U=s_{samp}=\sqrt{(s^2_{meas} - s^2_{anal})} \qquad (2.6.6)$$

2.6.2 Spatial Heterogeneity of Lead.

Lead is heterogeneously distributed in soils at most contaminated sites. Thomas *et al*., (2008) observed a spatial heterogeneity of Pb at two contaminated sites in Coseley and Nottingham (ranging from 2.3 to 57 % RSD). Argyraki, (1997) studied contaminated sites in Hounslow Heath, discovered high but varying concentration of Pb in the soil giving rise to high levels of uncertainty of 83.6% at the 95% confidence interval. These elevated values of measurement uncertainty are mainly attributed to the high degree of spatial heterogeneity of the soil at that site (Ramsey and Argyraki, 1997).

2.6.3 Methods of Quantifying Spatial Heterogeneity.

Spatial statistics include any of the formal techniques which study entities using their topological, geometric and geographic properties (Barnerjee *et al*., 2004). Complex issues arise in spatial analysis, many of which are often not clearly defined or resolved (Miller, 2004). Several statistical tools have been employed in quantifying heterogeneity and are discussed thus:

Variograms are widely used for geochemical mapping. The variogram is a graphical plot of variance as a function of distance. Spatial variability of target analytes over a geographical area can be characterised using variogram. It is based on the assumption that close spatially and temporally related samples exhibit similar values in concentrations (Myers, 1997). A theoretical variogram Zy (x,y) is a function describing the degree of spatial dependence of a spatial random field or stochastic process Z (x), defined as the variance of the difference between field values at two locations across realizations of the field (Cressie, 1993).

$$2y\,(h) = \frac{1}{n} \sum_{i}^{n} (g[x_i] - g\,[x_{i+h}]) \qquad (2.6.7)$$

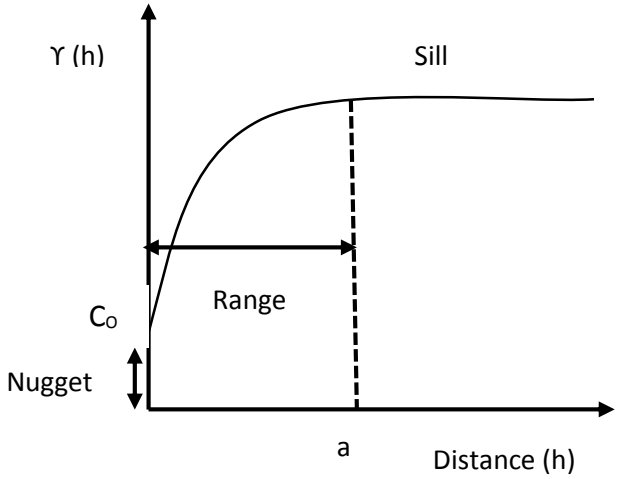


Figure 2.6.1: Illustration of an idealised variogram showing the range, sill and nugget effect (Adapted from Myers, 1997).

Bolviken *et al*., (1992) defined h as the distance between sample pairs, n is the number of possible samples pairs g (x_i) is the concentration of the element at point x_i and g (x_i + h) is the element concentration at distance h from point x_i. Variogram usually rises from the axis origin, the rate of increase reduces until it levels off (Figure 2.6.1). The range is the distance at which the graph flattens (Figure 2.6.1). The 'sill' represents the variance of the population and it is the height at which the plateau is reached (Figure 2.6.1). An intercept of the variogram on the y-axis is called the nugget effect (Figure 2.6.1). In most geological surveys, lag distances of 10 m to 1000 km are often used to construct Variograms. However, restricted by the typical sampling interval, variograms often fail to assess heterogeneity over the full range of scales e.g. 0.0001m-1000 m for a limited number of measurements (n=100) (Thomas *et al*., 2008). Gooverts, (1999) noted that local variability in data values is often misrepresented by the variogram. Myers, (1997) stated that factor such as nugget effect can increase the uncertainty attached to any estimated value.

The construction of kriged map contours requires variograms at four cardinal directions calculated from a minimum of 100 samples (Myers, 1997). Kriging refers to the construction of contour maps of estimated element concentrations across a study site. All kriging methods produce estimates of concentrations and uncertainty attached to those values for unsampled locations. With any interpolation method such as kriging, it may be difficult to quantify the uncertainty in the estimated values, for example, even if a variogram is adequate for a kriging analysis, it may not be adequate for assessing uncertainty of kriged estimates because uncertainty increases with increasing mean

values (Isaaks and Scrivastava, 1989). Woodbury (2003) reported that kriging models may overestimate or underestimate uncertainty to some degree and does not provide direct evidence for non–sampled sites. Kriging is usually time consuming.

A nested nine point sampling design has been used to characterize heterogeneity over the full range of scales (Taylor *et al*., 2005).The duplicate methods originally devised for the estimation of precision and uncertainty based on duplicate samples for some proportion of samples, has also been used to quantify heterogeneity (Ramsey, 1998). Miesch (1976) and Garret (1969) in Ramsey (1998) had originally suggested this experimental design for the estimation of sampling and analytical precision. Argyraki (1997), utilized a simple regular grid design, with and without composite samples for sampling contaminated sites.

According to Thomas *et al*., (2008), the specific method consisted of using the duplicate method at various scales 0.001m-100 m, followed by interpretation using robust ANOVA to subtract the analytical variance from the sum of the sampling and analytical variance (AMC, 1995, Ramsey *et al*., 1994). It is perhaps the simplest method that can be used to estimate heterogeneity, initially expressed as a sampling variance (Thomas *et al*., 2008). Field duplicate and sampling precision (s, the square root of the variance s^2) can be expressed as the relative standard deviation (RSD) for duplicates (CEN, 2005).

$$\text{RSD} = 100s/\bar{x}$$

Qualitative estimation of spatial heterogeneity has been made using the duplicate method with *in situ* analytical technique such as the P-XRF and X-ray microprobe for Zn and Pb at two contrasting sites (Taylor *et al*., 2005). Similarly, P-XRF was used to determine *in situ* measurement (i.e. sampling and analytical variance (Thomas *et al*., 2008).

2.6.4 Typical Values of Heterogeneity.

Typical values of heterogeneity are scale dependant characteristics. As a result of direct relationship between scale and heterogeneity, sampling results are only valid for the scale of sampling (CEN, 2005). Generally, the degree of heterogeneity will be higher for a smaller scale of sampling and lower for a larger scale (CEN, 2005; ISO, 2005; Thomas *et al*., 2008). The degree of heterogeneity can be expressed numerically in terms of relative standard deviations (% RSD), potentially as a function of scale. Typical values of heterogeneity expressed as $\%RSD_{samp}$ ranges from 4-55% across different sites for a range of elements (Taylor *et al*., 2005; Thomas *et al*., 2008; Ramsey *et al*., 2013).

However, %RSD assume normal distribution which is often found to break down at higher levels of heterogeneity. A new and alternative approach for reliably expressing heterogeneity as heterogeneity factor (HF) is discussed in Chapter 3.

2.6.5 Effects of Spatial Heterogeneity on plant uptake.

For all natural processes, heterogeneity exists at a variety of scales; the net effect of smaller-scale heterogeneity can have a significant effect on larger-scale predictions (Albert, 2000). The most important early studies on plant responses to spatial heterogeneity are those on nutrient heterogeneity (Drew, 1975; Hutchings and John, 2004). Studies by Jackson and Caldwell, 1989 ; Leichowicz and Bell (1991); Robinson, 1994; Gross *et al*. (1995) ; Jackson and Caldwell, 1996; found that spatially homogenous growing conditions are problematic because available resources in the natural environments are patchy at scales similar or smaller in size than individual plants. There is evidence that plants are strongly affected by heterogeneous conditions of available nutrient resources (Wijesinghe and Handel, 1994; Wijesinghe *et al*., 2001; Hutchings and John, 2004).

Plants in heterogeneous conditions could invest heavily in roots located where soil-based nutrient resources are most abundant (Hutchings and John, 2004). In many studies such as those of Drew and Saker (1975); Birch and Hutchings (1994); Stuefer *et al*. (1994; 1996); Alpert and Stuefer (1997); Wijesinghe and Hutchings (1997) reported that plants maximize resources acquisition from abundant locations in heterogeneous conditions. Wijesinghe and Hutchings (1999), studied the effect of nutrient heterogeneity on root growth and root/shoot ratio of *Glechoma hederacea* and discovered that total root mass increased with larger treatment patch and increase in root/shoot ratio as well. According to Birch and Hutchings (1994), plants may grow faster in heterogeneous condition of micronutrients.

Nutrient heterogeneity has similar application to contaminant heterogeneity as nutrient and contaminants are often both present in soil. Uptake of nutrient may result in the eventual uptake of contaminants from the soil by plants. Earlier studies (Haines, 2002; Millis *et al*., 2004; Manciulea and Ramsey, 2006; Thomas, 2010) have shown that contaminant heterogeneity can also influence plant uptake of contaminants from the soil. Significant impact (76 % changes in plant biomass and uptake) of Cd heterogeneity in soil on plant uptake has been reported in earlier studies by Manciulea and Ramsey (2006) at a scale of 0.03 m using a simplistic chequer board model. Thomas, (2010) also reported impact of Zn heterogeneity on plant uptake at a scale of 0.02 m. Spatially

heterogeneous distribution of contaminants in the soil might affect the amount of uptake, root development, root and shoot biomass, growth rate and period of growth (USEPA, 2000).

2.7 Summary of Review.

This chapter reviewed the forms, sources of Pb and showed from literature how they may influence uptake of Pb by plants and the effects of Pb on target receptors (e.g plants and human). It also reviewed spatial heterogeneity, its impact on plant uptake of contaminants from the soil and their potential application in phytoremediation of contaminated lands, Pb accumulator plant species, potential Pb contaminated sites with the view of selecting suitable field sites and plant species for the first pot trial.

CHAPTER 3: Measurement of *in situ* heterogeneity of Pb in soil at two contrasting field sites.

3.0 INTRODUCTION

This chapter discusses the experimental design, choice of field sites, sampling methods and results of the field investigation which covers the *in situ* measurement of Pb concentration and quantification of *in situ* heterogeneity in two sites (Gang Mine and BlackRock) in Derbyshire. Much of the content of this chapter has been published in Ramsey *et al*., 2013.

3.1 Background to Experiment.

Contaminants are generally heterogeneously distributed in soil. As discussed Chapter 2, Section 2.6, heterogeneity in contaminant concentrations varies with scale (Argyraki and Ramsey, 1997; Thomas *et al*., 2008; Ramsey *et al*., 2013). An examination of a field at a fine scale reveals a complex distribution of particles which vary in size, colour, shape, pore spaces and biotic constituents (Thomas, 2010). Spatial heterogeneity in contaminant distribution in soil can generate uncertainty in measurements of contaminant concentration during site investigations (Taylor *et al*., 2005). A highly heterogeneous distribution of contaminant in soil may result in greater risk of site misclassification against a threshold value (either as uncontaminated or contaminated). The consequences of such misclassification include the potential risk to human health, unrealistic human risk assessment models and creation of unnecessary remediation expenses.

Many sampling designs are aimed at reducing the impact of *in situ* heterogeneity by taking large composite samples for off-site homogenization (Gy, 1992; Reddy *et al*., 2001; Thomas *et al*., 2008; Jean-Phillipe *et al*., 2012). However, one limitation of these methods is that small scale heterogeneity that can have significant impacts on sampling strategies and exposure assessment are not often taken into account. Secondly, composite sampling and homogenization may not also produce results that can be used to realistically estimate the exposure to target receptors e.g plants or humans as the area of exposure of the target receptor can differ in scale from the scale often used in sampling designs (Thomas, 2010).

Heterogeneity can be viewed from a positive perspective. Ramsey *et al*., (2013) reported these three possible approaches to *in situ* heterogeneity, namely (i) reducing heterogeneity to an acceptable level by taking bigger composite samples, (ii) reporting

the effects as part of the full uncertainty of each measurement, (iii) evaluating the size of the *in situ* heterogeneity using analytical geochemistry and reporting the values to users of measurement. Once *in situ* heterogeneity has been quantified, it can be accepted as a source of additional information to improve the reliability, quantification and modelling of human risk and exposure (Ramsey *et al*., 2013). Such information is also useful in improving the accuracy of geochemical models.

Another consequence of spatial heterogeneity of contaminants in soil is a change in the extent of uptake of contaminants by plants. Contaminant uptake into food crops can be measured by the total concentration factor (discussed in Section 2.4.1). This is very useful in risk assessment to estimate exposure of humans to the contaminant via crops. Such data are usually based on pot trials in which the contaminants are homogeneously distributed. Earlier studies using the chequerboard style of distribution and simplistic binary model by Haines, (2002); Millis *et al*., (2004); Podar *et al*., (2004); Manciulea and Ramsey, (2006) showed that spatial heterogeneity of contaminants in soil has an enormous impact on metal uptake by plants. Work by Thomas, (2010) suggest that the degree and scale of heterogeneity with respect to root ball size can also be a key factor for some plant species.

A better insight and interpretation of *in situ* heterogeneity during site investigations can be made if methods which characterise *in situ* heterogeneity over a range of scales are employed. Some methods which can be used to quantify heterogeneity has been discussed in Chapter 2, section 2.6.3. The specific sampling design proposed by Thomas *et al*., (2008), used in this study is based on the duplicate method (Ramsey *et al*., 1992; AMC, 1995). It can be used to estimate the *in situ* heterogeneity, initially as a sampling variance. This can be achieved by taking duplicate field samples, each with two analytical duplicates. This enables the estimation of the two key components of the overall measurement variance (analytical and sampling variance). Analytical variance is generated from random error that occurs during chemical analysis, and can be subtracted from the measurement variance to give the sampling variance, and hence the heterogeneity. Sampling variance is the difference between two samples taken from the same nominal sampling location as a result of small scale heterogeneity (Ramsey and Argyraki, 1997).

3.1.1 Objectives

i. Determine *in situ* concentration and hence *in situ* heterogeneity of Pb in contaminated sites at scale of 0.02 -50 m using measurement techniques such as the P-XRF.
ii. Quantify spatial heterogeneity of Pb in the selected contaminated sites and express it effectively as a function of concentration (using a suitable statistical method).

Hypothesis

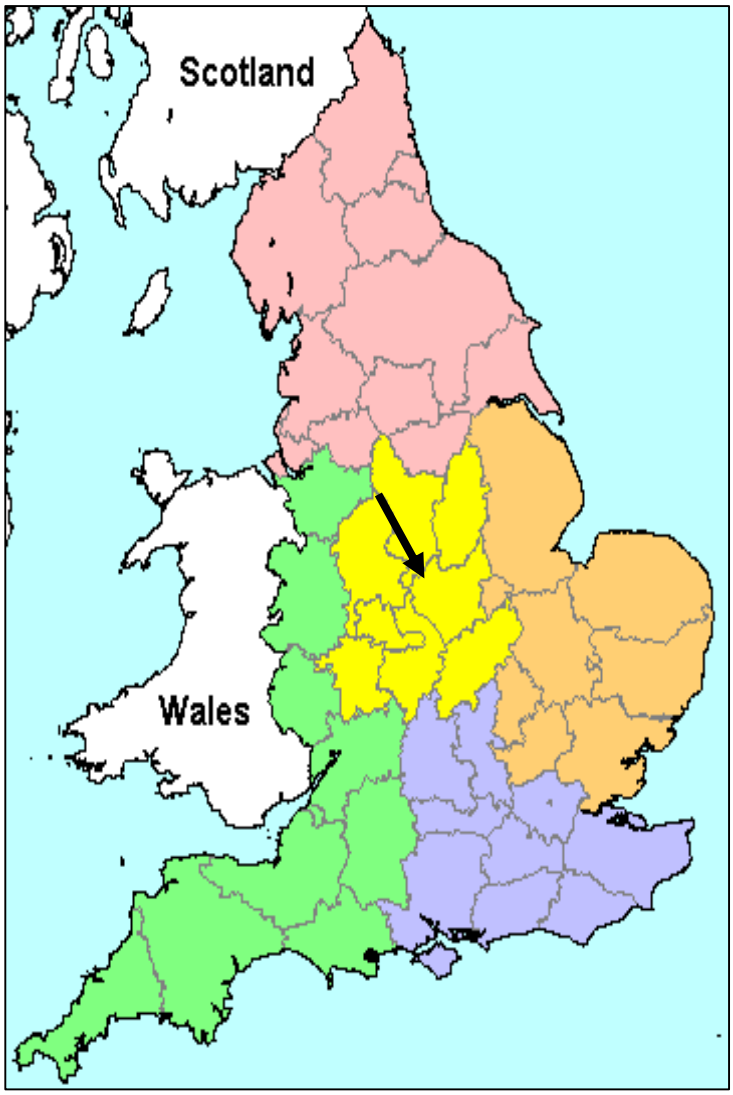
In situ heterogeneity can be quantified and modelled over a range of scale to describe the spatial distribution of Pb in selected field sites.

3.2 CHOICE OF FIELD SITES

A review of Pb contamination in the UK has been discussed in Chapter 2: Section 2.3.1. Reports by DEFRA (2007) indicate a high level of Pb contamination at some sites in Derbyshire. However, site selections were made based on the following criteria:

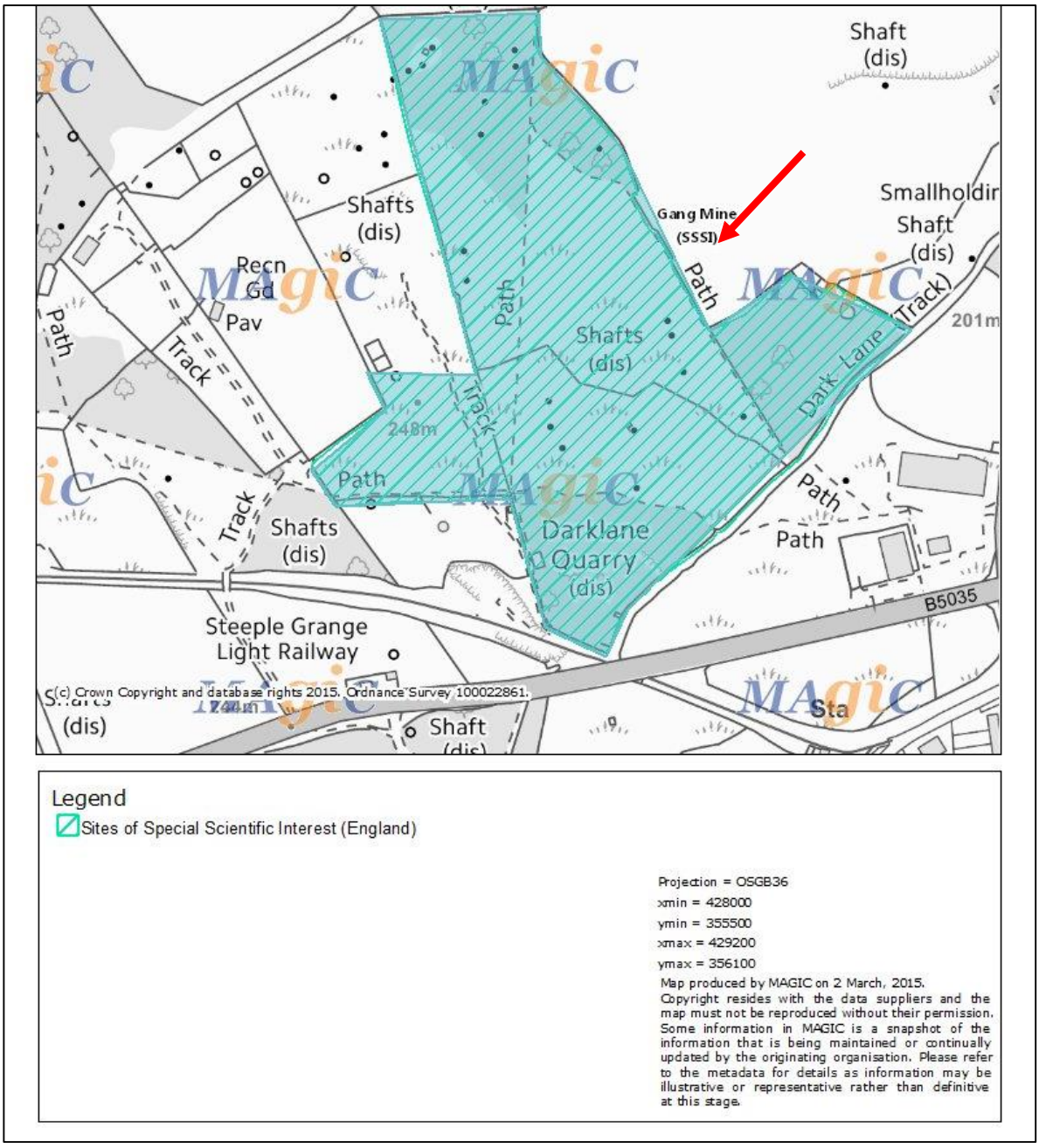
1. Recorded history of Pb contamination (Pb > 450 mg/kg).
2. Size or area of land in hectares (≥ 1 ha).
3. Extent of spatial heterogeneity and whether this value would be complementary to other sites in providing a range of values overall.
4. Presence of Pb accumulating plant species on the contaminated sites. This was a requirement because the effect of *in situ* heterogeneity on plant uptake was to be assessed in pot trials as part of this research.
5. Accessibility with respect to distance from Sussex, site ownership and permission to sample.

Based on these criteria, two Pb contaminated sites (Gang Mine and BlackRocks) in Derbyshire located in the Peak District were selected (Figure 3.2.3a & b).



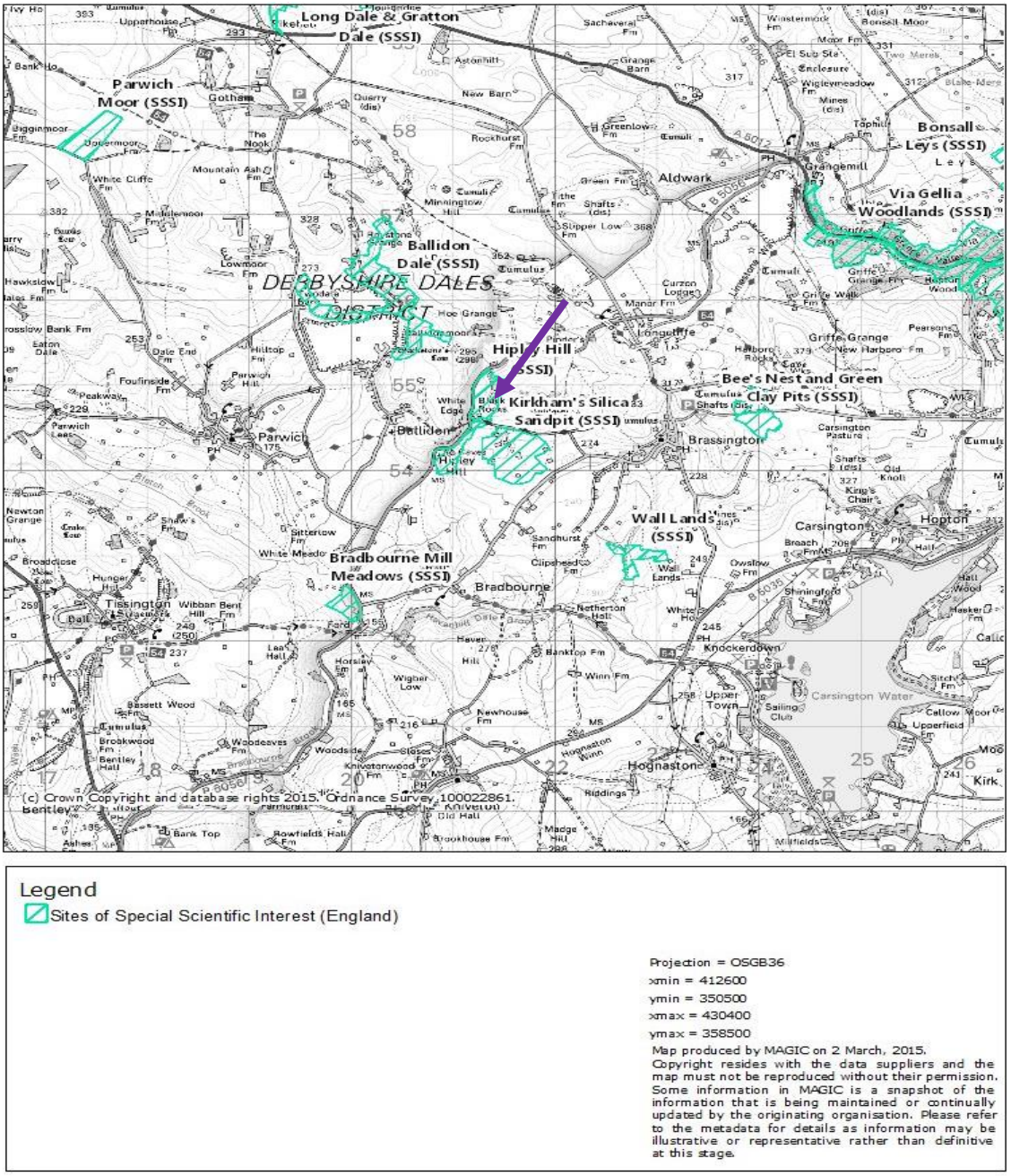
(a) Scale: 1:100000

(Source: magic.defra.gov.uk/Magic map)



(b) **Scale: 1:10,000**

(Source: magic.defra.gov.uk/Magic map)



(c) **Scale: 1:50,000**

Figure 3.2.1: (a) United Kingdom map showing location of Gang mine and Black Rocks (black arrow pointing to Derbyshire where both sites are located) Scale: 540 mm x 720 mm (Source: http://gwydir.demon.co.uk/jo/maps/ukindex.htm) (b) Map showing investigated field sites, (red arrow pointing Gang Mine location and (c) purple arrow pointing to Blackrock {Source: magic.defra.gov.uk/Magic map} (Ordnance Survey map with the permission of the Controller of Her Majesty's Staionery Office, Crown Copyright NC/ March/ 2015).

3.2.1 Site Description

3.2.2 Gang Mine

Gang Mine is designated a Special Area of Conservation (SAC) located on carboniferous limestone in Derbyshire North-West of Wirksworth (JNCC, 2011). Its National Grid Reference is SK 286557 (an approximate central point of the SAC. The sampling area was a 45 by 45 m. Grid references of the four corners were SK 28597 55649, SK 28638 55651, SK 28598 55694 and SK 28638 55696 at locations A1, J1, A10 and J10 of the sampling grid (Figure 3.2. 2.1) respectively. It lies on latitude 53^{o} 05 52' N and longitude 01^{o} 34 21' W and has an area of 8.26 hectares. It was an ancient mine in 1652, lying around the Southern point of the Eyam limestone series bordering the millstone grits to the east (JNCC, 2011). This limestone is still being mined by Dene quarry.

It is characterised by several spoil heaps. *In situ* measurements made during the field investigation showed that the spoil heaps contained high levels of Pb and Zn and support some unique plant species. Only a few plant species are able to tolerate the high mineral content of the spoil heap (Figure 3.2.2.1b) located in some part of the sampling area while areas outside the spoil support a wider range of plant species.

Gang Mine now belongs to the Derbyshire Wildlife Trust and is being developed as a nature reserve with funding under the English Nature's Reserves Enhancement Scheme. About one-fifth of the site is not grazed and the remaining area is well-grazed. There were wide variations in slope and soil contamination. The spoil heaps are unusual in the diversity of spoil materials of varying metal concentrations, from very fine spoil to large rock fragments or bare areas without vegetation. Some metallophytes species were present. The open spoil areas support large populations of *Thlaspi caerulescens* (alpine pennycress), *Rumex acetosa* (Sorrel), *Minuartia verna* (Spring sandwort), *Viola lutea* (mountain pansy), and some lichen species such as *Peltigera* and *Cladonia* thrive in some of the bare areas. Certain fungi, mosses and fern also grow in the highly metal contaminated spoil heap. They include *Dryopteris filix*-mas (male fern) and *Gymnoscarpium robertianum* (limestone fern).

(a)

20 m

Scale bar of 1 mm represents 20 m in real life (scale can be calculated as $\frac{Picture\ size\ or\ height}{Actual\ \ size\ or\ height}$ while scale bar = Picture size or height x (The length the scale bar represents/ Actual size or height of photo.

(b) 5 m

Figure 3.2.2.1: Gang Mine showing (a) field site with several spoil heaps and some vegetated parts amidst spoil heaps (b) Sampling location on spoil heap (high Pb 15800 mg/kg). A1, A10, J1 and J10 represent the orientation of grid of the four corners of the site.

3.2.3 Black Rocks

Black Rocks is about 0.8 km east of Middleton top, at National grid reference SK 293558 and on latitude 53º 05 55´ N and longitude 1º 33 50´ W. The site has an approximate area of 2 hectares (Natural England, 2014). The grid references of the four corners of 45 x 45 m grid were SK 292244 55728, SK 29286 557248, SK 29241 55686, SK 29284 55688 for locations A1, A10, J1 and J10 of the sampling grid respectively (Figure 3.2.2.2). It is close to the location of a former Pb mine which covers an area approximately 300 m^2 (Natural England, 2014). The scree slope around the rock supports some lead-tolerant plants. The scree slope has been washed off by erosion leaving the greater part of the slope bare without vegetation (Figure 3.2.2.2a).

This site has also been modified by trampling of human and animal feet over the foot paths running across the site. A small area of about 15 m^2 was fenced to protect against erosion. It supports plant species including *Rumex acetosa* (sorrel), *Agrostis stolonifera* (bent grass), *Gymncarpium robertianum* (limestone fern), *Thlaspi caerulescens,* (alpine pennycress), large strands of conifers such as *Pinus sylvestris* (scots pine), *Pinus pinus* (pines) and *Larix decidua* (European larch). The conserved area also contained low growing shrubs such as *Vaccinium myrtillus* (Bilbery). It is currently a popular place for recreation.

Figure 3.2.2.2: Black Rocks showing (a) bare areas (no vegetation) and (b) protected area (growth of some plant species). A1, A10, J1 and J10 represent the orientation of grid of the four corners of the sites area.

3.3 DESCRIPTION OF FIELD METHODS

Measurements: *In situ* sampling scales 0.02 m to 50 m.

The specific sampling method was laid out at both sites using a spaced regular grid of overall dimensions 45 m x 45 m (Figure 3.3.1). However, some grid dimensions were modified to adapt to the dimensions of the test sites. Sampling locations and duplicate sampling points were located using a 30 m tape, a compass and a hand held GPS. Bamboo canes of about 1m were placed at each sampling location. Surface vegetation and turf were removed using a spade. Mylar® film disc was placed over the sampling locations to protect the analyser window of the portable X-ray Fluorescence Spectrometer (P-XRF), and labelled containers placed alongside for core extraction after measurement with the P-XRF. Soil moisture measurements were taken *in situ* for top 1 mm using a TDR 100 soil moisture meter. *In situ* measurement of heavy metals in the topsoil was taken using P-XRF model Niton XL3t 900SHE with a battery powered X-ray tube as an excitation source (for operating techniques and specification, see Appendix I.1). Sample identities were entered into the P-XRF prior to use.

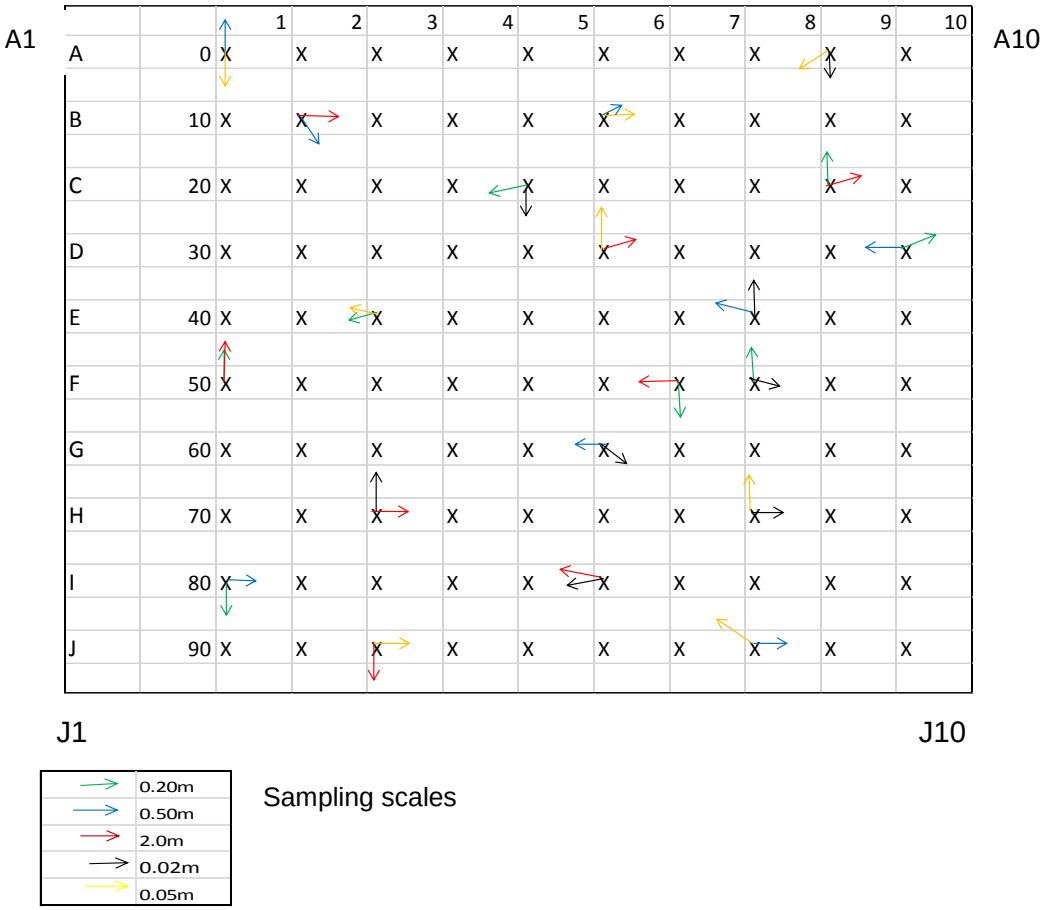


Figure 3.3.1: Specific sampling design (method proposed by Thomas *et al*., 2008) used at both sites for the quantification of contaminant heterogeneity over a range of scales for a site of 45 m x 45 m. X represents each sampling point at 5m spacing and arrows show locations chosen at random for duplicate sampling points at each sampling scale.

Key: Sampling points, locations and number of duplicates are original to the sites investigated in this chapter. 0, 10, 20……..represent sampling locations.

The P-XRF allowed a large number of *in situ* sample measurements to be made within a short period of time without disturbing the spatial heterogeneity of the soil. Soils were analysed to a depth of ~1 mm without any sample preparation except the removal of surface vegetation to a depth of ~1 mm using a spade. Before and after the whole session of taking measurements at the sampling locations, the P-XRF was used to take measurements of certified reference materials (CRMS) for the estimation of analytical bias (results in Appendix I.3). A 60 seconds count time was used at each measurement location to quantify target element concentration. This duration was selected to be long enough to ensure that the detection limit (0.02 mg/kg) was acceptable, whilst it was short enough to ensure that all measurements were completed within the two-day period.

After measurement with the P-XRF, extraction of a core with approximately 65 mm diameter and 50 mm depth was taken at each duplicate sampling location using a bulb

planting device. It was transferred into a 500 ml polypropylene straight–sided pot labelled with the sample identity. The X-ray microprobe was used to make X-ray map of Pb heterogeneity of some core soil samples from the field sites.

3.3.1 Full balanced and Simplified Design.

A full balanced design (Figure 3.3.2a) was used at the 0.2 m scale and a simplified balanced design (Figure 3.3.2b) was used at all other sampling scales. The balanced sampling design was used in the application of the duplicate method in an *in situ* investigation. Duplicate field samples can be taken at 10%, or a minimum of 8, sampling locations (Lyn *et al*., 2007b). The use of the balanced sampling design allowed the estimation of the two main components of the random error from sampling (s_{samp}), which is a measure of heterogeneity, and analysis (s_{anal}). The estimate of s_{anal} from the duplicate readings taken at the 0.2 m scale was assumed to be typical of measurements at all scales, and was therefore used to estimate s_{samp} for all other sampling scales, by rearranging equation 3.1.

$S_{meas} = \sqrt{s^2 samp + s^2 anal}$ .....Equation 3.1

$S_{samp} = \sqrt{s^2 meas - s^2 anal}$ .....Equation 3.2

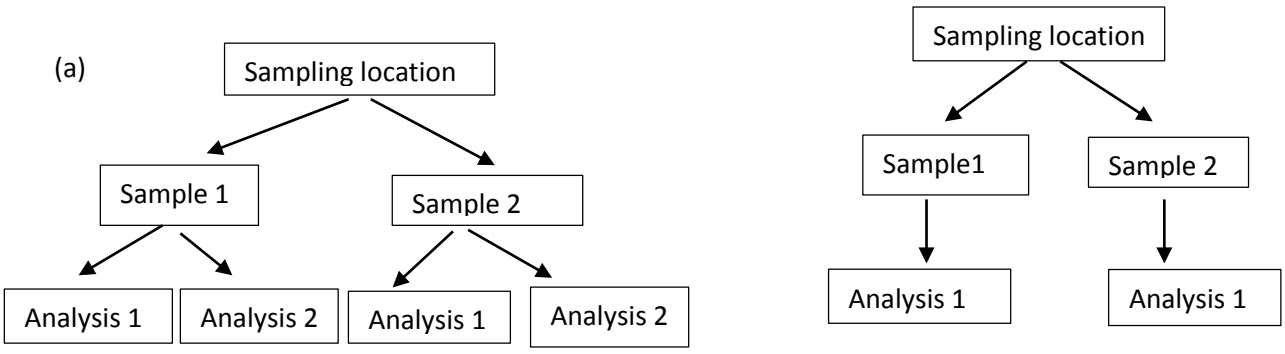


Figure 3.3.2:(a) Full balanced design used at sampling scale 0.2 m and (b) simplified balanced design used at the other sampling scales (Ramsey *et al*., 2013).

3.3.2 Data Analysis

Data was analysed using the robust analysis of variance (RANOVA). Robust ANOVA was preferred to the classical ANOVA because it can accommodate outlying values (≤10%) by down weighting the effects of these outliers. *In situ* heterogeneity was expressed as heterogeneity factor (HF). Heterogeneity factor is defined as ($10^{GSDsamp}$)

i.e. 10 to the power of the geometric standard deviation of sample {GSD_{Samp}}. If the GSD_{samp} is defined for log_e (natural logarithm), then the HF is equals to $e^{GSDsamp}$, but the calculated values of HF are identical to that calculated by $10^{GSDsamp}$. The geometric standard deviation of sample (GSD_{samp}) refers to the measure of scatter of a set of logarithmically transformed data whose preferred mean is the geometric mean, while the standard deviation of sample (S_{samp}) is the classic or arithmetic standard deviation of a set of data which is normally distributed. A log-transformed data results in a normal distribution and the measure of dispersion is the geometric standard deviation (Garret and Goss 1979; Kirkwood, 1979).

3.3.3 Data Quality.

Detection limit.

A number of methods are used in the calculation of the detection limit of a P-XRF. Clark *et al*., (1999) reported that 3 times the standard deviation of sample readings can be used to estimate P-XRF instrumental detection limit. Kalnicky and Singhi (2001) suggested the use of 3 times the standard deviation of 12 non-consecutive measurements of certified reference materials (CRM) such as National Institute of Science and Technology (NIST) 2709, 2711 and 2710 for low, mid and high concentrations respectively. The use of 3 times the standard deviation of measured concentrations of soil samples with low/background concentrations measured 5 times in succession was suggested by Vanhoof *et al*., (2004).

Standard deviation of the counts per second for each 60 second sample reading is recorded by the P-XRF. Thomas *et al*., (2008) used the median of 3 times the standard deviation of samples extrapolated to zero concentration. The median value of 3 times the standard deviation value for counts per second of each CRM readings extrapolated to zero concentrations was used to estimate the detection limit in this study (Appendix I.2). The estimated detection limit was 0.02 mg/kg which was low enough to not affect sample measurements in the range of 193 to 71000 mg/kg at both sites.

3.3.4 Analytical precision and bias.

Instrumental precision was estimated by two consecutive readings of the same sampling points to form analytical duplicates required as part of the balanced sampling design

using the duplicate method (Figure 3.3.2). Robust ANOVA was used to estimate the analytical precision expressed as standard deviation at the 0.2 m scale. This was expressed relative to the mean at 95% confidence.

In situ bias was estimated from repeated P-XRF analysis of six certified reference materials, National Institute of Standards and Technology (NIST) soil reference materials (2710a and 2711a), House reference material (HRM 31), and Canadian Certified Soil Reference Materials Project (CCRMP-TIL-4), North Carolina State soil reference material (NCS 73308), Resource Conservation and Recovery Act soil reference material (RCRA) and GBW 07411 were used estimate bias.

The estimated analytical precision for each site was 14.2% for Gang Mine, and 7.0% for Black Rocks at 95% confidence (Table 3.3.1). The monitoring certification scheme of the UK Environment agency (EA, 2006) in its published guidelines requires an analytical precision of less than 15% at 95% confidence for *ex situ* laboratory analytical methods. These values still compare reasonably to these published *ex situ* guideline, even though these measurements are made *in situ*.

Table 3.3.1: Summary estimates of data quality (Instrumental precision and bias at 95% confidence for P-XRF Pb measurements at Gang Mine and Black Rock.

	Instrumental precision %		**Instrumental bias %**
Element	**Gang mine**	**Black Rocks**	**Gang mine & Black Rocks**
Pb	±14.2	±7.0	-0.09

A statistically significant, but small bias of -0.09 % was found from the regression analysis of P-XRF Pb measurements against certified values (Appendix I.3). Soil moisture, surface roughness and pore spaces are potential sources of additional bias to *in situ* measurements. Earlier work (Argyraki *et al*., 1997) implicated surface roughness, soil moisture and pore spaces as potential sources of bias in field-based P-XRF measurements.

3.4 RESULTS OF *IN SITU* MEASUREMENTS.

An X-ray microprobe (EDAX Eagle II XMP, PV 8660/00) was used to produce a map of the relative Pb concentration within one of the *in situ* soil sample cores from Gang Mine (Figure 3.4.1). This is helpful in visualizing and explaining the concept of *in situ* heterogeneity. Quantification of the absolute concentration of Pb was however, not of sufficient quality to enable heterogeneity estimation at this finer scale. There were no equivalent soil cores or maps for Black Rocks because of the rocky nature of the sampling area, which prevented the taking of *in situ* cores.

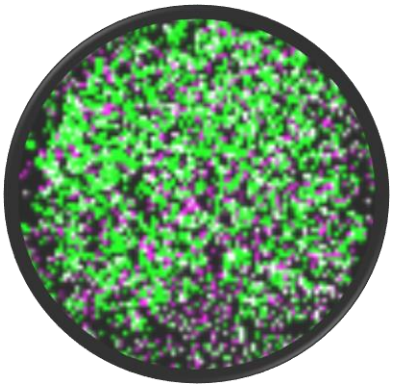


Figure 3.4.1:X-ray Microprobe (XMP) map of Pb heterogeneity in a soil core (E7) from Gang Mine field site (area 26 mm diameter by 9 mm depth taken from the core measured using the P-XRF with XMP spot size of 0.03 µm). More intense green shows areas of higher Pb concentration while areas in purple show soil particles or component (Source: *Ex situ* (XMP) analysis of selected *in situ* core soil samples.

Histograms of the *in situ* Pb concentration measured at both sites are shown in Figures 3.4.2a & 3.4.3a. The Robust ANOVA assumed that at least 90% of the measurement values either in their raw or log-transformed units are approximately normally distributed with < 10% of outliers.

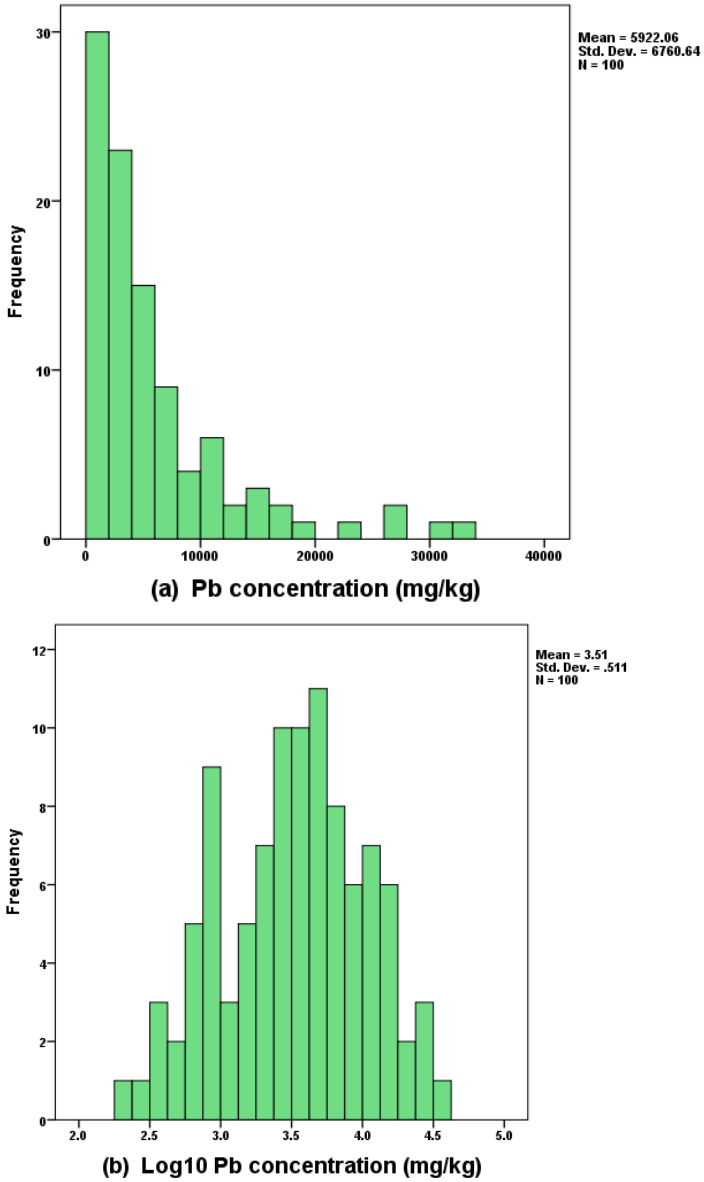


Figure 3.4.2: Frequency distribution of *in situ* Pb measurements in Gang Mine. (a) Positively skewed on a linear scale and (b) Near normal distribution on a log transformed scale.

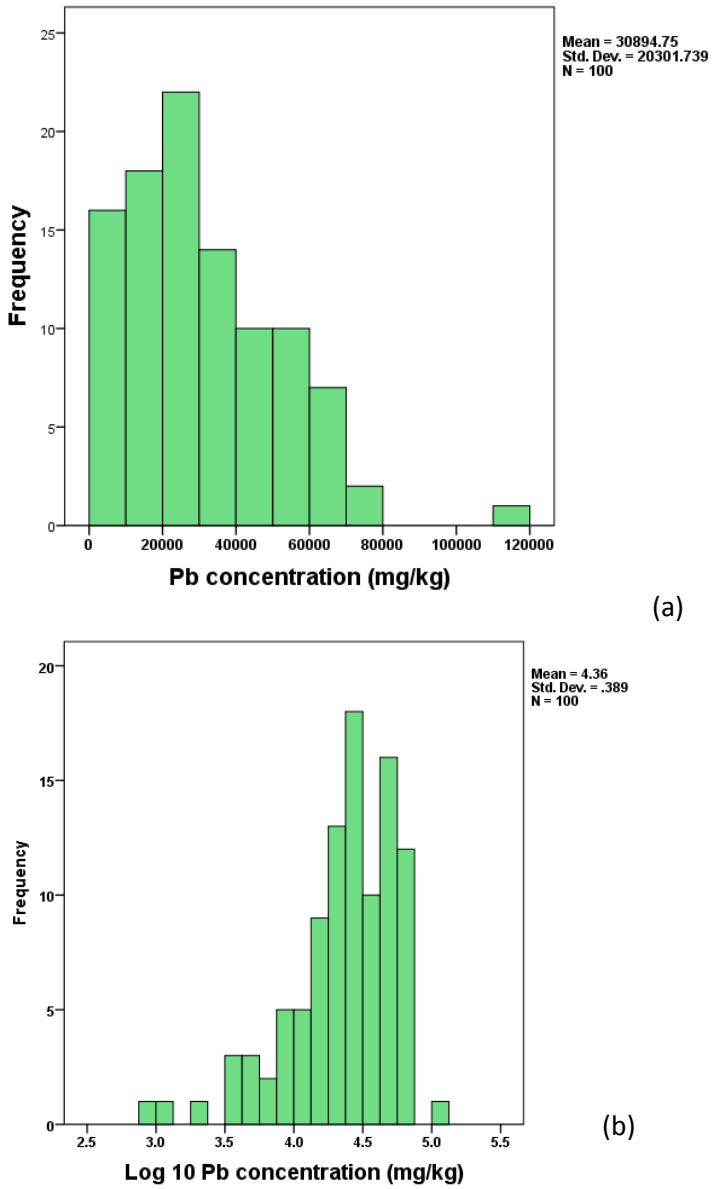


Figure 3.4.3: Frequency distribution of *in situ* Pb measurements in Black Rocks. (a) Positively skewed on a linear scale and (b) more nearly normal distribution on a log transformed scale.

The histogram of the frequency distribution (Figures 3.4.2a and 3.4.3a) showed that the assumption for robust ANOVA of an approximately normal distribution was not met. Logarithmic transformation of the concentration values produced a distribution an approximately normal distribution which fits the requirement of the robust ANOVA (Figures 3.4.2b and 3.4.3b: Appendix I.7). The central tendency of log transformed data can be estimated as the geometric mean (GMean) and the scatter expressed as geometric standard deviation (GSD) (Garrett and Goss, 1979).The mean is then expressed as the geometric mean (GM) and the scatter as the geometric standard deviation (GSD or GSD_{samp}). The confidence limits of the log-transformed data are no longer symmetrical to the linear limits of concentration. An example is the geometric mean value (3.512) and the geometric standard deviation (GSD) value (0.511) in the

units of $\log_{10}$ of the mg/kg concentration of overall geochemical distribution of Gang Mine. The log10 transformed geometric mean (3.512) is equivalent to a geometric mean of 3252 mg/kg in linear concentrations. The GSD is a measure of heterogeneity and can be termed a heterogeneity index. However, the GSD cannot be converted to meaningful concentration units (Ramsey *et al*., 2013). The upper confidence limit (UCL) can be calculated as 10555 mg/kg ($10^{4.023}$), and a lower confidence limit (LCL) of 1002 mg/kg ($10^{3.001}$) at the 68.3% confidence level (1 standard deviation). The effect of this is that heterogeneity can no longer be expressed as a fixed % RSD, so the best option is to express the heterogeneity as a heterogeneity factor HF which is calculated as $10^{GSDsamp}$ (see detail in Ramsey *et al*., 2013). Heterogeneity in this study was expressed as heterogeneity factor (HF).

The summary statistics of P-XRF Pb measurement are shown in Table 3.4.1 below. Gang mine had higher estimate (~20 to 45%) of Pb heterogeneity (HF=1.24 to 3.22) with RSD equivalent (13 to 107%) compared to Black Rock (HF 1.17 to 2.22) equivalent to an RSD of 15 to 33%. Results also showed that heterogeneity factor (HF) varied substantially between each sampling scale at both sites. Heterogeneity factor ranging from 1.17 to 1.4 (HF ≤ 1.4) can be classified as low heterogeneity, whilst high heterogeneity can be associated with heterogeneity factor greater than 1.4 (HF > 1.4). This is because the assumption of normal distribution by robust analysis of variance breaks down with increasing heterogeneity. When the result of this study was compared with previous studies using the HF approach, it was observed that when heterogeneity is greater than 30% equivalent to HF of approximately 1.4 (Published in Ramsey *et al*., 2013: Appendix I.8), it is more accurately expressed as heterogeneity factor HF.

In this order, 0.02 m and 0.05 m scales at Gang Mine had low Pb heterogeneity (HF = 1.24 and 1.44) respectively. High Pb heterogeneity (HF > 1.4) was recorded at scales 0.2 m, 0.5 m, 2 m, 5m and 20 m (Table 3.4.1). In contrast, at the Black Rock site, high Pb heterogeneities (HF > 1.4) were recorded at three scales (2, 5 and 20 m scales) whilst, 0.02, 0.05, 0.2, and 0.5 m scales had low Pb heterogeneity (HF < 1.4). Heterogeneity factor was significantly different ($p < 0.05$) between scales. It is an indication that heterogeneity varied at different scales. A comparison of heterogeneity of these sites to previously studied sites in the United Kingdom (Ramsey *et al*., 2013) showed that HF varied between scales across the different sites. However, HF increased as a function of increasing scale at current (Gang Mine and Black Rocks) and previously studied sites (Table 3.4.2). This is in line with earlier studies (Taylor *et al*., 2005; Thomas *et al*., 2008).

Table 3.4.1:Estimated robust and geometric mean (GMean) of Pb concentration and *in situ* heterogeneity expressed as RSD_{samp} and Heterogeneity factor (HF) for Gang Mine and Black Rocks. Gang mine generally has a higher level of Pb-heterogeneity, but a lower concentration of Pb.

Site	1.Gang Mine					2.Black Rocks				
Scale (m)	Mean (mg/kg)	RSD_{samp} (%)	GMean (mg/kg)	GSD_{samp}	HF	Mean (mg/kg)	RSD_{samp} (%)	GSD_{samp}	GMean (mg/kg)	HF
0.02	7991	13.5	6773	0.092	1.24	35107	15	0.07	32506	1.17
0.05	5362	36.8	4547	0.158	1.44	32781	22	0.09	28136	1.23
0.2	6401	60.9	3115	0.290	1.95	26631	12	0.116	19752	1.31
0.5	5907	50.1	3667	0.275	1.88	33100	22	0.084	27024	1.21
2	6720	107.4	3752	0.367	2.33	24627	33	0.295	18337	1.97
5	5443	70.6	3348	0.372	2.36	29829	40	0.258	23299	1.81
20	5617	85.9	3371	0.508	3.22	30013	59	0.346	23679	2.22

The robust mean Pb concentration of Black Rocks was ~ 5 times higher than that of Gang Mine. There was no significant relationship (R^2 =0.208 and 0.314) between Pb concentration and heterogeneity at both sites (Appendix I.6).

However, the site specific differences in Pb concentration could also be associated with the sources of contamination and land use. As mentioned earlier on in this chapter (section 3.2.2), Gang Mine is characterized by spoil heaps which are highly contaminated with Pb, and is currently used as a rural green space. Conversely, the scree slope in Black Rocks is covered by a thick layer of limestone spoil, presumably taken out of lead mine and dumped on the hillside. The lack of vegetation at some part of the site might have been caused partially by the more elevated Pb concentration. Some stones from limestone fragments in spoil heaps at both sites measured with the P-XRF had 2903 mg/kg and 194000 mg/kg Pb at Gang Mine and Black Rock respectively. High heterogeneity of these sites is a reflection of the mode of deposition and contamination source. Heterogeneity factor as a source of an additional about the mode of deposition of contaminant and contamination source can be seen in the heterogeneity of previously studied sites (Table 3.4.2) compared with the two sites investigated in this study. The heterogeneity of some previously studied sites initially expressed as RSD_{samp} and now expressed as heterogeneity factor (HF) is shown in the Table 3.4.2 below.

Table 3.4.2: *In situ* heterogeneity of Pb in soil (expressed as Heterogeneity factor (HF) values) increasing across sites (Source: Ramsey *et al*., 2013).

Sites	Location	Scale (m)	Mean (mg/kg)	GSD_{samp}	Heterogeneity (HF)
Sewage drying pans	Nottingham, Stoke Bardolph[c]	2	679	0.013	1.03
Playing field flood plain	Nottingham, River Trent[d]	0.5[b]	113	0.030	1.07
Landfill now camp site	Littlehampton[h]	2[b]	128	0.083	1.21
Field near Pb smelter	Avonmouth[f]	0.5	30448	0.097	1.25
Pb smelting site	Wirksworth[g]	2	4953	0.097	1.25
Garden & Allotment	South East London[a]	0.2[b]	942	0.121	1.32
Carnal dredgings site	Coseley, West[d]	0.5[b]	818	0.147	1.40
Landfill	Hounslow Heath, East[g]	2[b]	297	0.206	1.61
Canal dredgings site	Coseley, East[c]	2	467	0.257	1.81
Ex-firing range	Hounslow Heath, West[f]	2	756	0.379	2.39

Mean values are robust estimates and reported on a dry weight basis, except for [a] which is fresh weight,[b] values made using *ex situ* measurements ([c] Thomas, 2010, [d] Lee, 2002, [a] Boon, 2006, [f] Taylor and Taylor, 2003, [g] Argyraki, 1997, [h] Lyn, 2003).

Key: Heterogeneity values were originally expressed as RSD_{samp} in these previous works, and were converted to HF to enable comparison with the sites investigated (Gang Mine and Blackrock).

As stated earlier on, a new approach was used to estimate heterogeneity in this study. The use of heterogeneity factor (HF) was very useful in estimating and modelling the heterogeneity across different scales. A regression of HF against scale at both sites showed the variation of heterogeneity factor with scale. The linear regression model of HF against scale at Gang Mine showed a good fit with 93% of variance accounted for (Figure 3.4.4a).

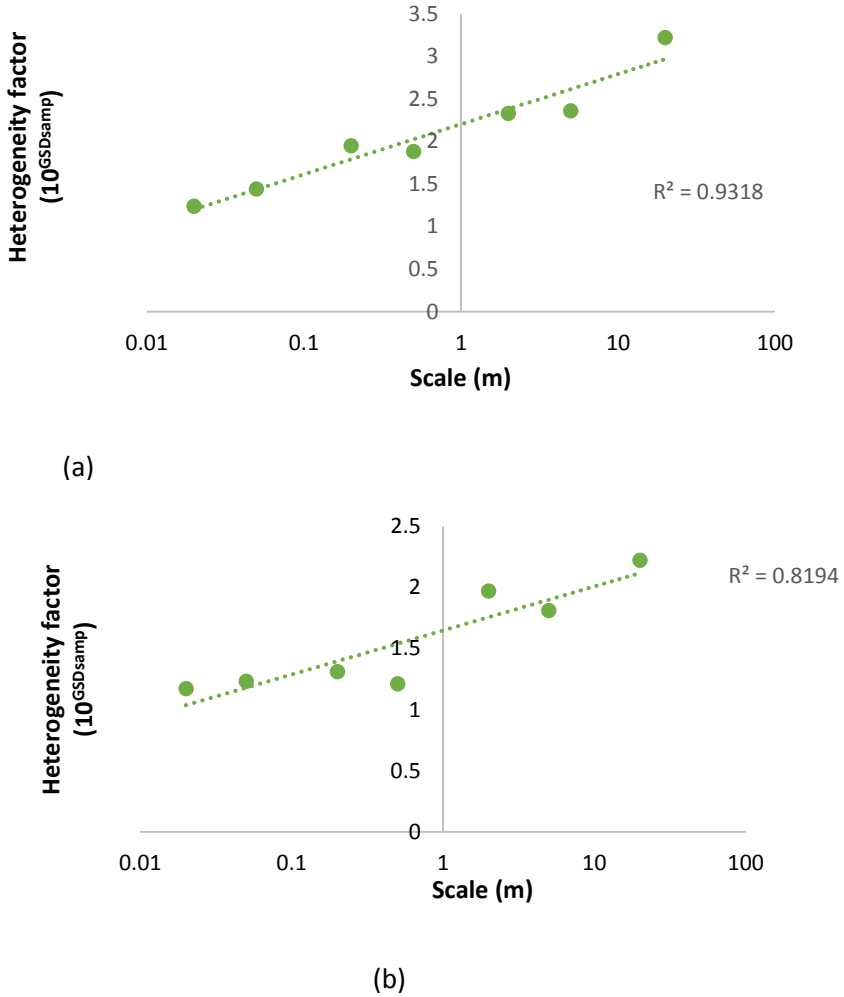


Figure 3.4.4: Regression model of heterogeneity factor (HF) against scale in (a) Gang Mine and (b) Black Rock.

Similarly 82% of variance was accounted for by the linear regression model of Black Rock (Figure 3.4.4b). However, only about 22-70% of variance is accounted for by the regression model of heterogeneity against scale when the heterogeneity was expressed as $\%RSD_{samp}$. It is an indication that HF more effectively models the *in situ* heterogeneity than %RSD in these cases. Heterogeneity factor (HF) may not only be more accurate in the description of high levels of heterogeneity, but also applicable to low levels (Ramsey *et al*., 2013). The HF approach has some advantages over the % RSD_{samp} (Discussed in Chapter 7). One of such advantages is in the ability to fit wide range of observed concentrations (e.g occasional value up to almost 40000 mg/kg in Gang Mine and over this range up to 71000 mg/kg in Black Rocks). It is also useful to express high heterogeneity values (e.g. over 50% at the 95% confidence level) as the lower confidence limit (LCL) never goes below zero, which is a problem that arises when $\%RSD_{samp}$ is used (Ramsey *et al*., 2013) and when heterogeneity is greater than 30%, it is more accurately expressed as HF than as %RSD.

3.4.1 Implications.

Evaluation of *in situ* heterogeneity using the specific sampling design based upon duplicated samples and heterogeneity factor (HF) was useful in setting up physical simulations of the effect of real-world heterogeneity on plant uptake in pot trial. It also has several uses for analytical geochemists discussed in Chapter 7.

Plant uptake models, evaluation and predictions of chemical processes that take place in contaminated environments can be more accurately made using heterogeneity values. Previous works on Zn and Cd showed that uptake of metals from the soil is greatly influenced by *in situ* heterogeneity. Studies by Millis *et al*., (2004) showed that the uptake of Cd by lettuce in the binary heterogeneity treatment was increased by 40% compared to the homogeneous Cd treatment with an estimated RSD of 200%. Thomas *et al*., (2008) observed 100% decrease in Zn uptake in the binary treatment compared to the homogeneous.

There is usually an assumption that *ex situ* laboratory measurements are more correct than *in situ* measurements, but *in situ* measurements are more realistic of contaminant concentration in the field and a closer representation of the true exposure experienced by living organisms (Ramsey, 2010). Estimation of *in situ* heterogeneity also enhances the reconstruction of such *in situ* heterogeneity at specified levels in pot trials to examine its effect on plant uptake in greenhouse experiments discussed in Chapter 6. Thomas, (2010) recreated *in situ* heterogeneity of Zn in pot trials in a slightly different approach. Plant uptake models will be useful in evaluating field heterogeneity. This general approach will help plant uptake in the field to be more accurately predicted.

3.4.2 Conclusions and further work.

Results showed that the specific sampling design, used in conjunction with the duplicate method, can be used to quantify spatial heterogeneity across a range of scales for Pb (or any other constituent of soil) at contrasting sites. The initial hypothesis that *in situ* heterogeneity can be quantified and modelled over a range of scale to describe the spatial distribution of Pb in selected field sites (Section 3.1 and summarised in Table 3.4.3 below) was accepted. The degree of heterogeneity can be expressed numerically as heterogeneity factor (HF) for each scale (i.e. sampling distance). This finding supports previous research into this area which links *in situ* spatial heterogeneity of Zn and scale (Taylor *et al*., 2005; Thomas *et al*., 2008)). Heterogeneity varied between sites, but did not increase as a function of scale. Results also showed that HF provides a better

estimate of heterogeneity (where heterogeneity > 30%) in contaminated site investigation when compared to the use of $\%RSD_{samp}$.

Table 3.4.3: Summary of hypothesis tested.

Hypothesis (alternative)	Species
1. (a) Heterogeneity quantified	Accepted
(b) Variation over a range of scale	Accepted

Probability P=0.05 of whether hypothesis is rejected or accepted.

Results suggest that the quantification of *in situ* heterogeneity expressed as HF can find useful application in the improvement of sampling strategies, reliability of risk and estimation of human exposure to Pb. However, for the purpose of this research, the results of the quantified spatial heterogeneity of Pb are fit for the purpose of designing greenhouse experiments to assess the impact of *in situ* heterogeneity of Pb on plant uptake discussed in Chapter 6.

CHAPTER 4: Pot trials to assess the effect of lead added treatments on selected species in one lead concentration, and over a range of concentrations.

4.0 INTRODUCTION

This chapter describes the design of the first and second pot trials which assessed the effect of Pb on selected plant species in Pb concentration of 1000 mg/kg and over a range of concentration (100 to 10000 mg/kg Pb added), compared to the control (0 mg/kg Pb added) treatment. These pot trials also compared within and between plant species to enable their selection for more focused hypothesis testing. It discusses the background to the experiment and justifies the subsequent selection of plant species from the first and second pot trials for further experiments. The discussion also addresses the choice of contaminant, Pb concentration levels and details of the experimental methods, seed germination, pot and growth media preparation, data analysis and interpretation.

4.1 Background to Experiments.

In the previous chapters (Chapters 2 and 3), reviews were made of the effect of spatial heterogeneity of Pb on plant uptake, factors affecting the uptake of Pb from the soil, Pb speciation, potential Pb accumulating plant species, justification for the selection of plant species for the first pot trial, and the finding from investigations of two field sites.

Several plants have been shown to accumulate Pb to varying extent. As discussed in earlier chapter (Chapter 2: Section 2.4.3), potential hyperaccumulator species such as *Armeria maritime* (sea pink)*, Arabidopsis halleri* (rockcress)*, Ambrosia artemisiifolia* (ragweed)*, Brassica napus* (oil seed rape)*, Brassica juncea* (Indian mustard)*, Brassica oleracea* (including common cultivars such as cauliflower, broccoli, cabbage, kale, Brussel sprout)*, Festuca ovina* (sheep fescue)*, Helianthus annus* (sunflower)*, Thlaspi rotundifolium* (round leaved pennycress), *Triticum aestivum* (bread wheat) and *Zea mays* (maize or corn) and *Aquilaria malaccensis* (Agarwood, Aloewood or Eaglewood) (Reeves and Brooks, 1983; Baker and Brooks, 1989; Baker *et al*. 1994; Deram *et al*. 1997; Bert *et al*., 2000; Kareem *et al*., 2013).

Plant species used in these pot trials were selected after a review of potential Pb accumulators in Chapter 2, Section 2.5. They were selected based upon their ability to accumulate Pb in their shoots and roots, root mass in comparison to scales of heterogeneity in the growth medium, status as either native or non-native species, and

practicability of obtaining and growing seeds in pot trials. Using these criteria, as explained in Chapter; Section 2.5, the study species used in these experiments include: *Brassica juncea, Brassica napus, Thlaspi caerulescens, Gentianna Penneliana, Zea mays* and *Bidens alba.*

4.2 Contaminant for use in pot experiment.

The choice of suitable speciation of Pb (contaminant) used in the growth medium for the pot trials was based upon the following two criteria:

1. The form and concentrations of Pb expected at the field site from literature survey.

2. The solubility and bioavailability of the particular form of Pb for plant uptake.

Two forms and speciation of Pb considered here are lead (II) oxide (PbO) and lead (II) nitrate (Pb (NO_3)), the use of which has previously been reviewed in the literature Chapter 2, Section 2.2. Lead oxide (PbO) was considered best for use in this experiment for these reasons: (i) to reduce the leaching and migration associated with Pb $(NO_3)_2$ (ii) being the most stable Pb species reported around investigated field sites (accounting for about 88-92% of Pb speciation), based on previous report (Gee *et al*., 1997), (iii) to avoid introducing nitrate with its nutrient proportion into the growth medium. Lead oxide is expected to be less bioavailable to the plants than Pb $(NO_3)_{2.}$, but this was not considered a major limitation to this research as PbO was viewed as more realistic of field conditions for both the first and subsequent pot trials that simulated *in situ* heterogeneity of Pb. The bioavailability of PbO was quantified experimentally (Section 5.5).

4.3 FIRST POT TRIAL.

The first pot trial was in done in two stages: (I) seed germination experiment and (ii) the first pot experiment. The seed germination experiment had 6 plant species made up of 16 varieties while the first pot trial had 4 species made up of 13 varieties selected after the seed germination experiment. As a result of these large number of species and varieties in the first pot trial, the number of replicates of each variety was limited to three. More replicates were maintained in subsequent pot trials after the final selection of the most suitable variety/species.

4.3.1 Objectives of the first pot trial.

The objectives of the first pot trial is to:

I. To quantify and compare Pb concentration in plant shoots, and hence potential for Pb uptake.
II. To assess the effects of Pb on plant growth and morphology in relation to uptake of Pb in pot trial.
III. Assess the viability of the seeds of these plant species for germination.
IV. Assess issues of seed availability in terms of obtaining seeds from suppliers.
V. Select most suitable species/varieties that can tolerate high Pb in soil for subsequent pot trials on the effect of heterogeneity of Pb in soil on uptake by plants by comparing different varieties within one species for between- treatment effects

Hypotheses

1. The 1000 mg/kg Pb in growth media has an effect on plant performance.

2. These species/varieties differ in their tolerance to Pb in the growth media at this concentration.

4.3.2 Seed Germination Experiment.

Methods

Prior to seed germination experiment, 18 seed trays (3 each for 6 plant species) were washed and sterilized with household bleach (one part to nine parts of water), thoroughly rinsed with tap water and finally with reverse osmosis water and air dried to ensure they are sterile for seed sowing. Trays were labelled with names of plants to be sown and date sown on them. Seed trays had drain holes to prevent water-logged conditions after seeds had been sown.

A light density fine grade, Sinclair® vermiculite of (grain size 2.0-5.0 mm) with neutral pH 7 (which is lighter and easier for seeds to breakthrough it) was used for sowing seeds. It was watered with tap water until evenly moist before sowing seeds and then placed in seed trays about 1cm below the rim. Small seeds were sprinkled thinly on the vermiculite, while large seeds were sown to a depth of about 1cm or according to supplier's instruction if present and covered thinly with vermiculite. After sowing, large trays with drain holes were used to cover trays to let in light and air, prevent medium from drying out and becoming damp as well. They were left to germinate in a glasshouse under a photoperiod of 16 hours natural light and maintained at a temperature of 20 C ± 5°C.

Trays were removed once germination occurred. Watering was done carefully when the top of the seed trays appeared dry using a fine spray watering can, and water sprinkled gently to avoid resetting or disturbing the seeds. The surface was kept evenly moist and never dried out. The record of seeds sown is shown in Table 4.3.1.

4.3.3 Result of the seed germination experiment (prior to 1st pot trial).

The result of the seed germination experiment is shown in Table 4.3.1. Sixteen varieties made of six species were sown. Four different varieties of *Brassica juncea*, two of *Brassica napus*, one of *Gentianna pennelianna* and *Biden alba*, four of *Zea mays* and four of *Thlaspi caerulescens*.

The following varieties had the highest germination rates, *Brassica juncea* (BJ 18) 88% among the *Brassica juncea* varieties, ZM OH43 95% among the *Zea mays*, BN SW 97% among *Brassica napus*, TC HS 95% among the *Thlaspi caerulenscens* (Table 4.3.1).

Gentianna pennelianna and *Biden alba* had low germination rates of 2% and 1% respectively. As a result of this poor germination rate and non-availability of an alternative source of seed of these species, they were dropped from the initial experiment.

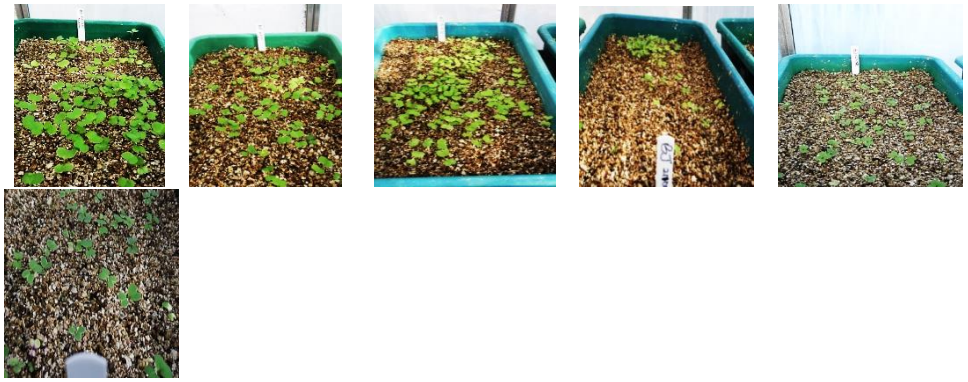
Thlaspi caerulescens (003045) supplied by KEW was also dropped due to its poor germination rate (5%).

Four species (*Brassica juncea*, *Brassica napus*, *Thlaspi caerulescens* and *Zea mays*) and 13 different varieties were considered for initial transplanting into unspiked growth medium after 7 days of germination to ensure proper growth and establishment before the actual transplant into the Pb spiked growth medium. Some of the varieties/species germinated before the initial transplant into unspiked growth medium are shown in Figure 4. 3.1.

Table 4.3.1: Result of the seed germination experiment. Note: *Thlaspi caerulescens* recently renamed *Noccaea caerulescens*

Seed type (Species)	Accession No/Abbreviation	Origin	Plant name/ common nane	Date sown	Estimated quantity sown	Supplier	No germinated	% Germination
Brassica juncea(BJ)	**P1 426308/ BJ 42**	Pakistan	K-100/ Indian mustard	1/8/2012	2.3 g (60 seeds)	USDA	40	67
	PI 173874/ BJ 17	India, Delhi	NA/Indian mustard	1/8/2012	2.3 g (60 seeds)	USDA	45	75
	PI 182921/ BJ 18	India, Gujarat	NA/Indian mustard	1/8/2012	2.1 g (60 seeds)	USDA	53	88
	PI 211000/ BJ 21	Afganistan, Badakhshan	NA/Indian mustard	1/8/2012	2.4 g (60 seeds)	USDA	25	42
Brassica napus (BN	**PI 601261/ BN SW**	Sweden, Malmohus	Crystal/ oil seed rape	1/8/2012	2.7 g (60 seeds)	USDA	58	97
	3045/ BN K	Algeria	NA/oil seed rape		2.3 g (60 seeds)	KEW	52	87
Zea mays (ZM) subs mays	**Ames 19288/ ZM OH 43**	USA, Ohio	OH43/ corn	1/8/2012	15.6 g (40 seeds)	USDA	38	95
	PI 550467/ ZM B 37	USA, Iowa	B 37/corn	1/8/2012	14.6 (40 seeds)	USDA	35	88
	PI 550473/ ZM B 73	USA, Iowa	B 73/corn	1/8/2012	15 g (40 seeds)	USDA	36	90
	PI 644101/ ZM 64	USA, Iowa	LH1/corn	1/8/2012	15.4 g (40 seeds)	USDA	33	83
Gentianna pennelianna (GP)	**Not applicable/GP**			1/8/2012	3.5 g (200 seeds)	Herbiseed	3	2
Biden alba (BA)	**Not applicable/BA**			1/8/2012	6.3 g (200 seeds)	Herbiseed	2	1
Thlaspi caerulescens (TC)	**Not applicable/ TC HS**	Not applicable	NA/Alpine pennycress	1/8/ 2012	9.2 g (80 seeds)	Herbiseed	76	95
	Not applicable/ TC BR	Black rocks	NA/Alpine pennycress	1/8/2012	3.8 g (60 seeds)	Claudia Harflett	54	90
	Not applicable/ TC GM	Gang Mine	NA/Alpine pennycress	1/8/2012	2.5 g (60 seeds)	Claudia Harflett	42	70
	8035/ TC KEW	Cameroun		1/8/2012	2.3 g (60 seeds)	KEW	3	5

USDA-United States Department of Agriculture. KEW—Royal Botanic Garden at KEW. Abbreviations representing species/varieties used in the first pot trial and subsequent pot trials in red. N/A—Not applicable



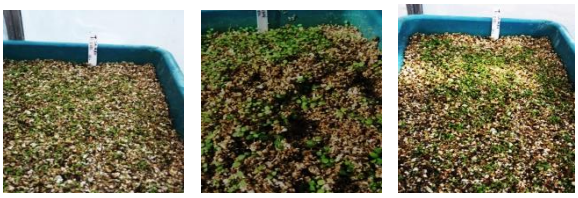
B. napus **(BN SW)** ***B. napus*** **(BN K)** ***B. juncea*** **(BJ 18)** ***B. juncea*** **(BJ 42)** ***B. juncea*** **(BJ 17)** ***B. juncea*** **(BJ 21)**

5 mm

Scale bar- **4 mm represents 5 mm**

Zea mays **(ZM B 73)** ***Zea mays*** **(ZM 64)** ***Zea mays*** **(ZM B 37)** ***Zea mays*** **(ZM OH43)**

5 mm Scale bar: 3.6 mm represents 5 mm



Thlaspi **(TC GM)** ***Thlaspi*** **(TC HS)** ***Thlaspi*** **(TC BR)**

5 mm

Scale bar--- 6 mm represents 5 mm

Figure 4.3.1: Some of the varieties of *Brassica napus*, *Brassica juncea*, *Zea mays* and *Thlaspi caerulescens* germinated (Species/varieties abbreviations are given in Table 4.3.1 above).

4.3.4 Growth Medium for Pot Trials (1st & 2nd).

The growth medium was a mixture of silver sand of grain size 0.063 - 0.2 mm and compost in the proportion (by volume) of 7 parts sand to 3 parts compost, which was spiked with total Pb concentrations of 1000 mg/kg (pot trial 1) and 100 to 10,000 mg/kg dry weight of Pb in the form of PbO for the second pot trial. Sand was used to allow for proper aeration. The ratio of sand to compost was as described in previous work (Thomas, 2010). Potting growth medium was chosen to best meet the needs of plant

roots of all species for air, water, nutrients and plant support. The nutrient rich compost combined with sand made an excellent growth medium for these plant species.

4.3.5 Moisture Determination

John Innes Compost No. 2 was used. Determination of moisture content of growth medium was done using 100 ml of both compost and sand from several lots placed into clear plastic bags. Fresh weights of compost and sand were recorded and then dried at 60^0 C in a fan oven overnight. These were useful in determination of the moisture content and estimation of the amount of sand and compost required for growth media in each experimental pot. The mean percentage moisture for sand and compost were 0.12% and 31% respectively (Appendix II.1: Tables B II.1 and CII.1).

4.3.6 Preparation of Growth Media for 1st pot trial.

A mass of 38.4 kg of silver grade sand was transferred into a concrete mixer to prepare a batch of growth medium (1000 mg/kg Pb). A volume of 13.5 L of John Innes Compost 2 was weighed and added to the concrete mixer (containing the silver grade sand) (Appendix II.1: Table DII.1).The content was thoroughly mixed using the concrete mixer to obtain a sufficiently homogeneous growth medium. Thirty-nine pots (3 replicates for 13 species/varieties) of 1000 mg/kg Pb added treatment were maintained in the first pot trial.

Five lots each of about 10 g of the mixed spiked growth media was sampled to check the Pb concentration of growth media. These portions were taken from randomly selected pots, dried in the oven at 110^{o}C and milled using the tema mill. A mass of 0.25 g of the milled sample was used to determine Pb concentration and (homogeneity) of the contaminant at each Pb concentration level using the Atomic Absorption Spectrometer (AAS) after acid digestion by nitric and perchloric acids. Certified reference materials (CRMS), duplicates and reagent blanks were used for quality control. Growth media actual Pb concentration for the first pot trial is shown in Table 4.3.2.

Table 4.3.2: Growth media Pb concentration check for pot trial 1.

Nos of replicates	Measured Pb concentration mg/kg	Nominal Pb concentration mg/kg
1	907	1000
2	943	
3	927	
4	940	
5	836	
6	914	
Mean	911	
STDEV	39.27	
SEM	16.03	

4.3.7 Transplanting of seedlings for the first pot trial.

After germination and the development of the first true leaves, plants of approximately equal size were selected and transplanted into the centre of separate circular 1- litre pots (15 cm deep and 12 cm wide) pots for each species containing unspiked growth medium (washed silver sand, John Innes compost II, 7 parts sand to 3 part compost). Forty seedlings per plant species were transplanted into pots (making a total of 240 seedlings) of unspiked growth medium first for two weeks and watered daily using a fine rose watering can. This was maintained under 16 hours of natural light at 20 ± 5 ^{o} C in the glasshouse. At two weeks after the first transplanting, three seedlings of each species were transplanted into the 39 pots containing growth medium spiked with Pb contaminant at concentration of 1000 mg/kg Pb added and another 39 in the 0 mg/kg Pb added.

A total of 78 pots were maintained (1000 mg/kg and 0 mg/kg added treatment and control of 4 species and 13 varieties) for 3 weeks under a photoperiod of 16 hours natural sunlight at 20 ± 5˚C in the glasshouse. These were maintained in 3.5-litre square pots (dimensions 17 cm x 24 cm) in a simple randomized block design both in 1000 mg/kg Pb and 0 mg/kg added Pb as control (Figure 4.3.2 and Appendix II.1: Table AII.1). Pots were rotated clockwise by 90^{o} weekly to reduce the effect of uneven environmental conditions within the glass house.

Randomized blocks were between species/varieties, because of the number of varieties and the available space/m^2 of greenhouse benches.

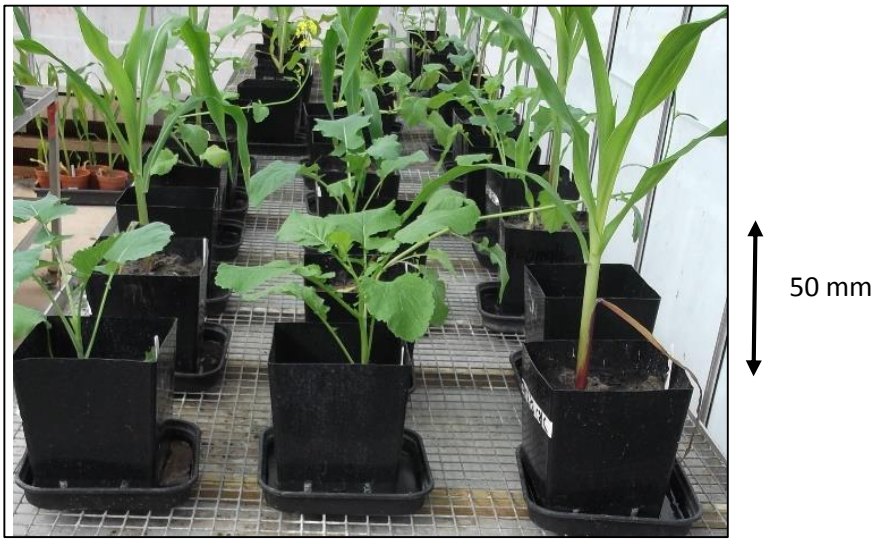


Figure 4.3.2: Randomized Block design for the first pot trial. Scale bar: 17 mm represents 50 mm

4.3.8 Data collection and analysis.

Growth data such as plant height, number of true leaves, number of dead leaves and the longest leaf length were taken at initial transplant (week 1) and at harvest (week 3). Stem height, leaf length and stem width of the different varieties were measured to the nearest ± 0.1 mm using a tape rule and caliper.

For the purpose of this experiment, growth rate was expressed in terms of Growth index (GI) (Keever, 1994 and Melannie *et al*., 2006), who estimated growth index in terms of measured plant height and width. However, GI was not a key variable in this experiment but merely an additional means of assessing growth rate during the growing period. Growth index was mathematically expressed as GI= height (mm) +width at widest point + width 90^0 to first width/3 (Keever, 1994).Growth index values are stated with 1 standard error on the mean.

Data were analyzed using IBM SPSS version 19 and Minitab 16 for windows. The Student t-test was used to test for between treatment effects for measured variables. Analysis of variance (ANOVA) and the Tukey HSD Post-hoc test were used to compare biomass and Pb concentration of shoots, roots and total plant Pb between species/varieties. This was used to study plants uptake and behaviour to Pb contaminant at the concentration applied. Results were applied in selecting plant species and Pb concentrations in further experiments.

At harvest, other observable effects such as leaf chlorosis were recorded when it occurred, which indicated a severe effect of the Pb added treatment on the species/varieties affected. Plants were harvested after three weeks of growth in the 1000 mg/kg Pb spiked growth medium. Dried and milled plant samples were analysed for shoot and root Pb concentration using the AAS (PerkinElmer AA Analyst 400) after acid digestion by nitric and perchloric acids.

4.3.9 Harvesting.

Plant stems were cut 0.01 mm above the soil surface for shoot harvest and soil removed from the roots using a sieve. Soil was removed from harvested plant materials by repeated washing using tap water and dried at 60^{o}C for 48 hours (Subramanian, 2011).This was milled (using a herbage mill) for acid digestion using nitric and perchloric acids (Thompson and Walsh, 1983; Subramanian, 2010) and analysed for Pb using the AAS (Acid digest method and quality control in Appendix II.14).

4.4 RESULTS OF THE FIRST POT TRIAL.

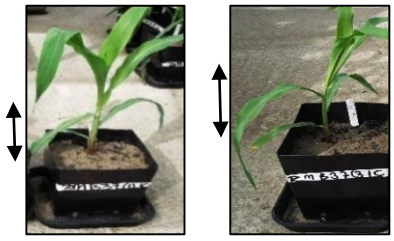
Visible significant differences within and between varieties and species were detected during the growth period. Adequate aboveground plant biomass (i.e. > 1 g FW) had been produced from 21 day growth in the spiked growth medium by most varieties when they were harvested. Survival rate was 100% for most species, except *Thlaspi caerulescens* (TC GM and TC BR). At harvest, a reduced root size was observed for all the *Brassica juncea* varieties in the 1000 mg/kg. Plants conditions at harvest in control and Pb added treatments are shown in Figures 4.4.1 to 4.4.4.

(a) BJ 42 ---------- (b) BJ 21---------- (c) BJ 17----------

(d) BJ 18----------

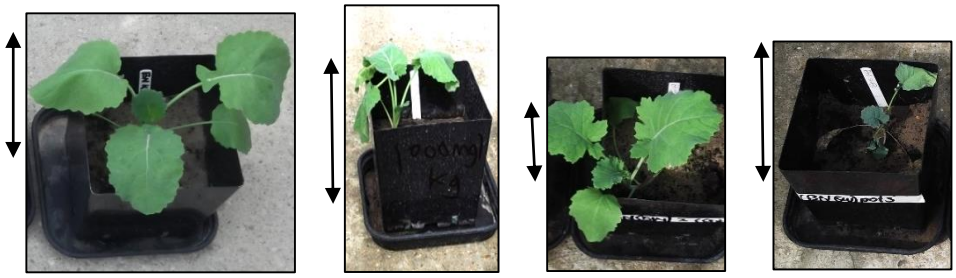
Figure 4.4.1: *Brassica juncea* BJ 42, 21,17 and 18 (from left to right) in the control (Left) and Pb added (right) treatments at harvest respectively. BJ 42, BJ 21 and BJ 18 showed chlorosis, reduced height and wilting of leavest. Arrow represents scale bar. See scale bar information on key below.

(a) ZM 64---------- (b) ZM B73---------- (c) ZM OH43----------



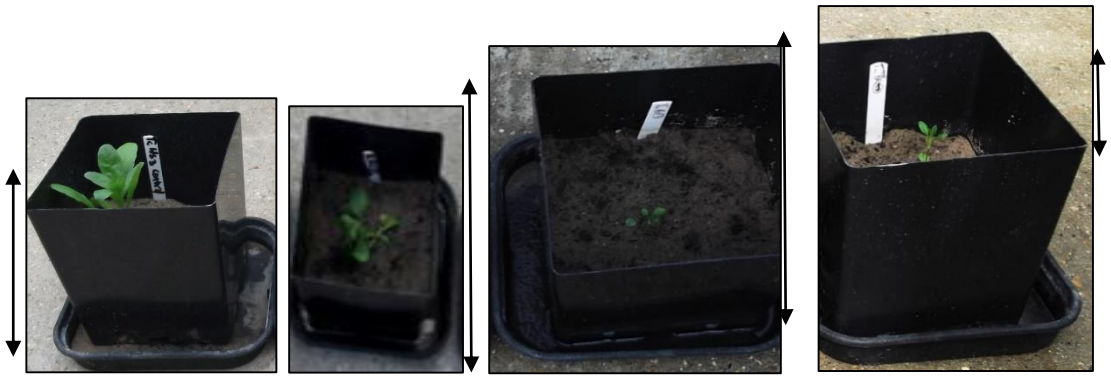
(d) ZM B37----------

Figure 4.4.2: *Zea mays* ZM 64, B37, OH43 and B73 varieties (from left to right) in the control and Pb added treatments at harvest respectively. Arrows represents scale bars. See scale bar information on key below.

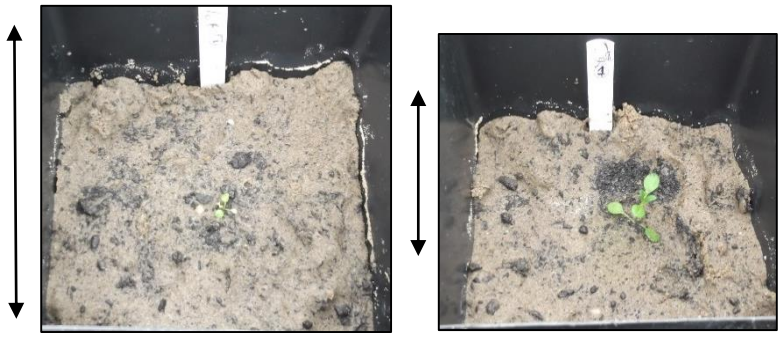


(a) BN K ---------- (b) BN SW----------

Figure 4.4.3: *Brassica napus* varieties, BN K and BN SW (from left to right) in the control and Pb added treatments at harvest respectively. Arrows represents scale bars.See scale bar information on key below.



(a) TC HS ---------- (b) TC BR----------



(c) TC GM----------

Figure 4.4.4: *Thlaspi caerulescens* (TC) varieties TCHS, TCBR, TCGM (from left to right) in the control and Pb added treatments at harvest respectively. Arrows represent scale bars. See scale bar information in key below.

Key: Scale bar information for Figures 4.4.1 to 4.4.4.

		Scale bar information	
Variety abbreviation	**Species name**	**Control**	**1000 mg/kg Pb added**
BJ 42	***Brassica juncea***	4 mm represents 20 mm	4 mm represents 20 mm
BJ 21	***Brassica juncea***	3 mm represents 20 mm	5 mm represents 20 mm
BJ 17	***Brassica juncea***	4 mm represent 20 mm	3 mm represents 20 mm
BJ 18	***Brassica juncea***	2 mm represents 20 mm	2 mm represents 20 mm
ZM 64	***Zea mays***	5 mm represents 20 mm	9 mm represents 20 mm
ZM B73	***Zea mays***	5 mm represents 20 mm	5 mm represents 20 mm
ZM OH43	***Zea mays***	3 mm represents 20 mm	3 mm represents 20 mm
ZM B37	***Zea mays***	4 mm represents 20 mm	5 mm represents 20 mm
BN K	***Brassica napus***	12 mm represents 20 mm	15 mm represents 20 mm
BN SW	***Brassica napus***	8 mm represents 20 mm	13 mm represents 20 mm
TC HS	***Thlaspi caerulescens***	15 mm represents 10 mm	50 mm represents 10 mm
TC BR	***Thlaspi caerulescens***	50 mm represents 5 mm	6 mm represents 5 mm
TC GM	***Thlaspi caerulescens***	50 mm represents 5 mm	19 mm represents 5 mm

4.4.1 Shoot, root and total dry biomass.

Comparison of the shoot, root and total dry biomass showed significant differences between treatments in these parameters for some species/varieties (Figures 4.4.5-4.4.7). Only those differences with statistical significance ($P < 0.05$) are discussed in detail.

The shoot dry biomass of *Zea mays* varieties ZM B73 and ZM 64 were significantly different ($P = 0.007$ and $0.036 < 0.05$) between treatments respectively (Appendix II.3: Tables CII.3 and DII.3). Similar trend of significant differences in shoot biomass between treatment were observed where $P = 0.012$ and $0.006 < 0.05$) respectively in BJ 18 and BJ 42 (Appendix II.3: Tables AII.3 & BII.3) among the *Brassica juncea* varieties and $P=0.012$ and 0.002 for BN K and BN SW respectively among the *Brassica napus* varieties (Appendix II.3: Tables EII.3 & FII.3). It implied that these differences were not random occurrences, but as result of the Pb treatment. The variety BJ 17 did not show chlorosis, while chlorosis and wilting of leaves were observed in BJ 42 (Figure 4.4.1.).

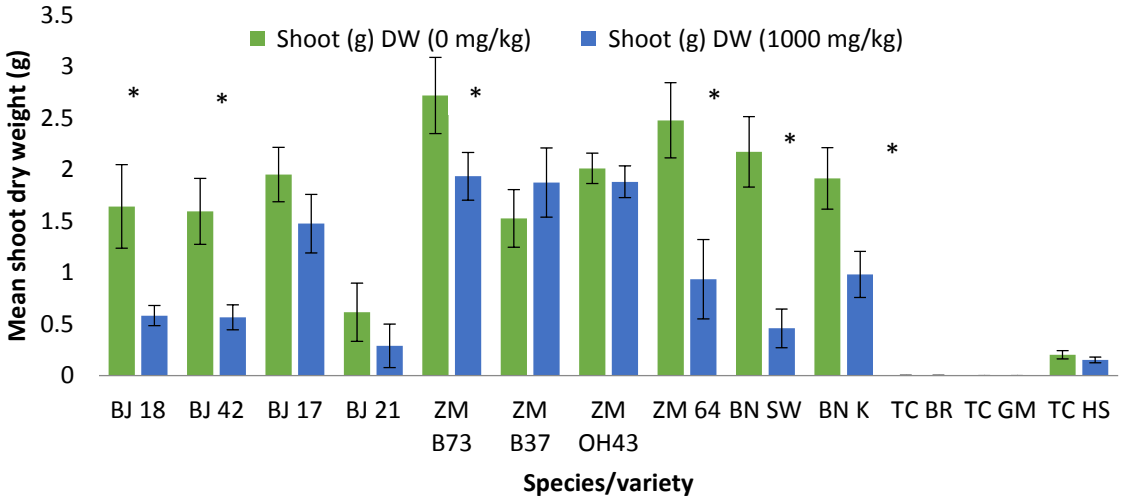


Figure 4.4.5: Mean shoot biomass DW between treatments for each species and variety in the 1st pot trial. Error bars represent 1 standard error on the mean where n=3. *--------Significant at P<0.05.

Root dry biomass was also significantly different $P=0.001$, 0.004, 0.002 and $0.03 < 0.05$) between treatments for BJ 18, ZM B73, ZM OH43, BN SW and TC HS respectively (Figure 4.4.6; Appendix II.4 : Tables A to DII.4).

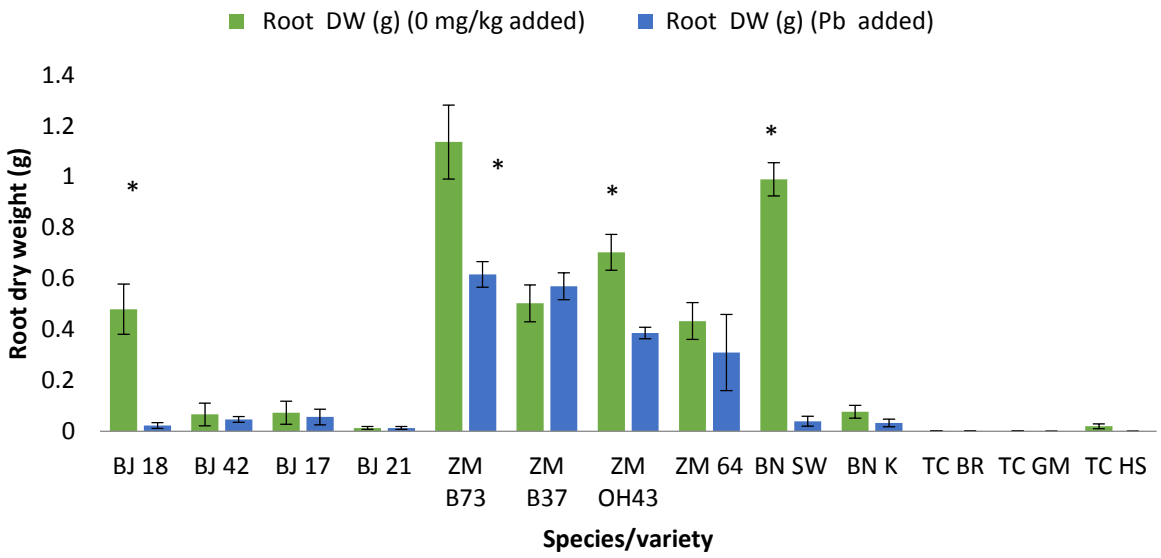


Figure 4.4.6: Root biomass DW between treatments for each species and variety in the 1st pot trial. Error bars represent 1 standard error on the mean where n=3. *--------Significant at P<0.05.

Similarly, the total dry biomass differed significantly between treatments for BJ 18, BJ 42, ZM B73, ZM 64, BN SW and BNK (Figure 4.4.7; Appendix II.4: Tables E to I-II.4).

The difference between the two treatments is an indication of the significant effect of Pb in the soil on biomass and plant performance. However, significant effect was not detected on the total dry biomass of some of the varieties and species, which suggest that not all species/varieties were negatively impacted by Pb or the experiment did not have sufficient power to detect such an impact.

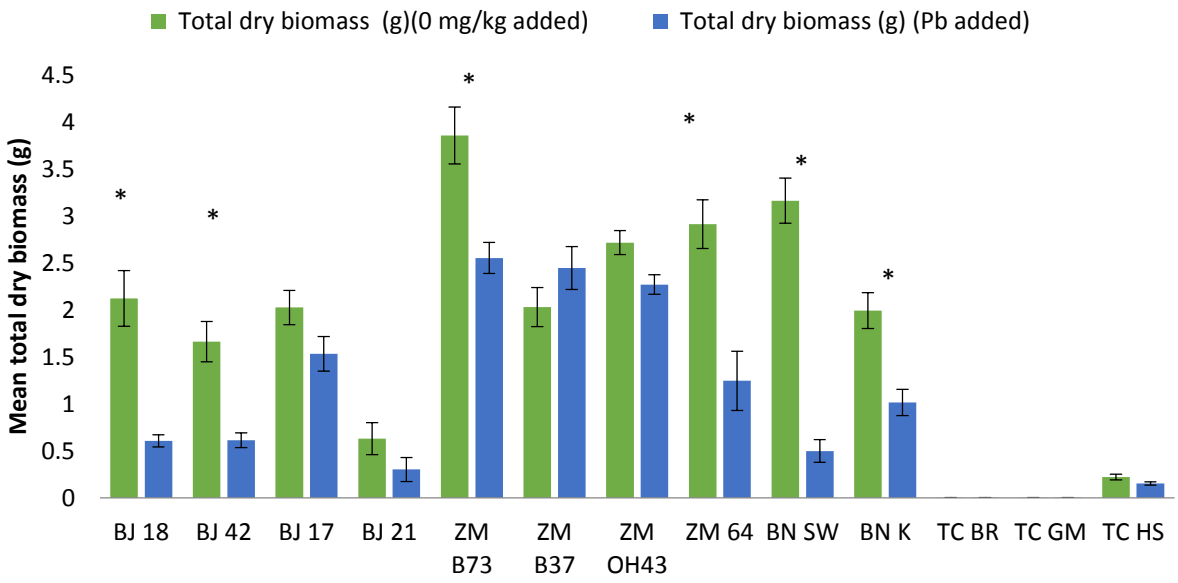


Figure 4.4.7: Mean <u>total dry biomass</u> DW between treatments for each species and variety in the 1st pot trial. Error bars represent 1 standard error on the mean where n=3. *--------Significant.

4.4.2. Comparison of shoot, root, total plant Pb (mg/kg) within species in the Pb added treatment (1st pot trial).

In line with stated hypotheses that some of the tested species/varieties are more tolerant to the Pb added treatment than the other, shoot, root and total plant Pb concentrations were compared within and between species/varieties. This comparison enabled selection of species/varieties which can tolerate high Pb in their shoots and roots without severe observable effect (e.g severe wilting of leaves and plant death) in the Pb added treatment.

The difference in the shoot, root and total plant Pb (mg/kg) dry weight was generally significant (P=0.000 < 0.05) within the *Brassica juncea*, *Zea mays* and *Thlaspi caerulescens* varieties (Figures 4.4.8 to 4.4.11; Appendix II.7: Tables A to L-II.7).

Mean shoot Pb concentration ranged from 83 to 144 mg/kg for BJ 17, 18 and 42. This difference was significant (Appendix II.6: Table BII.6). However the variety BJ 21 had 30 to 90% higher shoot Pb than the lowest and highest concentration within this range (Figure 4.4.8). A similar trend of increased total plant Pb (mg/kg) DW was recorded for this variety. The root Pb concentrations varied significantly within varieties and was highest (643 mg/kg) for BJ 18 and lowest (38 mg/kg) for BJ 21 (Figure 4.4.9).

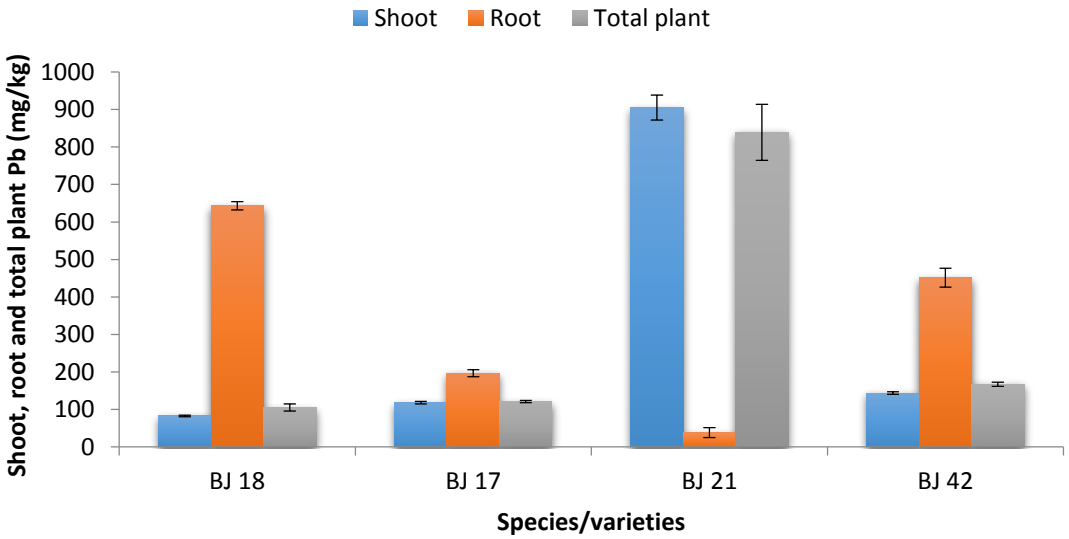


Figure 4.4.8: Mean shoot, root (mg/kg) and total Pb concentration (mg/kg DW) within varieties of Brassica juncea grown in the Pb added treatment in the first pot trial. Error bars represent 1 standard error on the mean where n=3.

The *Zea may* varieties ZM 64, ZM B 73, ZM OH43 and ZM B37 were significantly different (P = 0.000 < 0.05) in their shoot, root and total plant Pb concentrations (Appendix II.7:Tables F to HII.7). Lead concentrations for these varieties ranged from 45 - 126 mg/kg, 244 - 578 mg/kg and 79 – 203 mg/kg DW for shoot, root and total plant Pb respectively (Figures 4.4.9). The highest shoot Pb (578 mg/kg) was recorded for B73 and the lowest (244 mg/kg) for OH43 (Figure 4.4.9).

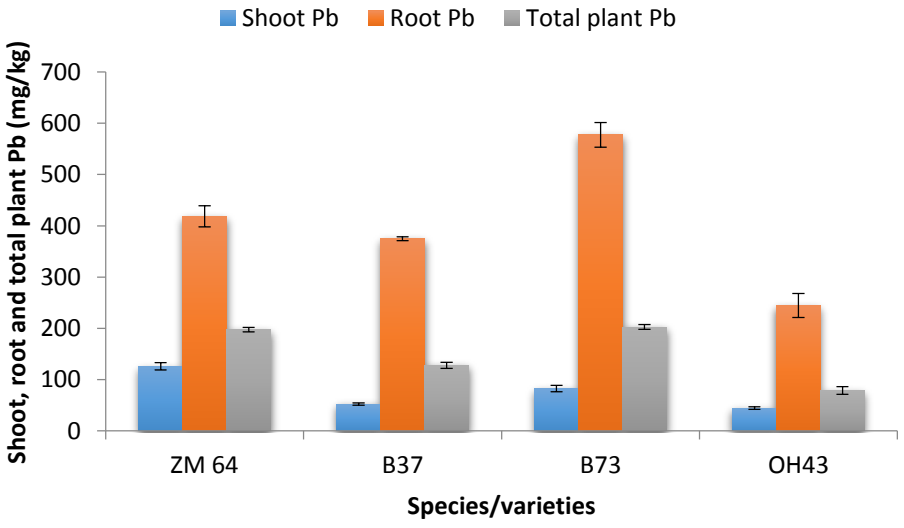


Figure 4.4.9: Mean shoot, root (mg/kg) and total Pb concentration (mg/kg DW) within varieties for Zea mays in the 1st pot trial. Error bars represent 1 standard error on the mean where n=3.

The two *Brassica napus* varieties (BN K and BN SW) were significantly different (P=0.03) in their shoot Pb concentrations (Appendix II.7: Table LII.7). The average shoot Pb

concentrations were 66 and 48 mg/kg respectively. There were no significant difference (P=0.417, 0.310) in their root (385 and 305 mg/kg) and total plant Pb (mg/kg) DW (77 and 69 mg/kg) respectively (Figure 4.4.10).

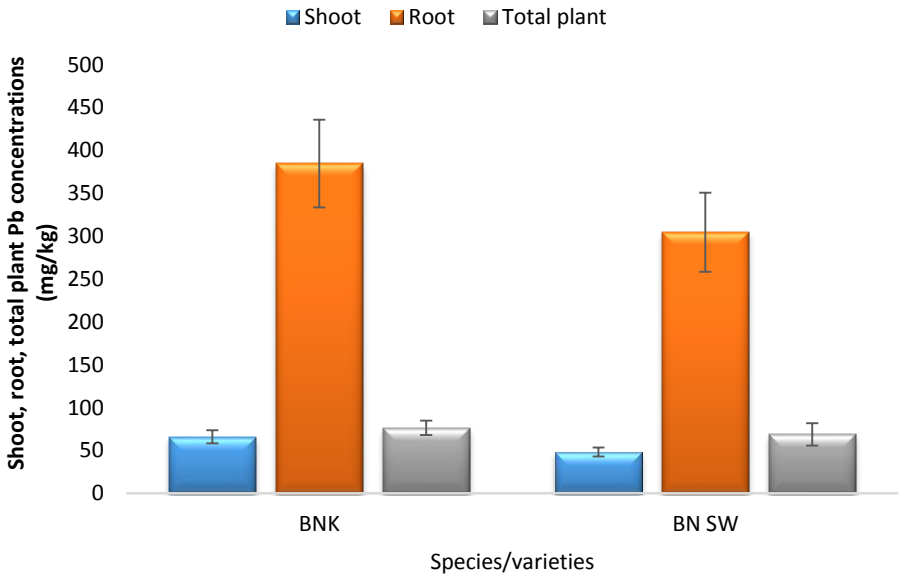


Figure 4.4.10: Mean Shoot, root (mg/kg) and total Pb concentration (mg/kg) within *Brassica napus* varieties in the 1st pot trial. Error bars represent 1 standard error on the mean where n=3.

Thlaspi caerulescens variety TC BR had the highest root Pb concentration (631 mg/kg) when compared to TC GM and TC HS (114 and 358 mg/kg) (Figure 4.4.11). This was 53 and 76% more than those of TC GM and TC HS respectively. The difference within these varieties was significant P=0.000 (Appendix II.7: Tables I II.7 to KII.7). TC HS had higher shoot Pb (264 mg/kg) when compared to TC GM and TC BR (Figure 4.4.11).

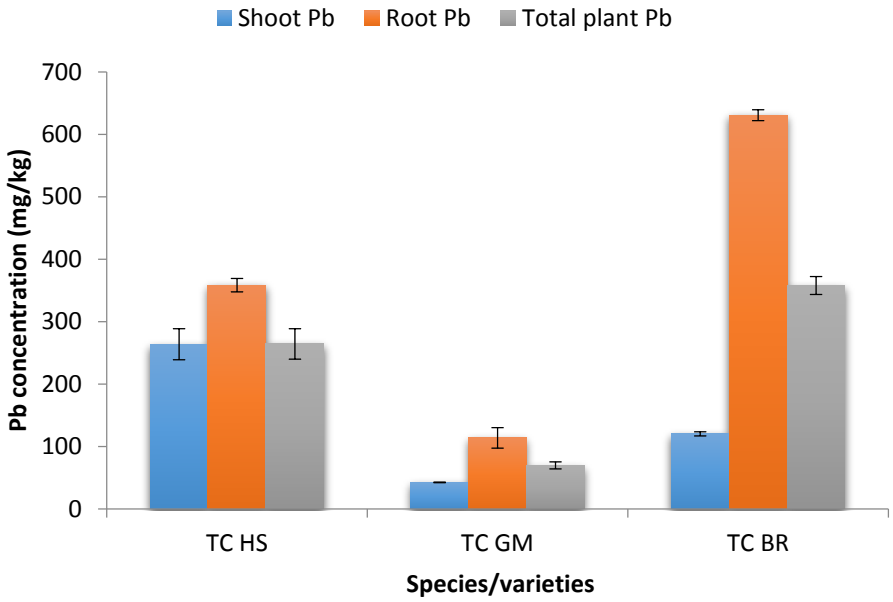


Figure 4.4.11: Mean shoot, root and total Pb concentration (mg/kg) within each Thlaspi caerulescens variety. Error bars represent 1 standard error on the mean where n=3.

4.4.3. Comparison of the shoot, root and total plant Pb between species/varieties of plants grown in the Pb added treatment (1st pot trial).

Comparison of the shoot, root and total plant Pb (mg/kg) DW between species/varieties are shown in Figures 4.4.12 to 4.4.14 below. Shoot, root, and total plant Pb concentrations (mg/kg) dry weight showed that the Pb added treatment had a significant effect (P = 0.000) on most of the plant species. However, the shoot, root and total plant Pb concentrations of some of the species were not significantly different (Figures 4.4.12 to 4.4.14; Appendix II.6: Tables A to I II.6).

Brassica juncea variety BJ 21 differ significantly (P < 0.05) from the others in its shoot, root and total plant Pb concentration with the highest mean shoot of 905 mg/kg and the lowest root Pb concentration of 38 mg/kg.

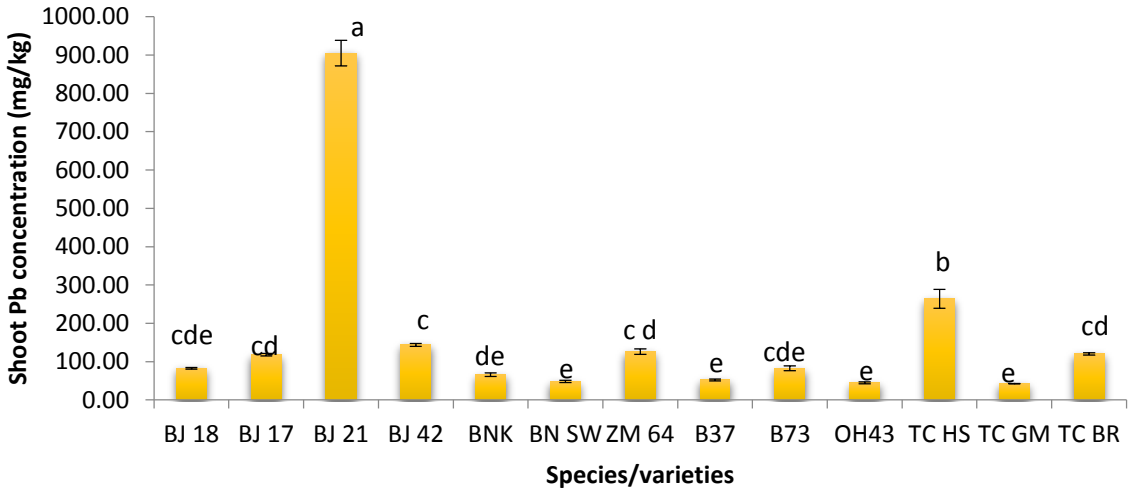


Figure 4.4.12: <u>Shoot Pb concentration</u> (mg/kg) across species and varieties in the 1000 mg/kg Pb added treatment. Tukey post-hoc test, sharing letters means not significantly different. Error bar represent 1 standard error on the mean where n=3).

Generally, more Pb was accumulated in the roots than shoots (by a factor of 2.5). Root Pb concentrations ranged from 114 to 642 mg/kg with the exception of the variety BJ 21 which had about 17 times lower root Pb than the highest root Pb concentration in this range (Figure 4.4.13). More Pb was accumulated in the shoot of same variety (BJ 21) (by a factor of 23.8) when compared to its root Pb concentration (Figure 4.4.12). Seed supplier's note on this plant suggest that BJ 21 seeds were collected from heavily Pb contaminated sites in Afghanistan. The exceptional Pb accumulating trait of this variety could be linked to its adaptation to Pb resulting in enhanced metal uptake and translocation to the shoot.

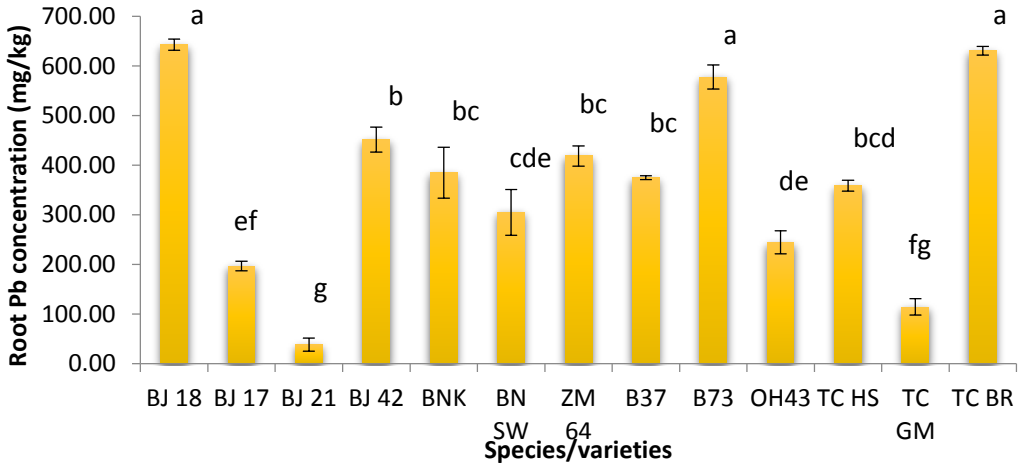


Figure 4.4.13: <u>Root Pb concentration</u> (mg/kg) across species and varieties in the Pb added treatment. Tukey post-hoc test, sharing letters means not significantly different. Error bars represent 1 standard error on the mean where n=3.

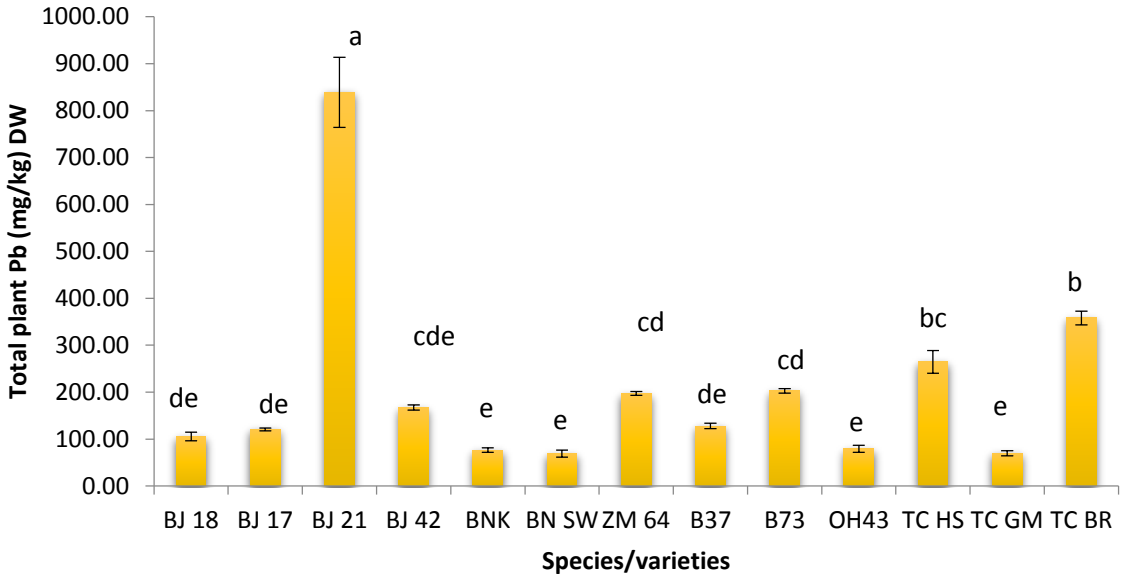


Figure 4.4.14: Total plant Pb concentration (mg/kg) dry weight across species and varieties in the Pb added treatment. Tukey post-hoc test, sharing letters means not significantly different. Error bars represent 1 standard error on the mean where n=3.

In contrast low shoot Pb concentrations were recorded for most species. Shoot Pb ranged from 42 to 263 mg/kg for most species/varieties. However, BJ 21 had shoot Pb concentration of 905 mg/kg, three times higher than the highest concentration and 21.5 fold higher compared to the lowest concentration in this range. Some of the varieties and species were not significantly different ($P > 0.05$) in their shoot, root and total plant Pb as judged by the Tukey HSD test (Figures 4.4.12 to 4.4.14). Varieties/species such as BJ 21, BJ 42, ZM B37, ZM 64, BN SW, BN K showed observable effects of Pb in the form of mild to severe leaf chlorosis and wilting of leaves (Figures 4.4.1 to 4.4.4).

4.4.4 Comparison of Concentration Factor between species and varieties.

As discussed in Chapter 2: Section 2.4.2, plant capacity to accumulate metals from the soils can be estimated by a Concentration factor (CF) (Safae *et al*., 2008) expressed as the ratio of the concentration of metal in shoots and roots mg/kg DW and the soil Pb concentration mg/kg DW. The shoot concentration factor was within the range of 0.05 to 0.99 (Figure 4.4.15) while the root concentration factor (CF_{root}) ranged from 0.04 to 0.70 (Figure 4.4.16). All species/varieties had CF_{shoot} less than 1, although it was very variable with 80% differences between the highest and lowest. Those of *Thlaspi caerulescens* TC HS and BJ 21 were significantly higher than most species/varieties (Figure 4.4.15; Appendix II.5: AII.5 and BII.5). The differences between some of the species were not significant. Shoot concentration factors for most species/ varieties were generally lower than the accumulator threshold of 1. It is an indication that most of these species/varieties

do not easily translocate Pb to the aboveground part of the plant from the root as a tolerance mechanism.

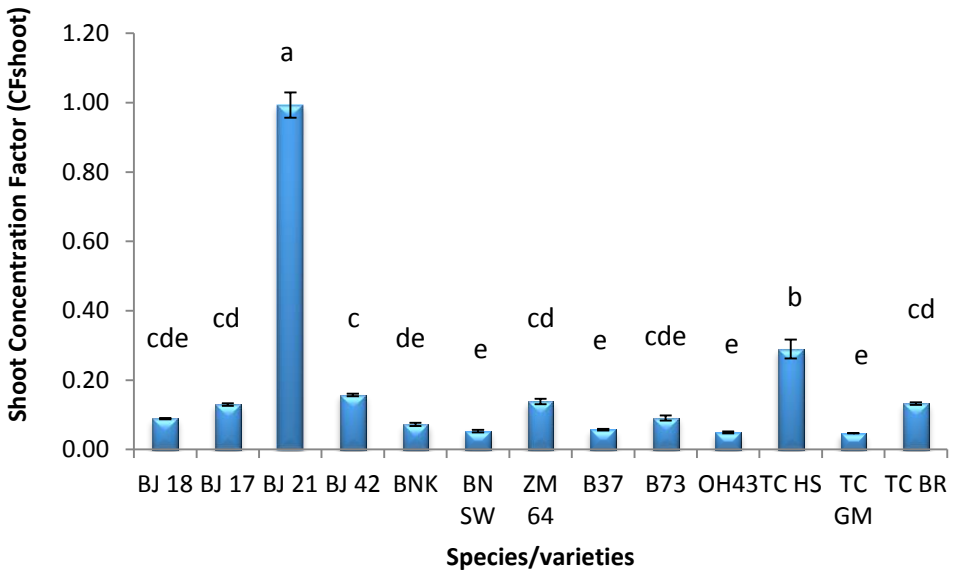


Figure 4.4.15: Mean Shoot Concentration factor (CF_{shoot}) between species and varieties in the Pb added treatment. Error bars represent 1 standard error on the mean where n=3. Means sharing letters are not significantly different as judged by the Tukey post-hoc test.

The CF_{root} of most species were generally higher than the CF_{shoot}, which was 73 to 75% higher (Figure 4.4.16) when compared to the CF_{shoot} for most species. There was an exceptional decrease (25 fold decrease) in CF_{root} of BJ 21. These values of CF_{shoot} and CF_{root} are similar to those of Pb accumulating species/varieties previously reviewed in literature.

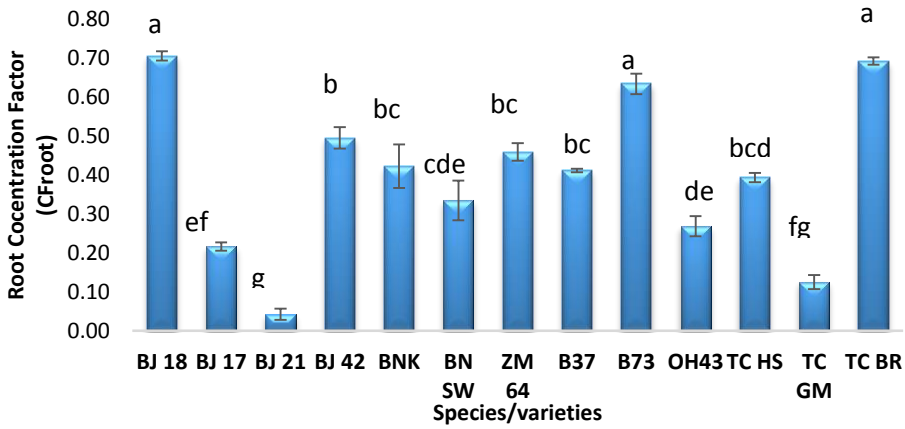
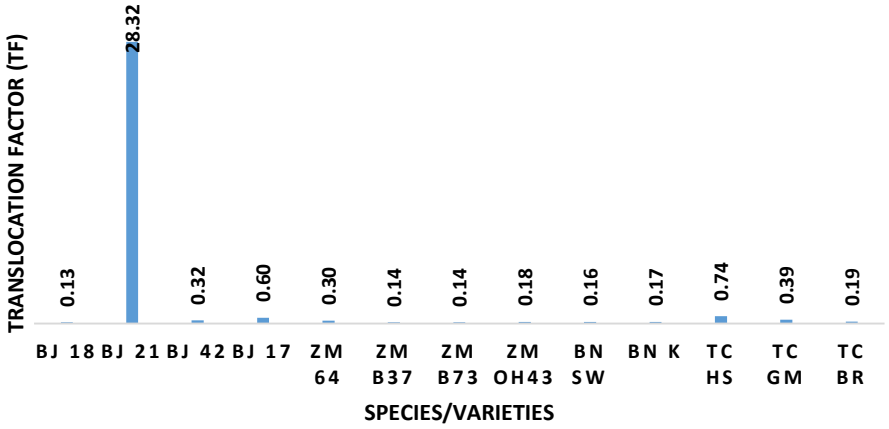


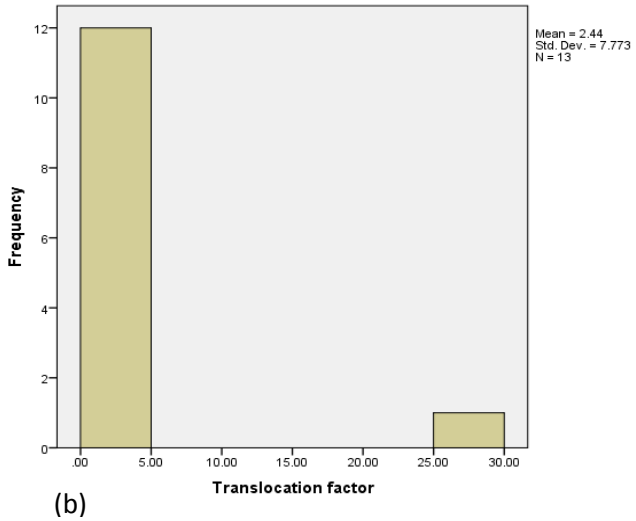
Figure 4.4.16: Root Concentration factor (CF_{root}) across species and varieties in the Pb added treatment. Error bars represent 1 standard error on the mean where n=3. Mean sharing letters means are not significantly different as judged by the Tukey post-hoc test.

4.4.5 Translocation factor of species/varieties in the first pot trial.

A general trend of low translocation factor (TF) was observed across species/varieties with an exception of the *Brassica juncea* variety BJ 21 (Figures 4.4.17a, 4.4.17b and 4.4.18). The TF of most species/variety ranged from 0.1 to 0.7, which were well below 1. This supports the evidence of poor translocation of Pb from root to the shoot suggested by the CF_{shoot} (Figure 4.4.15). The histogram of TF (Figure 4.4.17b) and the Log10 transformation of TF (Figure 4.4.18) divides these species into two main group, which could be seen as hyperaccumulator and accumulators.The variety (BJ 21) was clearly distinct from the other varieties/species as a Pb hyperaccumulator with TF varying by + 40 to 217 % from the other species/variety.



(a)



(b)

Figure 4.4.17: (a) Translocaton factor (TF) across species and varieties in the Pb added treatment. (Shoot Pb DW mg/kg/ root Pb concentration mg/kg) (b) Histogram of translocation factor.

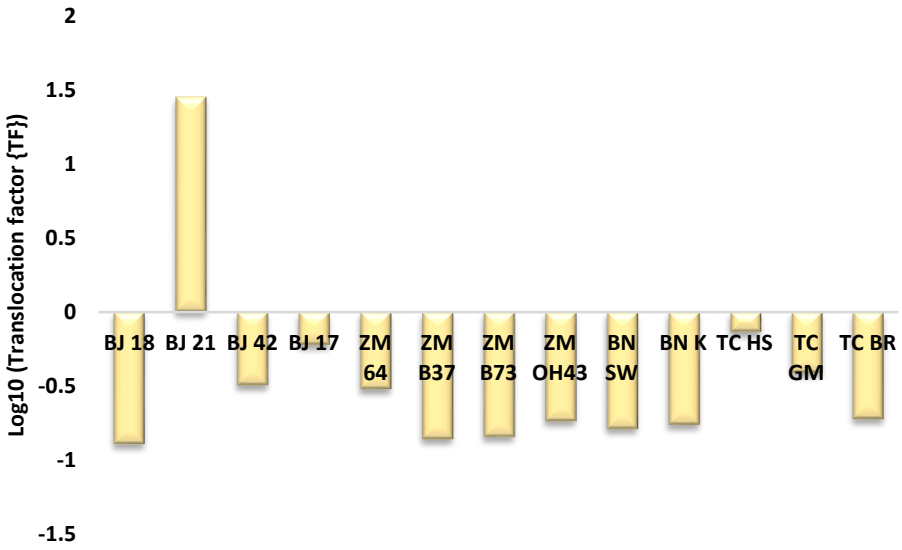


Figure 4.4.18 : Log10 transformation of the translocation factor (TF) across species and varieties in the Pb added treatment.

4.4.6 Growth parameters in the first pot trial.

Other growth parameters such growth index (GI), height, number of true and dead leaves and longest leaf length were used to study the behaviour of plant species/varieties to the Pb added treatment during growth period. The results are as shown in Figures 4.4.19 to 4.4.22 and their statistical significance are summarised in Appendix II.2: Table II.1. Plants height were higher (5 to 40%) in the control than the Pb added treatment for most species with the exception of the *Brassica juncea* variety BJ 17 and *Thlaspi caerulescens* varieties (TC BR and TC GM), which had higher height in the Pb added treatment than the control (Figure 4.4.19). However, the differences in plant height between the control and the Pb added treatment were only statistically significant for BJ 21, ZM B 37 and ZM 64. *Brassica napus* variety (BN K) had identical height in both treatments.

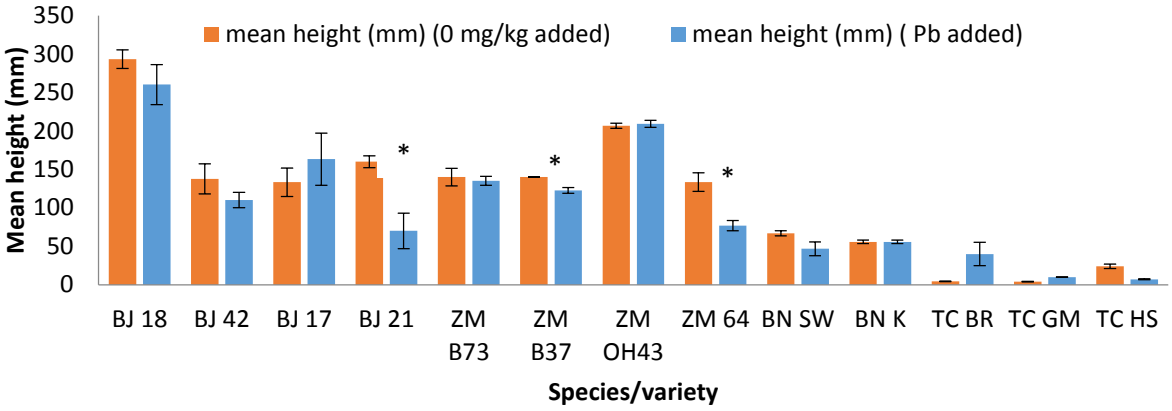


Figure 4.4.19: Height (mm) between treatments for each species/varieties. Error bar represent 1 standard error on the mean. *--- Significant.

Higher number of true leaves (3 to 40 %) were recorded in the control treatment for most species/varieties (Figure 4.4.20). However, some of the species/varieties e.g the *Brassica juncea* varieties (BJ 18, BJ 17), the *Zea mays* varieties (ZM B 73, ZM B 37) and the *Thlaspi caerulescens* varieties (TC BR, TC GM) had more number of true leaves (2 to 5%) in the Pb added treatment than the control. The differences in the number of true leaves between the Pb added treatment and the control of these exceptional species/varieties listed above were not statistically significantly (Appendix II.2: Table II.1). The *Brassica napus* varieties BN K and BN SW had same number true leaves in both treatments.

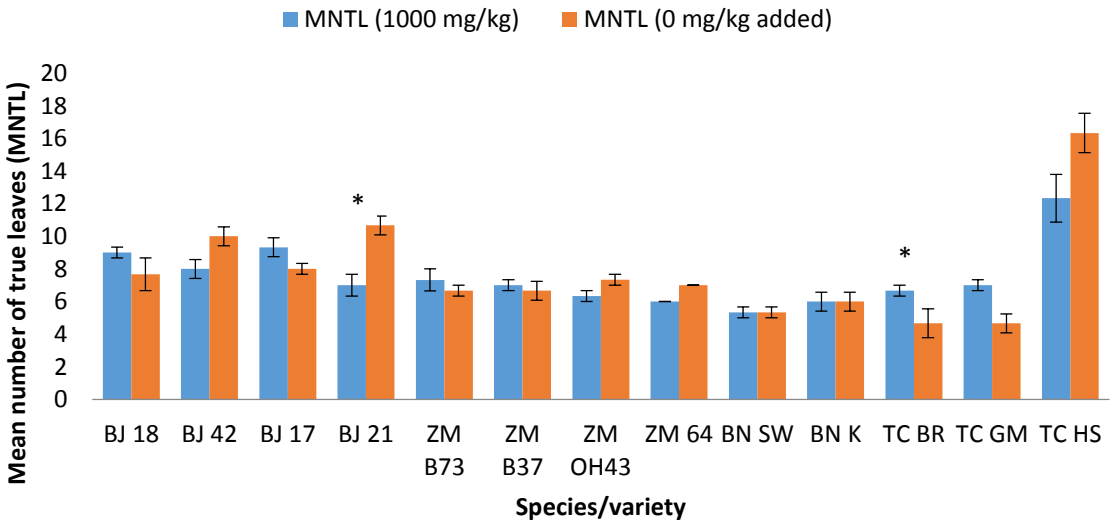


Figure 4.4.20: Mean number of true leaves (MNTL) between treatments across species and varieties in both treatments. Error bar represent 1 standard error on the mean. *---------Significant.

A trend of lower mean longest leaf length was observed across species/varieties in the Pb added treatment, when compared to the control (Figure 4.4.21). However, the *B. napus* variety BN K which had identical longest leaf length in both control and the Pb added treatment (Figure 4.4.21).

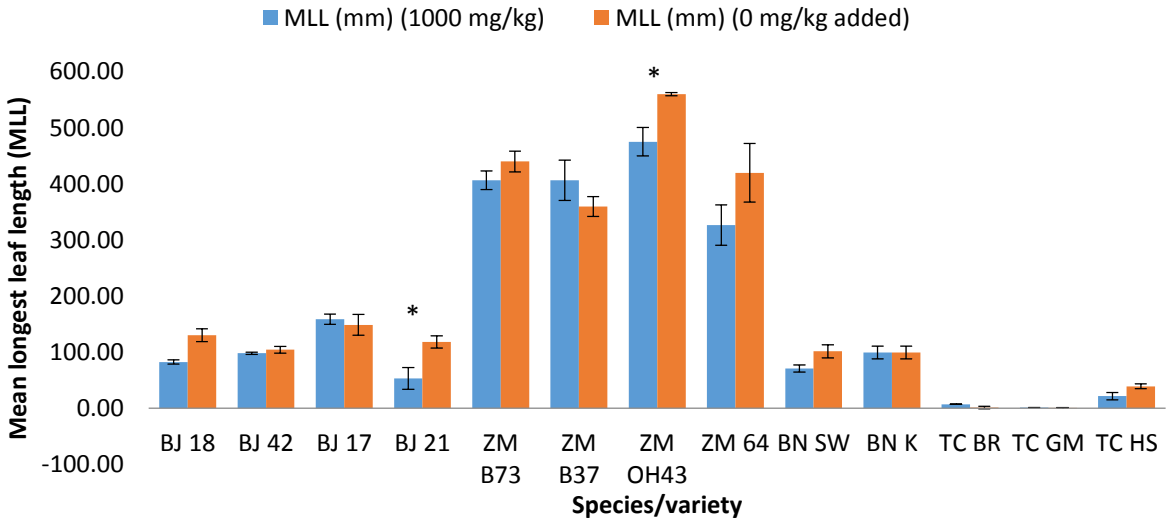


Figure 4.4.21: Mean longest leaf length (MLL) between treatments for each species and varieties in both treatments. Error bar represent 1 standard error on the mean. *-------- Significant.

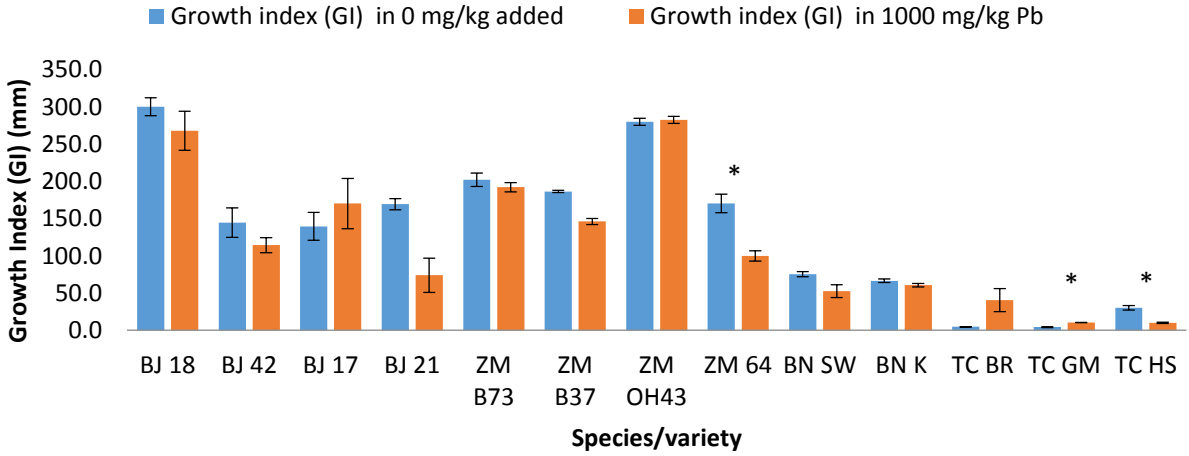


Figure 4.4.22 : Growth Index (GI) between treatments for each species/varieties in both treatments. Error bars represent 1 standard error on the mean. *------Significant

Growth index was highest (268 ± 26.2 mm) in BJ 18 among *Brassica juncea* varieties, ZM OH43 (283 ± 4.68 mm) among *Zea mays* varieties and TC BR (40.4 ± 15.2) among *Thlaspi caerulescens* varieties in the Pb added treatment (Figure 4.4.22). A general trend of decreased GI in the Pb added treatment was observed for most species except TC

BR which had better growth in the Pb added treatment when compared to the 0 mg/kg Pb added treatment.

The key variables compared in this experiment are summarised in Table 4.4.1 below with plant species selected for the second pot trial highlighted in red.

Table 4.4.1: Mean values of variables for each species/varieties compared in the first pot trial.

Variables	BJ 17	BJ 18	BJ 21	BJ 42	ZM 64	ZM B37	ZM B73	ZM OH43	BN K	BN SW	TC BR	TC HS	TC GM
Shoot biomass DW (g)	1.48	0.58	0.29	0.57	0.94	1.88	1.94	1.88	0.98	0.46	0.002	0.15	0.0017
Root biomass DW (g)	0.06	0.02	0.01	0.05	0.31	0.57	0.62	0.39	0.03	0.04	0.001	0.001	0.0010
Total plant biomass DW (g)	1.53	0.61	0.30	0.61	1.25	2.45	2.55	2.27	1.02	0.50	0.003	0.15	0.0027
Shoot Pb (mg/kg)	118	83	905	144	126	52	83	45	66	48	120	264	43
Root Pb (mg/kg)	197	643	38	451	418	375	578	244	385	305	631	358	114
Total plant Pb (mg/kg) DW	121	105	839	167	197	128	203	79	77	69	358	264	70
Shoot Pb (µg)	174	48	270	81	117	97	161	84	66	22	0.20	41	0.07
Root Pb (µg)	11	15	0.46	21	127	213	355	95	13	13	0.85	0.36	0.11
CF_{shoot}	0.13	0.09	0.99	0.16	0.14	0.06	0.09	0.05	0.07	0.05	0.13	0.29	0.05
CF_{root}	0.22	0.71	0.042	0.50	0.46	0.41	0.18	0.27	0.42	0.33	0.69	0.39	0.13

Key: Shoot, root and total biomass DW in blue, Shoot and root Pb (mg/kg) in purple, Shoot and root Concentration factors in green and species/varieties selected for the second pot trial highlighted in red.

4.5 DISCUSSION

Shoots, roots and total plant Pb (mg/kg) DW concentrations provided quantification of the effects of the Pb added treatment on these plants. The Pb added treatment at the concentration applied had a significant effect on growth and biomass of the most species/varieties with observed decrease in biomass in Pb added treatment, compared to the control. However, a few did not show significant change in biomass in the Pb added treatment with substantial Pb accumulation in shoots and roots. It is an indication that the presence of Pb in the soil may not necessarily cause poor plant growth. This is supported by earlier work on Cd (Millis *et al*., 2004) and on a range of toxic metals in soils (Anyanwu *et al*., 2008).

For most of the plant species, more Pb was accumulated in the root than in the shoot. This is in line with findings of Baker *et al*., (1994); Reeves and Brooks, (1989); Nabulo *et al*., (2008). Two of these plant species (BJ 21 and TC HS) were exceptions to this trend with more Pb accumulated in the shoot than in the root. *Brassica juncea* variety BJ 21 had a mean CF_{shoot} and TF of 0.99 and 28 respectively and this suggests a potential Pb hyperaccumulation by this variety. This ability to accumulate more Pb in the shoot is an advantage in terms of phytoremediation.

Moradi *et al*., (2010) stated that hyperaccumulators have potential roles in the mining industry where they may be found useful in phytoremediation/phytomanagement and phytomining. A few plant species such as *Parthenium hysterophorus* {L} (Whitetop weed or Santa Maria feverfew) and *Amaranthus viridis* {L} (Green or slender amaranth) have been shown to translocate high amount of Pb from their roots to shoots (Malik *et al*., 2010). Some of the plants studied showed potentials for Pb accumulation to varying extent. Low CF_{shoot} values between 0.05 and 0.29 were recorded for most varieties.

Comparisons within and between species/varieties suggest that the effect of the added Pb and uptake of Pb from the soil varied both within and between varieties/species of plants, though similarities in Pb concentrations were observed. However, observable effects of Pb on plant growth ranged from mild to severe chlorosis or none across species/varieties. Baker, 1981 and Baker *et al*., 1994 reported that plant species could respond to the presence of contaminant in the soil either by excluding or accumulating the contaminant.

Some of the species with Pb Concentration factor (CF) < 1 might be excluders, indicators or tolerant species whilst CF ≥ 1 might be classified as accumulators supported by literature. Earlier Chapter (Chapter 2) discussed criteria for classifying plant species as

excluders, accumulators or hyperaccummulators. However, there are no clear boundaries between these groups.

Current findings showed that significant amount of Pb was accumulated in roots of most plant species studied. This is an indication that classification of plants as excluders, accumulators or hyperaccumulators exclusively based on translocation and concentration factors might not be conclusive. Further experiments are required to investigate plants based on both *in situ* and pot trials as uptake of Pb may be influenced by bioavailable Pb in soil to plants. However, uptake and bioavailability of Pb in soil-plant system remains poorly understood (Robinson, 1998).

There was no significant effect of the Pb-added treatment on any of the biomass data of BJ 17 and no observable effect of the added Pb on that plant. This variety seemed to be unaffected by the Pb added treatment.

There was a significant effect of the added Pb on shoot dry biomass, total dry biomass and longest leaf length of BJ 18. However, BJ 18 showed tolerance to high Pb in the soil. The *Brassica juncea* varieties BJ 18 and BJ 17 were therefore selected because of their abilities to survive and thrive in high Pb in the soil without obvious stress compared to BJ 21 and BJ 42. Although, the total plant Pb of BJ 21 and BJ 42 were 70 to 80% and 14 to16 % higher, when compared to BJ 18 and BJ 17 respectively. Severe chlorosis, wilting of leaves and nearly plant death were observed in both BJ 21 and BJ 42 at the Pb concentration applied, which is an indication plant death might be recorded with higher Pb concentration (Figures 4.4.1 to 4.4.4).

Identification of a suitable plant species for further experiments also considered plants which can concentrate metal contaminant without completely inhibiting growth. Gregorio (2011) noted that prolific growth produces the necessary biomass to extract large amounts of metals per hectare that are commonly encountered in most contaminated sites. This first pot experiment shows that the amount of biomass these species/varieties produced affected the shoot and root Pb mass (µg) (Table 4.4.1), which was generally low (ranged from 0.11 to 95 µg), with the exception of BJ 17, BJ 21, ZM B37, ZM B73 and ZM 64. The duration of growth have partially contributed to the generally lower biomass of most species/varieties in the control and Pb added treatments, when compared to subsequent experiments. This is supported by findings in later pot trials (Chapter 4: Sections 4.7.1 to 4.7.2; Chapter 5: Section 5.4) , where some selected species with low biomass in this first experiment produced 30 to 60% bigger biomass in both control and Pb added treatments. However, TC BR consistently produced low

biomass in the second pot experiment irrespective of the longer growth period (Section 4.7).

Selection of plant varieties for further investigation was based initially on their ability to survive or tolerate high Pb in the soil. Biomass and growth data such as height, shoot, root, and total dry biomass, number of true and dead leaves and growth index were used to evaluate their performance and their ability to thrive in soil with high Pb.

The danger of losing replicates of those plants species (adversely affected by the added Pb in the initial pot trial) due to adverse effect of increased Pb concentration in further pot trials were also considered and so plant varieties that did not thrive well in high soil Pb or showed severe effect to added Pb were dropped from the first pot trial. This is an important consideration, as greater number of replicates will allow more reliable detection of statistically significant differences in the further experiments that simulate *in situ* heterogeneity. However, two replicates of *Thlaspi caerulescens* varieties TC GM and TC BR in the control treatment were lost in the first pot trial.

Similarly, ZM OH43 and ZM B 37 were also selected for the next stage. Though, the added Pb had a significant effect on the root dry biomass, shoot, root and the total dry biomass of ZM B73, it showed tolerance to high Pb in the soil. Their survival and growth in the Pb added treatment was not affected.

The varieties ZM B73 and ZM 64 had 56% and 50% higher total plant Pb (mg/kg) dry weight than the lowest concentration within the range respectively. These varieties ZM B37 and ZM 64 were dropped as result of the observable effects of added Pb such as chlorosis in ZM 64 and severe wilting of leaves in ZM B37. The Pb treatment also had an effect on their growth index, height and total dry biomass. This suggested that severer effect on these varieties might be seen at higher Pb concentrations in further experiments.

Brassica napus, BN K seemed less affected by the high Pb in the soil than BN SW, but BN K was not selected due to non-availability of its seeds for further experiments. However, both showed chlorosis and wilting of leaves, but to a greater extent in BN SW. Results showed no statistically significant differences ($P > 0.05$) in some of the growth data and Pb concentrations in roots and total plant between these varieties.

Thlaspi caerulescens TC BR seemed unaffected by the added Pb treatment. *Thlaspi caerulescens* variety TC BR had 50% and 35% higher total plant Pb (mg/kg) DW, when compared to TC GM and TC HS. Severe chlorosis and wilting of leaves was observed in TC HS as result of the added Pb. There was no significant effect of Pb on all the biomass

data of TC BR in the Pb added treatments. It grew well on the Pb added treatment when compared to the control.

The variety TC GM showed similar tolerance to high Pb in the soil, but TC GM was not selected due to non-availability of seedlings for the next experiment as most seedlings grown on unspiked growth medium, prior to transplanting into the spiked growth medium, died before they were transplanted. The few which survived grew better in the Pb added treatment than in the control.

4.5.1 Interpretation of results in relation to stated hypothesis.

The results were considered against stated hypotheses:

1. The 1000 mg/kg Pb in growth media has an effect on plant performance.
2. Some of the species/varieties differ in their tolerance to Pb in the growth medium at this concentration.

When a statistically significant difference ($P < 0.05$) is found in measured plant variables such as shoot, root and total dry biomass of the plant species between treatments, then hypothesis 1 is accepted, that the 1000 mg/kg Pb added treatment had a significant effect on such plant species.

Similarly, hypothesis 2 is accepted when a statistically significant difference ($P < 0.05$) in metal uptake is found within and between species/varieties. Summary of hypothesis testing for each species and varieties is shown in Table 4.5.1.

From the results of this first pot trial and in line with stated objectives i.e to select plant species/varieties for a further second pot trial with a range of Pb-concentration of 100, 300, 3000 and 10,000 mg/kg, 4 species made up of 6 varieties were selected for the next experiment.

The Four species made up of six varieties selected were BJ 18, BJ 17 (*Brassica juncea*), ZM OH43, ZM B73 (*Zea mays*), BN SW (*Brassca napus*) and TC BR (*Thlaspi caerulescens*).

These species/varieties were selected based on their ability to survive and tolerate high Pb in the soil and substantiated by the results of the biomass, growth rate and actual Pb concentrations in the above, below ground parts and whole plant. This is in line with studies by Gregorio (2011) who reported that the success of phytoextraction effort depends to a large degree on the identification of suitable plants that not only concentrate

metals to levels that would inhibit growth of most species, but demonstrate prolific growth in response to an established agronomic or horticultural practice.

The overall result of the first pot experiment informed the selection of the species/varieties for the second pot trial. The second pot trial further selected the most suitable plant species for pot trials simulating simplistic binary heterogeneity model and *in situ* heterogeneity.

Table 4.5.1:Summary of hypotheses tested in 1st pot trial for each species/varieties based upon independent sample t-test for (i) and Tukey H.S.D for (ii) comparison of means where p<0.05. Varieties selected for 2nd pot trial are highlighted in red.

Hypothesis	BJ 18	BJ 42	BJ 17	BJ 21	ZM B73	ZM B37	ZM OH43	ZM 64	BN SW	BNK	TC BR	TC GM	TC HS
(ia)Biomass	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept
(ib)Pb uptake	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept
(ii)Variation	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Accept	Reject	Reject	Accept	Accept	Accept

4.6 SECOND POT TRIAL.

4.6.1 Introduction

The second pot trial compared varieties and species as a function of Pb concentration. Six varieties of four species were selected after the first pot trial for transplanting into growth media containing a range of Pb concentration 100, 300, 3000 and 10000 mg/kg with 0 mg/kg added as control. The species and varieties selected are two *Brassica juncea* (BJ18 and BJ 17), two *Zea mays* (ZM B73 and ZM OH43), *Brassica napus* (BN SW) and *Thlaspi caerulescens* (TC BR).

4.6.2 Objectives

I. Assess the effect of the range of Pb concentrations on the selected plant species/varieties in the current experiment.
II. Determine suitable value for the Pb concentration in the growth medium that can be used in further experiments.
III. To select the most suitable species/varieties for subsequent pot trials simulating a simple design for the *in situ* heterogeneity of Pb in the growth medium.

4.6.3 Hypotheses

1. Changing Pb concentration in the growth medium has an effect on the biomass of selected plant species.

2. The amount of Pb accumulated by the different plant species is related to the Pb concentration of the growth medium across the range investigated.

3. There is a trend on the effect of the Pb concentration range on plant's biomass and uptake.

4.6.4. Methods.

Method of seed germination, transplanting, establishment in the unspiked growth media, were as described for the first pot trial. Concentration of contaminant, mass of sand and compost are shown in Appendix II.1: Tables BII.1 to EII.1.

4.6.5 Preparation of Growth Media for the 2nd pot trial.

A mass of 38.4 kg of silver grade sand was transferred into a concrete mixer to prepare each batch of growth medium (one for each treatment). A volume of 13.5 L of John Innes Compost 2 was weighed and added to the concrete mixer (containing the silver grade sand) (Appendix 4II.1: Table DII.1).The content was thoroughly mixed using the concrete mixer to obtain a sufficiently homogeneous growth medium.

These were repeated to make batches of growth medium spiked with PbO to make Pb concentration of 100 mg/kg, 300 mg/kg, 1000 mg/kg, 3000 mg/kg, and 10,000 mg/kg. Spiking the growth media with Pb contaminant was done using carrier sand of 6 kg (DW) mass, which was dried and thoroughly mixed in a dry bucket with pre-dried PbO dried to constant weight (checked at 0, 12, 18 and 24 hour duration (Appendix 4II.1: Figure AII.1). Dry carrier sand was used to ensure proper mixing of the PbO and the sand.

Masses of 5.4 g, 16.3 g, 54.3 g, 162.9 g and 542.9 g (Appendix II.1: Table DII.1) of PbO in dry carrier sands were mixed with sand and compost in the cement mixer to make batches of 100, 300, 1000, 3000 and 10,000 mg/kg (FW) Pb for all 6 species of plants (108 pots) and mixed until a homogenized mixture was obtained.

Similarly as in the first pot trial, five lots each of about 10 g of the mixed spiked growth media was sampled to check the Pb concentration of growth media. These portions were taken from randomly selected pots, dried in the oven at 110^{o}C and milled using the Tema mill of maximum grain size of < 8 mm. A mass of 0.25 g of the milled sample was used to determine Pb concentration and (homogeneity) of the contaminant at each Pb concentration level using the Atomic Absorption Spectrometer (AAS) after acid digestion by nitric and perchloric acids. Certified reference materials (CRMS), duplicates and reagent blanks were used for quality control. Growth media measured concentration for the first pot trial is shown in Table 4.6.1 below. Quality control for the first and second pot trials are reported in Appendix 4II.14: Tables AII.14 to KII.14.

Table 4.6.1 : Check for Growth media Pb concentration (Second Pot Trial).

S/N	Nominal Pb concentration mg/kg	Mean measured Pb concentration mg/kg
1	100	115±3.97
2	300	322±18.73
3	3000	3378±177.29
4	10000	10071±258.52

Plants were maintained at 20 ± 5^{O}C, at 16 hours photoperiod under natural sunlight in the glasshouse for six weeks as opposed to three weeks in the first experiment. Pots were arranged in randomized block design, randomizing between treatments and species/varieties (Figure 4.6.1 and Appendix 4II.8).

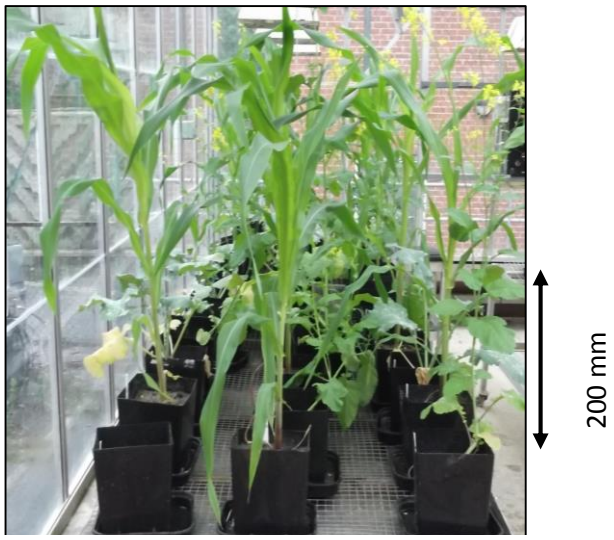


Figure 4.6.1: Plant species arranged in Randomized block design before harvest (2nd pot trial). Arrows represent scale bar. Scale bar length: 16 mm represents 200 mm.

Growth and biomass data such as height, number of true leaves, largest width, width 90 degrees to the largest width, longest leaf length, shoot dry biomass, root dry biomass, total dry biomass and growth index (height + largest width +width 90 degrees to the largest width/3) were recorded at transplanting (week 1), week 3 and week 6.

Plants were harvested in the sixth week (56 days of growth) as described for the first pot trial, as were measurement of these parameters.

These growth and biomass data were indices of plant growth and development. They were also used in evaluating the tolerance and ability of the plant species to survive the

different Pb concentrations. However, only the shoot, root, total dry biomass, shoot, root and total plant Pb (mg/kg) dry weight are presented in this chapter in line with stated hypothesis and are also used in the selection of plant species/varieties for further experiments.

4.6.6 Data Analysis.

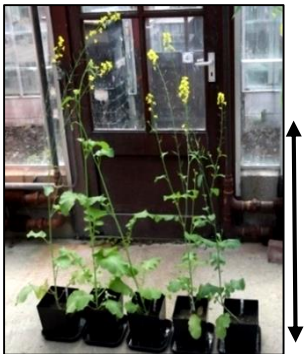
Data collected from this experiment were subjected to One-Way analysis of variance (ANOVA) and the Tukey HSD Post-Hoc test to compare the effects of Pb on the plant at the different concentrations applied. Statistical package IBM SPSS version 20 and Minitab version 16 for windows were used to carry out data analysis. The Tukey HSD Post –Hoc test was used, since equal variances were assumed. There were no surviving seedlings in the 0 mg/kg added, and one survivor in the 100 mg/kg for TC BR. As a result of this unequal observation, the Post-Hoc test for TC BR compared the 300 mg/kg added with 3000 and 10000 mg/kg Pb added. Post-hoc test could not also be used to compare shoot, root and total plant Pb concentration of TC BR as some of the samples were not analysed for Pb due to insufficient mass for analysis (mass was less than 0.01g).This was the minimum mass that could be used to prevent high level of uncertainty resulting from low sample mass. Concentrations sharing letters means they are not significantly different.

Letters were assigned based on the table for homogeneity of subsets (Appendices 4-II.9 and 4-II.10). Charts with error bars, which represent 1 standard error on the mean, were used to show biomass and uptake data at different concentrations.

4.7 RESULTS OF THE SECOND POT TRIAL.

Plants were harvested after 56 days, when sufficient biomass had been produced. Survival rate was 100% for most species except for *Thlaspi caerulescens* variety TC BR. Condition of plant species at harvest is shown in Figure 4.7.1.

(a) BJ 18 at harvest. Concentrations 0, 100, 300, 3000 and 10000 mg/kg Pb added (from left to right). Arrow represents scale bar. Scale bar: 18 mm represents 500 mm.



(b) BJ 17 at harvest. Concentration 0,100, 300, 3000 and 10000 mg/kg Pb added (From left to right). Scale bar: 24 mm represents 800 mm.

(C) BN SW at harvest. Concentration 0,100, 300, 3000 and 10000 mg/kg Pb added (From left to right). Scale bar: 5 mm represents 20 mm.

(d) TC BR at harvest. Concentrations (0,100, 300, 3000 and 10000 mg/kg Pb added (From left to right). 4 mm represents 2 mm.

(e) ZM OH43 at harvest. Concentrations (0,100, 300, 3000 and 10000 mg/kg Pb added (From left to right). Scale bar: 17 mm represents 200 mm.

(f) ZM B73 at harvest. Concentrations (0,100, 300, 3000 and 10000 mg/kg Pb added (From left to right). Scale bar; 12 mm represents 100 mm.

Figure 4.7.1: Condition of plants species at harvest (Second pot trial). Arrows represent scale bar for each figure.

The result of the analysis of variance of the growth and biomass data is summarized in Table 4.7.1.

Table 4.7.1: Statistical significance of biomass and growth data between-concentrations for each variety/species with ANOVA F-ratio and P values in bracket.

Biomass data	BJ 18	BJ 17	ZM B73	ZM OH43	BN SW	TC BR
Height (mm)	**S** (F=11.76; p=0.001)	**S** (59.47; p=0.000)	**S** (F=14.95; p=0.000)	**S** (F=10.53; p=0.001)	**S** (F=11.70; p=0.001)	**S** (F=10.33; p=0.011)
Number of true leaves	**S** (F=15.08; p=0.000)	**S** (F=10.74; p=0.001)	**NS**(F=2.35; p=0.125)	**S** (F=2.50; p=0.022)	**S** (F=7.063; p=0.006)	**S** (F=23.55; p=0.001)
Longest leaf length (mm)	**S**(F=5.12; p=0.017)	**S** (F=28.72; p=0.000)	**NS**(F=1.45; p=0.287)	**NS** (F=1.55; p=0.262)	**S** (F=3.77; p=0.040)	**S**(F=137.63; p=0.000)
Shoot dry biomass (g)	**S**(F=14.33; p=0.000)	**S** (F=5.62; p=0.012)	**S** (F=6.32; p=0.008)	**S** (F=14.12; p=0.000)	**S** (F=19.66; p=0.000)	**S**(F=107.97; p=0.000)
Root dry biomass (g)	**S**(F=79.95; p=0.000)	**S**(F=27.45 p=0.000)	**S** (F=12.11 p=0.001)	**NS**(F=0.538 p=0.711)	**S** (F=28.59 p=0.000)	**S** (F=12.80 p=0.007)
Total dry biomass (g)	**S** (F=21.87; p=0.000)	**S** (F=6.97; p=0.006)	**S** (F=7.46; p=0.005)	**S** (F=14.94; p=0.000)	**S**(F=21.49; p=0.000)	**S**(F=121.83; p=0.000)
Growth Index(mm)	**S**(F=10.14; p=0.002)	**S** (F=50.59; p=0.000)	**S** (F=24.61; p=0.000)	**S** (F=10.05; p=0.001)	**S** (F=12.21; p=0.001)	**S** (F=6.10; p=0.036)

Key: S—Significant at P < 0.05; NS—Not significant at P > 0.05; BJ 18---*Brassica juncea* 182921; BJ 17-- *Brassica juncea* 173874*;* ZM B73---*Zea mays* B73*;* ZM OH43---*Zea mays* OH43; BN SW---*Brassica napus* Sweden; TC BR---*Thlaspi caerulescens* BlackRock.

Significant differences were observed in the shoot, root and total dry biomass of some of the varieties/species at P = 0.000, 0.012, 0.005, 0.008, 0.006 (P < 0.05).

It is an indication that the effect of the added Pb on the shoot dry biomass differed at the different concentrations applied.

4.7.1 Results of Shoot, root and total dry biomass as a function of Pb concentration between species and varieties.

Figures 4.7.2—4.7.4 show the effect of Pb on shoot, root and total dry biomass between concentrations. The ANOVA result showed that shoot, root and total biomass of BJ 18, BJ 17, ZM B73, BN SW and TC BR were significantly different P =0.000, 0.001, 0.000 and 0.006 within and between varieties as a function of concentration respectively.

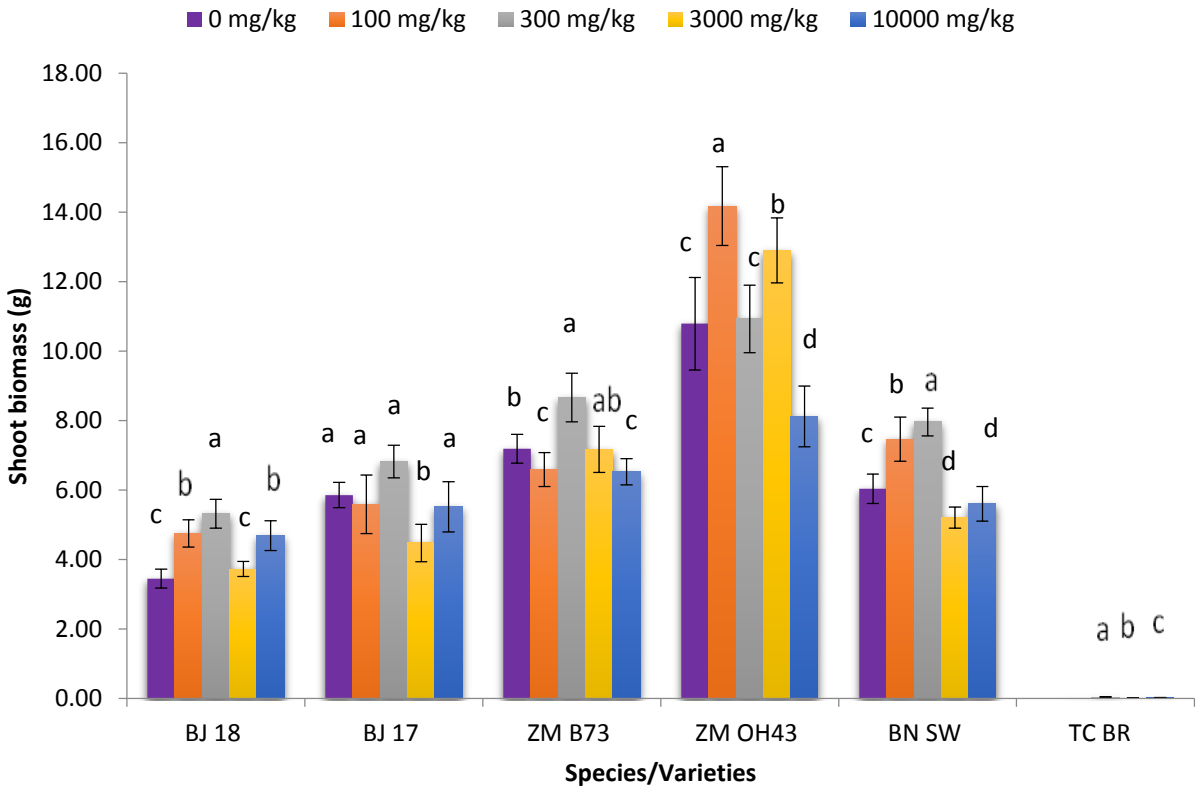


Figure 4.7.2: Shoot biomass DW over a range of concentration across species and varieties. Means sharing the same letter (for each plant species or variety) are not significantly different. Error bars represent 1 standard error on the mean (n=3).

The shoot and root biomass of BJ 18 increased with increasing soil Pb concentration with 5 to 8% lower biomass in the 3000 mg/kg concentration, when compared to the 0 mg/kg treatment (Figures 4.7.2-4.7.3). About 35% higher shoot biomass was recorded in 10000 mg/kg treatment when compared to the control (Appendix II.9: Tables AII.9 to BII.9). There were no significant differences in shoot biomass between some concentrations (e.g 0 mg/kg and 3000 and 100 and 10000 mg/kg). The root biomass of this variety decreased at higher Pb concentrations (3000 and 10000 mg/kg) when

compared to the lower concentrations (0, 100 and 300 mg/kg) (Figure 4.7.3). This is an indication that this *Brassica juncea* variety (BJ18) grew better in the Pb lower concentrations but was tolerant to higher Pb concentration.

There were no significant differences (as judged by Tukey HSD test) in the shoot and root biomass between the 0 mg/kg added and the 100, 300 and 10000 concentrations of BJ 17 (Figures 4.7.2-4.7.3). Root biomass of BJ 17 was lower by 37% in the 3000 mg/kg concentration and 10% in the 10000 mg/kg concentration, when compared to the 0 mg/kg concentration (Figure 4.7.3). It is an indication of the more severe effect of the added Pb on the root of this variety at higher concentrations. However, it showed no observable symptoms of Pb toxicity during the growing period. This suggest that BJ 17 was tolerant to Pb at the concentration applied.

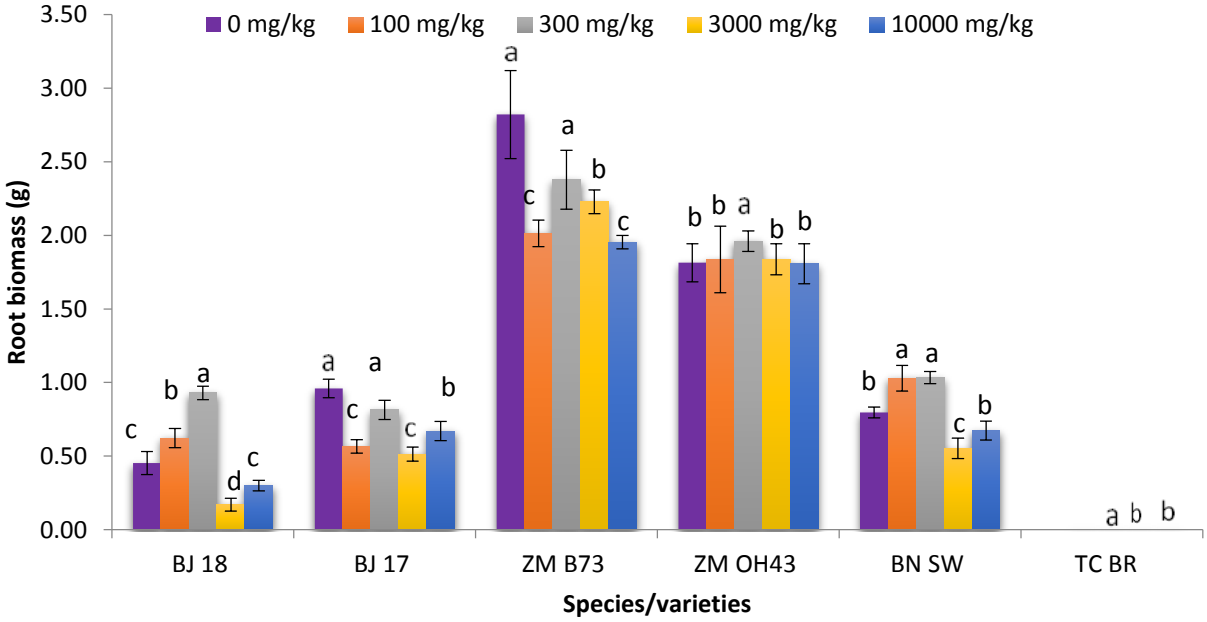


Figure 4.7.3: Mean root biomass DW over a range of concentration across species and varieties. Means sharing the same letter (for each plant species or variety) are not significantly different. Error bars represent 1 standard error on the mean (n=3).

Similarly, shoot, root and total biomass of ZM B73 decreased with increasing soil Pb concentration. Higher (18-44%) root and total biomass were recorded in the control treatment when compared to the other concentrations (Figures 4.7.3 and 4.7.4). Some concentrations were not significantly different in their shoot, root and total biomass. This is an indication of the effect of the varied Pb concentration. It also suggest that ZM B73 was tolerant to this Pb concentration range.

There were significant differences in shoot biomass between concentrations for the ZM OH43 variety (Figure 4.7.2). However, the root biomass of ZM OH43 were not

significantly different between concentrations (Figure 4.7.3). This suggests that the effect of the added Pb on this variety based on the root dry biomass might not have been detected within the scope of this experiment. The total dry biomass of ZM OH43 in the 10000 mg/kg treatment was lower by 18 % and 27%, when compared to the 0 and 3000 mg/kg concentrations respectively (Figure 4.7.4; Appendix II.9-Tables OII.9 to PII.9), which implied an effect of the Pb at the highest Pb concentration.

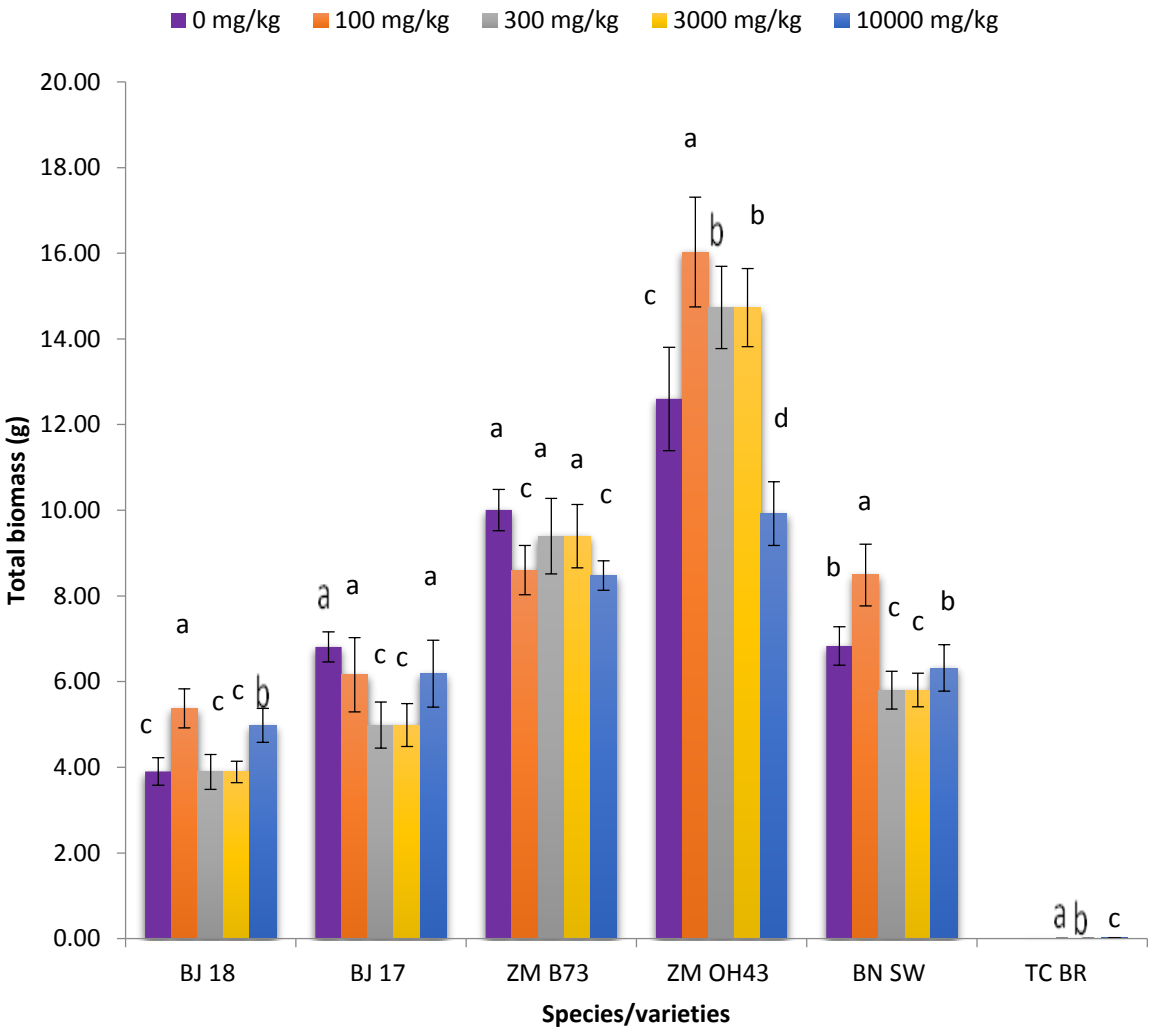


Figure 4.7.4: Mean total biomass DW over a range of concentration across species and varieties. Means sharing the same letter (for each plant species or variety) are not significantly different. Error bars represent 1 standard error on the mean (n=3).

Significant difference in shoot, root and total dry biomass between the 0, 100 and 300 mg/kg Pb added was observed in *Brassica napus* (Figures 4.7.2 - 4.7.4). The shoot and total dry biomass were 24 and 32% higher in the 100 and 300 mg/kg Pb added treatments (Appendix 4II.9: Tables EII.9 and FII.9). Similarly, the root biomass of *B.*

napus was higher by 29 and 34% in the 100 and 300 mg/kg concentrations, when compared to the 0 mg/kg concentration respectively (Appendix II.9: Table KII.9). It is an indication that this variety would grow better at lower Pb concentrations. A more severe effect of the added Pb was observed at higher concentrations.

Thlaspi caerulescens TC BR significantly differed in the shoot and root dry biomass at the different concentrations (Figures 4.7.2 and 4.7.3). Significant differences P = 0.000, 0.000 and 0.000 were observed in the total dry biomass of TC BR between 300, 3000 and 10000 mg/kg added (Figure 4.7.4).

4.7.2 Results of Shoot, root and total plant Pb (mg/kg) as a function of Pb concentration across species and varieties.

The Pb concentration of shoot, root and total plant Pb (mg/kg) dry weight provided further insight into Pb accumulating potentials and effect of the added Pb on the plant biomass of these plants species. The Tukey HSD comparison of varieties/species between concentrations is shown in Figures 4.7.5-4.7.7. *Thlaspi caerulescens* (TC BR) could not be compared using the Post-hoc test due to none or unequal number of replicates for some concentrations. There was a general trend of increasing shoot, root and total Pb concentration with increasing soil Pb concentration.

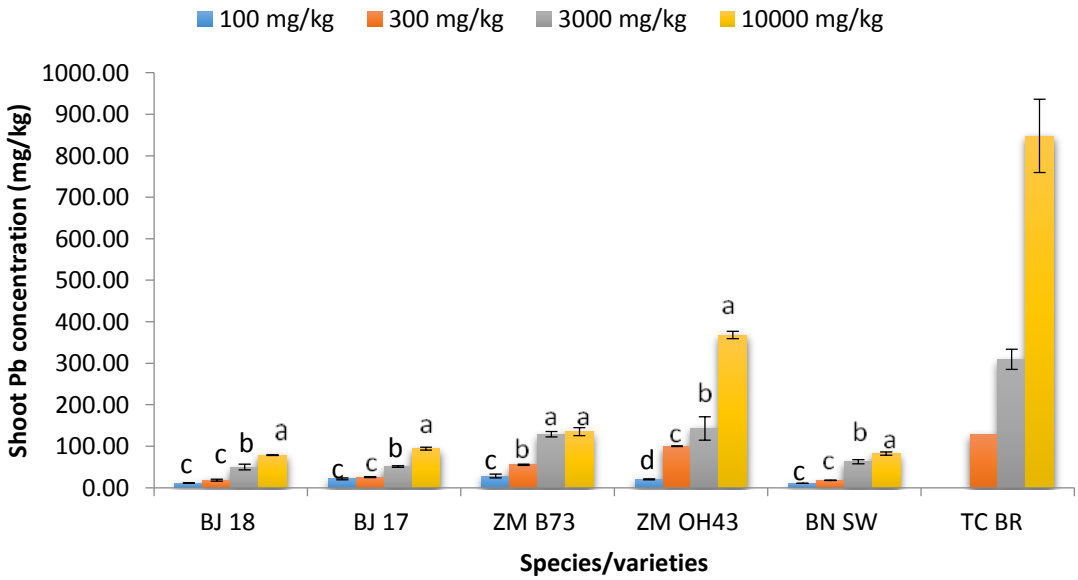


Figure 4.7.5: Mean shoot Pb concentration over a range of concentration across species and varieties. Means sharing the same letter are not significantly different, as judged by the Tukey HSD post-hoc test. Error bars represent one standard error on the mean (n=3). Post-hoc test not used for TC BR shoot Pb concentration to unequal observation (see Section 4.6.6).

Shoot Pb concentrations also varied between and within species. These differences were significant (P = 0.000 < 0.05). For BJ 18 and BJ 17, shoot Pb concentration ranged from 11 to 78 mg/kg and 21 to 94 mg/kg Pb. Shoot Pb in the 10000 mg/kg Pb added treatment of BJ 18 was 2 to 7 times higher when compared to the 100, 300 and 3000 mg/kg concentrations while BJ 17 had 2-4 times higher shoot Pb in 10000 mg/kg when compared to 100, 300 and 3000 mg/kg concentrations (Figure 4.7.5).

The *Zea mays* varieties ZM OH43 and ZM B73 shoot Pb was in the range of 20 - 368 mg/kg for all concentrations. Higher shoot Pb concentrations (40 to 80%) were recorded in the 10000 mg/kg added when compared to the other concentrations (Figure 4.7.5) for both varieties.

Similarly, BN SW variety had 30 to 80% higher shoot Pb in the 10000 mg/kg concentration, when compared to the 100, 300 and 3000 mg/kg concentrations. The difference between the 100 and 300 mg/kg concentrations was not significant {P > 0.05} (Figure 4.7.5).

This trend of increasing shoot Pb concentration with higher soil Pb concentration was also recorded for TC BR with a mean shoot Pb concentration of 848 mg/kg compared to 128 and 309 mg/kg in 100 and 300 mg/kg concentrations respectively.

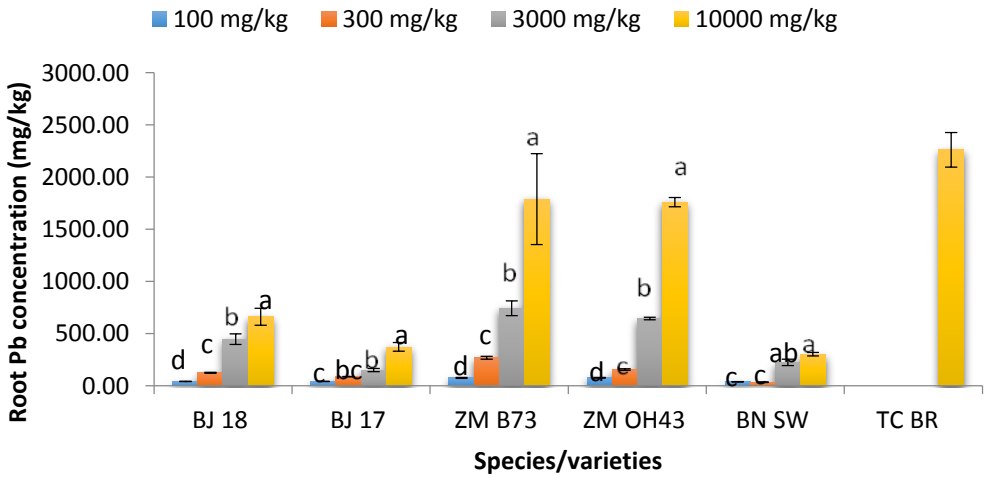


Figure 4.7.6: Mean root Pb concentration over a range of concentration across species and varieties. Means sharing the same letter are not significantly different, as judged by the Tukey HSD post-hoc test. Error bars represent one standard error on the mean (n=3). Post-hoc test not used for TC BR root Pb concentration to unequal observation (see Section 4.6.6).

There were significant differences (P = 0.000 < 0.05) in the root Pb concentration of all species and varieties. Similarities between lower concentrations (100 and 300 mg/kg) was observed for some species/varieties (Figure 4.7.6). The shoot and root Pb concentrations in the 10000 mg/kg treatment differed significantly from the other concentrations.

The total plant Pb concentration (mg/kg) dry weight varied with soil Pb concentrations between and within species. This difference was significant P = 0.000<0.05 (Appendix II.11: Tables AII.11 to CII.11). Tukey HSD test showed similarities between some of the species/varieties.

Total plant Pb concentration of BJ 18 and BJ 17 ranged from 15 mg/kg to 124 mg/kg DW.The *Brassica juncea* varieties BJ 18 and BJ 17 had higher total plant Pb (by a factor of 3 to 4) in the 10000 mg/kg concentration, when compared to the 100 mg/kg concentration. However, there was no significant difference between the 100 and 300 mg/kg treatments (Figure 4.7.7).

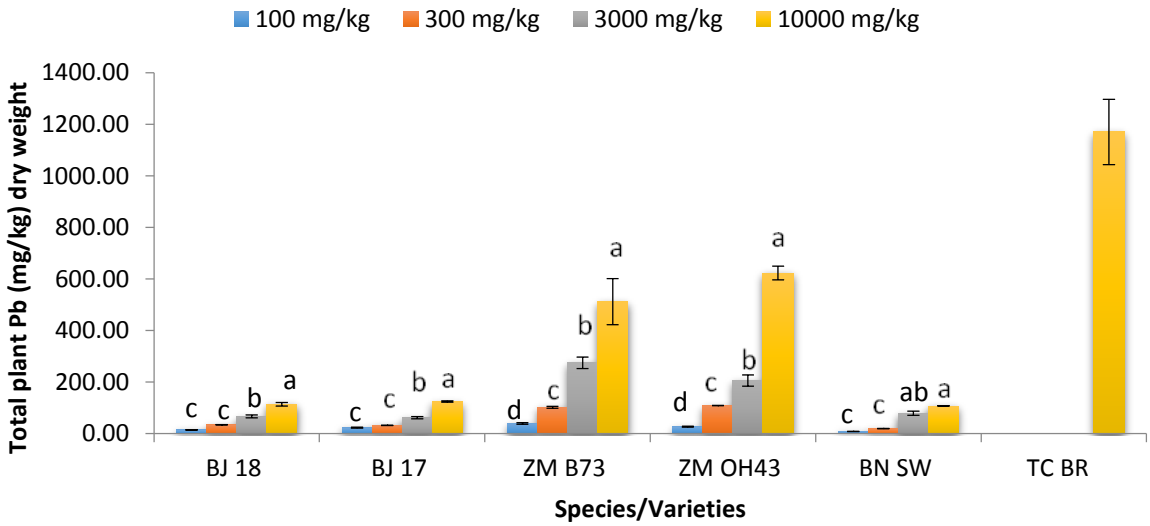


Figure 4.7.7: Mean <u>total plant Pb concentration</u> over a range of concentration across species and varieties. Means sharing the same letter are not significantly different, as judged by the Tukey HSD post-hoc test. Error bars represents one standard error on the mean (n=3). Post-hoc test not used for TC BR root Pb concentration to unequal observation (see Section 4.6.6).

For the *Zea mays*, there were significant differences in the total plant Pb (mg/kg) DW of ZM OH43 (Figure 4.7.7). Total plant concentration ranged from 27 – 622 mg/kg DW and 39 -512 mg/kg DW for ZM OH43 and ZM B7 respectively (Figure 4.7.7). Highest concentrations (622 and 512 mg/kg DW) were recorded for both varieties in the 10000 mg/kg Pb added treatment respectively which was 23 and 13 fold higher compared to the lowest concentration within the range.

Similarly, BN SW had the highest total plant Pb (107 mg/kg DW) in the 10000 mg/kg, which was 35 to 60% higher, when compared to 100, 300 and 3000 mg/kg treatments (Figure 4.7.7). The difference between concentrations was significant (Appendix II.11: Table 10).

Thlaspi caerulescens TC BR h also had high total plant Pb (mg/kg) dry weight (1170 mg/kg DW) in the 10000 mg/kg treatment (Figure 4.7.7).

4.7.3 Shoot and root concentration factor.

The shoot and root concentration factors (CF_{shoot} and CF_{root}), which is a measure of the Pb uptake showed decrease in CF with increasing soil Pb concentration for most varieties (Figure 4.7.8 and 4.7.9). The *Thlaspi caerulescens* variety (TC BR) had only one representative CF_{root} due to insufficient root mass of other two replicates for analysis as stated earlier in this chapter. The highest CFshoot was recorded for TC BR and the lowest for BJ 18 and BN SW, while those of BJ 17, ZM B73 and ZM OH43 were intermediate between the two extremes.

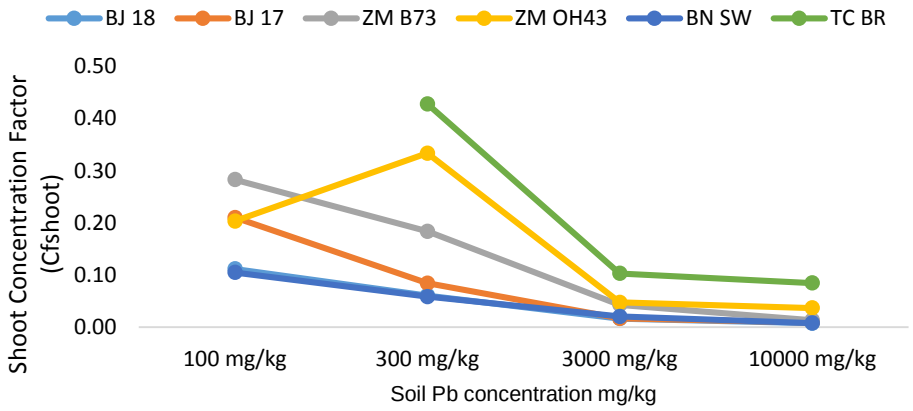


Figure 4.7.8: Shoot Concentration factor {CF_{shoot}} (Shoot Pb DW mg/kg/soil Pb concentration mg/kg) as a function concentration across species and varieties.

The root concentration factor (CF_{root}) also showed a similar trend of decreased CF with increasing soil Pb concentration (Figure 4.7.9). The variety TC BR had the highest CF_{root} in the 10000 mg/kg concentration and had no CF_{root} value for the other concentrations due to reason stated earlier in this section. *Brassica napus* (BN SW) had the lowest CF_{root} at nearly all concentration when compared to other varieties/species. However, BJ 17 had the lowest CF_{root} at the 3000 mg/kg concentration. The varieties BJ 18, ZM B73 and

ZM OH43 had intermediate CF_{root} between these two extremes with the highest CF_{root} in the 300 mg/kg.

Shoot and root concentration factors showed that most of the plant species excluded the Pb at higher soil Pb concentrations resulting in decreased CF with higher CF at lower soil Pb concentrations.

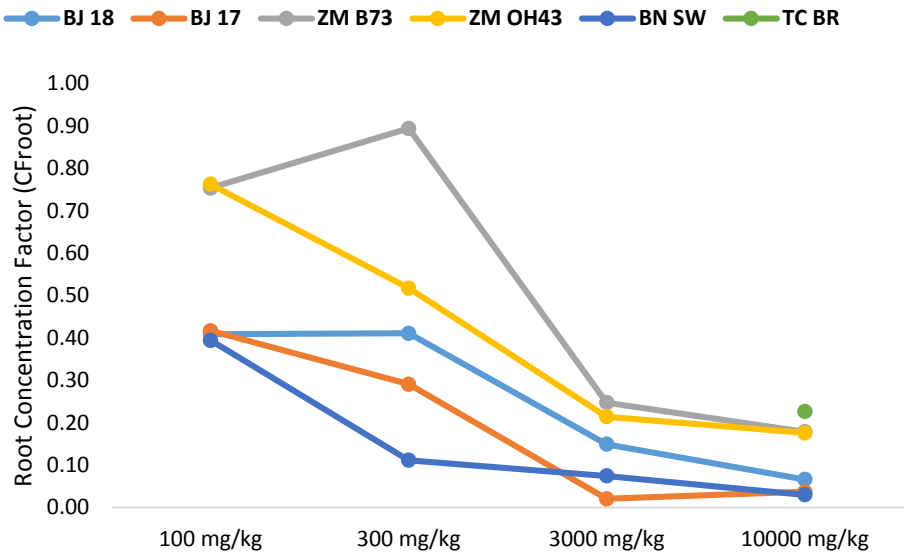


Figure 4.7.9: Root Concentration factor {CF_{root}} (Root Pb DW mg/kg/soil Pb concentration mg/kg) as a function concentration across species and varieties.

Scatter plots of both CF_{shoots} and CF_{roots} (Figure 4.7.10) implied a negative correlation or relationship between soil Pb concentration and concentration factors of shoot and roots for 99.9% of species/varieties with the exception of TC BR (1%). Increased soil Pb concentration resulted in decreased shoot and root CF. However, the increased CF_{shoot} and CF_{root} of TC BR at higher soil Pb concentration could be associated to the adaption of this variety to Pb contaminated soils. None of the seedlings of TC BR survived in the control with only one survival in the lowest soil Pb concentration (100 mg/kg) .The seeds of this variety TC BR used in pot trials were collected from Black Rocks (where they grew naturally), one of the heavily contaminated sites discussed in Chapter 3 of this thesis.

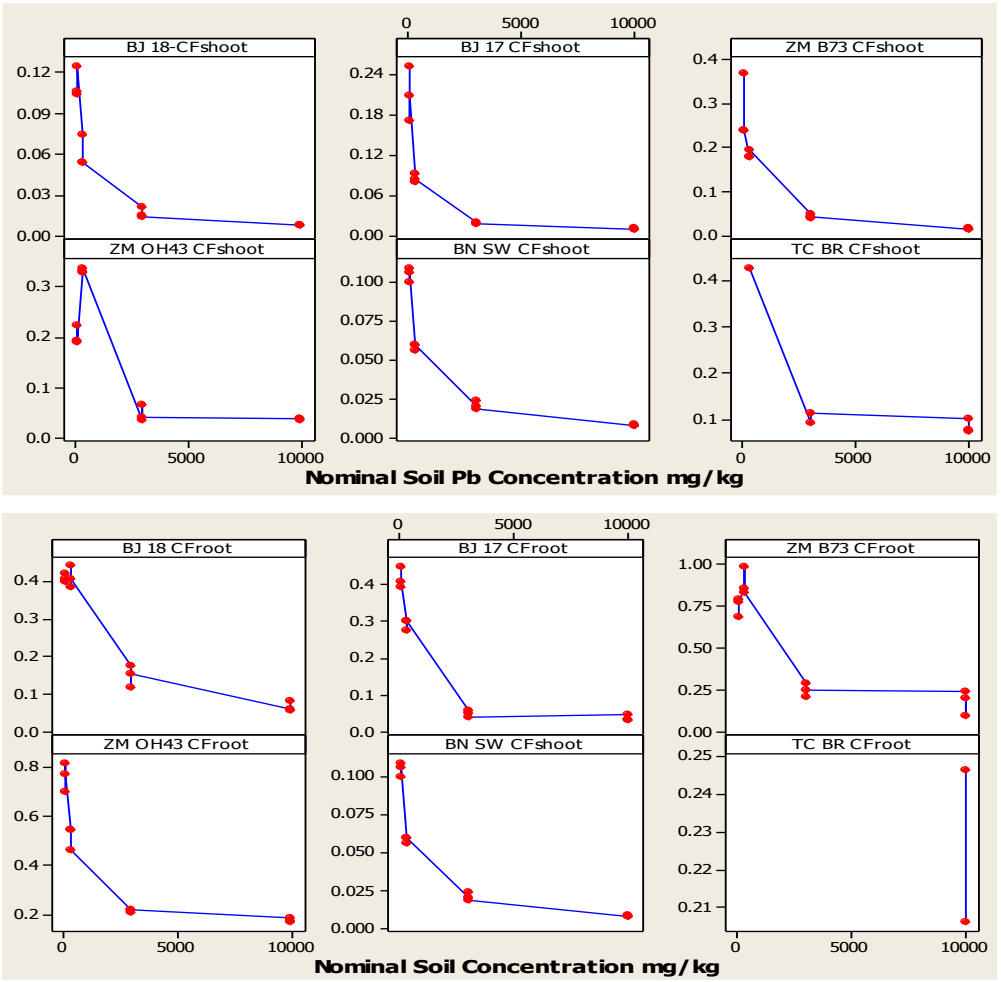
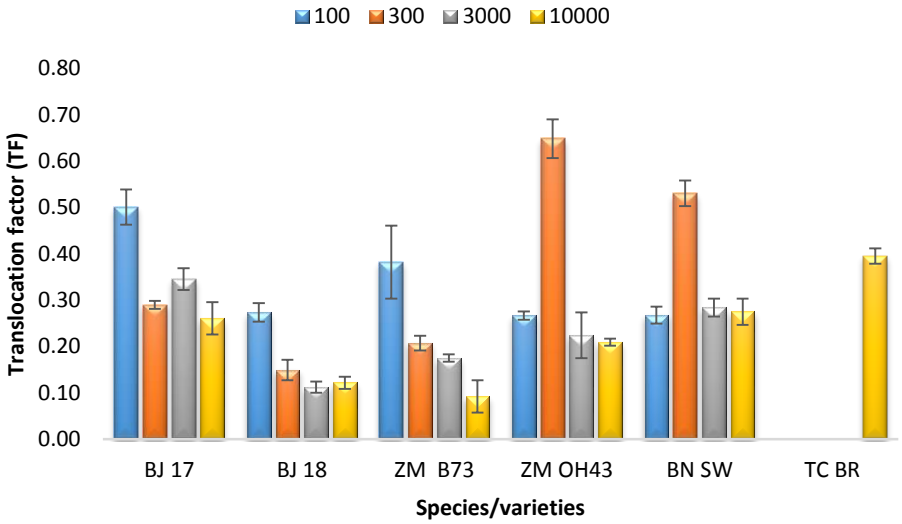


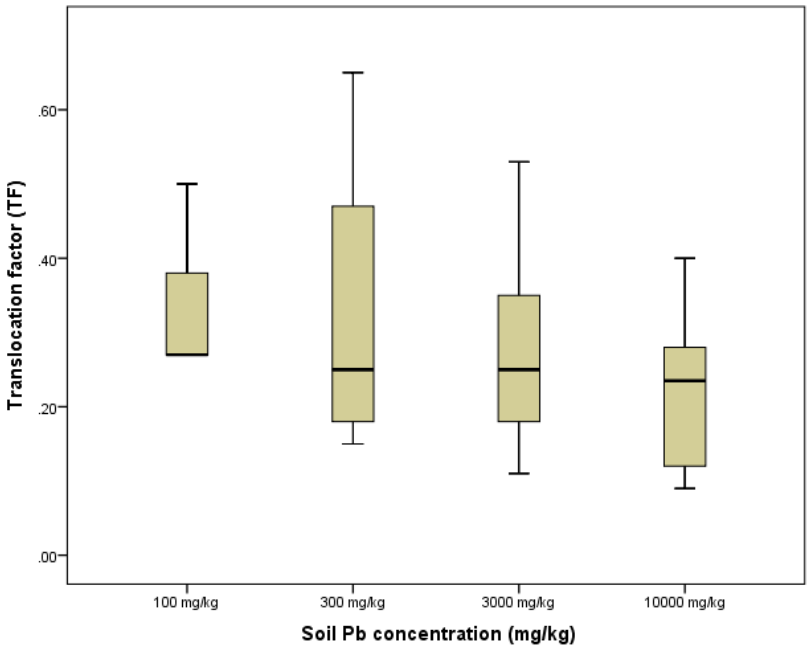
Figure 4.7.10: Scatter plot of CF_{shoot} and CF_{root} against nominal soil Pb concentration.

4.7.4 Translocation factor (TF) of species/varieties in the second pot trial.

The mean translocation factor (TF) of these species/varieties ranged from 0.1 to 0.7 (Figure 4.7.11). This is an indication of poor translocation of Pb from the roots to the shoots of most of the species/varieties studied. The rate of Pb translocation to the shoot of *Brassica juncea* variety (BJ 18) and the *Zea mays* variety (ZM B73) were lowered (by factors of 2 and 4) in high soil Pb concentrations of 3000 and 10000 mg/kg respectively. They both showed a trend of decreasing TF with increasing soil Pb concentration. An exceptionally higher (50 to 70%) TF was observed in the 300 mg/kg concentration of the *Zea mays* variety ZM OH43 and the Brassica *napus* variety BN SW, whilst their 100, 3000 and 10000 mg/kg were not significantly different. Translocation factor was higher (~42 to 92%) in the 100 mg/kg concentration of *Brassica Juncea* variety (BJ 17). The *Thlaspi caerulescens* variety had the highest TF in the 10000 mg/kg concentration. This is an indication of the tolerance and adaptation of TC BR to high Pb in the soil.



(a)



(b)

Figure 4.7.11: (a) Translocation factor {TF} (shoot Pb DW mg/kg/ root Pb concentration mg/kg), (b) Box plot of translocation factor as a function concentration across species and varieties. Error bar represents 1 standard error on the mean. TC BR had only one concentration (10000 mg/kg).

The box plot of translocation factor (Figure 4.7.11b) shows the overall effect of the range of Pb concentration on the TF of all the plant species/varieties in the second pot trial. Most of the plant species/varieties were similar in their TF in the 100 mg/kg concentration (Figure 4.7.11b). A greater percentage (~95%) of the species/varieties had their maximum TF (ranged from 0.3 to 0.7) in the 300 mg/kg concentration with ~5 % having

TF below this range. Translocation factor decreased with increasing soil Pb concentration. In the 3000 mg/kg concentration, ~60% of the plant species had TF > 0.2, whilst the remaining 40% had TF below 0.2. About 99% of plant species (e.g BJ 18, BJ 17, ZM B 73, ZM OH43 and BN SW) in the 10000 mg/kg concentration had TF below 0.3 with 1% (e.g *Thlaspi caerulescens* variety TC BR) having TF >0.3, which suggest a potential hyperaccumulation of the species. There was an overall ~30 to 90% variation in TF between species in the same soil Pb concentration and between the range of soil Pb concentrations. This is an indication that the amount translocated to the shoot from the root is species-specific and could be influenced by the soil Pb concentration. However, the amount taken up from the soil by the roots of these species/varieties is also influenced by the soil Pb concentration and the bioavailable fraction to the plants.

4.7.5 Growth parameters of species/varieties in the second pot trial.

Similarly as in the first pot trial, other growth parameters such growth index (GI), height, number of true and dead leaves and longest leaf length were used to study the behaviour of plant species/varieties to the Pb added treatment during growth period. The results are shown in Figures 4.7.12 to 4.7.15

Analysis of variance of the GI of all varieties/species showed significant differences P=0.002, 0.000, 0.000, 0.001, 0.001 and 0.010 was observed in the growth indexes of all varieties/ species (BJ 18, BJ17, ZM B73, ZM OH43, BN SW and TC BR) respectively. This is an indication that the different growth indexes were affected by the differences in the Pb concentrations.

The growth index (GI) across species/varieties over a range of concentration is shown in Figure 4.7.12 below. Comparison of GI between concentrations showed that the effect of the added Pb on the GI was not significantly different for most concentrations of BJ 18. It indicated that this variety had tolerance to high Pb concentration, but a slight decrease (1%) in GI was observed at the 10000 mg/kg added Pb compared to the 0 mg/kg added concentration.

Significant differences P=0.000 was observed between the 0 mg/kg added Pb and all other concentrations of BJ 17, which suggest different effects on the GI at the different concentrations applied. The growth index of this variety tends to increase with increasing Pb concentration. This is an indication of the tolerance of this variety to high Pb within this concentration range.

The lower concentrations of ZM B73 were significantly different from the 3000 and 10000 mg/kg Pb added (Figure 4.7.11).The effect of the added Pb on the GI differs at the different concentration range. It implies that, this variety had tolerance to high Pb as the GI tend to rise again at 3000 and 10000 mg/kg Pb added.

The 0 and the 10000 mg/kg Pb added of ZM OH43 variety were not significantly different (Figure 4.7.12). This is an indication of the significant effect of the added Pb on the growth of this variety and its ability to tolerate high Pb within this concentration range.

The *Brassica napus* variety BN SW grew well at Pb concentrations of 100 and 300 mg/kg with 19% lower GI at 3000 mg/kg and a further 26% lower in 10000 mg/kg Pb added treatment. It suggests that the added Pb had an effect on the GI at these concentrations, and that this variety will thrive well within the concentration range of 100 to 3000 mg/kg.

A definite pattern or trend in GI was not observed in TC BR. It grew well at 300, and then a 33% decrease at 3000 mg/kg and 16% increase at 10000 mg/kg. It showed an adaptation to high Pb, but the sudden decrease at 3000 mg/kg might be due to some other factors.

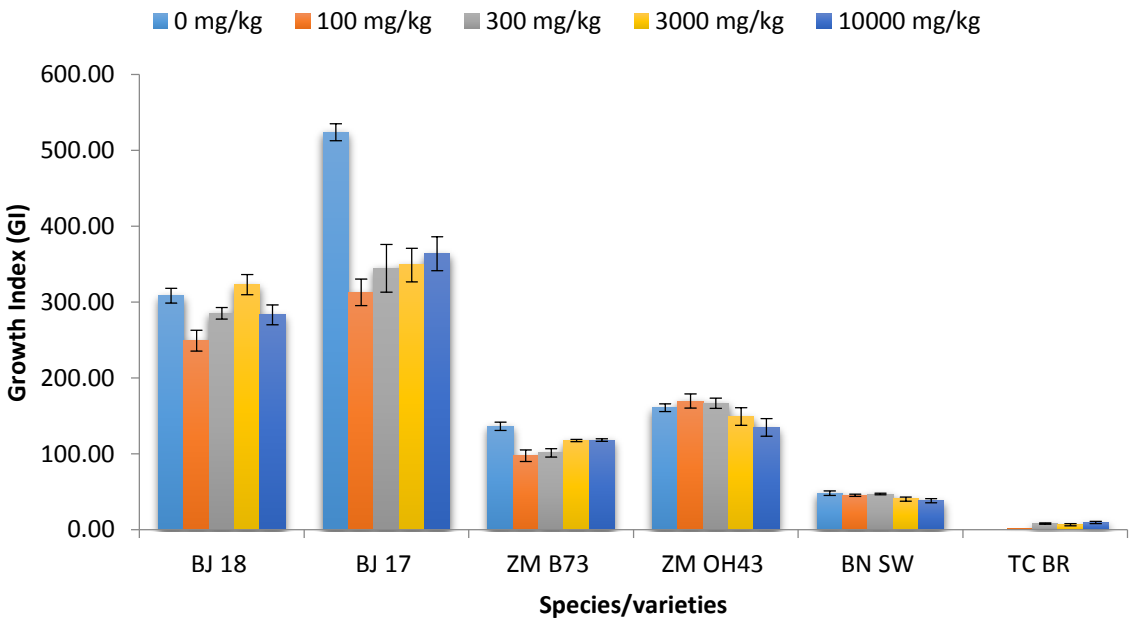


Figure 4.7.12: Growth index (GI) over a range of concentration across species and varieties in the second pot trial. Error bars represent one standard error on the mean n=3.

Significant differences P=0.000, 0.001, 0.022, 0.006 and 0.002 were observed in the number of true leaves of BJ 18, BJ 17, ZM B73, ZM OH43, BN SW and TC BR at the different concentrations applied (Figure 4.7.13).

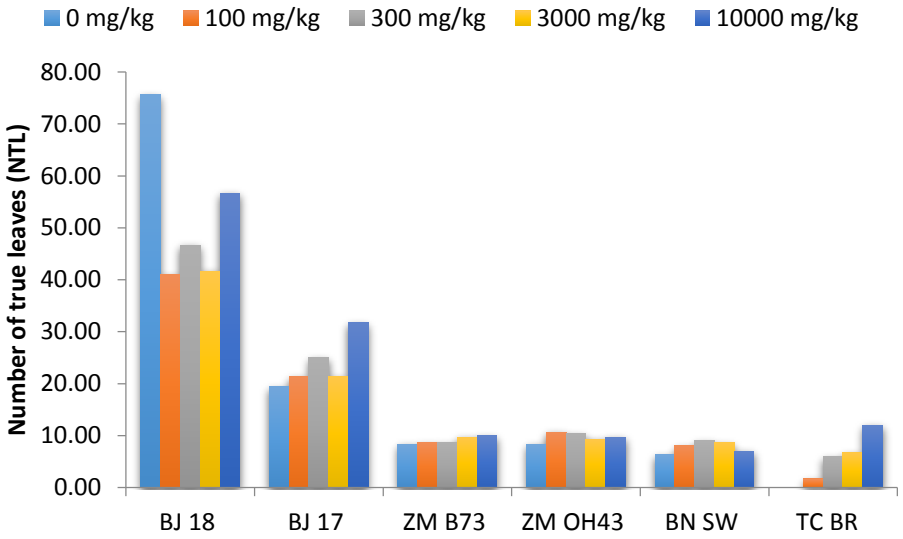


Figure 4.7.13 : Number of true leaves (NTL) over a range of concentration across species and varieties.

There were significant differences P=0.001, 0.000, 0.000,0.001,0.001 and 0.004 (P<0.05) in the height of all varieties (BJ 18, BJ 17, ZM B73, ZM OH43, BNSW and TC BR respectively (Figure 4.7.14). It is an indication that the differences in height of these varieties was as result of the varying Pb concentrations. Height of these species did not follow a particular trend. However, some of the varieties were not significantly different in their height at some concentrations (e.g the 300, 3000 and 10000 mg/kg concentration of the *Brassica napus* variety BN SW).

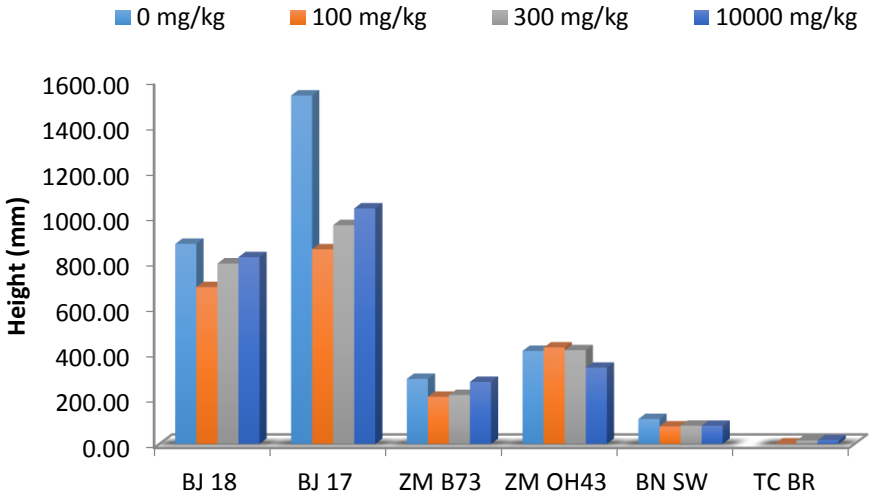


Figure 4.7.14 : Mean height over a range of concentration across species and varieties in the second pot trial.

The species/varieties (BJ 18, BJ, 17, BN SW and TC BR) differed significantly (P= 0.017, 0.000 0.040 and 0.000) in their longest leaf length respectively (Figure 4.7.15). It could

be inferred that the differing Pb concentrations were responsible for the different lengths observed.

There was no detected significant effect of the Pb added treatments at the different concentrations on the longest length of the leaves of both *Zea mays* varieties ZM B73 and ZM OH43 (Figures 4.7.15).

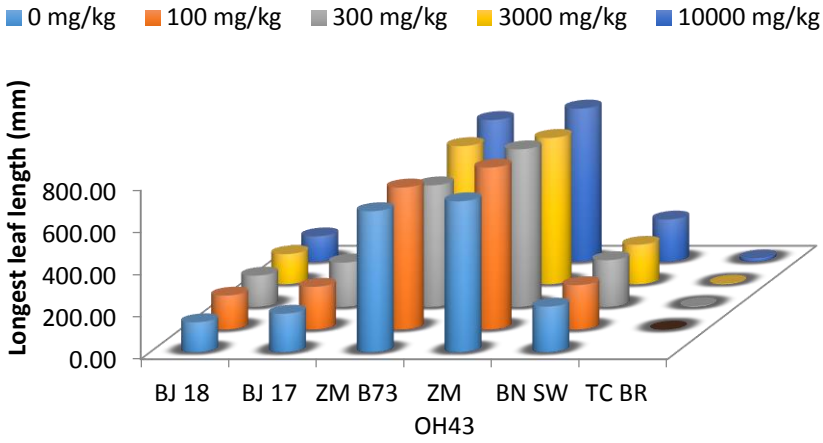


Figure 4.7.15: Mean longest leaf length over a range of concentration across species and varieties in the second pot trial.

4.8 DISCUSSION.

Some of the varieties and species showed signs of stress at some points (e.g. decrease in biomass and chlorosis) to high Pb concentrations especially at 10000 mg/kg treatment. A significant effect of the added Pb was detected in some varieties when compared to the control treatment (0 mg/kg Pb added concentration).The *Brassica napus* variety BN SW was stressed as a result of the increasing Pb concentrations (above 300 mg/kg). Severe wilting of leaves and chlorosis was observed in this variety at high Pb concentration. *Brassica juncea* variety BJ 17 did not show any observable effect of toxicity or stress to increasing Pb concentration.

Shoot Pb concentration for species/varieties in the 100 mg/kg concentration were in the order ZM B73 > BJ 17 > ZM OH43 >BJ 18 >BN SW. The 300 mg/kg shoot concentration were in this order TC BR >ZM OH43 > ZM B73 > BJ 17 > BJ 18 >BN SW whilst the 3000 mg/kg Pb concentration were in this order TC BR > ZM OH43 > ZM B73 >BN SW > BJ 17 > BJ 18). The shoot Pb concentration of the 10000 mg/kg treatment were in the order TC BR > ZM OH43 > ZM B73 > BJ 17 >BN SW > BJ 18. This is an indication that uptake of Pb by these plant is species specific, which is line with literature on potential Pb accumulator (Baker *et al*., 1981; Rotkttikhum *et al*., 2006). However, some varieties of the same species were similar while some differ significantly.

The shoot, root and total Pb concentrations (mg/kg) DW increased with increasing soil Pb concentrations for all species/varieties. This suggest that the amount of Pb uptake from the soil is dependent on soil Pb concentration. This is supported by works of (Morrey *et al*., 1988; Baker and Brooks 1989; Baker *et al*., 1994; Brown *et al*., 1995a; Brown *et al*., 1995b; Zhisvistoky *et al*., 2011). Significant effect of the added Pb on the shoot, root and total dry biomass at high Pb concentrations was also observed. This is an indication that the effect of the Pb treatment differed with varied Pb concentrations for most varieties.

Root Pb concentrations were in similar order for species and varieties as the shoot with TF ranging from 0.1 to 0.7 across species/varieties over a range of concentration. This TF range of values suggest poor translocation of Pb to the shoot, which could be considered as an adaptive mechanism of tolerance to Pb stress in these plant species. The roots of most varieties had 4 to 5 fold higher Pb concentrations than the shoots. This higher root Pb concentration is supported by literature. Results in the second pot trial show that over 95% of plant species can accumulate higher (3-5 times) Pb in their roots

than in shoots. This was also observed in the results of the first pot trial and in line with previous findings (Nabulo *et al*., 2008; Bothe *et al*., 2010).

The shoot and root concentration factors provided an insight into how Pb was taken over concentration gradient. The shoot concentration factor (CF_{shoot}) ranged from 0.02 to 0.43 with highest CF_{shoot} for TC BR in the 300 mg/kg concentration. A general trend of low CF_{shoot} was observed for most species. These CF_{shoots} also supported low shoot accumulation of Pb or poor translocation (TF ranged from 0.1 to 0.7) of Pb from the root to the shoot. This is very close to values reported in previous works from literature review (Chapter 2: Section 2.5). Low concentration factors is associated with plants having effective mechanism in place to exclude harmful contaminants (Daniela *et al*., 2010). All species/varieties had higher CF_{shoot} (5-28 times) and CF_{root} (3-13 times) in the lower Pb added concentrations and a general trend of decreased CF in shoots and roots with increasing treatment concentrations. This suggest that Pb uptake decreased with higher concentrations of Pb. This could explain why some of the plants in the higher Pb concentration seem to grow well as much as those in the lower Pb concentration, thus leading to a similarity in biomass between higher and lower concentration in some of the plant species. It also implied that most plant species excluded much of the Pb in the higher Pb concentrations (e.g 3000 and 10000 mg/kg).

Brassica species varieties BJ 18 and BN SW had similar (0.04-0.06%) shoot Pb concentration in the 100, 300 and 10000 mg/kg treatments. However BN SW had 25% higher shoot Pb in the 3000 mg/kg when compared to BJ 18. This similarity in shoot Pb concentration was also true for the *Zea mays* varieties ZM B73 and ZM OH43. All species/varieties seemed to tolerate the added Pb in the soil, but to differing extent. *Thlaspi caerulescens* TC BR had higher Pb concentration in the 10000 mg/kg concentration than any of the other varieties/species.

The change in biomass with respect to Pb concentration range did not follow a particular trend in most of the species/varieties. However, the biomass of TC BR increased with increasing Pb concentration. A regression model of the total biomass against concentration gradient for all plant species (Appendix II.16) shows that, there was no significant relationship between biomass change and concentration gradient. This suggest that the Pb concentration range could not be used to predict changes in biomass. This is in line with similar observation reported by Foroughi *et al*., (2014) with *Noccaea caerulencens* in hydroponic trial over a range of Zn concentration. In addition to inferred exclusion of Pb at higher Pb concentration suggested by decreased CF with increasing Pb concentration, there is also a possibility that the change in biomass might

have occurred randomly in some cases irrespective of concentration gradient. In such cases, it could be inferred that other factors might have influenced the total dry biomass. Work by Zhu *et al*. (2008) suggests that the efficiency of the captured solar energy and the actual conversion of the captured energy into vegetative tissues that constitute the bulk of plant may influence plant biomass.

In cases where the total dry biomass was decreased significantly due to increasing Pb concentration (e.g shoot Pb of BJ 18, ZM B73, ZM OH43, BN SW, TC BR and root Pb of BJ 17, ZM B73, TC BR in 10000 mg/kg), it could be inferred that the added Pb had influenced any of the metabolic activity chiefly accounting for biomass production, such as meristematic activities, cell elongation and the efficiency of photosynthesis. Studies by Baker, (1981); Baker *et al.,* (1994); Lasat (1996) suggest that the presence of high Pb and Zn in the soil may influence some of these metabolic activities in plants. Internal and external factors that influence plant growth might also have been responsible.

Work by Wayne and Ames, (2012) suggests that internal factors such as differentiation processes involved in the establishment of localized differences in biochemical, metabolic activity and structural organization in plants could be responsible for differences in plant structure in response to nutrient and contaminants in the soil. Some studies (Sharp, 1990, Deng *et al*., 1990) suggest that external environmental factors may affect specific shoot activity such as low water potential that could influence uptake of nutrient and contaminants at different concentrations. However, Waynes and Ames (2012) reported that certain environmental factors may influence developmental times and block particular stages resulting in differential response of individual plants to presence of contaminants in the soil. This may seem to explain the differential plant response to concentration gradient observed in this second pot trial.

Efforts were made to reduce the effects of uneven environmental conditions by rotating pots at 90 degrees weekly as described in earlier work with Zn (Thomas, 2010). This might not have completely eliminated this effect and thus, might have partially influenced the biomass data trend in this experiment. However, Pb concentration of shoots, roots and dry weight total plants compared to the 0 mg/kg concentration showed a significant effect of the Pb treatment on some of the plant species/varieties.

One other reason that might have affected the biomass trend in this experiment is the transition from vegetative to reproductive stage in some of the species/varieties. Study by Taku and Zheng-Hua, (2010) suggest that the transition from the vegetative growth to the reproductive growth phase of the plant can lead to decrease in shoot and root biomass.

Both *Brassica juncea* varieties were flowering and producing seeds from the fourth week after transplanting and notable changes such as reduced leaf area and thinning of stem were observed in some concentrations. This might have accounted for changes in biomass in higher Pb added concentrations and similarities in shoot dry biomass of lower and higher concentrations. The over expression of flowering time gene in some varieties or species have been shown to affect shoot dry biomass (Salehi *et al*. 2005). Hormonal regulation of root and shoot has also been reported (Sakamoto and Matsuoka, 2004) to play important roles in plant growth and development, including the regulation of meristematic activities, cell elongation, both of which are crucial for biomass yield.

This non-regular trend in biomass with Pb concentration gradient might also have been influenced by other factors. For example leaf eating caterpillars were found on the leaves of *Brassica juncea* varieties in the 3000 mg/kg treatment near harvest (Appendix II.12: Figure AII.12). They were immediately handpicked and destroyed before any damage was caused especially to plant biomass. This might have marginally impacted the biomass of this plant species in that concentration. The reason for the caterpillar attack is not known. Robinson *et al*., (1998) reported the defence against herbivores and pathogens as one reason to explain the advantages conferred on plants by heavy metal accumulation. However, Delorme *et al*., (2001) reported that despite the obvious appeal of the herbivore defence theory, some studies have shown that, in many cases, heavy metal accumulation does not protect the plant from herbivore attack which supports the observation of caterpillar attack in this study. The attraction of the caterpillar to a specific plant and concentration could be recommended for future research. However, the cause of variation in plant response to Pb concentration gradient was not investigated further in this pot trial.

Root biomass with respect to scale of heterogeneity is very crucial to selecting suitable plant species for further pot trials. When the total dry biomass of different species/varieties were considered, *Zea mays* varieties ZM B73 and ZM OH43 had higher root biomass (with highest mean root dry biomass of 1.95 g and 1.80 g respectively) than any of the other varieties/species, so not comparable to any other species for any single scale (e.g. 2 cm scale) of heterogeneity.

The *Brassica juncea* varieties differ slightly in their morphology, biomass and Pb concentrations. *Brassica juncea* BJ 18 was a tall creeping plant with highest mean root dry biomass of 0.62 g and total plant Pb of 114 mg/kg DW whilst BJ 17 was a tall erect plant and had higher root dry biomass and total plant Pb (0.96 g and 124 mg/kg). Root Pb concentration of BJ 18 was 2 times higher when compared to those of BJ 17.

Brassica napus BN SW was a short, thick sturdy plant with slightly higher root biomass (highest mean root dry biomass of 0.72 g) than the *Brassica juncea* variety BJ 18 and similar total plant Pb of 107 mg/kg DW.

Thlaspi caerulescens had the lowest root dry biomass (0.017 g) of all the varieties and highest mean total plant Pb of 1169 mg/kg DW. These contrasts in root biomass would affect the selection criteria. This is because different plant biomass (e.g root biomass) would recognize different scales of heterogeneity. The root balls of these varieties were also different. However, the total dimension (size) of the root ball was not taken into account in these earlier experiments. It was considered necessary to take into account the dimension (size) of the plant's root ball in further experiments. This was used to examine the relationship between the size of the root ball and the scale of heterogeneity on uptake of Pb from the growth medium by the selected plant species discussed in Chapter 5.

Zea mays varieties had the biggest biomass of all the other varieties/species (i.e. 10g DW). *Brassica napus* BN SW had similar biomass to the BJ varieties (i.e. 4 and 5 g), but severe observable effects of the added Pb at 3000 and 10000 mg/kg were recorded for BN SW, even though it was generally tolerant. The total biomass of BJ 18 was 28% higher in the 10000 mg/kg Pb added concentration when compared to the control (0 mg/kg). This is an indication of the tolerance of this variety to high Pb concentration.

Based on this pot trial, a concentration range of 100 and 10000 mg/kg Pb was used in subsequent trials. This is because most of the varieties in the second pot trial showed varied tolerance, though with significant detrimental effects of added Pb observed at highest concentrations of 10000 mg/kg Pb. Most of the varieties/species tolerated with light apparent effects, Pb added concentrations in the range of 100 to 300 mg/kg Pb. The total dry biomass of BN SW was 24 and 32% higher in the 100 and 300 mg/kg Pb added respectively, when compared to the 3000 and 10000 mg/kg concentration. It suggests that this variety grew better in the control and at lower Pb concentrations, though tolerant to high Pb (3000 and 10000 mg/kg).

The number of varieties/species for further research on the effects of heterogeneity was restricted to two contrasting species, to allow for more number of replicates to be employed in those experiments. Low number of replicates was one of the limitations to statistical power in the first and second trials. It was difficult to have more replicates, because of the large number of species and varieties involved. As a result of this factor, some interesting but subtle effects might not have been detected.

4.8.1 Discussion in relation to stated hypothesis.

The stated hypotheses sought to understand the impact of varied Pb concentration on plant biomass and uptake of Pb between species/varieties. Hypothesis were accepted when there was a significant difference between the measured values of the variables. Result showed that significant effect of the added Pb on most species/varieties occurred in the higher Pb treatments. There was a trend in uptake with respect to Pb concentration gradient. However no regular trend was observed in the biomass with respect concentration gradient. A summary of the hypotheses test framed as alternative hypotheses (listed in section 4.6.3) is shown in Table 4.8.2.

Table 4.8.2: Summary of results of hypotheses tested for each species between-treatments, based upon Tukey H.S.D. comparison of means statistical tests where P < 0.05.

HYPOTHESES	BJ 18	BJ 17	ZM OH43	ZM B73	BN SW	TC BR
(1)Shoot biomass DW	Accept	Reject	Reject	Accept	Accept	Accept
(2)Root biomass DW	Accept	Accept	Reject	Reject	Accept	Accept
(3) Total dry biomass DW	Accept	Accept	Accept	Accept	Accept	Accept
(4)Total plant Pb	Accept	Accept	Accept	Accept	Accept	Accept
(5) Trend in uptake versus concentration range	Accept	Accept	Accept	Accept	Accept	Accept
(6) Trend in biomass versus concentration range	Reject	Reject	Reject	Reject	Reject	Reject

Key: Plant species selected for the third and fourth pot trials are highlighted in red

In line with one of the stated objectives i.e to select the most suitable species for further pot trials, *Brassica juncea* variety BJ 18 and *Brassica napus* BN SW were selected for use in further trials. These two species had the most similar biomass when compared to *Zea mays* and *Thlaspi caerulescens*, and also responded similarly to same range of Pb concentrations. Although, BN SW had slightly higher biomass (by a factor of 1.4) than BJ 18. It is worth comparing these two species in response to same scale of Pb heterogeneity in further pot trials.

CHAPTER FIVE: An investigation of a simplistic binary model of heterogeneity of Pb on biomass and plant uptake by two selected plant species (*Brassica napus* and *Brassica juncea*).

5.0 INTRODUCTION AND BACKGROUND TO EXPERIMENT.

Two pot trials had been carried out and were discussed in earlier chapter (Chapter 4). These earlier pot trials tested and compared the behaviour of some Pb accumulating species, out of which *Brassica juncea* and *Brassica napus* were selected for further investigation on the effects of heterogeneity.

This chapter discusses the third pot trial that investigated the impact of a simplistic binary model of heterogeneity on biomass and Pb uptake of the selected plant species compared against homogeneous and control (0 mg/kg Pb added) treatments. It builds on our understanding of plant root responses to nutrient patches in previous works by Jackson and Caldwell, (1989); Hutchings *et al*., (2000); Wijensinghe *et al*., (2001); Haines, (2002). Root proliferation of *T*. *caerulescens* to Zn patches has been reported (Schwartz *et al*., 1999b, Whiting *et al*., 2000; Haines, 2002). The chapter also discusses the bioavailability experiment which determined the extractable Pb in the growth medium of pot trials and the *in situ* soil from Gang Mine.

Soil properties and constituents that affect plant growth are often heterogeneously distributed. According to Jackwell and Caldwell (1993); Wang and Cheng (2013), heterogeneity is regularly considered important for competitive interaction among plants. Significant variation was found in nutrient resources at different scales around a single plant (Jackwell and Caldwell, 1993; Wang *et al*., 2013). Previous works by Stuefer *et al*., 1994; 1996; Wijesinghe and Hutchings, 1997; 1999; Fransen *et al*., 2001; Wijesinghe *et al*., 2001; Wang *et al*., 2006;2013; Mou *et al*., 2013; Hu *et al*., 2014 reported a strong effect of nutrient heterogeneity on plant biomass and acquisition of nutrient resources.

The study of heterogeneity in the distribution of trace metals (e.g. Cd and Zn) in the soil has received some attention in recent years. Earlier studies by Millis *et al*., (2004), Haines (2002) and Thomas, (2010) using the simplistic binary ('hit and miss') heterogeneity in pot experiments showed significant differences in Cd and Zn concentrations of shoots and roots compared to those grown in homogenized growth media. Schwartz *et al*., (1999b); Whiting *et al*., (2000) and Haines (2002), observed a positive root proliferation in *Thlaspi caerulescens,* a Zn accumulator in response to substrate patches with high Zn concentration. Gray *et al*., (2005) and Bondada *et al*., (2007) reported a non-foraging but

positive response of *Pterris vittata* the arsenic hyperaccumulator plant, to spatial distribution of arsenic in soil. According to Banuelos *et al*., (1998), effects of heterogeneity may explain significant differences in plant uptake of contaminants between pot experiments in controlled (usually nominally homogeneous) environments, and *in situ* studies.

Differential root growth that might affect metal uptake has been shown in a number of plant species. Foraging traits, such as the localized root proliferation in patches of substrate with high metal concentrations may be important in enhancing heavy metal accumulation in hyperaccumulator species (Haines, 2002). Some plants are able to forage for patchily distributed resources by positioning or proliferating leaves, roots or ramets when patches of higher quality or greater resource is available (Hutchings and De Kroon, 1994; Birch and Hutchings, 1994; Wijesinghe and Handel, 1994). Previous studies (e.g Jackson and Caldwell, 1989; Wijensinghe *et al*., 2001; Hutchings and John, 2004) showed that foraging responses such as root proliferation in response to local nutrient enrichment had been observed in many plant species, and for some species, greater growth has been achieved in patchy habitats than in homogenous habitat. According to Robinson (1994) and Hutchings *et al*., (2000), patchy distribution of nutrients can influence plant performance as a result of altered resource acquisition, allocation patterns and changes in total biomass.

5.1 Objectives of third pot trial.

1. To examine and compare the response (positive and/or negative responses) of two selected plant species to a simple form of heterogeneity (a simplistic binary design) compared against a homogeneous treatment, before the fourth pot experiment more closely simulating the *in situ* heterogeneity seen in the field.

2. Examine root responses of the selected plant species to Pb in the homogeneous and the binary heterogeneous treatments of the growth medium.

5.1.1 Hypothesis

1. Simplistic binary design of Pb heterogeneity has a significant impact on (a) biomass and (b) Pb uptake, when compared against a homogeneous design and against a control treatment containing no added Pb.

2. Roots of plants will preferentially proliferate in patches with no added Pb in the heterogeneous design, to avoid the toxicity of the added Pb.

5.2 Study species for the third pot experiment and further pot trial.

5.2.1 *Brassica juncea* (L) Czern (Indian mustard).

Brassica juncea (L.) Czern (Indian mustard or brown mustard) belongs to the family Brassicaceae or family Cruciferae commonly known as the mustard family (Woods *et al*., 1991). *Brassica juncea* has pale green foliage, with few hairs on the first leaves and leaf blades that terminate well up the petiole with the lower leaves deeply lobed and the upper leaves narrow and entire (Raskov and Woods, 1987; Woods *et al*., 1991). It grows to a height of 1 to 2 m (Hemmingway, 1995). *Brassica juncea* has an annual growth habit (Raskov and Woods, 1987). It is one of the known accumulators of Pb and Zn (Bennett *et al*., 2003; Anjum *et al*., 2012). It is has been reported as accumulating 9580 mg/kg of Pb in roots and 3580 mg/kg in shoots (Meyers *et al*., 2008) (Section 2.5: Table 2.5.1). This suggest that *Brassica juncea* is a hyperaccumulator of Pb. Huang and Cunningham (1996) observed an uptake and localization of lead in the root system of *B. Juncea* when treated hydroponically. It is also a known hyperaccumulator of zinc (Baker and Brooks, 1989; Thomas, 2010).

It tolerates an annual precipitation of 500 to 4200 mm, annual temperature between 6 to 27^{o}C and pH of 4.3 to 8.3 ((Meyers *et al*., 2008). It is a hardy cool-season vegetable growing well at an average monthly temperatures of 15 to 18^{o}C and moderately tolerant to soil acidity preferring a pH of 5.5-6.8 and thrives well in areas of hot days and cool nights with fair resistance to drought (Duke, 1981). Its growing period is from 40 to 60 days depending on the variety and weather conditions (Duke, 1982). *Brassica juncea* may also be grown as a biennial plant with long erect branches, dense root mass and a rooting depth of 90 to 120 cm (Hemmingway, 1995). Figure 5.2.1 show *B*. *juncea* in the wild.

Figure 5.2.1: *Brassica juncea* L. Czern in the wild (490 mm x 640 mm). Source: (www.discoverlife.org).

5.2.2 *Brassica napus* (L) (Rapeseed, rape or oilseed rape)

Brassica napus (L.), (commonly called rapeseed, rape, oilseed rape), is a member of the family Brassicaceae (mustard or cabbage family) (Potts *et al*., 1999). Rape seed is grown for the production of animal feed, vegetable oil for human consumption and biodiesel (Suh *et al*., 1988). It grows in sandy loam to clay loam soils to a root depth of 36 cm (Chardin *et al*., 2001).

Brassica napus is one of the most common green leafy vegetable that is consumed in some households (Chimbira and Moyo, 2009). It has a branched tap root ball system with root diameter ranging from 150-170 mm and the entire plant is anchored by a single rigid tap root (Crook and Ennos, 1997; Chimbira and Moyo, 2009). However, Thomas, (2010) did not observe a tap root development in pot trials with *B. napus* (Variety ES Astrid, grade CS). The development of tap root in this plant may depend on the variety or the type of growth medium used. Its upright stems and shoot height ranged from 1.3-1.5 m, and the largest single root in this plant is the tapering tap root which grows to depth of 60-90 cm (Crook and Ennos, 1993). The mechanical role of the tap root is for effective anchorage below some critical depth, to give physical stability where plants can take up water, nutrients and incidentally heavy metals from the soil (Ennos and Filter, 1992; Ennos *et al*., 2001).

Chimbira and Moyo (2009) studied the uptake of Pb and Cd by *B. napus* in clayey soils and observed that an interaction between Cd and Pb in the soil reduced Pb uptake by *B. napus*. However, Carlson and Bassaz (1997) reported an uptake 984 and 354 mg/kg Pb in root and shoot by *B. napus* plants with increasing concentration of Cd in the soil. Figure 5.2.2 show *B*. *napus* in the wild.

Figure 5.2.2: *Brassica napus* .L. in the wild (375 mm x 500 mm). (Floridata. Com, 2003).

5.3 Experimental Design for the Simplistic binary heterogeneity experiment.

The experimental design was based upon the method described by Haines (2002) with modifications to identity (i.e Pb in place of Zn) and concentrations of the contaminant. Only a fraction of the Pb in the PbO used to spike the growth media is bioavailable to plants, therefore the concentrations of Pb chosen for this experiment allowed for the estimated bioavailability of Pb. However, an extraction experiment (Section 5.5) was carried out to determine the bioavailable Pb to plants using $MgCl_2$ extraction (Tessier *et al*., 1979; Chao *et al*., 2007; Zimmerman and Weidorf, 2010). This is reported in Section 5.5.4. Further estimates of bioavailability were made from the results of the herbage analysis from the second pot trial. The choice of Pb concentrations in this experiment was based on plant tolerance from the first and second pot trials.

Brassica juncea Accession PI 182921 {BJ 18} and *Brassica napus* Accession PI 601261 {BN SW}) were subjected to control conditions without additional Pb and to treatments in which Pb was added homogeneously or in a binary design. Simple randomized block design was used, with randomization between treatments as shown in Figure 5.3.1 and Appendix III.1: Table AIII.1.

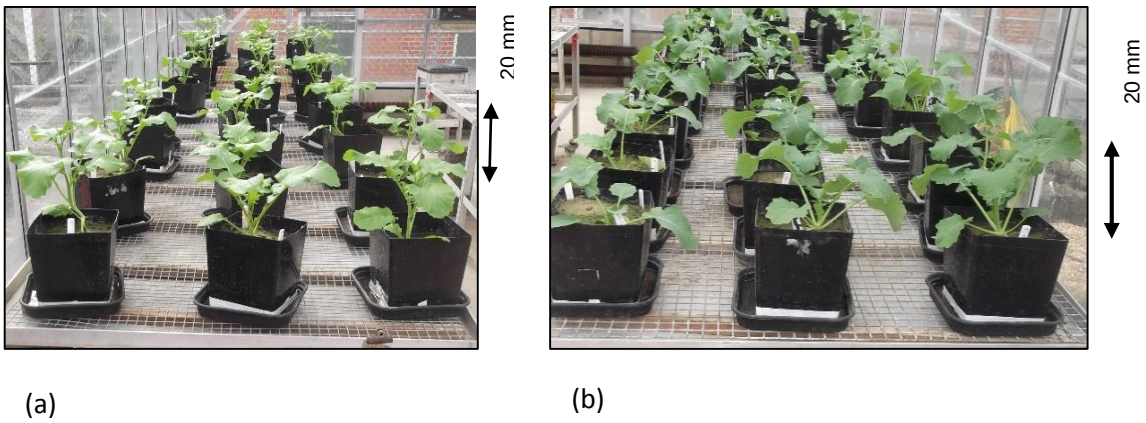


Figure 5.3.1: Randomized block design showing (a) *B. juncea* - left (Scale bar: 9 mm represents 20 mm) and (b) *B. napus* --right (Scale bar: 7 mm represents 20 mm). Arrows represent scale bars.

5.3.1 Method.

Germination of seeds, preparation of growth media, spiking of growth media with the PbO contaminant, transplanting of seedlings and harvesting, processing and analysis of herbage samples for Pb, were done as described in the first pot trial (Chapter 4: Section 4.3) except for changes in contaminant concentration, the amount of sand and compost

used and the use of a 4-way 40 mm by 80 mm and 170 mm deep binary pot divider (Figure 5.3.2).

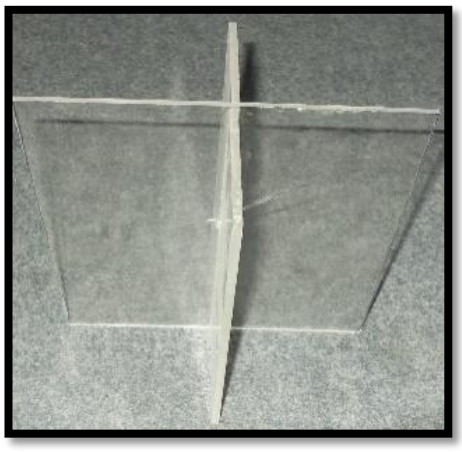


Figure 5.3.2: Binary pot divider used in the simplistic binary experiment (4-way 40 mm x 80 mm x 170 mm).

Six kg of dry carrier sand was spiked with PbO to make nominal concentrations of 1000 and 2000 mg/kg DW of Pb in the final growth media. Spiked carrier sand was thoroughly mixed with sand and compost in the cement mixer. The amount of PbO needed to make concentrations of 1000 and 2000 mg/kg Pb, and the estimated amount of sand and compost required (70% and 30% volume/volume of sand and compost respectively) has been calculated as shown in Appendix III.1: Table BIII.1. Selection of species/varieties for this experiment was based on their total dry biomass with respect to scale of heterogeneity (4 cm at a depth of 17 cm) and Pb concentrations of herbage samples discussed in Chapter 4.

The selected plant species are *Brassica juncea* (variety BJ 18) and *Brassica napus* (variety BN SW). Full names, accession numbers, origin and suppliers are shown in Chapter 4: Table 4.3.1. The study species' morphology, characteristics and growth requirements have been described in Section 5.2.

In the two treatments with added Pb, the pots were divided into quadrants. In the homogeneous treatment, all quadrants contained a nominal concentration of 1000 mg/kg (DW) Pb (Figure 5.3.3b) while for the binary treatment, a nominal concentration of 2000 mg/kg (DW) Pb was introduced into two opposite quadrants of the pot (Figure 5.3.3c). Plants were grown for six weeks under natural light (photoperiod of 16 hours) in a greenhouse at a temperature of 20±5º C. Power analysis used values for the variances of shoot Pb concentration (mg/kg) of both species taken from the second pot trial. Average shoot pooled standard deviation of 93 mg/kg and population mean difference of 4 mg/kg were used. The estimated minimum number of replicates at 95 % confidence

level and at 90 % probability of detecting a difference in population mean was 7.2. Using these data, a maximum number of 10 replicates (allowing for 20% failure rate or chances of detecting subtle differences than the number from the power analysis) per treatment, (3 treatments-Control, homogeneous and binary) for each species was used, making a total of 60 pots maintained in randomized block design (Figure 5.1.1; Appendix III.1: Table AIII.1). A diagrammatic representation of the experimental design is shown in Figure 5.3.3.

Dried and milled herbage samples were analysed for Pb. The growth medium was also analysed for its actual Pb concentration and reported in Table 5.3.1 below. Certified reference materials (NIST standard reference materials and house reference materials), duplicate samples and reagent blanks were used for quality control (Appendix III.4)

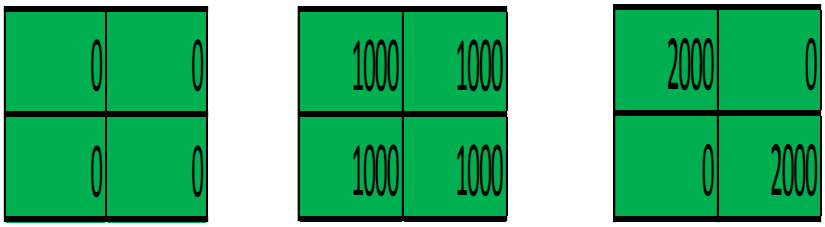
Table 5.3.1: Actual Pb concentration of growth media in pot trial 3.

Nominal Pb concentration mg/kg	Actual Pb concentration mg/kg	STDEV	SEM
0	24	6.1	2.5
1000	1012	190	72
2000	2418	693	309

Key: STDEV-Standard deviation. SEM—Standard error on the mean.

Plant growth information, such as growth index (GI) (discussed in chapter 4), height, number of true leaves, number of dead leaves, was recorded at initial transplant, in the third, fourth, fifth week, and also at harvest in the sixth week to assess physical variation between the treatments. Biomass data e.g. root and shoot dry biomass ratio were recorded at harvest. The approximate root ball diameter in all binary quadrants of the pot was also recorded.

Plant measurements such as height and root ball diameter were taken to the nearest 1 mm using ruler, measurement tape and Vernier calliper. Data were analysed using IBM® SPSS version 20 and Minitab 16 for Windows. Statistical tools such as the analysis of variance, independent-sample t-test and mixed model ANOVA (with treatment as fixed factor and block as random factor) were used. The Kolmogorov-Smirnov test was used test for normal distribution of data (Appendix III.2: Table LIII.2). The Tukey Post-hoc test was also employed for the comparisons between treatments. Graphs with error bars (representing 1 standard error on the mean) were prepared, in which a shared letter of the alphabet indicates that the mean values are not significantly different.



5.3.3a: Control **5.3.3b: Homogeneous** **5.3.3c: Binary**

Figure 5.3.3: Diagrammatic representation of the experimental design, values in mg/kg (mean nominally 1000 mg/kg for both treatments).

5.4 RESULTS.

There were visually observed differences between the treatments which gave an indication of the variation. During the growing period, clear visible differences such as decreased height, presence or absence of chlorosis were also detected between treatments for both plant species (Figures 5.4.1a to 5.4.1b and 5.4.2a to 5.4.2b). This qualitative observation was then confirmed quantitatively using ANOVA, which showed that the differential Pb treatments had a significant effect on most of the variables (shoot, root and total biomass, Pb uptake and root ball diameter).

(a) 5.4.1a **(b) 5.4.1b**

Figure 5.4.1: *Brassica juncea* {5.4.1a} *36* days after planting seedlings showing increased height simplistic binary (right- Scale bar: 13 mm represents 50 mm) compared against both the homogeneous (central- scale bar: 14 mm represents 50 mm) and to the control (left- Scale bar: 13 mm represents 50 mm), {5.4.1b}: *B. juncea* (Scale bar: 32 mm represents 1000 mm) in the binary treatment at harvest (56 days) showing healthy growth and no chlorosis (in contrast to *B. napus* in same treatment in Fig 5.4.2b. Arrows represent scale bars for each figure and information highlighted in blue.

(a) 5.4.2a **(b) 5.4.2b**

Figure 5.4.2: *Brassica napus* {5.4.1a} 36 days after planting seedlings showing decreased height in simplistic binary (right pot- Scale bar: 9 mm represents 20 mm) compared against both the homogeneous (central pot- Scale bar: 13 mm represents 20 mm) and to the control (Left pot: Scale bar: 9 mm represents 10 mm), {5.4.2b}: *B. napus* in the binary treatment at harvest (56 days) showing chlorosis and wilting of leaves (Scale bar: 9 mm represents 20 mm).

Biomass data in line with experimental hypothesis such as the shoot, root and total dry biomass and root ball diameter in binary quarters are discussed in detail. Summary of all variables including growth data such as the number of true and dead leaves, longest leaf length, height and growth index which are not part of the key hypothesis tested are presented in Table 5.4.1. A general trend was observed in some of these variables.

The raw measurement of each variable is presented in Appendix III.3.The summary of analysis of variance (ANOVA) result for all growth and biomass data showed significant differences between treatments for most of the variables except the longest leaf length and the root-shoot biomass ratio of *B. napus* (Table 5.4.1). It is an indication that the treatments had an effect on these plant species.

Table 5.4.1: Growth and biomass data from third pot trial (Simplistic binary heterogeneity experiment) showing Analysis of variance (ANOVA) result for growth and biomass data with mean (for control in blue, homogeneous in red and binary in purple) and P values in brackets.

Plant Species	Height(mm)	Growth index (GI)	Longest leaf length (LLL)	Number of true leaves (NTL)	Number of dead leaves (NDL)	Shoot dry biomass (g)	Root dry biomass (g)	Total dry biomass (g)	Root-shoot ratio (g)	Root ball diameter (mm)
Brassica juncea	S (0.002)	S (0.002)	NS(0.678)	S(0.000)	S(0.000)	S(0.001)	S (0.000)	S(0.002)	S(0.000)	S (0.000)
	1326:1121:1310	451:381:445	222:219:228	46:35:40	0:3:4	14:10:14	2.6:1.3:2.0	17:11:15	0.2:0.1:0.2	12:33,10
Brassica napus	S (0.000)	S (0.000)	NS (0.690)	S(0.000)	S(0.000)	S(0.000)	S (0.000)	S(0.000)	NS(0.11)	S (0.000)
	45;27:20	26:15:12	311:313:305	14:11:13	0:2.9:3.2	13:12:9	2.6:2:1.6	16:14:11	0.2:0.2:0.2	27:69:17

NS-- Not significant between treatments. S--- Significant at P<0.05 between treatments. P values in brackets.

5.4.1 Biomass results for *Brassica juncea.*

Plants were harvested after 56 days of growth when sufficient aboveground biomass had been produced, at which point there was a 100% survival rate. Mean shoot, root and total dry biomass for *B. juncea* increased by 31% in the binary treatment compared against the homogeneous. This difference was statistically significant {$F_{3,\ 26}$ =23.97; 64.11; 32.38, P<0.05} (Appendix III.2: Table AIII.2). Further comparison with the Tukey HSD post-hoc test confirmed this significance (Figure 5.4.3). This same trend was observed for the individual shoot and root dry biomass values, as shown in Figure 5.4.6 below. However, the apparent differences in the shoot, root and total dry biomass between the binary and control treatments were not significant. This implies that there is no significant effect on the biomass caused when the Pb is distributed in this heterogeneous way. At harvest plants in the binary treatment were healthy and generated substantial biomass, whilst those in the homogeneous treatment showed signs of chlorosis and reduced height. At 40 days, plants in the binary treatment had also begun flowering while those in the homogeneous treatment only began to flower after a further 7 days.

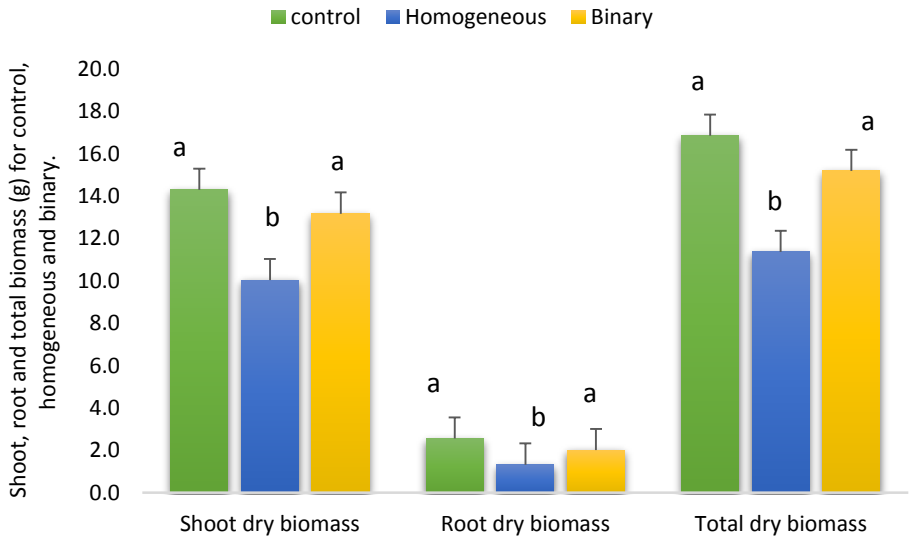


Figure 5.4.3: Mean <u>shoot, root and total biomass</u> (DW) between treatments of *B. juncea*. Means that share the same letters for each variable are not significantly different, as judged by the Tukey post-hoc test). Error bars represent 1 standard error on the mean for ten replicates (n=10).

5.4.2 Biomass results for *Brassica napus*.

Early visible response to treatments was observed for *B. napus* after 28 days growth (Figure 5.4.2a). Plants in the binary treatment were stunted with severe chlorosis (Figure

5.4.2b) at harvest after 56 days of growth. However, substantial biomass was generated and 100% survival rate was recorded. *Brassica napus* biomass did not show the same pattern of response to the treatments as *B. juncea*. There were clearly visible differences in the shoot, root and total dry biomass between treatments. These differences were statistically significant {$F_{3, 26}$ =48.97; 27.71; 64.78, P < 0.05} (Appendix III.2). Further comparison with Tukey HSD post-hoc test also confirmed significant differences in the above and below ground biomass between treatments (Figure 5.4.4). A trend of decreased total biomass in response to Pb treatment was observed with an approximately 70 % lower in the binary treatment compared to the control. A similar result was observed in the mean shoot and root dry biomass.

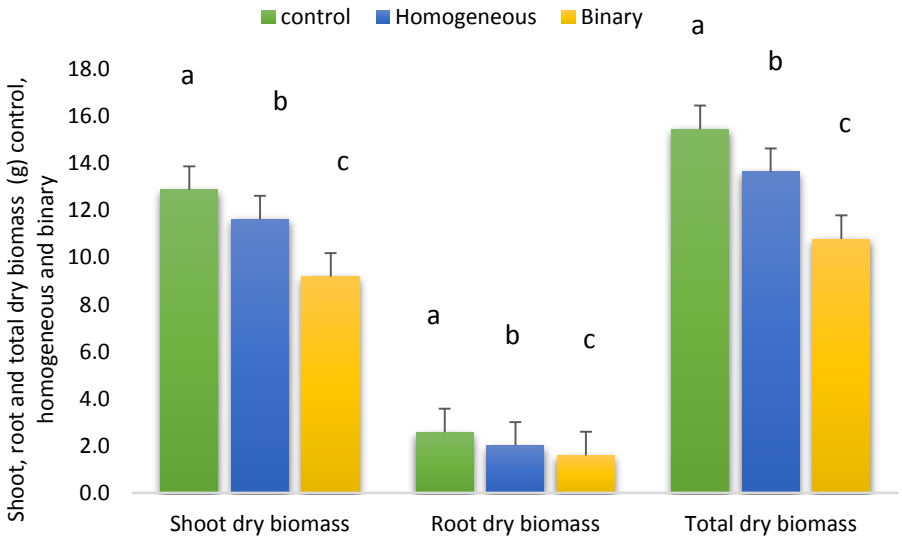


Figure 5.4.4: Mean shoot, root and total biomass DW between treatments of *B. napus*. Means that do not share letters for each variable are significantly different, as judged by the Tukey post-hoc test). Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

5.4.3 Root-Shoot biomass ratio of *B. juncea* and *B. napus*

The root-shoot biomass ratio of both plant species in control, homogeneous and binary is shown in Figure 5.4.5. The control treatment of both species had the highest root-shoot biomass ratio. This was decreased by 17% in homogeneous and binary treatments of *B. napus* and decreased by 38 and 20% in the homogeneous and binary treatments of *B. juncea* respectively. There was no significant difference {P=0.011>0.05} (Appendix III.2: Table DIII.2) in root-shoot biomass of *B. napus* between treatments, whilst the differences was statistically significant {P=000<0.05} (Appendix III.2: Table EIII.2) for *B. juncea*.

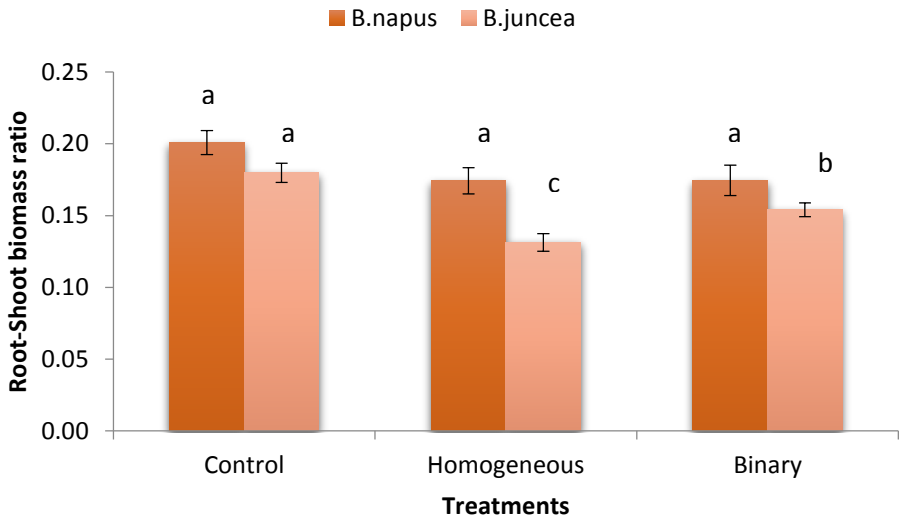


Figure 5.4.5: Mean root-shoot biomass DW between treatments of *B. napus* and *B. juncea*. Means that do not share letters for each species are significantly different, as judged by the Tukey post-hoc test). Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

5.4.4 Root response result for *Brassica juncea* and *Brassica napus*.

The diameter of each root ball was recorded to assess responses of plant species to patches of Pb in the binary treatment. The root ball diameter (Raw measurement in Appendix III.3) in the binary quarters showed that more roots were preferentially proliferated in the patches of no added Pb (0 mg/kg added) {70 mm and 33 mm} than in 2000 mg/kg Pb added {17 mm and 9.5 mm} in *B. napus* and *juncea* respectively (see Figure 5.4.6). Significant differences {$F_{2,\ 17}$ =17.72; 31.72, P<0.05} were recorded between species and binary patches respectively. The roots of both plant species, therefore avoided the Pb by a decreased root mass in the 2000 mg/kg Pb added patch.

The homogeneous patches had nearly equal distribution of roots in all quadrants compared to the binary treatment as shown in Figure 5.4.7.This suggests that in homogeneous growth media, roots are equally allocated to contaminants as was the case in this study.

The difference between root diameter in the homogeneous and binary treatments was also significant (P<0.05). Result showed both varieties have different root morphology (Figures 5.4.8a and 5.4.8b). A tap root was observed in *B. napus* (Figure 5.4.8b), whilst *B. juncea* lacked tap root (Figure 5.4.8a), but had a network of fibrous roots which was also observed in the fourth pot experiment discussed in Chapter 6.

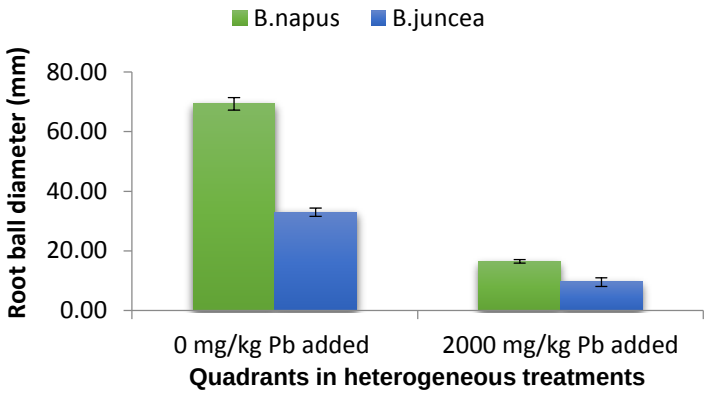


Figure 5.4.6: Root ball diameter between binary patches of *B. napus* and *B. juncea*. Error bars represent 1 standard error on the mean where n=10.

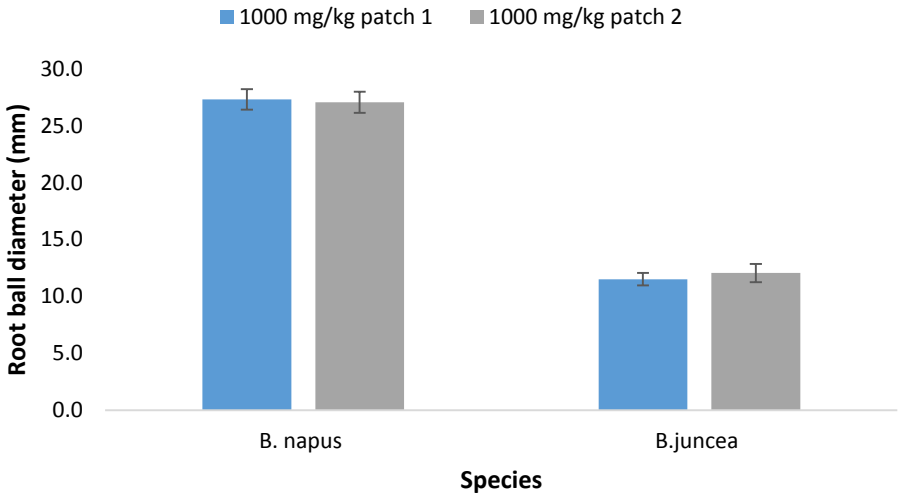


Figure 5.4.7: Comparison of the root ball diameter in homogeneous quadrants of *B. napus* and B. juncea. Error bars represent 1 standard error on the mean where n=10.

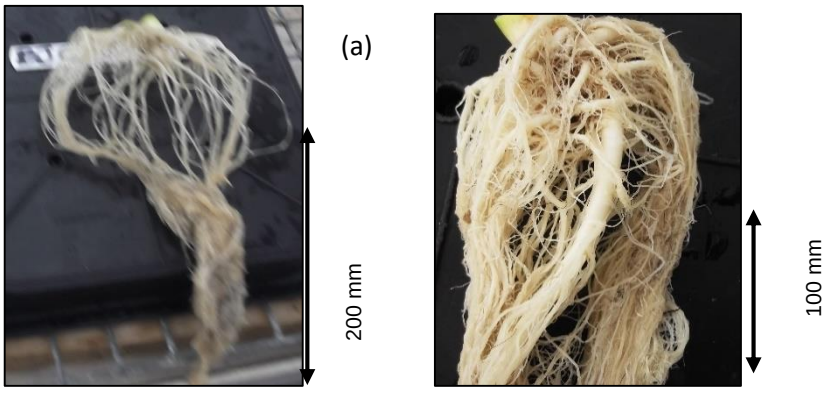


Figure 5.4.8: Roots of (a) *B. juncea* with no tap root (left- 2.9 mm represents 200 mm) and that of (b) *B. napus* showing a central tap root (right- 2 mm represents 100 mm).

5.4.5 Lead uptake results for *Brassica juncea*.

The difference in the total plant Pb concentration mg/kg (DW) between the binary and homogeneous treatments was significant (P = 0.002). Plants in the 0 mg/kg Pb added (control) were not analysed for Pb as this work compares the binary treatment against the homogeneous. Mean total plant Pb concentrations in the homogeneous treatment was 41% higher than that of the binary (see Figure 5.4.9). Similarly, shoot and root Pb concentration in the homogeneous treatment were twice and 57% higher than those of the binary treatment respectively (Figures 5.4. 9 and 5.4.10). This is in line with similar findings of reduced contaminant concentrations (40-200%) in simplistic heterogeneous (binary) treatment of Zn and Cd by Podar *et al*., (2004); Millis *et al*., (2004); Manciulea and Ramsey (2006); Thomas, (2010) and Moradi *et al*., (2009).

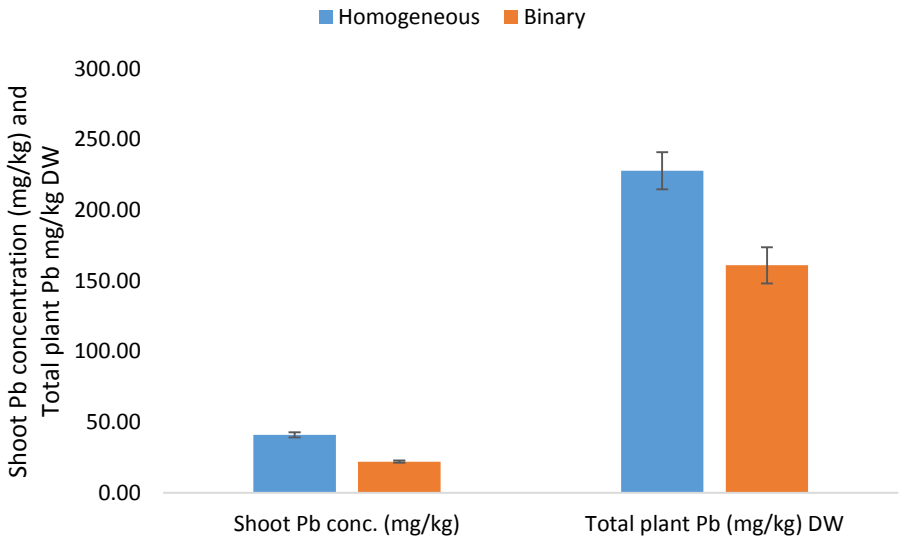


Figure 5.4.9: Mean shoot Pb concentration (mg/kg) and total plant Pb concentration {mg/kg DW} between treatments of *B. juncea*. Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

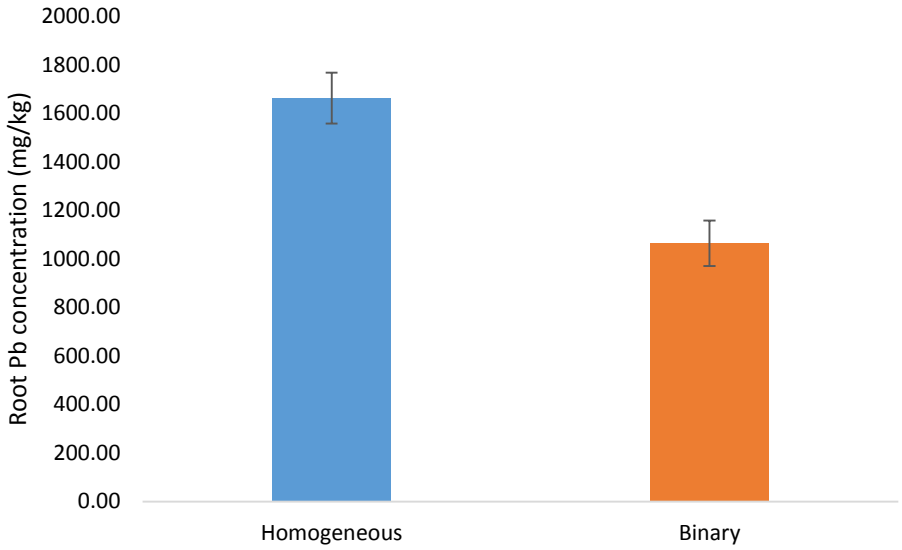


Figure 5.4.10: Mean root Pb concentration mg/kg between treatments of *B. juncea*. Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

5.4.6 Lead uptake results for *Brassica napus*.

The mean Pb concentrations of shoot, roots and total plant (mg/kg, DW) also decreased in response to heterogeneity in the binary treatment as did the dry biomass (see biomass in Figure 5.4.4), as opposed to the case of *B juncea* which had reduced uptake and increased biomass.

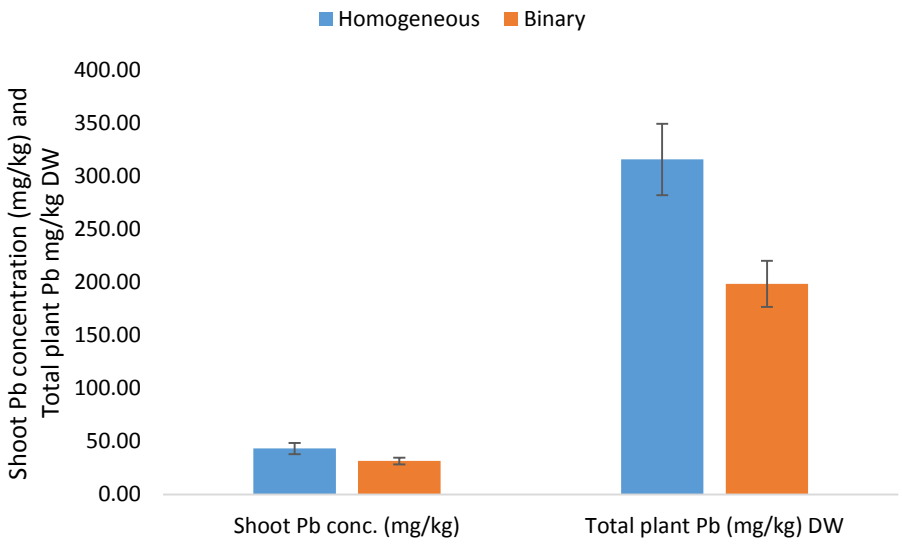


Figure 5.4.11: Mean shoot Pb (mg/kg) and total plant Pb concentration {mg/kg DW} between treatments of *B. napus*. Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

A highly significant difference ($p < 0.05$) in mean total plant Pb (mg/kg DW) was detected between the binary and homogeneous treatments with about 63% decrease in uptake in

the binary treatment compared to the homogeneous treatment. Similar trend was observed for the shoot and the root Pb concentrations (See Figures 5.4.11 and 5.4.12 below).

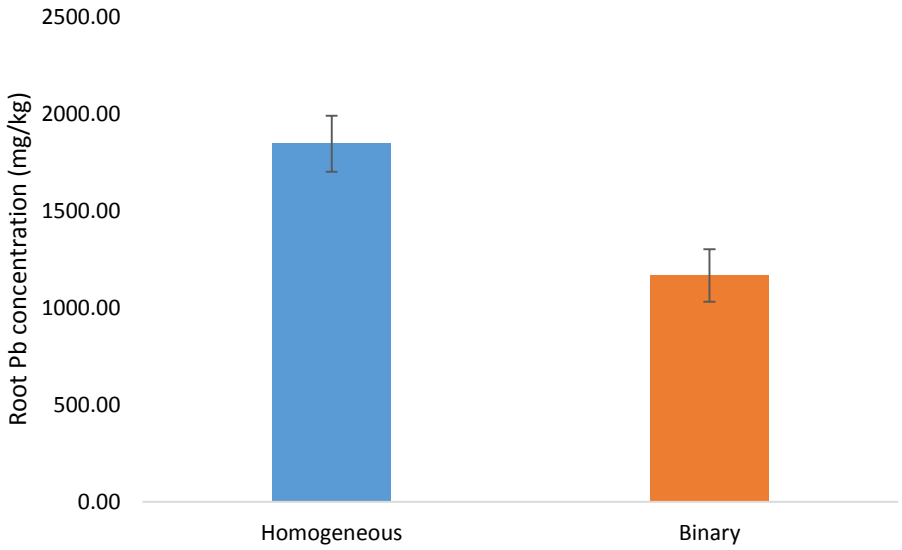


Figure 5.4.12: Mean root Pb concentration mg/kg between treatments of *B. napus*. Error bars represent 1 standard error on the mean (SEM), for ten replicates (n=10).

5.4.7 Uptake between species with respect to Concentration factor.

The shoot concentration factor (CF_{shoot}) for *B. napus* in the binary and homogeneous treatments were not significantly different whilst that of *B. juncea* was twice as low in the binary treatment when compared to the homogeneous treatment (Figure 5.4.13). The CF_{shoot} was generally low (0.02-0.09) for both species. The total concentration factor (CF_{total}) was 55% and 44% higher in the homogeneous than the binary treatment for *B. juncea* and *B. napus* respectively. There was a significant difference ($P < 0.05$) in CF_{total} between treatments.

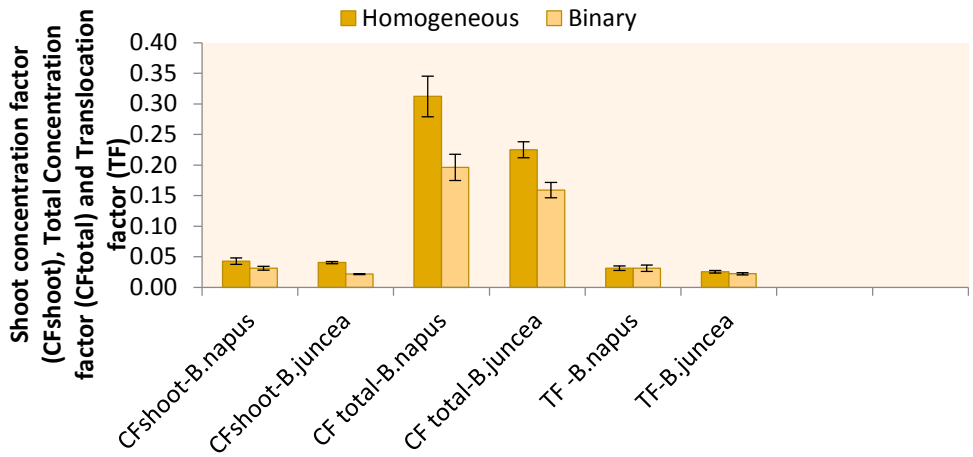


Figure 5.4.13: Mean total concentration factor (CFtotal), shoot concentration factor (CFshoot) and translocation factor (TF) of *B. napus* and *B. juncea* in homogeneous and binary treatments. Error bars represent 1 standard error on the mean where n=10.

The translocation factor (TF) for both species ranged from 0.02-0.04. Results suggest that about 75-95% of Pb was accumulated in the root with 5-25% accumulated in the shoot.

5.4.8 Uptake expressed as Pb mass (µg) for *B. juncea* and *B. napus*.

Shoot and root uptake of both plant species expressed as Pb mass (µg) are shown in Figures 5.4.14 to 5.4.15. The advantage of expressing uptake in (µg) and the difference between this form, and uptake expressed as concentration (mg/kg), is discussed in Chapter 6: Section 6.4.3.

Elevated shoot and root Pb masses in (µg) were observed in both treatments, when compared to uptake expressed as (mg/kg). However, reduced Pb mass was observed in the binary treatment of both species, when compared to the homogeneous as in uptake expressed in mg/kg concentration.

There was no significant difference between *Brassica juncea* and *Brassica napus* shoot Pb masses in the binary treatments, with *B. napus* having 21% higher Pb mass than *B. juncea* in the homogeneous treatment (Figure 5.4.14).There was no statistically significant difference ($P = 0.185$; $0.988 > 0.05$) in shoot Pb mass between species (Appendix III.5: AIII.5 and BIII.5). However, the differences in shoot Pb mass between the homogeneous and binary treatments were significant ($P = 0.005$; $0.0002 < 0.05$) respectively for *B. napus* and *B. juncea* (Appendix III.5: CIII.5 and DIII.5).

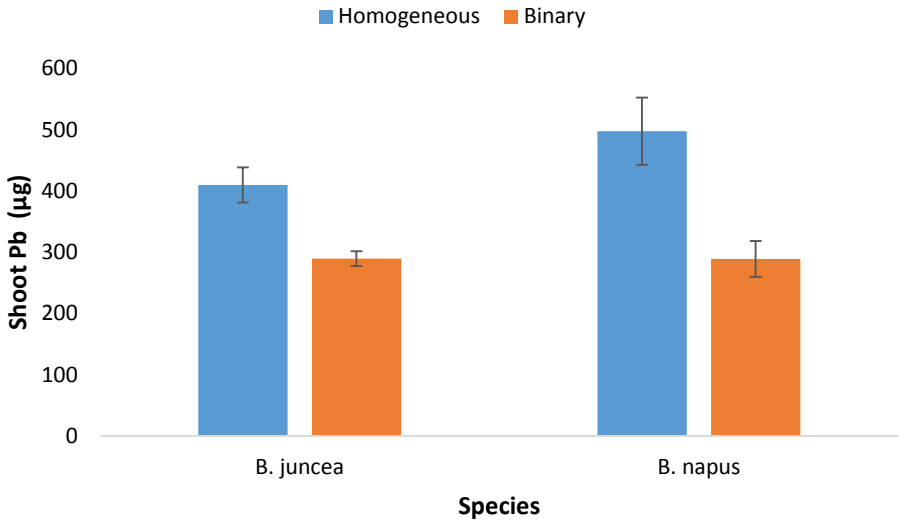


Figure 5.4.14: Mean shoot Pb mass (µg) for *B. juncea* and *B. napus* in homogeneous and binary treatments. Error bars represent 1 standard error on the mean where n=10.

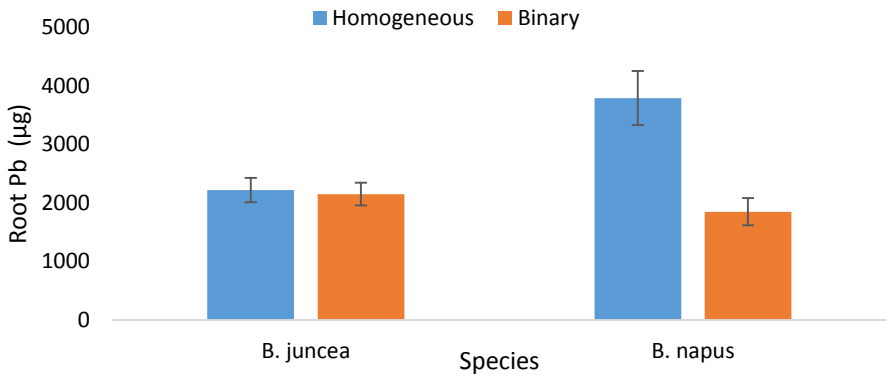


Figure 5.4.15: Mean root Pb mass (µg) for *B. juncea* and *B. napus* in homogeneous and binary treatments. Error bars represent 1 standard error on the mean where n=10.

Root Pb mass of *B. juncea* in the binary treatment was 16% higher than that of *B. napus*, whilst *B. napus* had 70% higher root Pb mass than *B. juncea* in the homogeneous treatment (Figure 5.4.15). The differences in root Pb mass between species in the homogeneous treatment was statistically significant (P=0.009 < 0.05), whilst the root Pb masses of both species in the binary treatment were not significantly different (P = 0.334 > 0.05) (Appendix III.5: CIII.5 and DIII.5). However, the difference in root Pb mass between the homogeneous and the binary was statistically significant (P=0.002 <0.05) for *B. napus*, and was not significant (P = 0.812 > 0.05) for *B. juncea* (Appendix III.5: EIII.5). Root Pb masses in both treatments were ~40 to 60% higher than the shoot Pb mass.

Results of the shoot and root Pb masses of both species in the two treatments show that heterogeneity has a significant impact on plant uptake expressed as Pb mass.

5.5 LEAD BIOAVAILABILITY IN POT TRIAL.

The section presents the results of experiment which determined the extractable Pb of *in situ* soils and growth medium used in pot trials. It introduces the concept of bioavailability of contaminants in soil to plants and the some methods of determination of bioavailable Pb fraction.

5.5.1 Bioavailability experiment.

An experiment to determine the extractable fraction of Pb in the growth medium and soil samples collected from Gang Mine field site was carried out using the modified Tessier method {$MgCl_2$ extraction} (Tessier *et al*., 1979) as described by Li *et al*., (1995).

Experimental objective: To determine the bioavailable fraction of Pb in soil from field sites and in growth medium of pot trials to plants.

5.5.2 Background to experiment.

It is known that a part of the total Pb in soils may be taken up by plants roots and transferred to the shoots. Some plants are more effective in translocation of Pb to shoots than others (Romeiro *et al*., 2006). According to Huang and Cunningham, (1996), the amount of Pb bioavailable to plants depends partially on the concentration of Pb in the soil and growing medium.

Bioavailability is defined as the degree to which chemicals present in the enviromment may be available for interaction with biological systems or ecological receptors such as plants (ISO, 2005). Bioavailability of contaminants is affected by several factors such as temperature, pH, chemical composition, cation exchange capacity and redox conditions (Kabata-Pendias, 2010). Other factors such as speciation or the form of Pb used may also affect bioavailability and uptake of contaminants by plants (Morrey *et al*., 1988). Uptake of Pb from the soil or growing medium varies with different species.

The determination of actual metal bioavailability in soils to plants remains an unresolved problem in most environmental contamination analysis (Anyanwu *et al*., 2008). There is no universally established quantitative method for determining directly the exact fraction bioavailable to plants (Erickson *et al*., 1990; Anyanwu *et al*., 2008). Several chemical extraction methods have been used to determine the bioavailable fraction of metals in

soil. These are indirect methods which gives an estimate of the fractional amount of metals available to plants (Elsokkary and Lag, 1978; Farago and Mehra, 1992).

Chemical extractants can be classified into the following groups: neutral salts dilute solutions of weak or strong acids and complexing agents (Harmsen, 2007). Such chemical extractants include ethylenediaminetetracetic acid (EDTA), diethylenetriamine pentacetic acid (DTPA), ammonium acetate, $NaNO_3$, NH_4NO_3, $CaCl_2$, CH_3COOH and $MgCl_2$ (McGrath, 1996; Brunoi *et al.*, 2004). Helgesen and Larsen (1998) and Menzies *et al*., (2007) reported that, neutral salt extractants such as 0.01 M $CaCl_2$ and 0.1 M $NaNO_3$ provide the most useful indication of metal phytoavailability while Gupta *et al*., (2006), recorded a better correlation (r=0.87) between extractable metals in different amendments of tannery sludge on soil using EDTA compared to $NaNO_3$ and $CaCl_2$ (r=0.53 and 0.60) respectively. Extractants used in single extractions are mainly to evaluate the exchangeable (bioavailable) fractions of elements in the soil (Mossop and Davidson, 2003: Sinha *et al*., 2006).

One of the earliest extraction methods for determining exchangeable or bioavailable fraction of metals in soil is the Tessier procedure (Tessier *et al*., 1979). A short extraction procedure which produced strong correlation data between the extraction method and amount of bioavailable fraction for many metals tested has been used (Maiz *et al*., 2000). The modified EDTA extraction method by the United Kingdom Ministry of Agriculture, Fisheries and Food (1986) has been reported by Anyanwu *et al*., (2008). The method described by Rauret *et al*. (1999) to determine the phytoavailable fraction of Pb, Zn, Cd and Cr in soil has been used by Olayinka *et al*. (2011). Two commonly used and accepted methods for extractable metals by the European Commission are $MgCl_2$ extraction (Tessier *et al*., 1979) and the EDTA (Quevaulier, 1998). Extraction methods used in different studies are summarised in Table 5.5.1.

Table 5.5.1: Summary of different extraction methods used for bioavailable Pb in previous works.

Plant species	Form of Pb in the soil	Total Conc.(mg/kg) in the soil	Extractable conc. (mg/kg)	% Extractable form	Extraction method	Heterogeneity	Source
Amaranthus viridis	Not known	86.59	4.52	5	EDTA	-	Olajire *et al*., 2006
Amaranthus viridis	Not known	86.59	3.54	4	Acetic acid	-	Olajire *et al*., 2006
Celosea argentea, Amaranthus viridis, Corchorus olitorius	Not known	387	21.3	6	Acetic acid	-	Olayinka *et al*., 2011
Oryza sativa	Not known	359	0.06	0.02	$CaCl_2$	-	Kashem *et al*., 2007
Oryza sativa	Not known	359	16.7	5	DPTA	-	Kashem *et al*., 2007
Oryza sativa	Not known	359	70	19	1 M HCl	-	Kashem *et al*., 2007
Food crops and vegetable	Not known	7103.05	1509.65	21	Ammonium EDTA	-	Anyanwu *et al*., 2008
Spinach, celery, garlic and cole	PbO	79.50	10	13	$MgCl_2$	-	Zimmerman and Weidorf *et al*., 2010; Chao *et al*., 2007

5.5.3 Methods.

Magnesium Chloride Extraction

Of the two commonly used and accepted methods ($MgCl_2$ extraction and the EDTA extraction) for extractable metals by the European Commission, $MgCl_2$ extraction was considered appropriate for this study for the following reasons (i) the possibility of exaggeration of the bioavailable Pb by EDTA- a chelating agent, (ii) the high solubility of PbO (the Pb species used to spike growth medium used in pot trials and the dominant Pb speciation at the two sites investigated in Chapter 3) in $MgCl_2$ extraction (Foster and Lott, 1980; Clevenger *et al*., 1991).

One gram of air dried soil sample was placed in 50 ml tube to which 8 ml of 0.5 M $MgCl_2$ was added at a temperature of 22±5ºC. The sample was continuously agitated or shaken for 1 hour on a shaker. The fraction was separated from the supernatant by centrifugation at 10,000 rotation per minutes (approximately 12, 000 gravity) for 30 minutes. The Two commonly used and accepted methods for extractable metals by the European Commission are $MgCl_2$ extraction (Tessier *et al*., 1979) and the EDTA (Quevaulier, 1998). The supernatant was collected, filtered using Whatman filter paper and analysed for Pb using the AAS. One gram of certified reference material (BCR), duplicate samples and reagent blanks were used for quality control (Appendix III.4).

5.5.4 Results of Lead Bioavailability Experiment.

The result of the analysis of growth medium and *in situ* soil samples are presented in Table 5.5.2 below. The result showed that on average, 18% of the growth medium total Pb is available for uptake, whilst 13% is available from the field site. This suggest that taking 13% of the mean measured total Pb concentrations of 5922 and 25212 mg/kg produces potentially bioavailable concentrations of 770 mg/kg for Gang Mine and when applied to Black Rocks 3278 mg/kg. When the percentage extractable is applied to growth medium range of Pb concentrations (100 to 10000 mg/kg), it provides a potentially bioavailable concentration range of 18 to1800 mg/kg.

Table 5.5.2: Summary of growth medium of pot trials and *in situ* soil sample from Gang Mine extractable Pb results.

Sample	Nominal Pb concentration (mg/kg)	Measured Pb concentration (mg/kg)	Extractable Pb (mg/kg)	% Extractable Pb
Gang Mine-C5	N/A	4020	507	13
Gang Mine-G6	N/A	11120	1571	14
Gang Mine-J8	N/A	8085	995	12
Gang Mine-I1	N/A	3700	489	13
			Mean	13
			STDEV	0.8
			SEM	0.4
Sample	**Nominal Pb concentration (mg/kg)**	**Measured Pb concentration (mg/kg)**	**Mean extractable Pb (mg/kg)**	**% Extractable Pb**
Growth medium	100	105	17	16
	300	366	74	20
	1000	956	174	18
	1000	1012	189	19
	3000	2943	475	16
			Mean	18
			STDEV	1.7
			SEM	0.8

Key: Gang Mine -C5; Gang Mine-G6; Gang Mine-J8; Gang-Mine-I1 represent soil samples from duplicate cores at sample locations C5, G6, J8 & I1 of Gang Mine field site.

5.6 DISCUSSION

Biomass results of *B*. *juncea* in this study is in line with previous works with Zn and Cd (e.g Millis *et al*., (2004); Podar *et al*., (2004); Manciulea and Ramsey, (2006); Menon *et al*., (2007); Moradi *et al*., (2009); Thomas, (2010) and support the findings of higher biomass and lower metal uptake in the binary treatment compared to the homogeneous treatment. However, lower biomass and lower Pb uptake expressed as concentration (mg/kg) and Pb mass (µg) was observed in *B. napus* in the binary treatment compared to the homogeneous treatment. *B*rassica *napus* grew better (26% higher biomass) in the homogeneous treatment than in the binary, whilst *Brassica juncea* had better growth in the heterogeneous (binary) treatment than in the homogeneous treatment. This contrasting behaviour of these two plant species in simplistic spatial heterogeneity is an indication that their responses to heterogeneity of Pb is species-specific.

The species-specific behaviour can also be seen in the effect of Pb on shoot, root and total biomass DW in binary and homogeneous treatments. For example, a more severe effect of the added Pb (visible severe chlorosis and wilting of leaves) was seen in the binary treatment of *B. napus* than that of *B. juncea*. This was also observed in the decreases (43% and 26%) in total dry biomass of *B. napus* in the binary compared to the control and homogeneous treatments respectively. Whereas, *B. juncea* had significantly ($p<0.05$) higher biomass (41%) in the binary treatment than in the homogeneous treatment. As earlier mentioned in this chapter, *B*. *juncea* biomass and uptake result is in line with earlier studies on the variation in dry biomass and metal uptake between different plant species in response to simplistic spatial heterogeneity of zinc (Thomas, 2010). Nabulo *et al*., (2008) reported variation in dry biomass to a similar extent between plant species in response to different treatments with Zn and Cd in a pot trial. Variation in Cd uptake to a lesser extent between some varieties of lettuce has been reported (Millis *et al*., 2004).

The root-shoot biomass ratio provides useful information on how these plants allocate carbon and resources to the above and below ground parts in the presence of contaminants in the soil. This has impact on the uptake of contaminants and nutrients in the soil. The root biomass ratios in this study showed that 80% higher biomass was allocated to the above ground part, compared to the root in both plant species. This is in line with studies by Mokany *et al*., (2006) which suggest that root biomass can influence plants uptake potential. Decreased root-shoot biomass ratio in the homogeneous and binary treatments of *B*. *juncea* and *B*. *napus*, when compared to the control is an indication of the effect of the Pb added treatment on the plants. It also suggest that roots

were decreased in response to the spatial distribution of Pb in the growth medium as *Brassica juncea* had 15 to 38% decrease in root-biomass ratio in the homogeneous, compared to the control and binary treatments. The effect of the varied Pb distribution on root-shoot biomass ratio was more pronounced in *B*. *juncea* than in *B*. *napus*, with 11 to 30% higher root-shoot biomass ratio recorded for *B*. *napus* in control, homogeneous and binary treatments. However, *B*. *napus* had same root-shoot biomass ratio in the homogeneous and binary treatments. This indicated that both plant species have specific adaptation and variation in growth pattern in response to Pb heterogeneity. There was no statistically significant difference in the root-shoot biomass ratio between treatments of *B*. *napus*. It also suggest that *B*. *napus* tends to ignore heterogeneity in allocation of biomass and resources in the presence of Pb and its spatial distribution in the soil.

A similar pattern of Pb uptake expressed as Pb concentration (mg/kg) was observed in both plants. *Brassica napus* and *B*. *juncea* had higher total plant Pb (316 and 227 mg/kg DW) in the homogeneous treatment, compared to the binary (199 and 161 mg/kg DW) respectively. This showed that that the simplistic binary treatment had lower Pb uptake (by 59 and 40%, respectively). Previous studies (Millis *et al*., 2004; Thomas, 2010), stated earlier in this section, had also observed lower contaminant concentrations in simplistic models when compared to the homogeneous patterns. *Brassica juncea* had 22% decreased uptake in the homogeneous treatment when compared to *B. napus*. These two plant species accumulated Pb to a different extent in the heterogeneous treatment when compared to the homogeneous treatment and also affected to a differing extent in the binary treatment. Kabata-Pendias and Pendias, (2001); Audet and Charcrest, (2007) reported a great deal of variation in the degree to which different plant species can accumulate different heavy metals from the soil.

Elevated Pb uptake expressed as Pb mass (µg) (twice higher) was observed in the homogeneous and binary treatments of both species, when compared to uptake expressed as concentration (mg/kg). Results also suggest that *B*. *napus* would accumulate more Pb in shoots and roots in the homogeneous treatment than *B*. *juncea*, whilst *B*. *juncea* has the tendency of accumulating more Pb in the root in binary treatment than *B*. *napus* judging from their shoot and root Pb masses. It also supports the fact that response of these plant species to simplistic heterogeneity is species-specific, which may be influenced by individual plant adaptation and tolerance to Pb in the soil. The differences in Pb masses between treatments also suggest that heterogeneity of Pb in the soil have a significant effect on plant uptake expressed as Pb mass, which could influence their choices for use in phytoremediation. This also provided an insight into how metal uptake can be enhanced in plants for phytoremediation by exploring the

uptake strength of homogeneous and heterogeneous treatments (discussed further in chapter 7).

Neither species are hyperaccumulators of Pb as judged by the observed total concentration factor (CF_{total}) (0.10 to 0.32), translocation factor (TF) (0.01 to 0.04) and the shoot concentration factor (CF_{shoot}) (0.01-0.09). Criteria for classifying plants into accumulators and hyperaccumulators has been discussed in Chapter 2. However, plants with Pb concentration greater than 1000 mg/kg are also classified as hyperaccumulators. The low CF_{shoot} recorded for both species is an indication that much of the Pb is excluded from the shoot in the homogeneous and binary treatments by both species. It implies that less Pb will be accumulated in their shoot. However, the amount accumulated in the shoot could be influenced by the soil Pb concentration and the bioavailable pool. Low shoot accumulation might have possible advantage in a way to consumers of leafy part of these plant species, if the concentration accumulated do not exceed Pb limit in vegetables. However, shoot Pb may be dependent on the soil Pb concentrations, soil characteristics and individual plant translocation mechanisms.

The shoot Pb concentrations of both species in the homogeneous and binary treatments were 39 to 81% lower than the experimentally determined extractable Pb, when compared to the predicted bioavailability of 18% (Section 5.5). The root Pb concentrations were 6-7 fold higher, when compared to the experimentally determined bioavailable concentration. This suggest that other factors which increase the mobility and uptake by roots might have influenced the Pb accumulation in the root other than the bioavailable pool. Such factors include pH, soil microorganisms, root exudates and plants mechanisms for coping with heavy metal stress and delocalisation of heavy metals in plant cells and tissues (discussed in detail in Chapter 2).

Higher proportion of roots were preferentially proliferated in 0 mg/kg Pb added patches (~70 mm and 33 mm) than in the 2000 mg/kg Pb added (17 mm and 9.5 mm) respectively, as shown by the root ball diameter for *B. napus and B. juncea* (Figures 5.4.6 and 5.4.7). The root biomass for the different quadrants were not taken, but this was an improvement implemented in the subsequent fourth pot trial. A significant difference between these quadrants was recorded for *B.napus* and similarly for *B.juncea* ($P < 0.05$). The roots therefore effectively 'avoided' the Pb. This result is in line with similar observation by Millis *et al*., (2004) of higher root proliferation in patches of lower concentration of another toxic element Cd, in pot trial. Results here also indicated that

responses to heterogeneity might be due to the nature, morphology and size of the root ball. A central tap root (Figure 5.3.8) was observed in *B*. *napus* variety used in this study, but was absent in *B*. *juncea*. The *Brassica juncea* variety used had several branched fibrous root networks.

A further experiment simulating a more realistic heterogeneity model (Chapter 6) confirmed this finding. It is highly unlikely that contaminant spatial heterogeneity in the field will have this simplistic distribution. The pot trial is just a way to try to understand mechanisms at work rather than being realistic for the real soil environment.

Similarly, earlier studies by Thomas, (2010) suggest possible root proliferation in response to patchy distribution of Zn in a pot trial. Results indicated that the variation in the response of these plant species to the different treatments might be due to the different pattern of root allocation to resources and contaminants. However, it was opposed to the foraging habit observed for Zn in *Thlaspi carulescens* in previous studies by Haines (2002).

Results of this experiment suggest that *B. napus* would be more sensitive to spatial heterogeneity than *B. juncea* and that *Brassica juncea* will therefore grow better than *B. napus* in soil that is heavily contaminated with Pb (i.e. > 1000 mg/kg) in a heterogeneous way. The reason for this sensitivity to spatial heterogeneity in *B*. *napus* is not known. However, it could be could be partially attributed to its root morphology and size.

Other factors might have influenced the different response of this species to treatments compared to *B*. *juncea* in this study and in earlier work with Zn. For example, variation in genetic, physiological or biochemical adaptations of plants to different contaminants might have influenced this plant response to Pb heterogeneity. Macnair and Baker, (1994); Guefarchi *et al*., (2013); Park and Ahn, (2014); Kumagai *et al*., (2014) suggest that genetic, physiological and biochemical adaptations of different plant species could influence uptake, tolerance, response to contaminants in the soil. Other factors that could produce elemental variability or variation in plant response to contaminants in soil such as transportation and deposition of contaminants within plant tissues, developmental stages, seasonal variation and differences in microclimatic/micro edaphic conditions has been reported by Farago and Mehra (1994); Lasat *et al*., (1996) Prado *et al*., (2010); Thomas, (2010).

Findings of this experiment provided an insight to the important role of spatial distribution of contaminants in metal uptake from the soil by plants, tolerance to contaminants in soil and growth and development in plants.

5.6.1 Interpretation of results in relation to stated hypotheses.

Hypothesis (1) is accepted that the simplistic binary design of Pb heterogeneity has a significant impact on (a) plant uptake of Pb and (b) plant biomass when compared to a homogeneous design (and in one case against a control treatment). Hypothesis (2) is accepted that roots of plants will preferentially proliferate in patches with no added Pb in the heterogeneous design, presumably to avoid the toxicity of the added Pb. The hypothesis tests are summarized in Table 5.6.1.

Table 5.6.1: Summary hypothesis for each species based on Tukey H.S.D comparison of means, Analysis of variance (ANOVA) and Independent sample t-test where P < 0.05.

Hypothesis	*B. juncea*	*B. napus*
1 (a) Plant Pb	Accept	Accept
(b) Biomass	Accept	Accept
2 Root proliferation	Accept	Accept

This study also showed that a simplistic binary heterogeneity model had a significant effect on plant uptake in comparison to homogeneous distribution.

In nature, contaminants are rarely distributed in either homogeneous or simplistic binary model (as shown in Chapter 3), therefore a more realistic simulation of *in situ* heterogeneity was carried out to assess the effect of more realistic patterns of heterogeneity of Pb on plant uptake in pot trials. This is described in detail in Chapter 6.

Chapter 6 – Experimental assessment of the effect of variable lead heterogeneity on lead uptake and biomass of two *Brassica* species.

6.0 INTRODUCTION

This chapter describes the design of the pot trial to simulate a more realistic *in situ* heterogeneity of Pb based upon that measured in the field investigation (Chapter 3) at the scale characteristic of the selected plant species (i.e. 2 cm). It discusses the background to the experiment and the details of experimental design, methods and results.

This fourth pot trial was based on simulations of *in situ* Pb heterogeneity in a range of treatments. It was used to assess the effect of changing Pb heterogeneity on biomass (shoot and root), uptake of Pb and root placement of the two selected plant species (*Brassica napus* PI 601261 and *Brassica juncea* PI 182921.

6.1.1 Objectives of experiment.

i. To design a pot trial to mimic the range of *in situ* Pb heterogeneity found in the field.

ii. To assess whether the variation in spatial heterogeneity of Pb in soil has a significant impact on Pb uptake by these plant species, and their biomass.

6.1.2 Hypotheses

1. The degree of spatial heterogeneity of Pb in the growth medium have an impact on (a) the extent of uptake of Pb (b) the plant biomass, by each species (*B. juncea* and *B. napus*.) between treatments.

2. Each effect differs between various levels of heterogeneity (e.g. low, medium and high heterogeneities) compared with homogeneous treatment with the same overall concentration of Pb.

3. These plant species respond to changing heterogeneity using modified root placement.

4. Responses to changing heterogeneity differ between *Brassica juncea* and *Brassica napus*.

6.2 BACKGROUND TO THE EXPERIMENT

In situ heterogeneity of Pb in soil was estimated over a range of scales from two site investigations using *in situ* measurement techniques and the specific sampling design proposed by Thomas *et al*., (2008). The heterogeneity of these two sites (reported in chapter 3: Section 3.3) was compared with those of other previously studied sites by other workers (Chapter 2 Section 2.6.2 and published in Ramsey *et al*., 2013) upon which the range of heterogeneity in this pot trial was simulated. The degree of heterogeneity was expressed as a heterogeneity factor (HF), where a homogeneous distribution would result in a HF factor of 1 and heterogeneous distribution in a value of HF > 1 (Chapter 3: Section 3.3).

Lead concentrations were measured at all sampling locations and results presented in Chapter 3: Table 3.2 shows how the spatial heterogeneity of Pb expressed as HF varied between sites and scales. Heterogeneity values used in this pot trial reflect the scale of heterogeneity that can be potentially seen by the selected plant species in the volume of soil contained in the pot. *In situ* heterogeneity of Pb at the 2 cm scale was chosen for the purpose of this experiment. Earlier work by Thomas, (2010) with zinc heterogeneity also used the 0.02 m (i.e. 2 cm) scale that can be replicated within a pot trial. Similarly, Manciulea and Ramsey, 2006 used a scale of 0.03 m (3 cm) with the simplistic chequer board models and showed that changes in heterogeneity of Cd can have a significant effect (+76%) on plant uptake (discussed in more detail in Chapter 2; Section 2.6).

6.3 EXPERIMENTAL DESIGN

Four heterogeneity models were simulated here (using excel computer models with a combination of the Robust ANOVA- a visual basic programme developed based on a FORTRAN programme {(Ramsey, 1998} and previous work {AMC, 1989}), which generated the levels of heterogeneity similar to those that had been found in field sites and previous field studies. The scale of heterogeneity used, the plant species selected, and the mean Pb concentration chosen, were based upon field experiment and conclusions of earlier pot trials in this thesis (Chapters 3, 4 and 5).

The sample size was determined using power analysis to estimate the minimum number of replicates required to detect a statistically significant difference between means of different treatments based on the assumption that data are normal in their distribution (Zar, 1999). Data from the third pot trial used for power analysis were confirmed normally distributed using the Kolmogorov Smirnov test (Appendix III.2: Table LIII.2). The power analysis was done as described by Zar, 1999 and Thomas, 2010. Power analysis used values for the variances of shoot Pb concentration (mg/kg) taken from the third pot trial. Mean shoot pooled standard deviation of 117 mg/kg and population mean difference of 12 mg/kg were used. The estimated minimum number of replicates at 95 % confidence level and at 90 % probability of detecting a difference in population mean was 6.3. Using these data, a maximum number of 10 replicates per treatment (allowing for 20% failure rate and a chance of detecting smaller differences than the number from power analysis) was used. Four treatments – homogeneous (HO), low (LH), medium (MH) and high heterogeneity {HH}) for each species making a total of 80 pots were maintained in randomized block design (Figure 6.3.2; Appendix IV.1: Table AIV.1).

It is impossible to simulate the exact *in situ* heterogeneity (real life situation). The actual spatial heterogeneity of contaminants can only be estimated by sampling at the field site, and it is practically impossible to recreate the exact *in situ* heterogeneity in pot trials. In view of this potential complexity, the model of heterogeneity was designed to simulate as closely as practicably possible the *in situ* heterogeneity of Pb measured at this scale in field sites (Chapter 3: Table 3.4.1) including some previously studied sites by other workers (Chapter 3: Table 3.4.2) with a range of intermediate HF (HF ranged from 1 to 3.22 (3.22 at the 20 m scale). The simulated heterogeneity factors (HF) were 1.00, 1.25, 2.00 and 3.19 while an overall mean concentration of approximately 1000 mg/kg in all treatments was maintained (Figure 6.3.1a-d). The simulation is based on the log-normal distribution observed in the field sites, with increasing values of geometric standard deviation (GSD) and hence the values of HF. The central cell (C3) of all treatments was

also maintained at 1000 mg/kg Pb. This is to ensure that the heterogeneity treatment did not differentially affect the early establishment of the seedlings. The amount of Pb required to make each concentration was calculated as shown in Appendix IV.10: Table BIV.10).

Cells	1	2	3	4	5
A	1000	1000	1000	1000	1000
B	1000	1000	1000	1000	1000
C	1000	1000	1000	1000	1000
D	1000	1000	1000	1000	1000
E	1000	1000	1000	1000	1000

Figure 6.3.1a: Homogeneous---GSD 0.0; robust mean=1000; HF=1.00

Cells	1	2	3	4	5
A	900	700	900	1100	900
B	1100	1100	1400	1400	1400
C	1100	700	1000	900	900
D	1100	900	1100	1800	900
E	900	1100	900	1100	700

Figure 6.3.1b: Low heterogeneity--GSD 0.1 Robust mean =1029; HF=1.2

Cells	1	2	3	4	5
A	500	300	500	1100	500
B	1100	1100	2200	2200	2200
C	1100	300	1000	500	500
D	1100	500	1100	4000	500
E	500	1100	500	1100	300

Figure 6.3.1c: Medium heterogeneity-- GSD=0.30; robust mean=962; HF=1.99.

Cells	1	2	3	4	5
A	300	100	300	1000	300
B	1000	1000	3000	3000	3000
C	1000	100	1000	300	300
D	1000	300	1000	10000	300
E	300	1000	300	1000	100

Figure 6.3.1d: High heterogeneity---GSD=0.50; robust mean=947; HF=3.19

Figure 6.3.1: Four models of *in situ* heterogeneity for 4th pot trial.

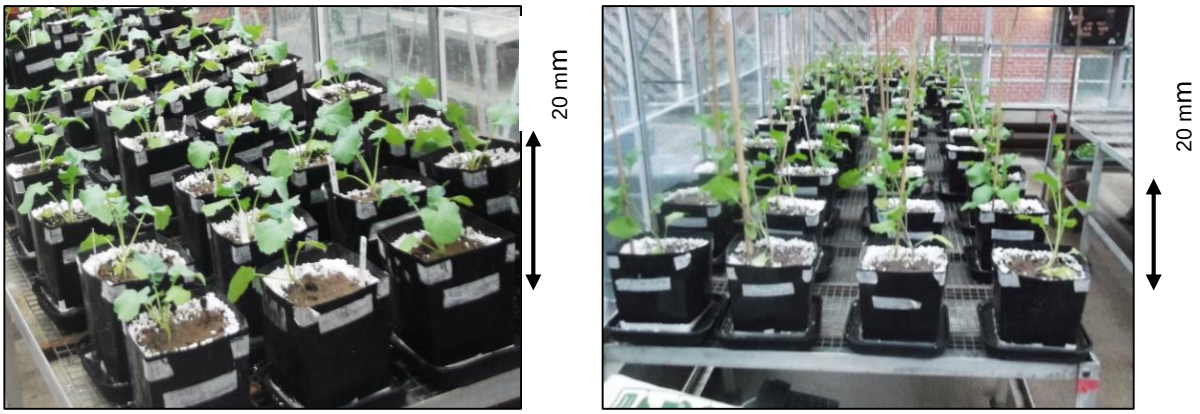
6.3.1 Methods

Eighty (80) rigid square pots (14 X14 cm and 17 cm deep) were thoroughly washed with detergents and labelled with names of plant species (BN & BJ) and four treatments e.g. Homogeneous (BNHO; BJHO), low heterogeneity (BNLH; BJLH), medium heterogeneity (BNMH; BJMH), high heterogeneity (BNHH; BJHH) (Appendix IV.1).

The method used in the pot trial was based on the understanding from previous work by Thomas, (2010) with modifications to certain stages of the experiment, dimensions of pots, pot trial equipment (Appendix IV.1: Figure AIV.1) and identity of the contaminant. A customized cell divider made from a 1 mm clear polyethylene terepthalate glycol (PTEG) sheet was inserted into the pots to produce a 5 by 5, 2-dimensional grid with each cell measuring 25 mm square and 170 mm deep. This was used to create the designed heterogeneity models. The relatively thin PETG helped to maintain the heterogeneity design by reducing the collapse of each column after its removal. Labelled paper liners were inserted into each cell while filling cells with growth media. It provided a filling template, helped maintain the structural integrity of the divider and minimized spillage from adjacent cells.

The gap between the paper liners and the outer edge of the pot were packed with an inert Sinclair Perlite (grain size 2.0-5.0 mm) because of the non-vertical sides of pots. Cells were filled according to the particular designed model of heterogeneity. Filling of the pots (Appendix IV.1: Figure AIV.1) was done in two stages to ensure that equal volume of growth medium goes into the cells and that the growth medium is evenly distributed throughout the pot. The gently compacted growth medium was measured with a 100 ml customized container into each cell according to the design. The growth medium was tapped down before an additional 50 ml was added and tapped down again.

Completed pots were placed on drip trays and arranged on benches in the randomized block design (Figure 6.3.2 and Appendix IV.1: Table AIV.1) with blocks of 4 rows and 10 columns.



(a) (b)

Figure 6.3.2: Plants arranged in randomized block design showing (a) *B. napus* (Scale bar: 15 mm represents 20 mm) and (b) *B. juncea* (Scale bar: 9 mm represent 20 mm). Arrows represent scale bars.

The growth medium was moistened from below by capillary action before transplanting seedlings already established in an unspiked growth media for two weeks. Tap water was applied using a fine rose watering can. This ensured that the heterogeneity was disturbed to a minimal extent.

Seed germination, preparation of growth medium, transplanting of seedlings and harvesting were done as described in earlier experiments (Chapter 4: Section 4.3.3). The percentage moisture content of the growth medium was originally 8.5%. The pH of the growth media and field site (Gang Mine) soil were determined using pH meter {model: Hanna 209} (Appendix 6IV.10: Tables GIV.10 and HIV.10). Growth medium mean pH in pot trials was 6.44 ±0.05 whilst that of field site (Gang Mine) soil was 6.32 ± 0.12.The established seedlings of the selected plant species *Brassica napus* (BN SW) and *Brassica juncea* (BJ 18) were transplanted into the centre of each treatment after two weeks growing in the unspiked growth media. Ten replicates of each treatment were maintained in the greenhouse for six weeks under simulated sunlight using light-emitting diodes (LED) lights (under a photoperiod of 12 hours) at 20 ±5 C.

6.3.2 Harvesting.

Harvesting was done after 60 days of growth. Data such as the longest leaf length, number of true leaves and height (to the nearest 1 mm) were collected after 14, 28 days and at harvest, to assess growth variation between treatments (Appendix IV.7). Plant biomass data such as root and shoot biomass (FW and DW) in all pots in homogeneous, low and medium, heterogeneity treatments were collected at harvest to assess the impact of heterogeneity on the plant species. Shoots of all treatments were harvested as described in earlier experiments (Chapter 4: Section 4.3.7). However, for the high

heterogeneity treatments, the roots in each cell were harvested as described below, the biomass measured, and used to test the root placement hypothesis (Section 6.3. 3).

6.3.3 Chemical Analysis.

Roots were carefully washed to remove soil particles that could introduce potential bias in measurements of metal concentration. Harvested roots and shoots were dried at 60ºC for 48 hours in a fan oven, weighed for DW, and analysed for Pb concentration using an Atomic Absorption Spectrometer (AAS) after acid digestion using nitric and perchloric acids (Thompson and Walsh, 1983). Thompson and Walsh (1983) reported that a biomass of 1 gram (DW) was ideal for chemical analysis, but did not preclude the use of smaller masses, with suitable checks on data quality. In this study, an analytical test portion of 0.5 g was used, but for roots within single cells of some pots, a mass of 0.2 g was used generally, and 0.1 g exceptionally, but always with matching analytical quality control (Appendix 6IV.11:Tables AIV.11 to GIV.11).

The growth medium was also analysed for their actual Pb concentration and result is presented in Table 6.3.1 below. This analysis did not show any significant difference between the nominal and actual soil Pb concentration. The regression analysis showed a strong positive relationship (r^2=0.99) between the actual and nominal soil Pb concentration and the regression model accounted for 99% of variance (Appendix IV.10: Figure AIV.10).

Table 6.3.1: Growth media actual Pb concentration for pot trial four.

Nominal soil Pb concentration	Actual Pb soil concentration	STDEV	SEM
100	105	7.01	2.48
300	366	60.60	27.05
500	534	51.06	22.79
700	681	52.90	21.59
900	837	21.12	9.03
1000	956	30.46	12.43
1100	1198	45.90	19.61
1400	1408	157.67	70.39
1800	1782	116.83	47.69
2200	2229	241.83	98.70
3000	2943	225.34	91.97
4000	4072	192.93	86.13
10000	9670	495.82	221.35

Key: STDEV- Standard deviation
SEM—Standard error on the mean

6.3.4 Data analysis.

Data were analysed using statistical software Minitab 16 and SPSS 21 for Windows. Statistical tools such as analysis of variance (ANOVA), Tukey post-hoc test and the mixed model ANOVA (treatment used as fixed factor and block as a random factor) were used to test for significance of measured variables (Appendices IV.2 to IV.6) whilst Kolmogorov-Smirnov test was used to test for normal distribution of data (Appendix IV.9: Tables AIV.9 and BIV.9). Raw data of measured variables are presented in the Appendix (Appendix IV.8: Tables AIV.8 and BIV.8).

6.3.5 Root Placement sub-experiment.

The high heterogeneity treatment was used for this experiment, to represent the extreme case of heterogeneity. The roots were extracted by placing each of the 40 cubes of growing medium in a wooden box. The customized sleeve was removed. The cube was held securely in position with a holding block (Appendix IV.1: Figure AIV.1). A customized blade was used to divide growth medium into the 25 original individual cells, using measured grooves on top of the holding block. Roots were harvested from each cell and the dry biomass recorded. Root extraction device and pot divider were constructed as described in earlier work on zinc heterogeneity by Thomas, (2010) with slight modifications to dimensions of the pot equipment and methods as stated earlier on in this chapter.

For the high heterogeneity treatment, harvested roots were weighed and those with equal nominal soil concentration from each pot were combined and analysed for Pb. The recorded mass of root biomass were subsequently combined (mathematically) for the measurement of total root biomass in each pot. The raw measurements were used to assess root distribution in response to heterogeneity.

6.4 RESULTS.

Both species (*B. juncea and B. napus*) had 100% survival rate until harvest. Sufficient biomass suitable for individual analysis of the shoots and roots were produced. Visible signs of chlorosis such as loss of green colouration were observed in both species. Curly thinner stems, random turning of stem clockwise or anticlockwise to corners of pots and reduced leaf area were observed in *B. juncea* in low, medium and high heterogeneity treatments with severity in the HH treatment (Figure 6.4.4). *Brassica napus* had broader leaves and sturdier stem than *B. juncea* in all treatments (Figure 6.4.3). The result for each plant *species* is discussed separately for each variable. Condition of plants at harvest is shown in Figures 6.4.1 and 6.4.2.

Figure 6.4.1: *B. napus* in order of increasing heterogeneity (HO, LH, MH & HH) {scale bar- ~8 mm represents 10 mm; Scale bar: 8 mm represents 10 mm; Scale bar: 6 mm represents 10 mm; Scale bar: 12 mm represents 10 mm for HO, LH, MH and HH respectively} from left to right generally showing decreased in biomass except with increasing heterogeneity, except for the MH treatment.

Figure 6.4.2: *B. Juncea* in HO, LH, MH & HH treatments from left to right (Scale bar: 42 mm represents 500 mm; Scale bar: 48 mm represents 800 mm; Scale bar: 49 mm represents 900 mm; Scale bar: 51 mm represents 1000 mm for HO, LH, MH and HH respectively) showing narrower leaves, slimmer stem in LH, MH & HH (decreased biomass) compared to HO.

Key: Black arrows represent scale bars for each Figure and scale bar information highlighted in red.

6.4.1 Biomass results of *Brassica napus*

Biomass generally decreased with increasing heterogeneity (Figures 6.4.3-6.4.5) with a 4.72 fold decrease in total dry biomass of *B. napus* in HH when compared to the HO treatment. However, the peak biomass was in the MH among heterogeneous treatments which had significantly higher total dry biomass (2 and 4) fold higher, when compared to LH and HH treatments respectively. This trend was similarly recorded for both shoots and roots. The highest total biomass (mean 9.7 g) was produced in the homogeneous treatments when compared to all the spatially heterogeneous treatments. This difference was statistically significant ($F_{3, 36}$ = 687.40: P = 0.000 < 0.05). ANOVA and Tukey HSD test showed a statistically significant difference between the 3 heterogeneity treatments (Appendix IV.2). The overall differences between treatments was also statistically significant ($F_{3, 36}$ = 917.05; P=0.000 P < 0.05). This is an indication that the degree of spatial heterogeneity of Pb had an impact on the biomass of *B. napus*. The mixed model ANOVA (Appendix IV.3: AIV.3 and Table BIV.3) showed statistically significant effect P < 0.05 of spatially heterogeneous Pb treatments on plant biomass.

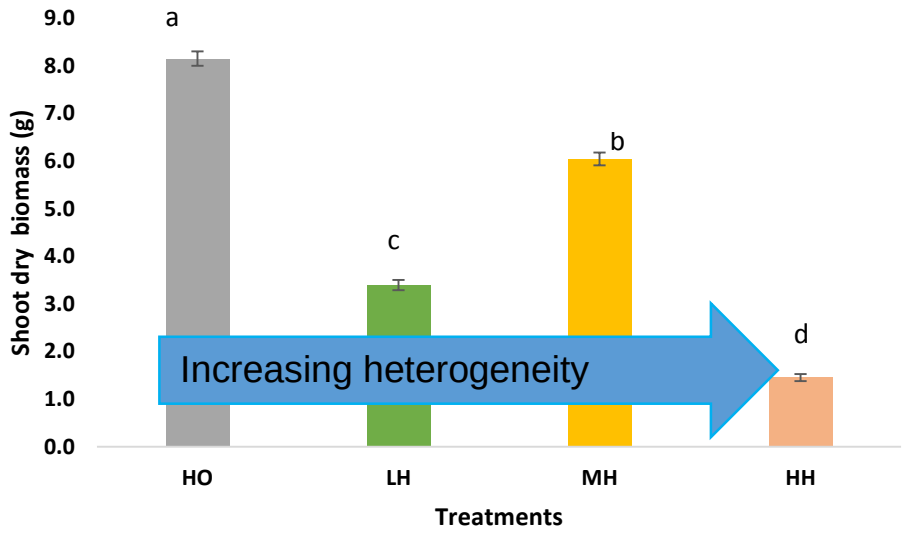


Figure 6.4.3: Shoot biomass (DW) between treatments of *B. napus*. Means that do not share the same letter are significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean, n=10.

HO---Homogeneous (grey), LH-----Low heterogeneity (green), MH----Medium heterogeneity (yellow), HH—high heterogeneity (peach) for all captions in this section.

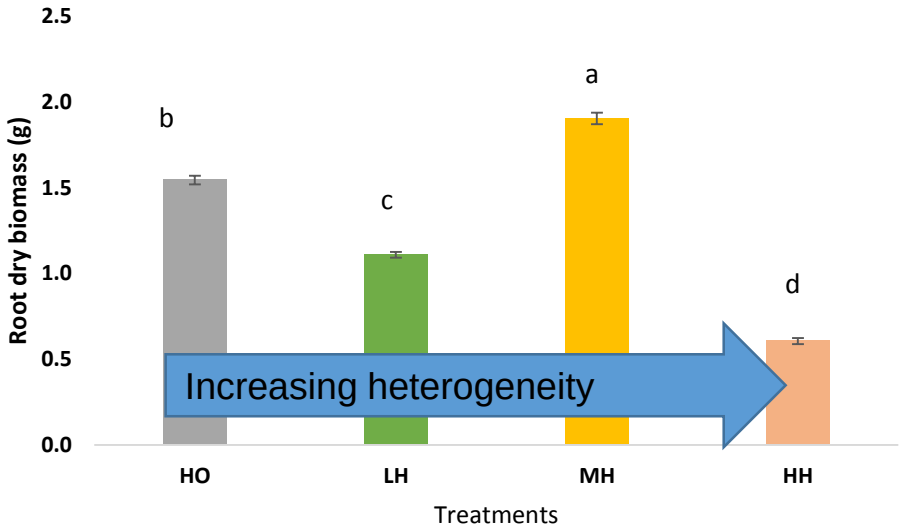


Figure 6.4.4: Root biomass (DW) between treatments of *B. napus*. Means that do not share the same letter are significantly different as judged by the Turkey Post hoc test. Error bars represent 1 standard error on the mean, n=10.

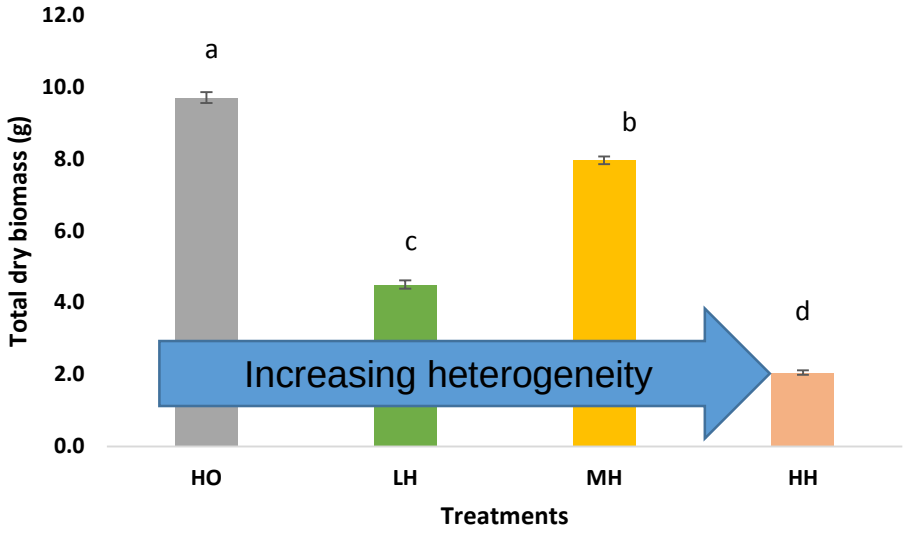


Figure 6.4.5: Total plant biomass (DW) between treatments of B. napus. Means that do not share the same letter are significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean, n=10.

6.4.2 Biomass result for *Brassica juncea*.

Similarly for *B. juncea*, biomass decreased with increasing heterogeneity with a 3.95 fold decrease in total dry biomass in the HH treatment than the HO treatment (Figures 6.4.6-6.4.8). Shoots and roots in the HH had 4.50 and 2.51 lower when compared to the HO treatment respectively.

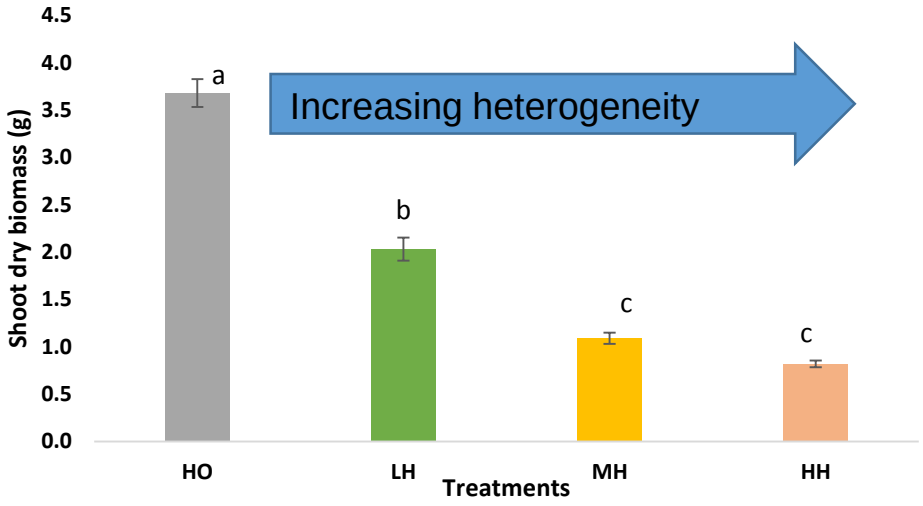


Figure 6.4.6: Shoot biomass (DW) between treatments of *B. juncea*. Means that share a letter are not significantly different as judged by the Turkey Post hoc test. Error bars represent 1 standard error on the mean n=10.

HO---Homogeneous (grey), LH-----Low heterogeneity (green), MH----Medium heterogeneity (yellow), HH—high heterogeneity (peach) for all captions in this section.

The LH treatment had significantly higher total dry biomass (1.66 and 2.34 fold higher) than the MH and HH treatments respectively. This was similarly recorded for shoots and roots (Figures 6.4.6-6.4.8). The HO treatment had an overall highest biomass (4.45 g).

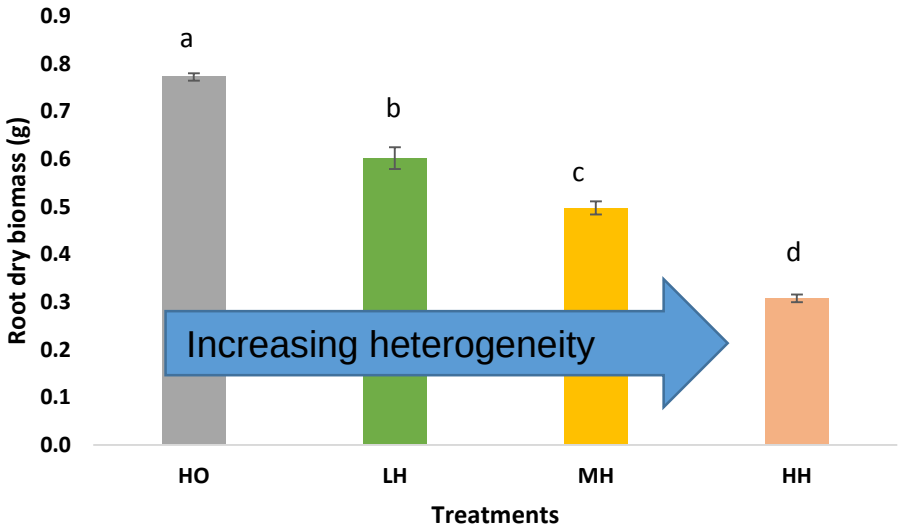


Figure 6.4.7: Root biomass (DW) between treatments of *B. juncea*. Means that do not share the same letter are significantly different as judged by the Turkey Post hoc test. Error bars represent 1 standard error on the mean, n=10.

The differences between treatments were statistically significant (F = 202: P=0.000 < 0.05). ANOVA and Tukey HSD test also showed a statistically significant difference in shoot, root and total dry biomass between the 3 levels of heterogeneity (Appendix IV.4). However, the difference in shoot dry biomass between the MH and HH treatments was not statistically significant. The mixed model ANOVA showed a statistically significant impact (P = 0.000 < 0.05) of spatially heterogeneous Pb treatments on plant biomass. This confirmed that the degree of spatial heterogeneity of Pb had an impact on the biomass of *B. juncea*.

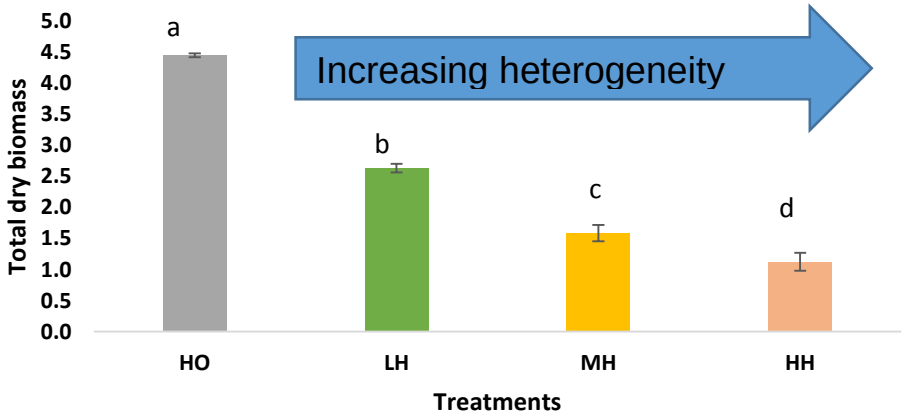


Figure 6.4.8: Total biomass (DW) between treatments of *B. juncea*. Means that do not share the same letter are significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean n=10.

6.4.3 Pb uptake results.

Shoot and root uptake for both plant species are presented and discussed in terms of both Pb concentration in mg/kg and Pb mass per plant in µg (Pb mass (µg) used in phytoremediation studies e.g Husnain *et al*., 2013). Both methods of quantifying uptake showed varied effects on the plant from different perspectives. Lead concentration (in mg/kg) is often used in the estimation of human exposure to Pb and concentration of contaminant in herbage and soil, while the mass concentration (in µg), which compensates for simultaneous changes in both biomass and concentrations, finds useful application in estimating uptake for phytoremediation purposes. Both were used in this research because of the potential implications of this study for both human risk assessment and phytoremediation of Pb contaminated land.

6.4.4 Pb uptake results for *B. napus* expressed as Pb concentration (mg/kg).

The shoot and root Pb concentration in mg/kg increased with increasing heterogeneity with a peak uptake in the LH treatment, 2 fold higher than the HO treatment for roots (Figures 6.4.9 to 6.4.10). Shoot Pb concentration in the HH treatment decreased by 30% when compared to the HO treatment. A similar trend was observed in the root Pb concentration mg/kg with 40% lower root Pb in the HH treatment when compared to the HO.

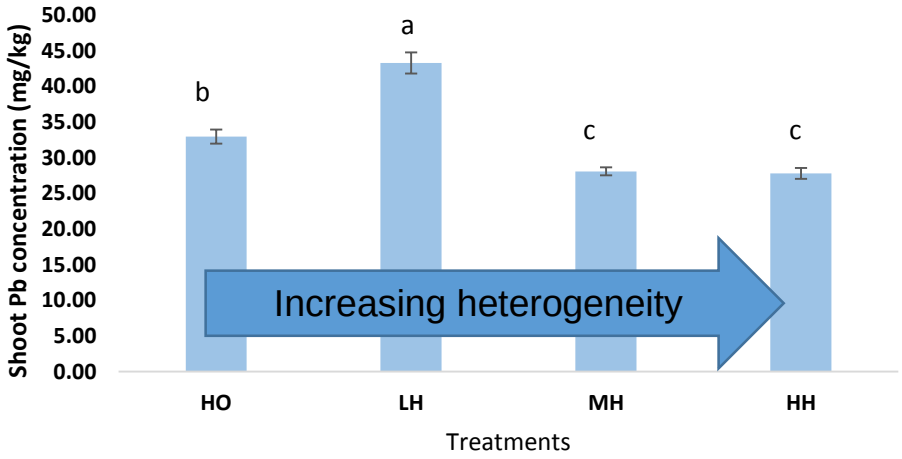


Figure 6.4.9: Shoot Pb concentration (mg/kg) between treatments of *B. napus*. Means that share the same letter are not significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean n=10.

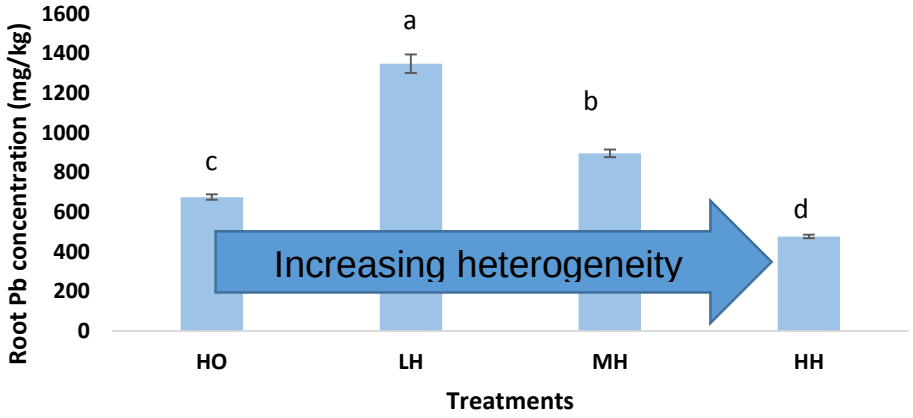


Figure 6.4.10: Root Pb concentration (mg/kg) between treatments of *B. napus*. Means that do not share letter are significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean n=10.

6.4.4.1 Uptake expressed as Pb mass (µg) for *B. napus*.

The shoot and root Pb mass µg had 40-80% decrease in the HH treatment when compared to the HO (Figures 6.4.11-6.4.12).These two approaches showed differences in peak uptake. For example, the root Pb mass had peak uptake in the MH treatment which was 40% higher, compared to the HO treatment while shoot and root Pb concentration in mg/kg had its peak uptake in the LH treatment, 2 fold higher than the HO.

However, the shoot Pb concentrations mg/kg of MH and HH treatments were not significantly different as judged by the Tukey HSD comparison. Shoot peak uptake was recorded in the HO treatment when Pb mass was used (Figures 6.4.11 and 6.4.12). There was a statistically significant ($F_{3, 36}$ =164.38: P=0.000 < 0.05) difference in shoot Pb mass between treatments. The shoot Pb mass of the LH and MH treatments were not significantly different as judged by the Post-hoc comparison. The varied dry mass of shoot and roots recorded at harvest had influenced the uptake when expressed in terms of Pb mass.

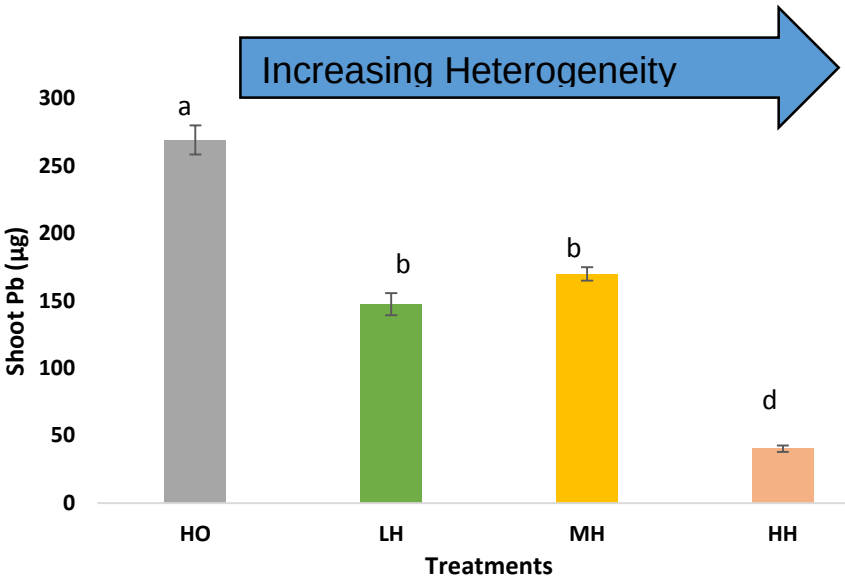


Figure 6.4.11: Shoot Pb mass (µg) between treatments of *B. napus*. Means that share the same letter are not significantly different as judged by the Tukey Post hoc test. Error bar represent 1 standard error on the mean, n=10.

HO---Homogeneous (grey), LH-----Low heterogeneity (green), MH----Medium heterogeneity (yellow), HH—high heterogeneity (peach) for all captions in this section.

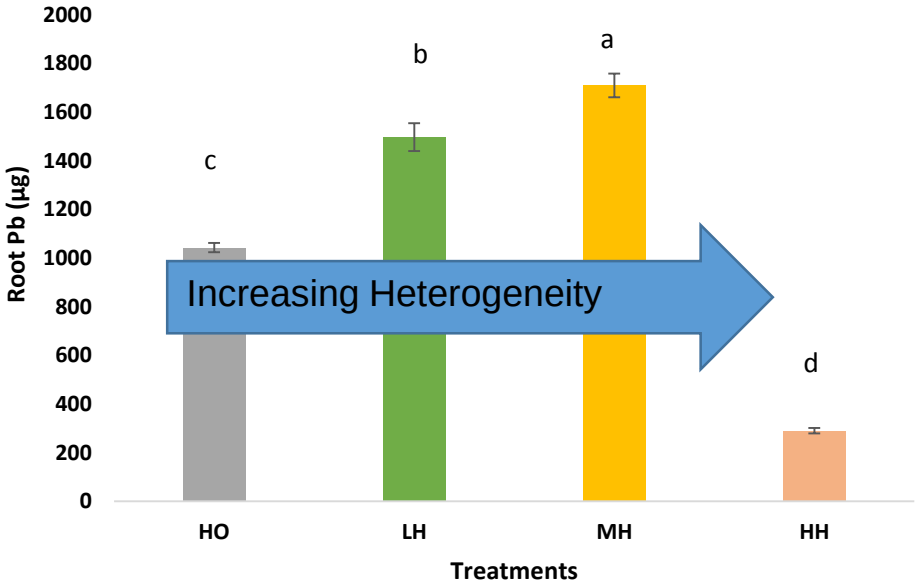


Figure 6.4.12: Root Pb mass µg between treatments of *B. napus*. Means that share the same letter are not significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean, n=10.

Lowest mean shoot and root Pb mass (40 and 289 µg respectively) were recorded in the HH treatments compared to the other spatially heterogeneous treatments (LH & MH) and the HO treatment. Similar trend was observed with the root Pb (mg/kg) but the shoot Pb (mg/kg) did not follow this trend.

ANOVA and Tukey HSD test showed a statistically significant difference in shoot and root Pb uptake between the 3 heterogeneity treatments, and between the 3 heterogeneity treatments and the HO treatment.

The Mixed model ANOVA result also showed a significant effect of spatial heterogeneity on Pb uptake where P = 0.000 < 0.05 (Appendix IV.3: Table BIV.3). This clearly suggest that the degree of spatial heterogeneity had an impact on the extent of Pb uptake in *B. napus*.

6.4.5 Pb Uptake result for *B. juncea* expressed as Pb concentration (mg/kg).

Shoot Pb uptake in mg/kg concentration of *B. juncea* increased with increasing heterogeneity with a peak uptake in the HH treatment and higher by 20% than the HO shoot Pb (Figure 6.4.13).

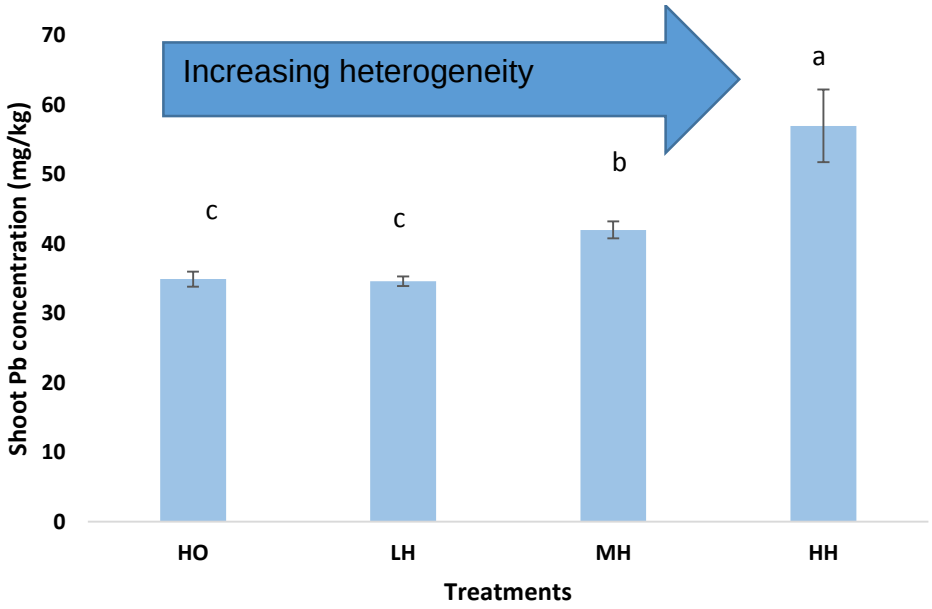


Figure 6.4.13: Shoot Pb concentration between treatments of *B. juncea*. Means that do not letter are significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean n=10.

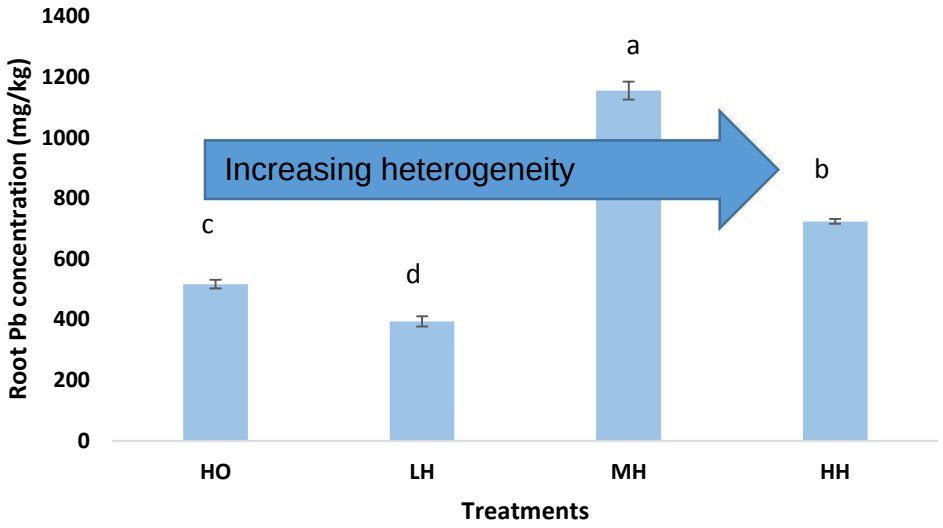


Figure 6.4.14: Root Pb concentration between treatments of *B. juncea*. Means that share the same letters are not significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean, n=10.

A similar trend of increased root Pb concentration mg/kg with increasing heterogeneity was also observed (Figure 6.4.14). However, the peak root Pb concentration was recorded in the MH treatment which was 2.2 fold higher than HO root Pb.

6.4.5.1 Uptake expressed as Pb mass (µg) for *B. juncea*.

Shoot Pb uptake (Pb mass µg) decreased with increasing heterogeneity with a peak uptake in the HO treatment. (Figure 6.4.15). The peak shoot Pb mass in the HO treatment was 2.8 fold higher than that of HH treatment (Figure 6.4.15). The shoot Pb mass was maximum in HO where the highest shoot biomass was recorded. The shoot Pb mass in MH and HH were not significantly different (Figure 6.4.15).

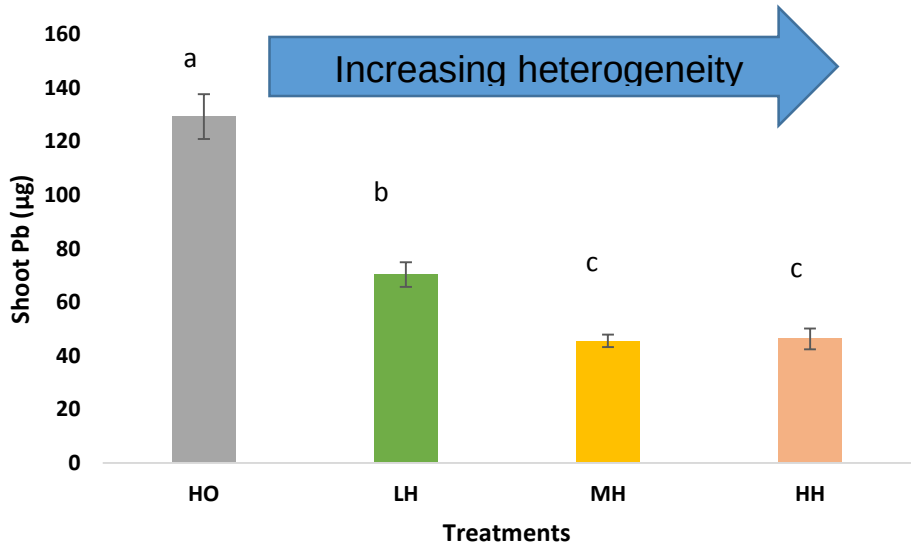


Figure 6.4.15: <u>Shoot Pb mass</u> µg between treatments of B. juncea. Means sharing letters are not significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean n=10.

The root Pb mass increased with increasing heterogeneity with a peak uptake recorded for MH treatment, which was 45% higher than HO root Pb mass (Figures 6.4.16). The HH treatment had significantly lower (by factors of 2.8 and 1.8) shoot and root Pb mass when compared to the HO treatment respectively (Figures 6.4.15 and 6.4.16). However, the lowest shoot and root Pb concentrations (mg/kg) for *B. juncea* was recorded in the LH treatment, whilst the lowest mean root Pb mass (222 µg) was recorded in the HH treatments when compared to the other treatments.

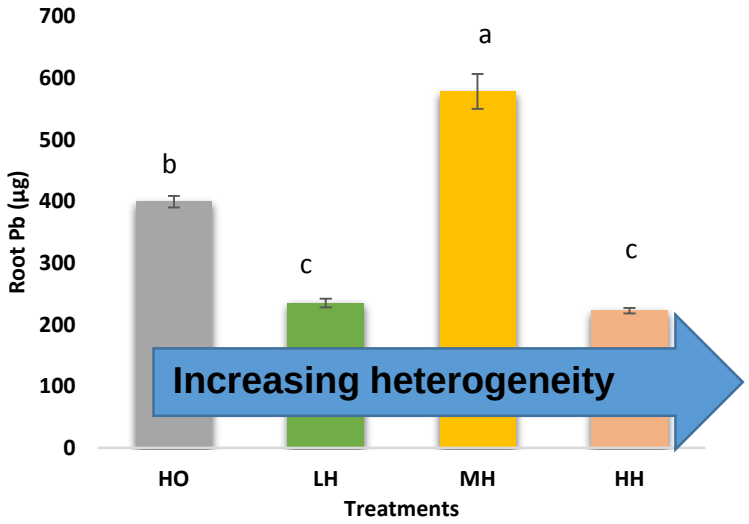


Figure 6.4.16: <u>Root Pb mass</u> µg between treatments of *B. juncea*. Means that share the same letter are not significantly different as judged by the Tukey Post hoc test. Error bars represent 1 standard error on the mean n=10.

The MH treatment had significantly higher mean root Pb mass compared to HO, LH and HH treatments. However, the MH treatments had lower shoot Pb mass (by factors of 2.8 and 1.5) when compared to the HO and LH treatments respectively (Figure 6.4.16). Generally the difference in shoot and root Pb mass between treatments was statistically significant ($F_{3, 36} = 55.37$: $P = 0.000 < 0.05$).

A statistically significant difference in Pb uptake was observed between the 3 heterogeneity treatments from the ANOVA result, further comparison with Tukey HSD test also showed that some treatments were significantly different, whilst some did not differ significantly (Appendix IV.5). The mixed model ANOVA result also showed a significant effect ($F_{3, 36} = 284.29$: $P = 0.000 < 0.05$) of different treatments on Pb uptake (Appendix IV.5: Tables DIV.5). It is an indication that these levels of spatial heterogeneity had an impact on the extent of Pb uptake in *B. juncea*.

6.4.6 Shoot and Root Concentration Factors (CF_{shoot} and CF_{root}) of *B. napus* and *B. juncea*.

The shoot and root concentration factor used in this thesis is calculated as Pb concentration of shoot or root (mg/kg DW)/ soil Pb concentration) as discussed in Chapter 2. Rotkttikhum *et al*., (2006), Baker *et al*., (1994) and Thomas, (2010) suggest that the concentration factor (CF) increases where mechanisms that exclude contaminants are weaker and subsequently result in contaminant accumulation into plant tissues. The CF_{shoot} and CF_{root} of *B. napus* and *B*. *juncea* are compared in Figures 6.4.6.1 and 6.4.6.2 below.

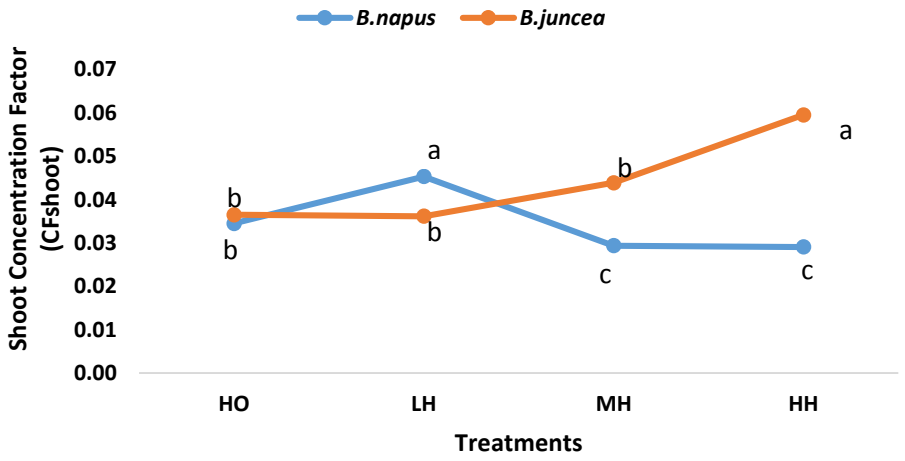


Figure 6.4.17: Shoot Concentration Factors (Pb concentration in shoot (mg/kg DW)/mean soil concentration of Pb) for *B. napus* and *B. juncea* grown in 4 different treatments of Pb spatial heterogeneity. Where CF is greater than 1 (accumulator threshold) Pb is accumulated. Means that share the same letter are not significantly different for each species as judged by the Tukey Post hoc test.

Generally, CF_{shoot} for *B.napus* and *B*. *juncea* ranged from 0.03 to 0.06 with 1.7 and 1.5 fold rise in LH and HH treatments, when compared to the HO treatment respectively. Peak CF_{shoots} were recorded in LH and HH treatments of *B*. *napus* and *B*. *juncea* respectively (Figure 6.4.6.1). Shoot concentration factors in all treatments were below the accumulator threshold of 1 by factors of 16 to 25 for B. *juncea* and 20 to 33 for *B*. *napus*.

Brassica juncea had 1.5 fold higher CF_{shoot} in the HH treatment when compared to the HO treatment whilst *B*. *napus* had similar CF_{shoot} in the HH as the HO treatment. This is an indication of increased shoot uptake, but not in terms of Pb mass of *B. juncea* in the HH treatment whilst *B*. *napus* might generally exclude Pb from the shoot in all treatments with an exception of the LH treatment.

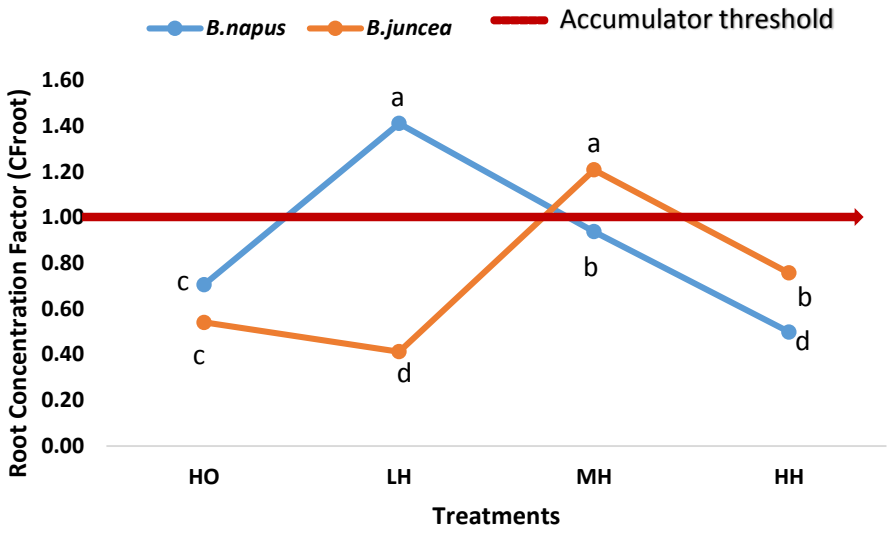


Figure 6.4.18: Root Concentration Factors (Pb concentration in root (mg/kg DW)/mean soil concentration of Pb) for *B. napus* and *B. juncea* grown in 4 different treatments of Pb spatial heterogeneity. Where CF is greater than 1 (accumulator threshold) Pb is accumulated. Means that share the same letter are not significantly different for each species as judged by the Tukey Post hoc test.

The root concentration factors (CF_{root}) of both species ranged from 0.41 to 1.41 (Figure 6.4.6.2). The CF_{root} of *B. juncea* increased with increasing heterogeneity with 1.59 fold decrease in the HH treatment when compared to the peak CF_{root} in MH treatment. The CF_{root} of *B. juncea* in the HH was 1.41 fold higher than that of the HO treatment and 1.59 fold lower than the peak CF_{root} in MH treatment.

Brassica napus had a peak CF_{root} in the LH treatment which was 2 fold higher than the HO CF_{root}. However, CF_{root} was lower by factors of 1.4 and 2.8 in the HH treatment when compared to the HO and LH treatments of *B. napus* respectively. This decreased CF_{root} of both species in the high heterogeneity treatment supports earlier findings for Zn (Thomas, 2010) of reduced root CF (10 fold decrease) in highly heterogeneous Zn treatment with *B. napus* and *juncea.*

Brassica juncea and *B. napus* had CF_{root} above the accumulator threshold of 1 in MH and LH treatments respectively. This implies that higher amount of Pb was accumulated in the roots of these species in the LH and MH treatments when compared to the other treatments. A significant variation ($P = 0.000 < 0.05$) in CF was observed between treatments for both species. This suggests that spatial heterogeneity had a significant effect on the ability of plants to accumulate or exclude Pb.

Results showed high Pb accumulation in roots of *B. napus* in the LH treatment with a substantial drop in Pb uptake in the highly heterogeneous treatment. In contrast, roots

in MH treatment of *B. juncea* showed high Pb accumulation. The CF_{shoot} of both species were generally well below the accumulator threshold, which suggests that root Pb uptake is about 4-5 times higher than shoot Pb uptake. The HO, LH and HH treatments had CF_{root} below the accumulator threshold.

Based on the exclusion principles proposed by Baker, (1981) and from previous work (Mishra *et al*., 2006), both species can be seen to possess strong mechanism of excluding Pb from its tissues across treatments. Results also indicated that both plant species will actively exclude Pb from the root in the highly heterogeneous treatment (with a decrease in CF_{root} in the HH treatment by factors of ~ 3 and 2 when compared to the peak CF_{root} in LH and MH treatments of *B*. *napus* and *B*. *juncea* respectively). Both species tend to actively exclude Pb at nearly all levels of heterogeneity to varying extent with a peak accumulation in the MH and LH treatments for *B*. *napus* and *B*. *juncea* respectively. Similar trend of decreased CF_{root} has been reported in earlier studies (Thomas, 2010) with *Plantago lanceolata* in Zn spatial heterogeneity treatments. However, for pot trial four, *B*. *juncea* had higher CF_{shoot} and CF_{root} (by a factor of 1.5 and 2 respectively) in the HH treatment than *B*. *napus*. This suggests that Pb can be more easily accumulated in roots of *B*. *juncea* than *B*. *napus* in the HH treatment.

6.5 Root placement sub-experiment results.

A further sub-experiment into the root placement of plant species in high heterogeneity treatment was carried out in order to investigate the varying plant response to Pb spatial heterogeneity. The LH & MH treatments were not included in the root placement as the HH treatments was considered to represent extreme case of spatial heterogeneity (high heterogeneity). The HH treatment, had the highest patch contrast (cells with varied Pb distribution), was considered useful in investigating root response to heterogeneity that can provide useful insights into plant root response to heterogeneity. This was also supported by biomass and uptake results which showed highly significant difference between HH and the other treatments. Typical root morphology for the two species are shown in Figure 6.5.19.

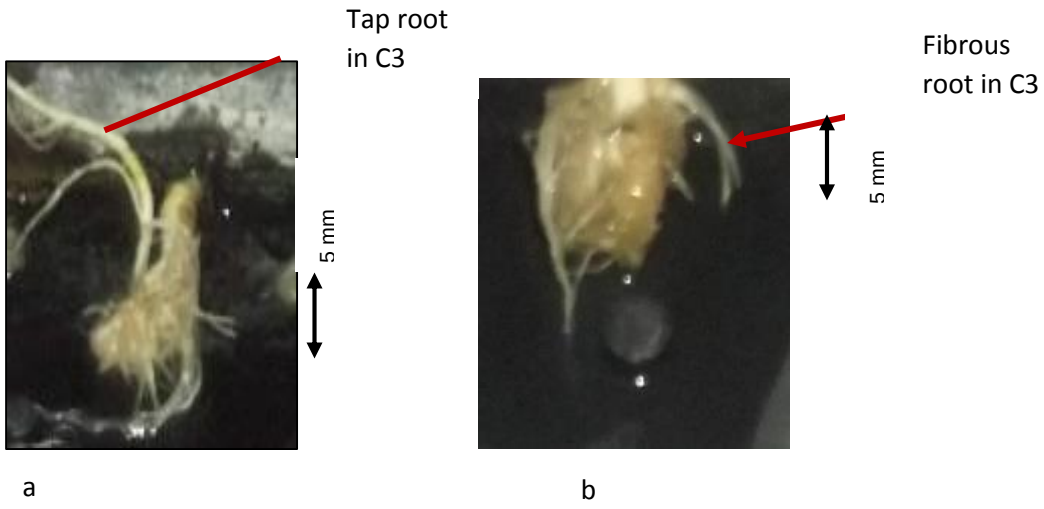


Figure 6.5.19: Extracted roots showing (a) a tap root in C3 of *B. napus* (4 mm represents 5 mm) and (b) a fibrous root in *B. juncea* (4 mm represents 5 mm).

6.5.1 Root placement result for *B. napus*

Dried, extracted roots from each cell were weighed and roots from cells of same nominal Pb concentration were combined and analysed for Pb. Figure 6.5.21 below shows mean root biomass DW in cells within the same concentric patches {outer, middle and central} (Figure 6.5.20) with same nominal soil Pb concentration of *B. napus*.

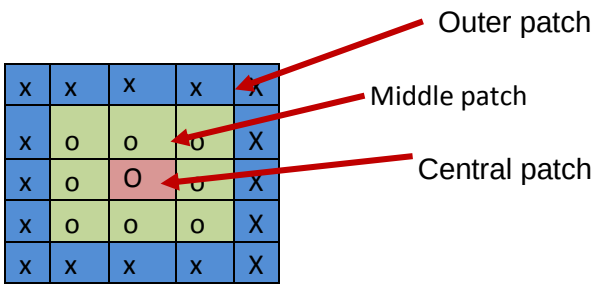
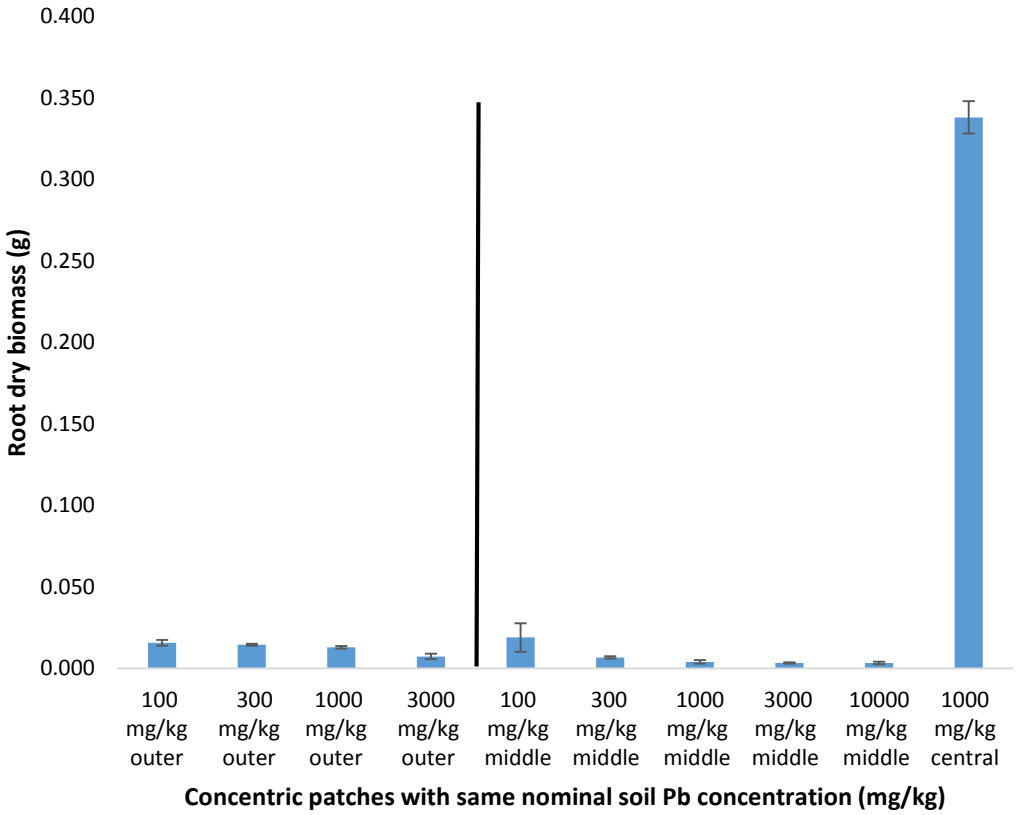


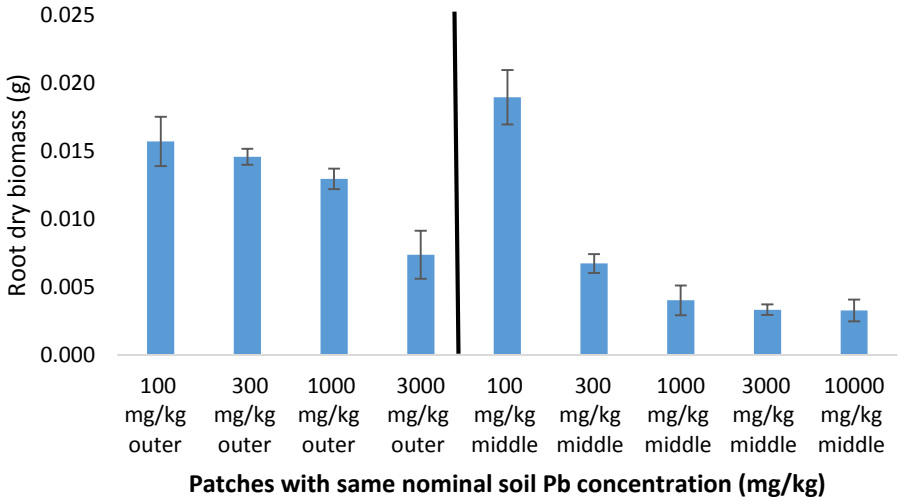
Figure 6.5.20: An illustration of concentric patches.

The ANOVA result show significant differences (F = 585.02; P = 0.000 < 0.05) between root biomass DW in concentric patches of varied soil Pb concentration (Appendix IV.6). The 1000 mg/kg concentration in the central patch had 13 to 94% higher root biomass, when compared to the all patches with the same nominal concentrations.

Root biomass of the 300 mg/kg outer and middle patches were significantly different (T= -8.60; P = 0.000 < 0.05) with the outer having 10% higher biomass than the middle patch. The root biomass of the 100 and 300 mg/kg outer and middle patches did not differ significantly (T = 1.48; P=0.154 > 0.05). Patches with higher Pb concentrations (3000 and 10000 mg/kg) were not significantly different in their root biomass (T= 2.24; P=0.066 > 0.05). However, they differed significantly from the 100, 300 and 1000 mg/kg patches in their root biomass. The root biomass has been decreased by 17 to 90% in the patches with higher Pb concentrations (3000 and 10000 mg/kg). This is an indication that the root biomass had been impacted on by the patch contrast (heterogeneous distribution of Pb in cells). There was a trend (r^2=0.996) of decreasing root biomass with increasing patch soil Pb concentration in the outer concentric patches (Figure 6.5.21a below: Appendix IV.6; Figure AIV.6). However, there was no significant linear relationship (r^2=0.2364) between decreasing root biomass (6.5.21b) with increasing patch Pb concentration of the middle concentric patches (Appendix IIV.6: Figure BIV.6).



(a)



(b)

Figure 6.5.21: (a) Root biomass DW in concentric patches of same nominal soil Pb concentration of *B. napus* in the HH treatment with the central 1000 mg/kg patch (b) Root biomass DW in the outer and middle concentric patches with same nominal soil Pb concentration of *B. napus* in the HH treatment without the central 1000 mg/kg patch.

The root Pb concentration in patches with same nominal concentration increased with increasing nominal soil Pb concentration of patches (Figure 6.5.22). There was a strong positive relationship (R^2 = 0.98) between cell root Pb concentration and the nominal soil Pb concentration with the ~99% of the variance accounted for by the regression model of cell root Pb concentration against nominal soil Pb concentration (Figure 6.5.23) which suggest that the soil Pb concentration had an impact on root uptake in patches. It is also an indication this plant species responded to the increasing root uptake of Pb by decrease in the proportion of root biomass in patches with high Pb concentration.

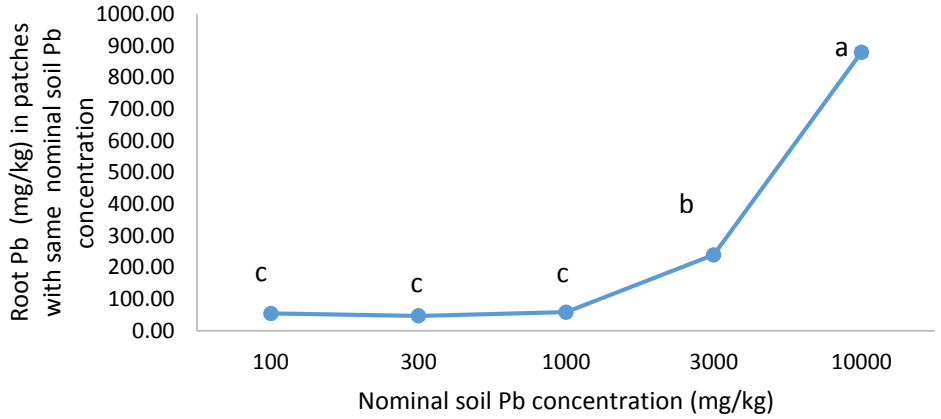


Figure 6.5.22: Root Pb concentration against nominal soil Pb concentrations of *B. napus* in the HH.

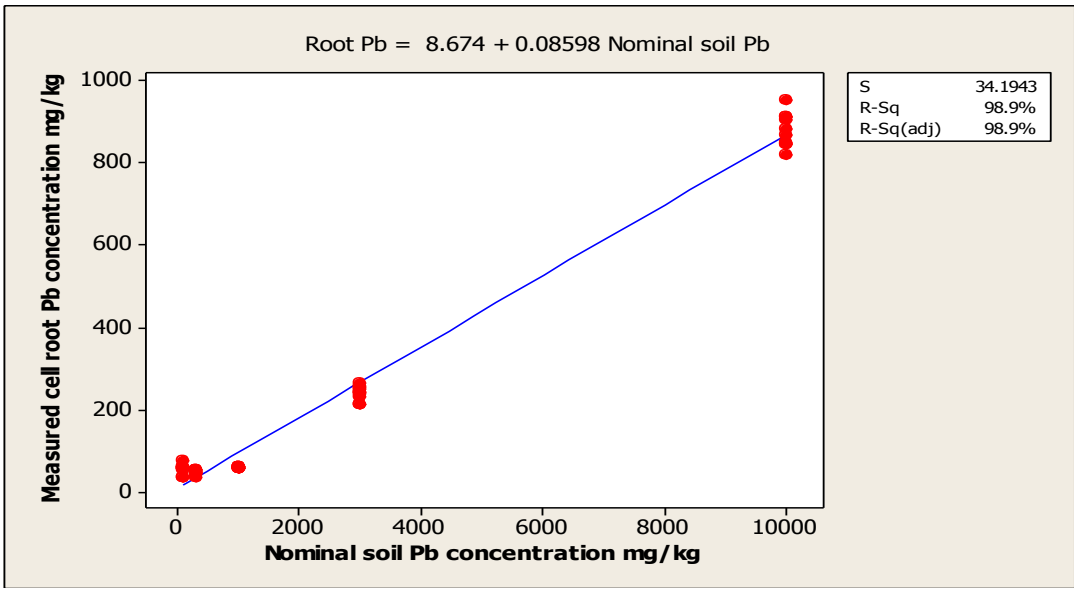


Figure 6.5.23: Regression model of measured root Pb concentration of patches with same nominal soil Pb) against nominal soil Pb concentration of *B. napus* in the HH treatment.

6.5.2 Root placement Result for *B. juncea*.

Figure 6.5.24 below show the root biomass DW in patches with same nominal soil Pb concentration of *B. juncea*. The ANOVA result also showed significant differences (F = 13.77; P = 0.000 < 0.05) between cells of varied soil Pb concentration. The 1000 mg/kg central patch had 6 to 98% higher root biomass, when compared to the other concentric patches with same nominal soil Pb concentrations.

The 100 and 300 mg/kg did not differ significantly (T= -0.80,-0.28; P=0.445, 0.784 > 0.05) in their outer and middle root biomasses with 50 and 5% higher root mass in middle and outer patches of the 100 and 300 mg/kg respectively.

Patches with higher Pb concentrations (3000 and 10000 mg/kg) were significantly different from the 100, 300 and 1000 mg/kg outer and middle patches in their root biomass. The 100, 300 and 1000 mg/kg patches were similar in the distribution of root in the outer and middle patches. The root biomass was decreased by 11 to 95% in the patches with higher Pb concentrations (3000 and 10000 mg/kg). Result showed that the root mass of *B. juncea* also decreased with increasing soil Pb concentration of cells which implied an ability of the root to detect patch contrast and contaminant heterogeneity. There was a trend (R^2=0.7050 and 0.6589) of decreasing root biomass with increasing patch Pb concentration in the outer and middle concentric patches respectively (Figure 6.5.24; Appendix IV.6: Figures C -IV.6 and D-IV.6).

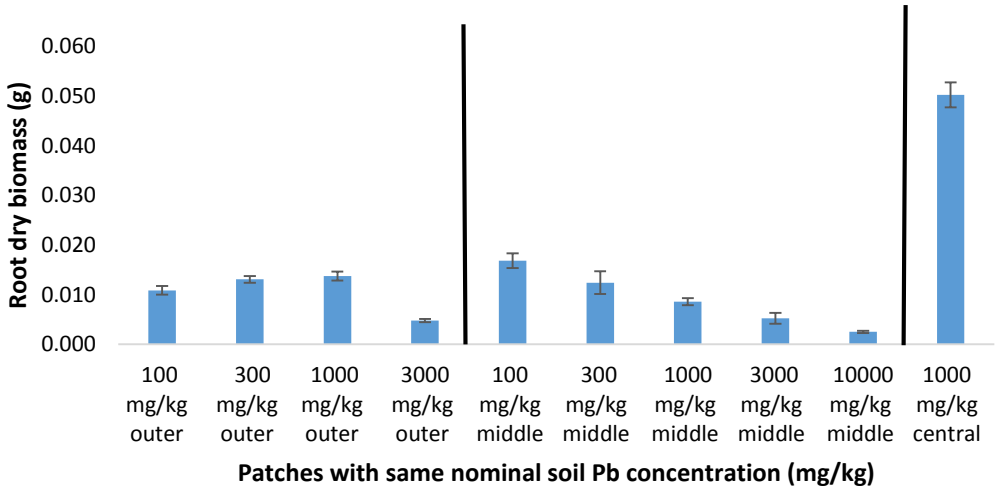


Figure 6.5.24: Root biomass DW in concentric patches of same nominal soil Pb concentration of *B. juncea* in the HH treatment.

A similar trend of increased root Pb concentration with increasing patch nominal Pb concentration was also observed in *B. juncea* (Figure 6.5.25). Cell root Pb concentrations did not differ significantly between cells with 100, 300 and 1000 mg/kg Pb concentrations. There was a strong positive relationship (R^2=0.97) between cell root Pb concentration and the nominal soil Pb concentration with 97% of the variance accounted for by the regression model of cell root Pb concentration against nominal soil Pb concentration (Figure 6.5.26). This implies that individual cell root uptake has been influenced by the soil Pb concentration in the HH treatment. The slope of the regression model shows that root Pb uptake in *B. juncea* was approximately 2 times higher than that of *B. napus*.

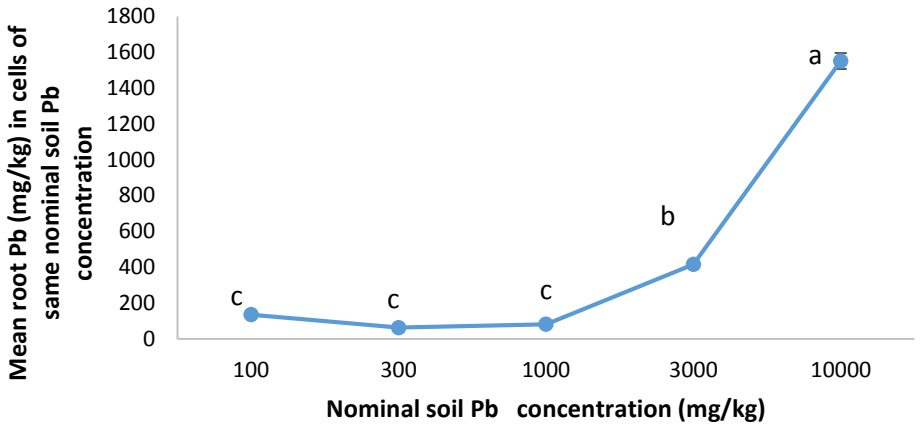


Figure 6.5.25: Mean root Pb concentration against nominal soil Pb concentrations of *B. juncea* in the HH treatment.

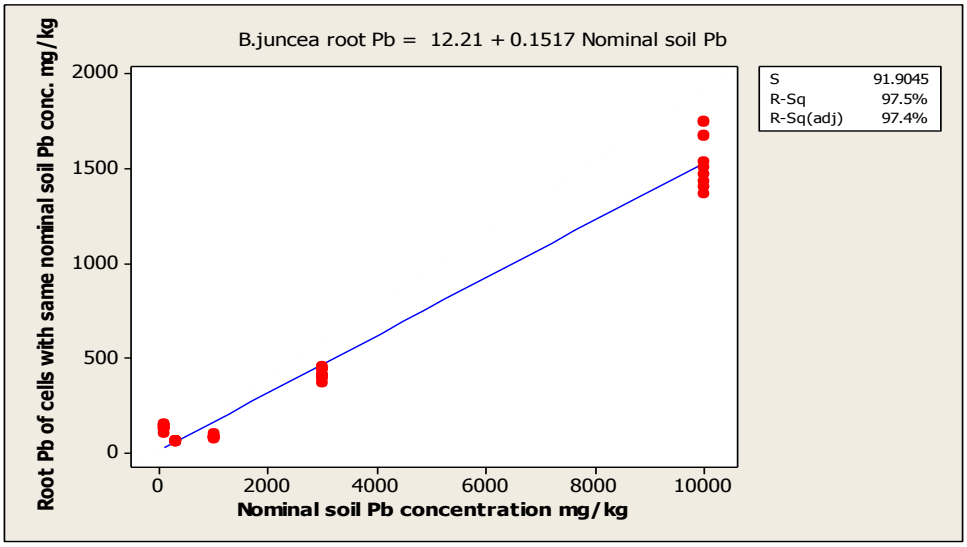


Figure 6.5.26: Regression model of mean root Pb concentration (Patches of same nominal soil Pb) against nominal soil Pb concentration of *B. napus* in the HH treatment.

6.5.3 Comparison of plant response to root placement.

These results suggest that plant roots for both species have shown a response to changing heterogeneity. It also showed similarities in response to changing heterogeneity between the two plant species, though significantly impacted to varied extent. However, both plant species were significantly different (T = 2.68; P = 0.009 < 0.05) in the proportion of root mass allocated to cells of varied Pb concentration (Appendix IV.6: JIV.6).

Visible differences were observed in the root morphology of these species. Figure 6.5.27 compares the root biomass of the 1000 mg/kg central patches of both species. The total root mass of *B*. *napus* and *B*. *juncea* varied by a factor of 2. Result suggest that *Brassica napus* had 53% higher root biomass in the central patch than *B*. *juncea.* This difference was statistically significant (T = -28.26; P = 0.000 < 0.05) (Appendix IV.6: I IV.6). This contrast in root morphology is due to the presence of tap root in *B*. *napus* and its absence in *B*. *juncea*. The differential root structure and morphology of these species might have contributed to the varied extent of the effect of the heterogeneity.

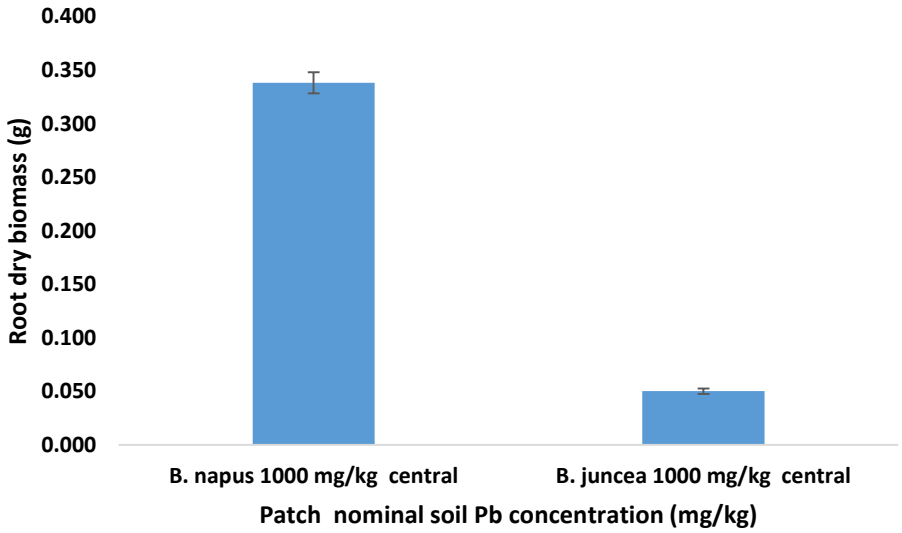


Figure 6.5.27: Comparison of the central root biomass of *B. napus* and *B. juncea*.

Root placement in the HH treatment provided an insight into the behaviour of these plant species in heterogeneous media. Decreased root mass was observed in patches with increased soil Pb concentration which suggest that both plant species were able to detect patch contrast (heterogeneity) of Pb concentration in the growth medium. Increased root Pb concentration with increasing soil Pb concentration of each cell, with the highest root Pb concentration in the 10000 mg/kg concentration is an indication that the contrast in Pb distribution across cell in each heterogeneity treatment could influence root Pb uptake

in each cell and subsequently the overall Pb uptake with impact on the proportion of roots. This might have partially influenced the instances of peak uptake at different levels of heterogeneity observed in these species.

The similarities in how these species respond to heterogeneity despite their contrasting root morphology suggest an avoidance response to the toxicity of Pb irrespective of root morphology. However, it will take a multidisciplinary approach often involving physiological and biochemical investigations to confirm this inference and to provide further insights on varied plant response (see Chapter 8: Section 8.2).

6.5.4 Growth parameters of *Brassica napus* and *Brassica juncea* in the fourth pot trial.

Other growth parameters such growth index (GI), height, number of true and dead leaves and longest leaf length were used to study the behaviour of these species during the growth period in a more realistic heterogeneity treatment as used in earlier pot trials.The results are as shown in Figures 6.5.28 to 6.5.31.

B. napus

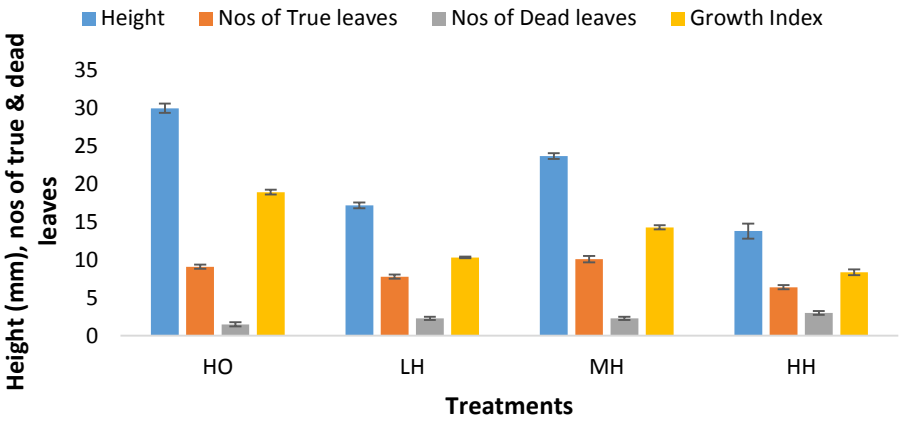


Figure 6.5.28: Mean height, number of true and dead leaves and growth index of *B. napus*. Error bars represent 1 standard error on the mean (n=10).

The height of *B. napus* was maximum in the HO treatment, which was two fold higher than the HH treatment (Figure 6.5.28). A trend of decreased height with increasing heterogeneity was observed between treatments. A peak height was recorded in MH among heterogeneity treatments.

A Similar trend of decrease with increasing heterogeneity was observed in the growth index (GI) of *B. napus* (Figure 6.5.28), which supports better growth in the HO treatment,

when compared to the other treatments. This is an indication of its significant impact on the overall biomass.

The number of true leaves were 20 to 50 % lower in the HH treatment, compared to the other treatments (Figure 6.5.28). The maximum number of true leaves were recorded for the MH treatment.

The number of dead leaves were not significantly different between treatments. However, fewer number of dead leaves (10%) were recorded in the HO treatment, when compared to the LH, MH and HH treatments (Figure 6.5.28).

The longest leaf length was recorded in the HO treatment, which was 40 % longer than the HH treatment (Figure 6.5.29). Leaves were narrower with increasing heterogeneity with an exception of the MH treatment.

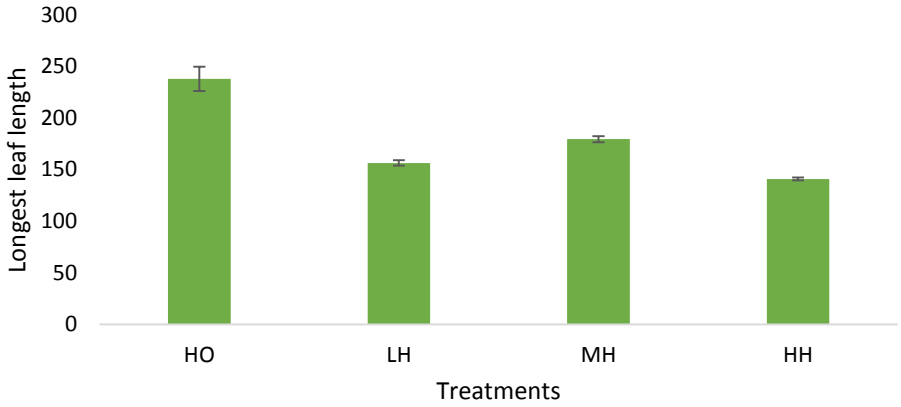


Figure 6.5.29: Mean longest leaf length of *B. napus*. Error bar represent 1 standard error on the mean (n=10).

B. juncea

Conversely, the height of *Brassica juncea* increased with increasing heterogeneity. Maximum height was recorded in the HH treatment, which was two higher than the HO height (Figure 6.5.30).

A similar trend of increased longest leaf length with increasing heterogeneity was observed in *Brassica juncea* with a maximum leaf length in the HH treatment (Figure 6.5.30).

However, the growth index did not follow this trend. The GI of *B. juncea* was not significantly different between treatments (Figure 6.5.30).

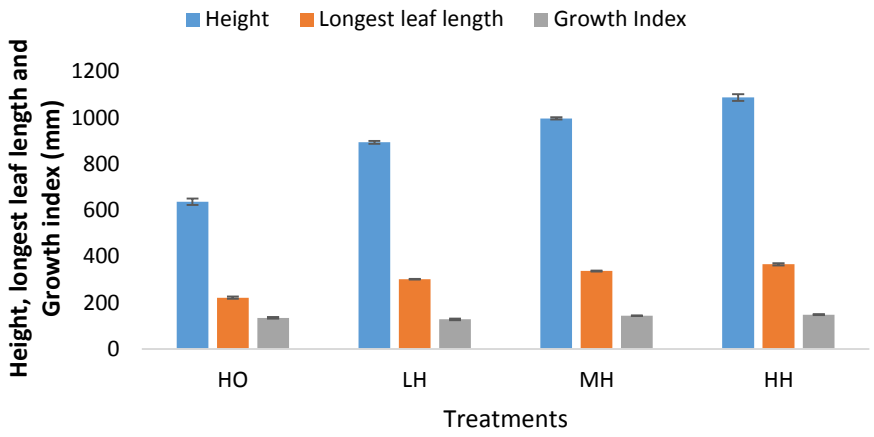


Figure 6.5.30: Mean height, longest leaf length and growth index of *B. juncea*. Error bars represent 1 standard error on the mean (n=10).

The number of true leaves also increased with increasing heterogeneity with peak number of true leaves in the HH treatment, which was 2% higher than the HO treatment (Figure 6.5 31).

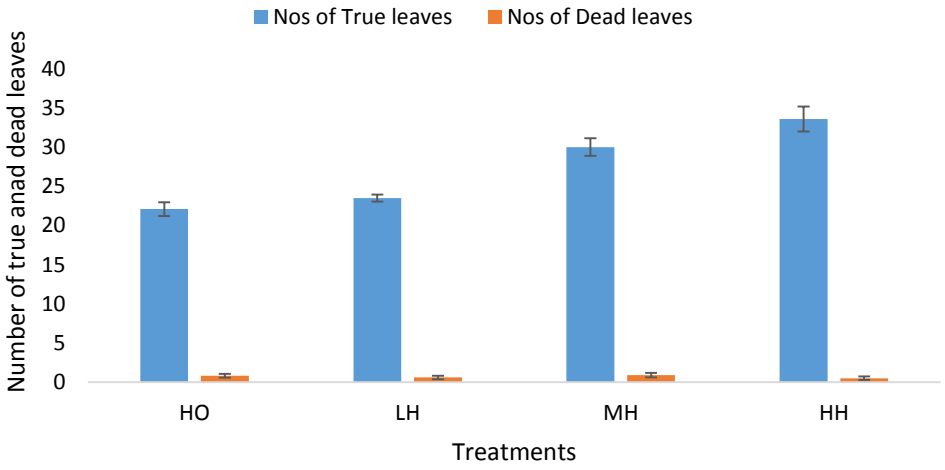


Figure 6.5.31: Mean dead leaves between treatments of *B. juncea*. Error bar represent 1 standard error on the mean (n=10).

The number of dead leaves were not significantly different between HO and LH treatments (Figure 6.5.31). However, ~3 % higher number of dead leaves were recorded in MH treatment, whilst the lowest was recorded in the HH treatment.

Comparatively, fewer (~25%) number of dead leaves were recorded for *B. juncea* than *B. napus*. This suggests that reduction in the number of dead leaves might be an adaptation of *B. juncea* to tolerating heavy metal stress, while *B. napus* will shed more leaves as an adaptive mechanism to withstand heavy metal stress.

6.6 Discussion in relation to stated hypothesis.

Hypotheses 1 and 2 in their alternative form stated that (i) The degree of spatial heterogeneity of Pb in the growth medium will impact upon (a) the extent of uptake of Pb and (b) the plant biomass, by each species (*B. juncea* and *B. napus*.) between treatments.

(ii) This effect differed at various levels of heterogeneity (e.g. low, medium and high heterogeneities) compared with the homogeneous treatment.

The result showed that there was a significant effect of spatial heterogeneity on biomass and plant uptake of both species, but to a differing extent. The differences between treatments were also statistically significant.

Uptake (Pb concentrations mg/kg) was maximum at the LH and MH treatments for both plant species and this was about 1.4 to 2.0 fold higher when compared to HO treatments of *B. napus and B. juncea* (Figures 6.4.11-12 and Figures 6.4.15-16). *Brassica napus* generally had thrice higher Pb uptake (as Pb mass) in HO, LH and MH treatments in comparison with *B. juncea*, but had 1.2 fold higher Pb mass in HH than *B. juncea*.

Uptake increased with increasing heterogeneity for *B. napus* with the exception of the HH treatment where a decrease in Pb concentration (mg/kg) and mass was observed. This confirmed similar findings of increased uptake of Zn with increasing (µg) heterogeneity (by a factor of 1.2-1.6) for *B. napus* (Thomas, 2010).

B. juncea which had decreased Pb uptake (expressed as Pb mass) with increasing heterogeneity with an exception of the MH treatment where a significantly higher uptake was recorded. This is in contrast to earlier findings for Zn (Thomas, 2010) of increased uptake of Zn with increasing heterogeneity with an exception in the binary simplistic treatment of *B. juncea*. This exceptional difference in Pb mass of MH treatment might have been influenced by the scale of heterogeneity, size of the root ball, biomass and root response to the spatial distribution of Pb.

Brassica napus had approximately twice the biomass of *B. juncea* irrespective of the levels of heterogeneity. However, both species were affected by a similar factor of ~ 1.4 to 2 in their peak Pb uptake (expressed in both Pb concentration and mass) compared to a homogeneous treatment. Similarly, *B. napus* and *juncea* showed decreased uptake in the HH treatment, but to varying extent. *Brassica napus* had 5 and 4 fold decrease in shoot and root Pb uptake (expressed as Pb mass) respectively in HH when compared to the HO treatment. Shoot and root uptake (Pb concentration mg/kg)

of *B. juncea* were higher by factors of 1.6 and 1.4 in HH when compared to HO treatment while *B. napus* was 1.2 and 1.4 fold lower respectively.

Both plant species showed decreased biomass with increasing heterogeneity with an exception of the MH treatment of *B. napu*s. Approximately 5 and 4 fold lower biomass were recorded in the high heterogeneity treatment when compared to the homogeneous treatment of *B*. *napus* and *juncea* respectively. This is in contrast to increased biomass with increasing Zn heterogeneity in earlier work by Thomas, (2010). The decreased biomass with increasing Pb heterogeneity as opposed to increased biomass with increasing Zn heterogeneity might be linked to the phytotoxicity of Pb, whilst Zn is an essential element to plants at low concentration levels. That study (Thomas, 2010) found no phytotoxic effect in *B*. *juncea* at root Zn concentration of 2500 mg/kg.

Several factors might have be responsible for the contrast between the two plant species in response to spatial heterogeneity of Pb e.g. the scale of heterogeneity, the size of root ball and differing concentration factors (CF) when compared to the threshold CF of 1.

Hypothesis 3 of this experiment states that the response of plant species to changing heterogeneity is associated with differences in root placement. There are two assumptions associated with this hypothesis (i) roots of these plants will be able to detect the degree of contrast between patches and (ii) that roots of these plant species will preferentially proliferate in patches with lower Pb concentration, presumably to avoid the toxicity of Pb

Earlier (third) experiment with the simplistic binary model of Pb heterogeneity (Chapter 5: Section 5.4.4) suggested that the roots of these plant species will proliferate in patches of zero added Pb. Two – fold increase in root diameter was observed in the zero Pb mg/kg added patch compared to the binary patch (2000 Pb mg/kg added) in the binary treatment (Chapter 5). Significant differences were observed in root ball diameter of these species in the binary model in comparison to the homogeneous treatment. This is an indication of an avoidance response to the toxic Pb.

This (fourth) experiment, based on simulation of more realistic model of *in situ* heterogeneity, suggests that roots responded to more complex degrees of heterogeneity. Both plant species detected the differences in patches and responded to the different degrees of patch contrast (i.e. spatial distribution of contaminant). Results showed that plant root mass will change with the degree of patch contrast to a varying extent and in different ways e.g. by decreased root biomass in patches of high Pb concentration, for both species. Thomas, (2010) found differences in the response of these plants' roots to Zn heterogeneity which suggested that root placement response

to heterogeneity may be plant specific. Results of this study showed that those same plant species though differed in morphology, both had similar response to Pb spatial heterogeneity. However, they differed in the proportion of root mass allocated to cells of varied soil Pb concentration. It supports the prediction of an avoidance response of roots presumably to Pb phytotoxicity by the binary simplistic model of Pb heterogeneity.

Hypothesis 4 stated that there is a difference between responses to changing heterogeneity between the two plant species (*Brassica juncea* and *Brassica napus*). A comparison of biomass and uptake of both plant species showed a statistically significant difference (P = 0.000 <0.05) between both species. The summary of the hypotheses tested is shown in Table 6.6.1.

Table 6.6.1: Summary of hypothesis tested.

Hypothesis (alternative)	**Species**	
	B. napus	***B. juncea***
2. Biomass	Accepted	Accepted
(b) Pb Uptake (mg/kg and mass)	Accepted	Accepted
3. Between heterogeneity levels (a) biomass	Accepted	Accepted
(b) uptake	Accepted	Accepted
4. Root response to heterogeneity	Accepted	Accepted
5. Between species	Accepted	Accepted

Probability P < 0.05 of whether hypothesis is rejected or accepted.

6.6.1 Interpretation of results.

The root placement experiment found three distinct regions of root distribution as a function of soil Pb concentration in both species. The highest proportion of *B*. *napus* roots (26 to 113%) were proliferated in the 1000 mg/kg central patch, when compared to the other patches. Results show that roots were selectively placed in the low Pb patches (100 and 300 mg/kg patch) with approximately 80% of roots proliferated in the central 1000 mg/kg patch. This is an indication that the tap root which was located in the central cell (C3) of *B*. *napus* where greater proportion of the roots were placed has influenced the overall mass of roots in the 1000 mg/kg patches. This suggests that the tap root is a big proportion of root mass and might have influenced the proportion of root proliferated to other patches in response to spatial heterogeneity. It is also an indication that the contaminant concentration and the range at which these patches are located in relation to the root could play an important role in determining the distribution of roots into patches with similar or different contaminant concentrations.

There was a continuous decrease in root biomass (~16 to 90 %) with increasing soil Pb concentration in the middle patches of *B*. *juncea*. The heterogeneity design (Chapter 6: Section 6.3) shows that cells with elevated Pb concentrations (patches with 3000 and 10000 mg/kg) were located in the middle patch. It is a pointer to the fact that these patches with elevated Pb concentrations might have influenced the proportion of roots proliferated in the middle patches with an exception of the 100 mg/kg middle patch of *B*. *napus*.

The 3000 outer patch had 20 to 80% lower root mass than the 100, 300 and 1000 mg/kg outer patches. Rather than a decreased root biomass in the outer 1000 mg/kg patch of *B*. *juncea*, the root biomass in the 100, 300 and 1000 mg/kg outer patches were not significantly different. It is an indication of the adaptation of this plant species to this Pb concentration range. The highest proportion of roots (10 to 50%) were proliferated in the 1000 mg/kg central, when compared to the other patches. This denser root at 1000 mg/kg represent the central cell (C3) where the seedling was transplanted originally and the additional root mass in *B*. *napus* due to the tap root. This suggests that the root morphology of both plant species plays an important role in the root response of this plant to Pb spatial heterogeneity, particularly in the proportion of roots proliferated into patches. Results also show that roots were selectively placed in the low Pb patches (100 and 300 mg/kg patch).

B. napus and *juncea* generally showed a decreased biomass with increasing heterogeneity. The root mass in patches with the same Pb concentration also varied for both species in the high heterogeneity treatment. This is similar to findings of Menon *et al*., (2006) of decreased root mass in heterogeneously Zn contaminated loamy soil.

However, the mechanism of root proliferation in cells is not yet understood. Studies by Barber and Silverbrush, 1984; Pierret *et al*. 2005 suggested that the mechanism of root proliferation involve interactions between the roots and the soil, based on root functional architecture. Some previous studies expressed varied opinion on the mechanism of root response in heterogeneous media.

For example, Caldwell, (1994) suggested that proliferation of roots into patches can be influenced by the density of the plant tissue. Studies by (Hodge, 2004) suggested that root proliferation as a measure of increased root biomass does not give a complete picture of the change in the plant root system, as alterations in the architecture of root system can occur without a change in the biomass. Eissenstat and Caldwell (1988) argued that the proliferation of roots in patches may be related to the specific root length (SRL) or root length per unit mass (cm/g) which varies with root diameter and often used as a substitute to root diameter will provide relevant insight into root response. Menon *et al*., (2006) suggested that the use of neutron radiography to study live plant roots in heterogeneously contaminated soil provides a source of valuable information to explain root response in pot experiments. The short-fall of neutron radiography include problems of visibility and recognition of root orientation. Based on the nature of the root placement experiment, it was more practically possible to use the root biomass that has been widely used in previous studies (Haines, 2002; Millis *et al*., 2004; Schwartz *et al*., 2004; Thomas, 2010). Results show that these plants will preferentially proliferate roots in patches with low Pb concentration in response to Pb heterogeneity to avoid Pb toxicity.

Results also indicated that *B. napus* and *B. juncea* are tolerant Pb accumulators using classification by Baker, (1981) of three categories of plant in response to increasing metal concentrations namely, accumulators, indicators and excluders. Both species had CF above accumulator threshold at peak uptake. Concentration factor below the accumulator threshold in some treatments, suggests strong exclusion mechanism at those levels of heterogeneity. This is in line with findings of Baker (1978, 1981) that all tolerant species show some degree of heavy metal exclusion from the shoot with varying degrees of accumulation in the root and some population showing reduced metal uptake. Lasat *et al*., (1996) and Fahr *et al*., (2013) also reported the ability of some plant species

to resist Pb concentration build up in certain parts of the plants by reduced movement of contaminant across the plasma membrane through the formation of a callose barrier. However, it is not known if these species are capable of forming a callose barrier in their roots.

A trend of 4 to 7 fold increases in the root Pb concentration and mass was observed across treatments in both species, which is in line with previous studies on Pb accumulator plant species reviewed in literature (Chapter 2). The contrast in response of *B. juncea* and *B*. *napus* to the different levels of heterogeneity also showed that these plants have evolved diverse mechanisms to cope with heavy metal stress. Such mechanism of metal tolerance may be a complex syndrome of cellular, physiological and biochemical levels of adaptations the study of which is beyond the scope and objectives of this thesis. Baker *et al*., (1994) and Fahr *et al*., (2013) reported that tolerance mechanisms in excluder and accumulator species are largely internal with differing sites of detoxification of metal ions in the different parts of the plants.

Whilst both plant species have shown the ability to recognise Pb patch contrast, it is essential to note that *B. napus* tended to be more sensitive (by factors of 1.2 to 2) in detecting patch contrast than *B. juncea*. However, *B*. *juncea* seemed to have stronger mechanism of excluding the Pb from the root with CF (0.4 to 0.8) below accumulator threshold at two levels of heterogeneity. As mentioned earlier on in this discussion, varying plant response to spatial heterogeneity, and the behaviour of the roots in response to changing heterogeneity, might also be associated with the evolution of diverse complex mechanisms by these plants to withstand heavy metal stress in the soil.

CHAPTER 7: DISCUSSION.

7.0 INTRODUCTION

This Chapter discusses the findings from the thesis. It also addresses the wider significance of the research results and their implications for both phytoremediation of Pb contaminated land and for the estimation of human exposure to Pb.

7.1 Discussion.

The field study reported in Chapter 3 addressed objective two of this thesis and found that the differences in heterogeneity factors between sites at some sampling scales could be quantified and might be associated with the sources of contamination and mode of deposition of Pb. A review of contaminants heterogeneity across those sites, together with those previously reported, showed that high values of heterogeneity factor (HF > 1.4) occurred at sites where these were sources that would be expected to give rise to spatially erratic distribution of Pb such as mine wastes, canal dredging, firing range or landfill, whilst low values of heterogeneity (HF ≤ 1.3) occur in those on sites where the sources results in the homogeneous distribution of Pb such as sewage drying pans, smelter fume or flood plains that are greater than 20 km downstream of Pb mines (Chapter 2: Section 2.4.2; already published in Ramsey *et al*., 2013). Sites histories discussed in Chapter 3 showed that both field sites studied here were abandoned Pb mining sites which have become modified by previous and current land uses (See Chapter 3: Section 3.1).

The quantification of *in situ* Pb heterogeneity expressed as a heterogeneity factor was used to construct more realistic field conditions in pot trials, which provided insights into the impact of heterogeneity on plant uptake. This is useful for improved modelling of environmental processes such as Pb uptake, and potentially for improving the reliability of risk assessment and models of human exposure to Pb.

The results of this study have shown that *in situ* heterogeneity of Pb could be a very significant factor affecting the effectiveness of phytoremediation and phytoming. This is in line with work by Robinson *et al*., (2006) which suggested that metal heterogeneity might be a key component of their proposed phytoremediation equation for contaminated land that used spatial position in form of longitude and latitude to account for metal heterogeneity. This phytoremediation equation in Robinson *et al*., (2006) which

incorporated metal heterogeneity in a qualitative way, did not include the quantification of the actual *in situ* metal heterogeneity. The review of earlier studies on Pb accumulating plants suggests that some of the plant species used in this thesis have phyto-management potential. This has implications for remediation of contaminated land discussed in Section 7.2).

The first and second pot trials with a wide range of plant species/varieties (reported in Chapter 4) showed varied Pb uptake rates between and within species, both at one Pb concentration and over a range of soil Pb concentrations. A range of 11 to 70% variation in Pb uptake rate was observed between BJ 21 and other *Brassica juncea* varieties. The *B*. *napus* variety BN K differed from BN SW variety in its Pb uptake rate by 21%, while the *Zea mays* varieties varied by 44 to 55%. The *Thlaspi caerulesens* varieties varied in Pb uptake by 34% within varieties. A variation of 20 to 100% in Pb uptake was observed between different species/varieties. This is a pointer to sub-specific adaptation and tolerance to contaminants in the soil with striking variation within varieties. Earlier work on Cd uptake with several varieties of lettuce (Millis *et al*., 2004), and for a range of contaminants (Nabulo *et al*., 2008), also found over a 100% variation between and within plant species/varieties.

In the current study, whilst some of the varieties used in this study appeared to have actively excluded Pb, others took up approximately 20 to 70% Pb over a range of concentrations, and a few showed hyperaccumulating tendencies (with Pb ≥1000 mg/kg) or adaptation to high Pb in the soil. This was in the case of *Brassica juncea* variety BJ 21 and *Thlaspi caerulescens* variety TC BR respectively. Different varieties of the same species have varied characteristics in terms of nutrient requirement, growth rate and size which might have influenced their ability to accumulate Pb and other heavy metals from the soil. Individual plant species/varieties showed variation in uptake of contaminants based on variation between cultivars. Differences in genotypic, physiological and biochemical adaptation could potentially influence Pb uptake between and within species/varieties. Establishing a causal mechanism between these parameters and plant growth and Pb uptake is beyond the scope of this study and could form a basis for further studies.

Results from this study suggest that plants used in pot trials showed natural variability in plant growth and Pb uptake due to complex interplay of plant specific characteristics, site-specific heterogeneity and potentially environmental factors. This may also be applicable to plants growing on Pb contaminated sites such as the two investigated field

sites in Chapter 3. This variability might have further impact in estimation of human exposure to Pb and other contaminants. Work by Moir and Thornton, (1989) and Millis *et al*., (2004) suggest that the variability between plants of the same species and between different plant species contributes to the overall variance in uptake of contaminants, which is a source of uncertainty in modelled predictions of plant concentration factor and human exposure. These reports provide substantiation of the variation in plant growth and uptake observed in this study, and the inferred consequences of such variation.

Results from growing plant species/varieties in a range of concentration showed a near linear positive relationship between the nominal soil Pb concentration and the plant Pb concentration for nearly all species. The shoot and root Pb concentrations (mg/kg) increased with increasing soil Pb concentration, but the concentration factor decreased as a function of increasing soil concentration, which is in line with findings of Baker *et al*., (1994). The differences in shoot and root Pb concentration were statistically significant ($P<0.05$) between and within species, which implied that uptake will vary depending on the variety/species used. However some plant species were not significantly different in their shoot and root Pb concentration. For instance the *Brassica juncea* variety BJ 18 and *Brassica napus* BN SW) had similar total plant Pb concentrations (15 to 113 mg/kg DW and 14 to 107 mg/kg DW) respectively in a range of soil Pb concentration, whilst BN SW had slightly higher biomass than BJ 18. However, clear distinctions between these two plant species were observed in further pot experiments using simplistic binary model of heterogeneity and field modelled heterogeneity (Chapters 5 and 6).

Results in Chapter 4 indicate that variations in uptake between plant species are influenced by soil Pb concentration. This might have an influence on what the actual and predicted risk to humans is from consumption of Pb contaminated food crops based on plant concentration factor. This suggests that the prediction of one concentration factor at one pH for any soil Pb concentration often assumed by generic assessment criteria for estimation of human exposure to contaminants of interest might not be correct in many cases. However, further work may be required to assess how a range of Pb soil concentration may influence plant Pb concentration and subsequently concentration factors. Such information could be useful in improving Pb uptake models used for human risk assessment and the quality of predictions made from such models.

Elevated soil Pb concentration is often thought to impair plant growth and development. In this study, the biomass of some of the plant species/ varieties were not affected in the

presence of high soil Pb concentration (with significant plant Pb concentration) showing an absence of an observable effect of Pb toxicity (e.g BJ 17 and ZM OH43 of pot trial 2). This suggests that moderately elevated soil Pb concentration may not necessarily cause poor plant growth and health in some species/varieties. This might be advantageous from the phytoremediation point of view.

The first and second pot trials were not only designed to use the variability between and within plant species/varieties to test specific hypotheses, but also to select suitable plant species for further pot trials in this overall study. They have been fit-for-purpose in selecting two *Brassica* species. However, more significant differences, and better delineation of between and within species/varieties effects, might have been obtained by increasing the number of replicates used in these first two pot trials. Never the less, results from the first and second pot trials have clearly shown that the Pb added treatments have statistically significant effects on uptake/biomass and also significant variation in these variables within and between the selected plant species/varieties.

The third objective of this thesis investigated the effect of Pb-heterogeneity on plant uptake, firstly using the simplistic binary model and subsequently with a more closely field-modelled heterogeneity, and was addressed in pot trials 3 and 4 (Chapters 5 and 6). Result from these pot trials showed that both the presence of heterogeneity and different levels of Pb heterogeneity have significant effects on plant Pb concentration, concentration factor and biomass of two plant species (*Brassica napus and Brassica juncea*). The effect of the binary simplistic model of heterogeneity varied in both species. Decreased uptake expressed as concentration in (mg/kg) and Pb mass (40 - 86%) was observed in the binary treatment over the homogeneous treatment for both species. This is in line with findings of Millis *et al*., 2004 who reported a 40% lower Cd concentration in a simplistic binary heterogeneity pot trial. However, shoot and root biomass was lower by 55 and 26 % for *B. napus* and higher by 31 and 24% for *B*. *juncea* in the binary treatment, when compared to the homogeneous treatment. Shoot uptake expressed as Pb mass (µg) was 40 to 70% higher in the homogeneous than the binary treatment of both species, whilst the root Pb mass was ~3 to 10% higher in the homogeneous as well (Chapter 5: Section 5.4).These results suggest that the homogeneous Pb distribution (mg/kg) and Pb mass (µg) may result in elevated Pb uptake for these plant species. Although better than assuming a homogeneous spatial distribution, this binary model is not very representative of the real field distribution of Pb. This may have serious implication for risk assessment models that use data from homogeneous pot trials as this

experiment suggests possible tendencies of over- prediction of risk using the homogeneous pot trial (as discussed further in Section 7.2.2).

The result of the pot trial which simulated more realistic *in situ* heterogeneity (Chapter 6) showed a different trend. Shoot and root uptake (expressed as Pb concentration mg/kg) peaked in the low heterogeneity treatment for *B*. *napus*. *Brassica juncea* had shoot and root Pb uptake peaked in the high and medium heterogeneity treatments respectively. It is interesting to know that uptake expressed in Pb mass (µg) showed different effects than that expressed as Pb concentration for both plant species. The shoot and root peak Pb mass was in the homogeneous and medium heterogeneity treatments respectively for both species. This is an indication that the increased plant yield (biomass) in the above-ground part (i.e. shoot biomass) in the homogeneous treatment have influenced the shoot Pb mass. It also suggests that *in situ* heterogeneity of Pb may produce higher root Pb mass, which could affect the overall total plant Pb mass, depending on the level of heterogeneity. In this case study, higher Pb masses were recorded in the medium heterogeneity treatments for both species.

The high heterogeneity treatment had significant decrease in shoot and root Pb mass when compared to the peak Pb mass found in the medium heterogeneity and homogeneous treatments. This might have been influenced by the root response to spatial heterogeneity, which showed that high proportion of roots are proliferated in low concentration patch. This suggests that plants with low biomass growing on contaminated land with highly heterogeneous distribution of Pb is expected to have low Pb mass. The low biomass in the high heterogeneity treatment has resulted in reduced Pb uptake expressed in terms of Pb mass. Results showed that differing levels of heterogeneity have a significant effect (between 60 and over 100%) on both uptake and biomass. This has implications for phytoremediation. Findings from this study suggest that predictions of plant uptake and plant performance for use in phytoremediation from homogeneous pot trials may not be accurate representations of plant uptake and performance under field conditions. Therefore, studies concerned with identification of suitable plant species for phytoremediation should take into consideration the spatial heterogeneity of contaminant in the field. This may be useful in making more effective selection of suitable plant species for specific contaminated sites.

The shoot concentration factor (CF_{shoot}) in all pot trials showed that about 4-5 times higher Pb was accumulated in the root than in the shoot for most plant species/varieties used.

This is in line with previous works of Baker *et al.,* (1994); Reeves and Brooks (1989). Feleafel and Mirdad, (2013), who also reported that 90% of Pb is accumulated in the root and that increasing accumulation of Pb in the roots can cause some ultra-cellular changes within plant tissues. Symptoms of such ultra-cellular changes include chlorosis, wilting and death of the plant (Feleafel and Mirdad, 2013). Severe chlorosis and wilting of leaves were observed in *B*. *napus* and some of the other species/varieties in the earlier pot trials. Similar observations were made by Thomas, (2010). However, the cause of these symptoms was not established in this study. Lead hyperaccumulation (≥1000 mg/kg Pb) was shown only in *Brassica juncea* variety BJ 21 and *Thlaspi caerulescens* variety TC BR in the first pot trial (Chapter 4: Section 4.7). Hyperaccumulator species for Pb are capable of accumulating high levels (≥1000 mg/kg) of metal in their above-ground biomass (Brooks *et al*., 1977; Baker and Brooks, 1989; Baker *et al*., 1994; Huang and Cunningham, 1996; Baker and Whiting 2002).

Results suggest that both species could exclude Pb at nearly all levels of heterogeneity to varying extents, with a peak Pb uptake in the medium and low heterogeneity treatments for *B*. *juncea and B*. *napus* respectively. It is an indication that they possess strong mechanism for excluding Pb from their tissues. The exclusion principles (Chapter 2: Section 2.5) proposed by Baker, (1981), and substantiated in later work (Mishra *et al*., 2006) describe well these findings in this thesis. The results also indicated that both plant species will actively exclude Pb from their roots in the highly heterogeneous treatment (with a decrease in CF_{root} in the HH treatment by factors of ~ 3 and 2, when compared against the peak CF_{root} in LH and MH treatments of *B*. *napus* and *B*. *juncea* respectively). A similar trend of decreased CF_{root} has been reported in earlier studies (Thomas, 2010) with *Plantago lanceolata* in Zn spatial heterogeneity treatments. However, *B*. *juncea* had higher CF_{shoot} and CF_{root} (by factors of 1.5 and 2 respectively) in the HH treatment than *B*. *napus*. This suggests that Pb can be more easily accumulated in roots of *B*. *juncea* in the HH treatment than those of *B*. *napus* in the same treatment.

Higher concentration factors suggest that these plants possess weak mechanisms to exclude heavy metals and so contaminant build up occurs in plant tissues especially in the roots), whilst lower CF, as mentioned earlier on in this discussion, suggests that plants have effective mechanisms to exclude heavy metals. Baker, (1981) described exclusion as one of plants' responses to the presence of heavy metals in the soil. Exclusion is also one of the five theories postulated (discussed in Chapter 4: Section 4.9) to explain why plants take up Pb from the soil, despite its widely proven toxicity to plants. The others include tolerance, accumulation, indication and defence mechanism against

herbivory. Plants species/varieties in this study exhibited exclusion, accumulation and tolerance to high Pb in the soil in some cases. However, *Thlaspi caerulenscens* variety TC BR exhibited tolerance and accumulation, whilst its behaviour in a Pb-added treatment was typical of an indicator species. Most of the species/varieties studied in this thesis exhibited a combination of these traits with generally observed exclusion of Pb from the shoot.

7.1.1 Spatial heterogeneity and root placement.

The earlier pot trial with *B*. *napus* and *B*. *juncea* using the simplistic binary heterogeneity investigated root distribution in the binary treatment (Chapter 5) found that 78 to 80 % of root production was in the Pb-free (0 mg/kg Pb added) patches and the other 20 to 22% in the 2000 mg/kg Pb-added patches for both plant species. This is an indication that *B*. *juncea* and *B*. *napus* were either preferentially proliferating roots in patches away from Pb contamination or that root growth was stunted in the Pb rich patches presumably due to Pb toxicity. This could explain why *B*. *juncea* had better growth and bigger biomass by 30% in the binary treatment, when compared to the homogeneous. These findings are in line with studies on Cd and Zn in simplistic binary model (Millis *et al*., 2004 and Thomas, 2010). However, this does not seem to explain the stunted growth and reduced biomass observed in *B*. *napus* binary treatment when compared to the homogeneous and control treatments. The reason for this observed stunted growth in *B*. *napus* is not known. It suggests that other factors might have influenced this species-specific difference in response to simplistic heterogeneity. For instance, root morphology (e.g the presence of tap root in *B*. *napus* and its absence in *B*. *juncea*) might have contributed to the differences in plant growth and biomass between species in the binary treatment.

An investigation of the root distribution and behaviour in the high heterogeneity treatment in the fourth pot trial which modelled field heterogeneity, also found some differences in response to more realistic heterogeneity between both species (Chapter 6).

In this study, decreased root mass was recorded in patches with high Pb concentration, when compared with patches with low Pb. This suggest preferential proliferation of roots in low Pb patch. An earlier pot experiment with *T*. *caerulescens* in Haines (2002), suggested that some plant species could discriminate between patches with different Zn-contaminant concentrations within the range of their root system. A comparison of the root biomass of the central 1000 mg/kg patch of both species shows that *B*. *napus* had 53% higher root mass in the central cell than *B*. *juncea*. This supports the fact that the higher central root biomass in *B*. *juncea*, when compared to the other patches did not

indicate a tap root as in *B*. *napus*. A network of fibrous roots were observed in *B*. *juncea* (Chapter 5: Section 5.4). This clearly supports the inference that the differential root morphology between species might have influenced root placement in patches of varied Pb concentrations.

The regression model of root Pb concentration in patches against nominal soil Pb concentration for both species showed that there was a near linear positive relationship between root Pb concentration and nominal soil Pb concentration, which implies that root Pb concentration increased as a function of soil Pb. The slope coefficient of this regression indicates that the the root-Pb concentration is present at ~9% and 15% total Pb concentration of the growth medium. The experimental determined percentage extractable Pb was 18 % of the total Pb, added as PbO (Chapter 5: Section 5.5). This suggest that *B*. *napus* might have excluded approximately half of the bioavailable Pb while *B*. *juncea* accumulated around 80% of the bioavailable Pb. However, this is a rough estimate of the plant Pb uptake. It is an indication that the bioavailable pool of contaminants in the soil may not be completely taken up by plants and the amount taken up is partially dependent on plant species. The percentage extractable Pb in this study is higher by a factor of ~2, when compared to the predicted 10% of bioavailable Pb in the soil to plants reported by Chaney *et al*., (1984), but similar to reported values of 19 and 21% by other workers (Kasheem *et al*., 2007; Anyanwu *et al*., 2008) respectively. This result suggest that percentage bioavailable Pb to plants could be higher than earlier predicted values, although there are other factors to consider. Bioavailability of Pb in soil to plants may be influenced by a number of factors. From literature, it is known that the bioavailability of Pb in soil to plant may be influenced by soil type, pH and Pb speciation (e.g. PbO in these pot trials) and of course, the estimation may also vary with the reagent used for extraction.

The differences in root behaviour to Pb spatial heterogeneity and placement of roots in a heterogeneous distribution of contaminant might also have contributed to their varied response, for example, the peak effects observed at different levels of heterogeneity. Further work in this area may provide useful insights that could help enhance the success of phytoremediation.

7.2 IMPLICATIONS AND POTENTIAL APPLICATIONS OF THE STUDY.

7.2.1 Implication for measurement of contaminant concentration in soil.

The heterogeneity factor (HF) may have useful applications in the design of cost-effective measurement strategies for contaminated land. Estimation of the effects of measurement uncertainty and *in situ* heterogeneity can be made for the interpretation of measured concentration values, which enable users of this information to assess the effects of both uncertainty of measurement and *in situ* heterogeneity. This information can be useful in risk assessment in terms of determining whether the contaminant concentration exceeds the threshold value. Prior studies (Ramsey and Argyraki, 1997; Environment Agency, 2009) have noted the importance of propagation of full uncertainty in the minimisation of the rates of false positive and false negative decisions as to whether the concentration of a contaminant {c} is greater than the threshold {T} (c >T) at any location is enhanced by estimates of full uncertainty.

Estimation of *in situ* heterogeneity can also help to gain understanding of the relationship between *in situ* heterogeneity of contaminants and uncertainty of measurement. The equation $U_{meas} = 200\ s_{meas}/\text{mean}$, can be used to show the close relationship between *in situ* heterogeneity (RSD_{samp}) and uncertainty of measurement. Ramsey *et al*., (2013) used this equation to show how both affect each other. At very low *in situ* heterogeneity, the measurement uncertainty is mainly dominated by that arising in the chemical analysis, whilst at high *in situ* heterogeneity, the measurement uncertainty is totally dominated by *in situ* heterogeneity at a particular scale of measurement (Ramsey *et al*., 2013). The analytical techniques used in this study (i.e. PXRF and AAS) can be widely applied in contamination studies with this limitation.

The specific experimental design in conjunction with the measurement technique such as the P-XRF used in the field investigation enabled the quantification of *in situ* heterogeneity over a range of scales and enhanced measurements, within a two day period. It proved to be very useful in this study. It is therefore recommended for use in routine and preliminary contaminated land investigations. This will help improve sampling strategies as the specific sampling design has been developed with no spatial bias in locating contaminant hot spots and does not require a prior knowledge of the sites for investigation (non-targeted or non-judgemental). In addition to these advantages, it adheres to the current recommendations on sampling strategies (EA, 2014) in the following ways viz (i) sampling areas are divided into definite (regular) sub-areas with

at least one sampling location within each area, (ii) It is systematic in its approach using the full balanced and simplified design (Chapter 3: Section 3.2.1).

The use of heterogeneity factor to express *in situ* heterogeneity can be regarded as new information to aid interpretations in geochemical investigations. It is recommended for use in contaminated land and geochemical investigations. It has wider application in the diagnosis of the source of contamination and mode of deposition of contaminants using the values of heterogeneity. As stated earlier on in this section (Section 7.1), high values of heterogeneity (HF>1.4) occur in sites where the contaminant sources produce spatially uneven pattern of Pb distribution such as mine waste, canal dredgings, firing range and landfill, and low values of heterogeneity (HF<1.3) occur at sites where contaminant sources result in a homogeneous distribution of Pb (e.g sewage drying pans, smelter fume or flood plains (> 20 km) downstream from Pb mine (Chapter 2: Section 2.6.2). Heterogeneity factor is also useful in improving the estimation of human exposure to contaminants and modelling of environmental processes (e.g. plant uptake), reliability of risk assessment and models.

Spatial heterogeneity has a significant impact on the reliable assessment and sampling of contaminated site investigations. As discussed earlier in this thesis, contaminated sites with highly heterogeneous distribution of Pb stand a risk of potential misclassification of land as either contaminated or not contaminated, which could generate further problems of unnecessary remediation expenses, create dispute that could require legal action on eventual finding of the true status of such land and in the case of inability to detect hot spots, risk of exposure to human.

7.2.2 Implications for human health risk assessment and phytoremediation.

A generic assessment criteria for contaminated land uses the relationship between the Pb concentrations in the soil and the plant, expressed as concentration factors, to estimate the potential human exposure and therefore the risk to human health from consumption of vegetables grown on contaminated land (DEFRA and EA, 2002c). Variations in the levels of heterogeneity of Pb has been shown in this study to have significant impact on uptake of Pb and also on plant growth. A range of plant species/varieties were used in this study, some of which are typical food crops that have been able to accumulate substantial amount of Pb in their shoots and roots above the

WHO and EU limits of Pb in food crops. This implies that these plant species may pose human health risk if they are grown on contaminated land and consumed by humans.

The World Health Organization (WHO) maximum limit of Pb in fruits and vegetables is 0.30 mg/kg FW, whilst the UK Food Standard Agency {FSA} is 0.1 mg/kg FW (CODEX, 2001; FSA, 2007). Shoot Pb concentration in this study ranged from 0.9 to 30 mg/kg FW which have exceeded these maximum limits of Pb in vegetables (3 to 100 fold higher than the World Health Organisation (WHO) limit), and 9 to 300 fold higher than the European Union (EU) limit. This supports earlier work (Moir and Thornton, 1989, Fytianos *et al*., 2001; Adekunle *et al*., 2009; Opajobi *et al*., 2011 Jiang *et al*., 2013) suggest that some temperate and tropical vegetable plant species could accumulate Pb from the soil above these limits. However, the primary route of Pb exposure to humans is via ingestion of Pb contaminated soil (ATSDR, 2012). Results from this research indicates that there could also be potential health hazard to humans via consumption of crops grown on land heavily contaminated by Pb. This in line with studies by Finster *et al*., 2003; Anyanwu *et al*., 2008; Li *et al*., 2012; Oti-Wilberforce and Nwabue, 2013a; 2013b on potential health risk from consumption of herbs, seeds and fruits of plants grown on Pb contaminated soils. Vegetables form a substantial part of man's diet, therefore the mass and frequency of consumption of contaminated vegetables may also play a role in determining the level of risk. In this study, the seeds and fruits were not assessed for their level of contamination, therefore future work may consider this aspect to evaluate the potential risk posed by the consumption of the seeds and fruits of edible plant species (e.g. rape seed oil from *Brassica napus* and mustard seeds from *B*. *juncea*).

A range of Pb concentrations were reported for different plant varieties/species used in this study. This has implications for human risk assessment. The variation between and within species/varieties could be a possible source of uncertainty in uptake models used to estimate Pb uptake from the soil for human health risk assessment. Studies concerned with the estimation of risk could consider using more consumed varieties of a particular species in plant uptake models (assuming homogeneous spatial distribution of contaminant in soil), to improve predictions for any species of interest.

This study found that spatial heterogeneity of Pb had a significant impact on plant uptake, compared against uptakes measured for homogeneous spatial distributions. This also has implications for plant uptake models used for estimation of human exposure to contaminants in risk assessment.

The results of this study suggest that uptake models based on more realistic field-modelled heterogeneity could therefore help improve the accuracy and reliability of risk

assessment models used to estimate human exposure to toxic contaminants. This was identified for Zn heterogeneity (Thomas, 2010), which supports the finding in this study, that concentration factors predicted by models that assume homogeneous distribution of contaminant in the soil are not realistic.

Realistic field-modelled heterogeneity could find useful application in phytoremediation by physically changing *in situ* heterogeneity of Pb in soil at contaminated sites prior to phytoremediation through specifically designed ploughing to mix the soil and thereby increase the mass of Pb taken up by specifically chosen plant species, thus increasing the success of phytoremediation of contaminated land based on the findings in Chapter 6: Section 6.4). The variation between – and within -plant species/varieties can be explored and harnessed to determine the most suitable varieties or species for phytoremediation of contaminated land by this approach.

Based on the results of this research, it is recommended that this new technique of assessing the impact of different levels of heterogeneity be employed in research on plant uptake of contaminants for assessment of risk to human health or for preliminary trials for other potential plants suitable for phyto-management or phytoremediation, and for a wider range of contaminants. This will be a source of tremendously useful information which could provide a wide range of plant concentrations within which on-site plant concentration might fall, especially at sites with unknown spatial distribution of the contaminant. Results for both plant species provide very strong support for growing plants in different levels of heterogeneities in pot trials and in a field trial, as a more robust way of comparing the effectiveness or efficiency of the different plant species prior to on-site phytoremediation.

CHAPTER 8: SUMMARY OF FINDINGS, SUGGESTIONS FOR FURTHER WORK AND CONCLUSION.

8.0 INTRODUCTION

This Chapter summarizes the main findings from the thesis in relation to stated aims and objectives. It also addresses the strengths, limitations, conclusion, and makes suggestions for further research work.

8.1 Summary of findings.

This thesis introduced a new way of quantifying and expressing *in situ* spatial heterogeneity of contaminant concentration as Heterogeneity Factor (HF), and applied it to Pb at two heavily contaminated sites. A previously employed site-specific sampling design, and the *in situ* measurement technique of P-XRF, were employed for the *in situ* measurement of concentrations and *in situ* heterogeneity of Pb in soils in their undisturbed state.

Thirteen varieties of six species of plant were selected to assess the impact of soil Pb on plant biomass and Pb-uptake in a first pot trial. Based on the results of this first pot trial, six species of four varieties were selected for a second pot experiment to examine this effect over a range of growth medium Pb concentrations. Results of this second pot trial were used to select two plant species for two further pot trials to investigate the effects of two models of *in situ* heterogeneity, that were initially a simplistic binary and finally a more realistic field-modelled design. A sub-experiment of the final pot trial assessed the effect of patchy distribution Pb on root growth and placement in the high heterogeneous treatment.

This section brings together the main findings and conclusions from each chapter of this thesis, in order to assess their overall significance.

- Chapters 1 and 2 identified the objectives of this research, and addressed the first objective, with an overview of soil pollution, heavy metal contamination and soil Pb contamination.
- Chapter 2 also found Derbyshire to be a suitable area for the selection of field sites, and identified *Brassica juncea*, *Brassica napus*, *Thlaspi caerulescens*, *Zea mays*, *Biden alba* and *Gentianna penneliana* as candidate species for use in the first pot trial.

Chapter 3 described the quantification of *in situ* spatial heterogeneity of Pb at two heavily contaminated sites in the United Kingdom (Gang Mine and BlackRock). The specific sampling design which comprised the balanced sampling design and the duplicate method was used in conjunction with the measurement techniques of P-XRF to quantify heterogeneity over a range of scale (0.02 to 50 m).

- The term Heterogeneity Factor (HF) was proposed as a better way to quantify *in situ* heterogeneity than the previous use of RSD. This is because HF allows for the non-normal frequency distribution of the Pb at these sites, but is equally applicable to sites that do have normal distributions. The use of HF, after a log transformation, also provided a better model of how the heterogeneity varied as a function of measurement scale.
- The field investigations established that *in situ* heterogeneity differed significantly between the two sites and also varied with spatial scale at both sites. For example, heterogeneity of Pb in Gang mine site (an abandoned Pb mine characterised with several spoil heaps of mine tailings distributed randomly around the site) was highly heterogeneous in its Pb distribution (HF values were 6-45% higher) when compared to Black Rock (abandoned Pb mine without such distribution of spoil heaps).
- Heterogeneity factor (HF) ranged from 1.17 to 2.22 in Black Rock and 1.24 to 3.22 in Gang Mine. Spatial heterogeneity changed as a function of scale. Both sites had similar frequency of distribution of Pb, with heterogeneity peaked at the highest spatial scale which was 60 to 90 % higher at the 20 m scale, compared to the 0.02 and the 2 m scale for Gang Mine and Black Rock respectively. Mean Pb concentration of contaminant also differed between sites with Black Rock having ~5 times higher mean than Gang Mine. However, there was no significant relationship between Pb heterogeneity and concentration at both sites.
- The use of heterogeneity factor (HF) to express *in situ* heterogeneity in this study has shown that heterogeneity of contaminated sites could be more accurately quantified and can be useful in improving the reliability of contaminated land investigation, risk assessment and modelling of geochemical processes such as plant uptake.

Chapter 4 presented two pot trials whose results answered their own scientific objectives, made suitable selections of plant species for further pot trials and secondly helped with the design of subsequent experiments.

- The first pot trial found that a fixed Pb concentration of 1000 mg/kg has a significant effect on biomass and Pb uptake of four *Brassica juncea* varieties (BJ 17, BJ 18, BJ 21 and BJ 42), two of *Brassica napus* (BN SW and BN K), three of *Thlaspi caerulescens*, (TC HS, TC GM and TC BR), four of Zea mays (ZM B37, ZM B73, ZM OH43 and ZM 64), *Gentianna pennelianna* and *Biden alba*. It also found ~20 to 100% variability in uptake rates between and within species comprising varieties with 10 to 70% variability in biomass from the first pot trial.
- The first pot trial selected four species of six varieties suitable Pb accumulators for the second pot trial in a range of Pb concentration based on this variability in biomass and uptake rates.

The second pot trial assessed the effect of a range of Pb concentrations on the uptake and biomass of the selected plant species.

- It found significant effect of the varied Pb concentration on these plant species with 20 to 70% variability in plant uptake and up to 60% in biomass between and within species and varieties.
- This pot trial showed that the selected plant species could thrive within this range of Pb concentration with more severe effects of the added Pb in the highest Pb concentration for most species.
- Having compared between- and within - species/varieties based on the observed variability in biomass and uptake, it found *B*. *juncea* and *B*. *napus* suitable for the subsequent pot trials using the simplistic binary and field models of heterogeneity.
- The first and second pot trials were not only fit for purpose in selecting suitable plant species for further experiments, but also provided insights on the adaptation and tolerance of these plant species to Pb added treatments at varied concentrations, which was helpful in choosing Pb concentrations in subsequent experiments.

In Chapter 5, the simplistic binary model of heterogeneity was used to assess the effect of heterogeneity on plant biomass, Pb uptake and root response to heterogeneity, compared to the homogeneous and control treatments for the two selected plant species.

- This experiment found reduced metal uptake for both species with ~ 20 to 40% increased biomass for *B*. *juncea* and ~ 30 to 60 % decreased biomass for *B*. *napus* in the binary treatment.
- For *B*. *juncea* shoot and root Pb uptake was reduced by 86 and 56 % in the binary treatment respectively compared to the homogeneous treatment. By contrast, the shoot biomass increased by 9 and 31 % and the root biomass increased by 17 and

52%, when the binary treatment was compared to the control and homogeneous treatments respectively.

- Shoot and root uptake in *B*. *napus* decreased by 37% and 58% respectively in the binary treatment, when compared to the homogeneous treatment.
- Conversely, for *B*. *napus* shoot biomass decreased by 40% and 26% and root by 61 and 26 % in the binary treatment, compared to the control and homogeneous respectively.
- Four times more roots were proliferated in the 0 mg/kg binary patches of both species which indicated an avoidance response presumably to the toxicity of Pb.

Chapter 6 addressed the effects of more realistic field-modelled heterogeneity. The experiment in Chapter 5 compared a simplistic binary model of heterogeneity to homogeneous treatment and found significant differences in plant growth and Pb uptake. However, none of these models represented the field heterogeneity found in nature and experienced by most plant species. The three models of Pb heterogeneity used in this experiment were based upon the findings of the field investigations, reported in Chapter 3. The heterogeneity design described in Chapter 6 ensured that Pb is heterogeneously distributed while the average contaminant concentration in all pots remained constant at all levels of heterogeneity, making heterogeneity as the key factor. This experiment therefore bridges the gap between the homogeneous and the simplistic binary model of heterogeneity. The same two plant species and varieties (*Brassica juncea* and *Brassica napus*) were selected for this experiment, as were used for the previous experiment (reported in Chapter 5). Results showed varied plant response to the different treatments and findings for each species is summarised below.

Brassica napus

- There were significant differences in both biomass and Pb uptake between treatments. Shoot and root dry biomass decreased with increasing heterogeneity with a peak biomass in the medium heterogeneity treatment. The shoot and root biomass in the homogeneous treatment was significantly higher (5 fold), than the high heterogeneity treatment.
- Lead uptake expressed in units of concentration (mg/kg) generally increased with increasing heterogeneity but with a peak uptake at the low heterogeneity, which was 2 times higher than that of the homogeneous treatment.
- Shoot uptake expressed in units of Pb mass (µg) decreased with increasing heterogeneity, with a peak shoot uptake in the homogeneous treatment which was

higher by a factor of 7 than the high heterogeneity treatment. This leads to the suggestion (In Section 7.2.2) that the mixing of soil *in situ* by ploughing may increase the efficiency of phytoremedation by this species at the concentration of Pb in this pot trial.

- However, root uptake expressed in Pb mass (µg) increased with increasing heterogeneity with a maximum root uptake in the medium heterogeneity treatment which was 2 and 6 fold higher than the homogeneous and high heterogeneity treatments respectively. Results suggest that realistic spatial heterogeneity of Pb is a significant factor influencing growth and Pb uptake for this species.

Brassica juncea

- Similarly, significant differences in both biomass and Pb uptake were also found between treatments for *B juncea*.
- Shoot and root biomass decreased with increasing heterogeneity with maximum biomass in the homogeneous treatment which was 4 fold higher than the high heterogeneity treatment.
- Uptake expressed as concentration (mg/kg) increased with increasing heterogeneity with peak uptake in the high heterogeneity treatment for the shoot and medium heterogeneity for the root, which were twice as high as the homogeneous treatment.
- Shoot Pb uptake expressed as Pb mass (µg) decreased with increasing heterogeneity with a peak shoot Pb mass in the homogeneous treatment, which was about twice as high as the high heterogeneity treatment. Similarly, this leads to a suggestion that ploughing contaminated sites to reduce heterogeneity should increase the amount of Pb removed by this plant species (Section 7.2.2).
- However, the root Pb mass increased with increasing heterogeneity with a peak Pb mass in the medium heterogeneity treatment. Results also suggest that spatial heterogeneity of Pb is also a fundamental factor affecting plant growth and uptake of Pb in this species.
- The impact on both species is an indication that site specific heterogeneity is an important factor in producing reliable estimates of Pb uptake and growth compared to the homogeneous and binary simplistic model.
- However, both plant species differ in morphology and size, both were affected by Pb heterogeneity to differing extent and peak effect was maximum at different levels of heterogeneity (Table 8.1.1 below summarises the similarities and differences between the two plant species in terms of the effects of realistic *in situ* heterogeneity). This suggest that the effect of Pb heterogeneity is plant specific. It implies that results

will vary for other different plant species and at different concentration of Pb potentially.

Table 8.1.1: Summary of the similarities and differences between *B. napus* and *B. juncea* on the effect of realistic in situ heterogeneity.

B. napus	***B. juncea***
• **Biomass** decreased with increasing heterogeneity with the exception of the MH treatment.	• **Biomass** decreased with increasing heterogeneity without an exception.
• Pb uptake (mg/kg) increased with increasing heterogeneity with a **peak uptake in LH** treatment for shoot and root by a factor ~2, compared to the HO treatment.	• Increased Pb uptake (mg/kg) with increasing heterogeneity with a peak uptake in the **HH treatment for shoot** and **MH treatment for the root** by a factor of ~2, compared to the HO treatment.
• Had **~5 times** lower biomass in **HH** than **HO**	• Had **4 times** lower biomass in **HH** than **HO**.
• **Root uptake expressed as Pb concentration** (mg/kg) was~ 2 times lower than that of *B. juncea* in the HH treatment.	• **Root uptake expressed as Pb concentration (mg/kg**) was ~2 times higher than *B. napus* in the HH treatment.
• **Shoot uptake expressed as Pb mass (µg**) decreased with increasing heterogeneity with a **peak** shoot Pb mass in the **HO** treatment.	• **Shoot uptake expressed as Pb mass (µg**) decreased with increasing heterogeneity with a **peak** shoot Pb mass in the **HO** treatment.
• **Root uptake expressed as Pb mass (µg)** increased with increasing heterogeneity with a **peak** root Pb mass in the **MH** treatments.	• Increased **root Pb mass (µg)** with increasing heterogeneity with a **peak** Pb mass in the **MH** treatment.
• Shoot and root Pb mass **(µg**) was approximately **50% lower** in the **HH** treatment, compared to HO.	• Shoot and root Pb mass **(µg) approximately 20 to 70% lower** in the **HH** treatment, compared to HO.

Root Response of both species to Pb heterogeneity.

The root placement investigation was a sub-experiment in Chapter 6 which addressed one of the objectives of the pot trial simulating *in situ* heterogeneity of Pb.

- An earlier experiment in Chapter 5 with the simplistic binary model of heterogeneity showed that roots of both species had root ball diameter in the 0 mg/kg quadrant twice as large as that of the 2000 mg/kg quadrant of the binary treatment which suggest a change in root morphology in response to Pb distribution in the binary treatment.
- The simplistic binary experiment found differing root morphology in both species with *B*. *napus* having four times as large root ball diameter as *B*. *juncea* in both binary quadrants. For example, a tap root was observed in B. *napus*, whilst *B*. *juncea* had no tap root, but a complex network of fibrous roots.
- The root placement experiment in the high heterogeneity treatment found similar morphology (presence of tap root in *B*. *napus* and absence in *B*. *juncea*).
- There was a definite pattern of root response typically of decreased root mass to increasing patch soil Pb concentration.
- However, the proportion of roots proliferated in concentric patches (outer, middle and central) differ significantly between species and in most cases between patches.
- Results suggest that Pb heterogeneity has a significant effect on root proliferation in heterogeneous environment and provided insights into these species behaviour in heterogeneous patchy distribution of Pb by proliferation of more roots in patches with low Pb concentration.

8.2 Suggestions for further work.

Having discussed the implications and potential applications of this research (Chapter 7: Section 7.2), this section makes suggestions for further work on possible aspects of future research.

1. This study did not quantify Pb heterogeneity at the micro scale. **Further work could be developed to overcome this limitation (e.g. going down to lower scales of heterogeneity with micro scale measurement) with comparable data quality (precision and bias) to the macro scale measurement used in this study (with the P-XRF).** This will be useful to see if linear function continues down to smaller scales.
2. **Spatial patterns of different contaminants (e.g. Cr, Ni, As etc) can be used for quantification of their *in situ* heterogeneity**. This will be enormously useful in building predictive models of geochemical processes and in the remediation of contaminated land. Further research is required in quantification of *in situ* heterogeneity both at macro, micro and nano scales for different elements and not just for Pb. Other effects and uses of *in situ* heterogeneity may well be discovered as more values of heterogeneity are reported.
3. One of the pot trials assessed the effect of Pb heterogeneity on biomass and plant uptake of Pb. Lead is one of the toxic contaminants that can be taken up by plants from the soil. There is a need for further work on **the effect of *in situ* heterogeneity of other contaminants in soil on plant uptake** either as individual study or a combination of contaminants in soil as these metals do not often exist in isolation in field scenario. The antagonistic and synergistic effects of such contaminants could be studied to improve the understanding of their geochemical pathway. Studies can also be expanded to essential trace elements or nutrients necessary for plant growth taking advantage of plant root response. This will be useful in increasing the uptake of such essential elements in food crops and vegetable plant species with the aim of improving human and animal dietary needs for essential microelements.
4. The first and the second pot trials which assessed the effect of a fixed and range of Pb concentrations on uptake and biomass of selected plant species also compared uptake rates between and within species/varieties. Further experiments in this area can **explore such intra and inter–specific comparison to select food plant varieties with low Pb uptake and high metal excluding potential**.

Such information will be useful to farmers and vegetable growers in highly contaminated regions of the world where immediate remediation is not imminent to ensure that varieties cultivated are safer for human and animal consumption.

5. This research has shown that spatial heterogeneity of Pb has significant impact on plant uptake of Pb. Further work could seek to **incorporate Pb heterogeneity parameter into the generic assessment criteria used to estimate the risk of Pb to human health** from consumption of food crops and vegetables which assumed homogeneity in concentration of Pb and other potentially toxic heavy metals. This will be useful in improving the reliability of future estimates of human exposure to Pb and other contaminants of interest.
6. The pot experiments did not **explore the physiological and biochemical basis of varied plant responses to heterogeneity**. This is an interesting interdisciplinary area of research that could provide further insights into varied plant response and unravel the internal mechanisms behind plant behaviour to metal heterogeneity.
7. The root placement experiment needs to be expanded to include other levels of heterogeneity in future work. This is to ensure that root responses in the other levels of heterogeneity are explored to provide supporting evidence for results found in the high heterogeneity treatment. This will be useful in understanding further the concept of root response to contaminant heterogeneity. Developing experimental techniques such as neutron radiography, tomography and image processing tools should be used in future experiments that will **enhance the study of whole root system in different levels of heterogeneity to obtain qualitative data** useful in making accurate whole root system model in contaminant transport.
8. Further work with contaminants other than Pb should incorporate the site-specific geochemical mineral phases and speciation of such elements. This might provide insights into **link between mineral phases of the different contaminants, spatial distribution in the soil and behaviour during uptake by plants**.
9. Heterogeneity in the pot trials undertaken in this research reflect the small volume of soil in the pot that can be potentially seen by plants roots. It might be worth **comparing future work in practical field trials with results obtained from pot trials.** Field trials might allow future research to explore effects of larger scales of heterogeneity and the potentials of using larger plant species (e.g using trees for metal uptake). **Ploughing of experimental field sites could be used to reduce heterogeneity in the field, as a control to such experiment**. Such ploughing could also provide homogeneous field condition for plants species,

whose Pb uptake is maximum or enhanced in homogeneous media, thus improving the success of phytoextraction.

10. This research explored the effect of spatial heterogeneity at an average Pb concentration of 1000 mg/kg. **Further work will be required in higher average Pb concentrations (e.g 5000 mg/kg and above).** This will be useful in assessing the effects of Pb spatial heterogeneity at higher concentrations, which could form a very vital stage in preliminary investigations prior to phytoremediation.
11. The field modelled heterogeneity pot trial used two species *B*. *napus* and *B*. *juncea* which showed species-specific differences in response to heterogeneity. **Future studies could explore a wide range of plant species**. Results from such research could be used in building geochemical baseline data on plant uptake (that incorporate metal heterogeneity) for many plant species, which will be tremendously useful in improving geochemical models of plant uptake for risk assessment, phyto-management and phytoremediation.
12. An observation made during the growing season of the field-modelled heterogeneity experiment showed that flowering time was delayed in *B*. *juncea* in the homogeneous treatment, but earlier in the heterogeneity treatments especially in the high heterogeneity treatment, whilst *B*. *napus* never flowered. This suggest a probable link between contaminant heterogeneity and reproductive stages of these plant species. **Future work might be required to investigate and validate this potential link**. Knowledge from such studies will provide insights on how contaminants interfere with plants reproduction and consequently potential contamination of seeds and fruits produced, which are consumed by humans. Such knowledge will be useful in improving plant yield and quality of farm products.
13. The variation in Pb masses (µg) between treatments of both species show that plant biomass could influence the amount of Pb uptake, consequently affecting their selection for phytoremediation. **Future research work could look at ways of increasing biomass of these plant species (e.g exploring the biomass increasing potential of mycorrhizae in roots of *B*. *juncea* and some other plant species),** thus improving their efficiency for use in phytoremediation.

In conclusion, this study has evidently demonstrated that spatial heterogeneity of Pb plays a key role in plant Pb uptake and growth. Future research on plant uptake should adopt the realistic heterogeneity models.

References.

Abdul, H.B. & Bivin, V.T. (2009). Translocation and bioaccumulation of Trace metals in deserts plants of Kuwait Governorates. *Research Journal of Environmental Sciences* 3: 581-587.

Adekunle, I.M., Olorundare, O. & Nwange, C. (2009). Assessments of lead levels and daily intakes from green leafy vegetables of Southwest Nigeria*. Nutrition and Food Science*, 39 (4): 413-422.

Agency for Toxic Substance and Disease Registry (ATSDR) (2012). Lead toxicity. *Case Studies in Environmental Medicine* WB 11O5:3-18.

Agency for Toxic Substances and Disease Registry (ATSDR) (1988).The nature and extent of lead poisoning in children in the United States. *Congress Report*.

Agency for Toxic Substances and Disease Registry (ATSDR) (1990). Case studies in Environmental Medicine: Lead toxicity. *ATSDR* 16.

Agency for Toxic Substances and Diseases Registry (ATSDR) (2007). Priority List of Hazardous Substances. Available at: http://www.atsdr.cdc.gov/ (Date retrieved 8th August 2012).

Agency or toxic substances and Disease Registry (ATSDR) (1992).Toxicological profile for lead. *ATSDR* 17: 88.

Ahmed, M., Singh, V.K. & Upadhyay, R.S. (2012). *Brassica* rhizosphere-microbe interactions and their role in phytoremediation In Anjum, N.A., Ahmad, I., Pejeira, M.E., Duarte, A.C., Umar, S. & Khan, M.C. Eds. *The plant family Brassicaceae-Contributions towards phytoremediation*. Springer Dordrecht Heidelberg, New York. PP 139-152.

Airst, R.L. (1996). Turning Brown fields to Greenbacks. *Environmental Protection* 28-35.

Akinci, E.I., Akinci, S. & Yilmaz, K. (2010). Response of tomato (*Solanum lycopersicum* L.) to lead toxicity: Growth, element uptake, chlorophyll and water content. *African Journal of Agricultural Research* 5(6): 416-423.

Albert, M.R. (2000).Notes on current techniques in modeling spatial heterogeneity. *57th Eastern Snow Conference* Syracuse, New York USA.

Aldler, T. (1996). Botanical clean-up Crews. *Science News* 150:42-43.

Allen, D.A. & Hattfield, G. (2004). *Medicinal plants in folk tradition: An Ethnobotany of Britain and Ireland*. Timber Press, Cambridge.

Alloway, .B.J (1990). Introduction In: B.J. Alloway (Ed). *Heavy metals in soils*. Blackie Academic Professional.

Alpert, P. & Stuefer, .J.F. (1997). Division of labour in clonal plants In: *The ecology and evolution of clonal plants*. Backhuys Publishers, Leiden, The Netherlands pp137-154.

AMC {Analytical Methods Community}, (1989) Robust statistics-how not to reject outliers-Part 1. Basic concept. *Analyst* 114:1693-1697.

AMC {Analytical Methods Community}, (1995). Uncertainty of Measurement Implications of Its Use in Analytical Science. *Analyst* 120: 2303-2308.

AMC {Analytical Methods Community}, (2009). Portable X-ray fluorescence analysis. *AMC Technical Briefs*. Analytical Methods Committee AMCTB No. 41.

Ander, E.L., Johnson, C.C., Cave, M.R., Palumbo-Roe, B., Nathanail, P. & Lark, R.M. (2013). Methodology for the determination of normal background concentrations of contaminants in English Soil. *Science of the Total Environment*. 454-455:604-618.

Anderson, C.W.N., Brooks, R.R., Chiarucci, A., LaCoste, C.J., Leblanc, M., Robinson, B.H., Simcock, R. & Stewart, R.B. (1999). Phytomining for nickel, thallium and gold. *Journal of Geochemical Exploration.* 67: 407-415.

Andra, S.S., Datta, R., Sarkar, D., Makris, K.C, Mullens, C.P., Sahi, S.V. & Bach, S.B.H. (2009). Induction of lead binding phytochelatins in vetiver grass (*Vetiveria zizanioides* L.) Using liquid chromatography and mass spectrometry. *Environmental Pollution* 157(7): 2173-2183.

Andra, S.S., Datta, R., Sarkar, D., Makris, K.C, Mullens, C.P., Sahi, S.V. & Bach, S.B.H.(2010a).Synthesis of phytochelatins in vetiver grass upon lead exposure in the presence of phosphorus. *Plant and Soil* 326 (1-2): 171-185.

Andra, S.S., Sarkar, D. Datta, R. & Saminathan, S. (2010b) .Chelant –assisted phytostabilization of paint- contaminated residential soils. *Clean Soil, Air Water* 38 (9): 803.

Andra, S.S., Sarkar, D., Datta, R. & Saminathan, S. (2006). Lead in soils in paints contaminated residential sites at San Antonio, Texas, and Baltimore, Maryland*. Bullettin of Environmental contamination and Toxicology* 77(5): 643-650.

Andra, S.S., Sarkar, D., Datta, R. & Saminathan, S. (2011).Predicting potentially plant – available lead in contaminated residential sites. *Environmental Monitoring and Assessment* 175(1-4):661-676.

Anjum, N.A., Ahmad, I., Pejeira, M.E., Duarte, A.C., Umar, S. & Khan, M.C (2012). *The plant family Brassicaceae-Contributions towards phytoremediation*. Springer Dordrecht Heidelberg, New York. PP 2-18.

Antiocha, R., Campanella, L., Ghezzi, P. & Movassaghi, K. (2007). The use of vetiver for remediation of heavy metal soil contamination. *Analytical and Bioanalytical Chemistry* 388(4):947-956.

Anyanwu, E.C., Ijeoma, K., Ehiri, J.E & Saleh, M.A (2008). Bioavailable lead concentration in vegetable plants grown on soil from reclaimed industrial site: health implications*. Internet Journal of Food Safety*.6:31-34.

Argyraki, A. (1997). Estimation of Measurement Uncertainty in the Sampling of Contaminated Land. Ph.D. Thesis. Department of Environmental Science and Technology, Imperial College, London. *British Library EthOS*.

Argyraki, A. (2014). Garden soil and house dust as exposure media for lead uptake in the mining village of Stratoni, Greece. *Environmental Geochemistry and Health* 36:677-692.

Argyraki, A., Ramsey, M.H. & Potts, P. (1997). Evaluation of portable X-ray Fluorescence Instrumentation for in situ measurements of lead on contaminated land.*The Analyst* 122:743-749.

Ashagre, H., Derara, A. & Tesfaye, F. (2013). Effect of copper and zinc on seed germination, phytotoxicity, tolerance and seedling vigor of tomato (*Lycopersicon esculentum*) L. cultivar Roman VF. International Journal of Agricultural Science Research 2(11):312-317.

Assche, F. & Clisjster, .H. (1990). Effects of metals on enzyme activity in plants. *Plant Cell Environment* 24:1-15.

Atila, A. & Mathe-Gaspar, G. (2005). Factors affecting heavy metal uptake in plant selection for phytoremediation. *Naturforsch* 60c:244.

Audet, P. & Charcrest, C. (2007). "Heavy metal phytoremediation from a meta- analytical perspective." *Environmental Pollution,* 147(1): 231-237.

Aziz, R.A., Kamarudzaman, A.N., Kamaruddin, N.A., & Salleh, M.N. (2011).Study on phytoremediation and heavy metals uptake in leacheate by reed beds plan. Proc. Int. Conf. Environ. *Biomedical. Biotechnology* 16:47-51.

Babel, S. & Kurniawan, T.A (2003). Low cost adsorbents for heavy metals uptake from contaminated water: a review. *Journal of Hazardous Materials* 97(1):219-243.

Bacic, A., Fincher, G.B. & Stone, B.A. (2009). *Chemistry, biochemistry and biology of 1-3 Beta glucans and related polysachharides*. San Diego, CA: Elsevier Science. 23- 47.

Baker, A.J.M & Whiting, S.N. (2002). In search of the holy grail- a further step in understanding metal hyperaccumulation. *The New Phytologist* 155:1-7.

Baker, A.J.M. & Walker, P.L. (1989). Eco-physiology of metal uptake by tolerant plants In: *Heavy metal Tolerance in plants- Evolutionary aspects* Shaw, A. (ed) CRC Press pp 155-177.

Baker, A.J.M. & Whiting, S.N. (2002). In search of the holy grail- a further step in understanding metal hyperaccumulation. *The New Phytologist,* 155:1-7.

Baker, A.J.M. (1981). Accumulators and Exuders-strategies in response of plants to heavy metals. *Journal of Plant Nutrition,* 3: 643-654.

Baker, A.J.M., & R.R. Brooks. (1989). Terrestrial higher plants which hyperaccumulate metallic elements: A review of their distribution, ecology and phytochemistry. *Biorecovery* 1:81-126.

Baker, A.J.M., McGrath, S.P., Reeves, R.R. & Smith, J.AC. (2000). Metal hyperaccumulator plants: a review of ecology and physiology of biological resource for phytoremediation of metal polluted soils In: Terry, N., Banuelos, G. (Eds), *Phytoremediation of contaminated soil and water*. Lewis Publishers, Florida pp 85-107.

Baker, A.J.M., Reeves, R.D & Hajar, A.S.M (1994). Heavy metal accumulation and tolerance in British Populations of the metallophyte *Thlaspi caerulescens* J&C.Presl (*Brassicaceae*). *New Phytologist* 127:61-68.

Baker, AJ.M. (1978). Ecophysiological aspects of zinc tolerance in *silene maritime* with. *New Phytol*. 80:635-642.

Banuelos, G.S., Ajwa, H.A., Wu, L. & Zambrzuski, S. (1998). Selenium accumulation by *Brassica napus* grown in Se-laden soil from different depths of Kesterson Reservoir. *Journal of Soil Contamination*, 7(4):481-496.

Banuelos, G.S., Ajwa, S.H., Terry, N. & Downey, S. (1997). Phytoremediation of Selenium –laden Effluent. *Fourth International in situ and on-site Bioremediation Symposium*, New Orleans, LA 3: 303.

Barber, S.A & Silverbrush, M. (1984).Plant root morphology and nutrient uptake. *In Roots, nutrient and water influx, and plant growth*. Eds. Soil Science Society of America. Crop Science Society of America and American Society of Agronomy. Madison 65-86.

Barkerly, J.B. (1978). Lead nitrate as an oxidizer in blackpowder. *Pyrotechnica Publications* IV: 16-18.

Barnajee, S.B.P., Carlin, A. & Gelfand, A.E. (2004). *Hierarchical Modeling and Analysis for Spatial Data*. Chapman and Hall/ CRC Press.

Barry, S.A.S. & Clark, S.C. (1978). Problems of interpreting the relationship between the amounts of lead and Zinc in plants and soil in metalliferous wastes. *The New Phytologist* 81:773-783.

Bassirirad, H. (2000). Kinetics of Nutrient uptake by roots responses to global change. *New Phytologist* 147: 155-169.

Basta, N.T., Ryan, J.A. & Chaney, R.L. (2005). Trace element Chemistry in residual-treated soil: Key concepts and metal bioavailability. *Journal of Environmental Quality* 34:49-63.

Bennett, L.E., Burkhead, J.L., Hole, K.L., Terry, N. & Pilon-Smits, E.A.H (2003). Analysis of transgenic Indian mustard plants for phytoremediation of metal contaminated mine tailings. *Journal of Environmental Quality* 32:432-440.

Bert, V. Macnair, M.R., De Langurie, P., Saumitoi-Laprade, P. & Petit, .D. (2000). Zinc tolerance and accumulation in metallicolous and non-metallicolous populations of *Arabidopsis halleri* (Brassicaeae). *New Phytologist* 146: 225-233.

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

Birch, C.P.D & Hutchings, M.J (1994).Exploitation of patchily distributed soil resources. *Journal of Ecology* 82:653-664.

Birkefield, A., Schulin, R., & Nowack, B. (2007). In situ transformations of fine lead oxide particles in different soils. Environmental Pollution 145:554-561.

Bisby, F.A., Roskov, M.A, Ruggiero, T.M., Orell, L.E., Paglinawan, P.W., Brewer, N. & Bailly, J. van Hertum (Eds) (2007). *Species 2000&ITIS Catalogue of life: 2007 Annual Checklist*. Species 2000: Reading, U.K.

Blacksmith Institute {BI} (2011). UNICEF programme Cooperation Agreement. Environmental Remediation—Lead poisoning in Zamfara. *Final Report*. Pp 57.

Blake, R.C, Choate, D.M, Bardihan, S., Revis, N., Barton, L.L., & Zocco, T.G. (1993). Chemical transformation of toxic metals by a pseudomonas strain from a toxic waste site. *Environ. Toxic and Chem*. 12: 1365-1376.

Blaylock, M.J. & Huang, J.W. (2000). Phytoextraction of metals In: *Phytoremediation of toxic metals using plants to clean the environment* (Eds) Raskin, I., Ensley, B.D. John Wiley and Sons. Inc. New York. PP 53-74.

Body, P.E., Dolan, P.R., & Mulcahy, D.E. (1991). Environmental lead –A review. *Critical Reviews in Environmental Control* 20:229-310.

Bolviken, B., Stokke, P.R., Feder, J. & Jossang, T. (1992). The fractal nature of geochemical landscapes. *Journal of Geochemical Exploration* 43(2):91-109.

Bondada, B.R., Underhill, R.S., Ma, L.Q., Guyodo, Y., Mikhaylova, A., Davidson, M.R., & Duran, R.S. (2007). Spatial distribution, localization, and speciation of arsenic in the hyperaccumulating fern *Pteris vittata* L. *Trace Metals and other Contaminants in the Environment*, 9: 299-313

Boon, K. (2006). Optimisation of investigation Strategies for contaminated land. PhD Thesis. Department of Biology and Environment Sciences, University of Sussex. *Sussex Research Online.*

Boonyapookama, B., Parkian, P., Techapinyawat, S., Delaune, R.D. & Jugsujinda, .A. (2005). Phytoaccumulation of lead by sunflower (*Helianthus annus*), Tobacco (*Nicotiana tobacum*) and Vetiver (*Vetiveira zizanoides*). *Journal of Environmental Science Health* 40:117-137.

Bothe, H., Regvar, M. & Turnau, K. (2010). Arbuscular mycorrhiza, heavy metal and tolerance In: Sherameti, I., Varma, .A (Eds) *Soil Heavy Metals*. Springer, Heidelberg, pp 87-111.

Bowen, H.J.M. (1979). *Environmental Chemistry of the Elements*. Academic Press, New York. 333.

Brady, N.C. & Weil, R.R. (1999). *The nature and properties of soils*. Prentice Hall, Inc. Upper Saddle River, New Jersey.

Brennan, M.A. & Shelley, M.L (1999). A model of the uptake, translocation and accumulation of lead (Pb) by maize for the purpose of phytoextraction. *Ecol. Eng* 12: 271-272.

British Geological Survey (BGS) (2012). *The Advanced Soil Geochemical Atlas of England and Wales*. NERC PP100-102.

Brooks, R.R. (1994). *Plants that hyperaccumulate Heavy metals* In Farago, M.E. (1994). Eds. *Plants and the chemical elements: biochemistry, uptake, tolerance and toxicity*. New York: Basel: VCH Weinheim.Pp 90-102.

Brooks, R.R. (1998). Phytochemistry of hyperaccumulators In: *Plants that accumulate heavy metals*. CAB International, Wallingford 15-53.

Brooks, R.R., Anderson, C., Stewart, R.B. & Robinson, B.H. (1999). Phytomining: growing a crop of a metal. *Biologist* 46(5): 201-205.

Brooks, R.R., Chambers, M.F., Nicks, L.J. & Robinson, .B.H. (1998). Phytomining. *Trends in Plant Science* 3(9): 359-362.

Brooks, R.R., Lee, J., Reeves, R.D & Jaffre, T. (1977). Detection of nickeliferous rocks by analysis of herbarium species of indicator plants. *Journal of Geochemistry Exploration,* 7:49-57.

Brown, G. (1995).How do earthworms affect microflora and fauna community diversity? Plant Soil 170:209-231.

Brown, S.L., Chaney, R.L, Angle, J.S. & Baker, A.J.M. (1995a).Phytoremediation potential of *Thlaspi caerulescens* and bladder campion for zinc and cadmium contaminated soil. *Journal of Soil Science Society of America* 59:125-133.

Brown, S.L., R. Chaney, J.S. Angle, & A.J.M. Baker (1995b). Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris* grown on sludge-amended soils. *Environ. Sci. Technol*. 29:1

Brunoi, C.L., Galleti, M. Cremishi, C. & Morabito, R. (2004).Comparison of three sequential extraction procedures (original and modified steps BCR procedure) applied to sediments of different origins. *Ann. Chimm*.94:409-419.

Buchauer, M.J. (1993). Contamination of soil and vegetation near a zinc smelter by Zinc, Cadmium and Lead. *Environmental Science and Technology* 17:121-123.

Burkarf, M.R. & Kolpin, D.W. (1993). Hydrologic and land-use factors associated with herbicides and nitrates in near- surface aquifers. *Journal of Environmental Quality* 646-656:22.

Buszewski, B., Jastrzebska, T., & Kowalkowski, A.G. (2000). Monitoring of Selected Heavy Metals Uptake by Plants and Soils in the Area of Torun, Poland. *Polish Journal of Environmental Studies* 9(6):511-515.

Caldwell, M.M. (1994). Exploiting nutrients in fertile soil microsites In. Caldwell, M.M. Pearcy, R.W eds. *Exploitation of environmental heterogeneity of plants*. New York, NY, USA: Academic Press, 325-347.

Canfield, R., Henderson, C., Slechta, D., Cox, C., Jusko, T. & Lanphear, B. (2003). Intellectual impairment in Children with 10µg per Decilitre. *The New England Journal of Medicine* 348 (16): 1517-1526.

Carlson, R.W. & Bazzaz, F.A. (1997). Growth of rye grass and fescue as affected by lead-cadmium –fertilizer interaction. *Journal of Environmental Quality* 8:348-352.

Catmak, I., Ozturk, L., Karanlik, S., Marschner, H. & Ekiz, H. (1996a). Zinc –efficient wild grasses enhanced release of phytosiderophore under Zn deficiency*. Plant Nutrition* 19:551-563.

Catmak, I., Sari, N., Marschner, H., Ekiz, H. & Kalayi, M. (1996b). Phytosiderophore release in bread and durum – what genotypes differing in Zinc deficiency. *Plant Soil* 180: 183-189.

Centers for Disease Control and Prevention {CDC} (2005b). Third National Report on Human Exposure to Environmental Chemicals. Atlanta Georgia. PP 12.

Centre for Disease Control (CDC) (1991). Preventing lead poisoning in young children. *Fact Sheet 91*.

Centre for Disease Control (CDC) (2005a). Blood lead levels- United States, 1999-2002.Centre for Disease Control and Prevention. *MMWR Morb. Mortal Wkly Rep* 54 (20): 513-516.

Centre for Disease Control (CDC) (2007 Lead exposure among child bearing age-United States. *MMWR Morb Mortal Wkly Rep* 56 (16):397-400.

Chandrasekaran, S. & Swamy, P.S. (2010). Growth Patterns of *Chromolaena odorata* in varied ecosystems at Kodayar in the Western Ghats, India. *Acta Oecologica* 36:383-392.

Chaney, R. L., Sterrett S. B. & Mielke, H. W. (1984).The potential for heavy metal exposure from urban gardens and soils. Proceedings of the Synopsium on Heavy Metals in Urban Gardens, Washington D.C: Agricultural Experiment Station, University of District of Columbia.

Chaney, R.L. (1983). Plant uptake of inorganic waste constitutes In: Parr, J.F, Marsh, P.B., Kla, J.M. eds. *Land treatment of Harzardous Wastes*. Park Ridge, NJ: Noyes Data Corp.50-76.

Chaney, R.L., Malik, M., Li, Y.M., Brown, S.L., Angle, J.S & Baker, A.J.M (1997).Phytoremediation of soil metals. *Current Opinions in Biotechnology* 8:279-284.

Chao, W., Chen-Xiao, L., Li-min, Z., Pei-fang, W. & Zhi-yong, G. (2007). Lead, copper, zinc and nickel in vegetables in relation to their extractable fractions in soils in suburban areas of Nanjing, China. *Polish Journal of Environmental Studies*, 16(20):199-207.

Chao-Wen, C., Yun-Wei, Y., Hur-Sheng, L., Yeou-Guang, T. & Men-Chi, C. (2006). A novel function of abscisic acid in the regulation of rice (*Oryza sativa* L.) root growth and development. *Plant Cell Physiology* 47(1):1-13.

Chardin, H. Mayer, C., Senechal, H., Tepfer, M., Desvaux, F.X. & Peltre, G. (2001). Characterization of high-molecular-mass allergens in oilseed rape pollen. *International Archives of Allergy and Immunology 125 (2):128-134.*

Chardon, E.S, Bosbach, D. & Bryan, N.D (2008). Reactions of the Feldspar surface with metal ions: Sorption of Pb^{2+}, U^{4+}, N^{5+} and surface analytical studies of reaction with Pb^{2+} andU^{4+}.*Geochim Cosmochim Acta* 72:288-297.

Charles, X. (1992). Impact of Lead –contaminated Soil on Public Health. *US Department of Health and Human Services*. Atlanta, Georgia 30333.

Chaudhry, Q., Blom-Zandstra, M., Gupta, S. & Joner, E.J. (2005). Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the Environment. *Environmental Science and Pollution Research* 12 (1): 34-48.

Chemical and Environmental Sampling (CEN) (2005). Quality through Accreditation, Certification and Industrial Standards. *CEN Trend Analysis Workshop*. 1-32.

Chen, H. & Cutright, T. (2001). EDTA and HEDTA effects on Cd, Cr and Ni uptake by *Helianthus annus*. *Chemophere* 45(1):21-28.

Chen, Y., Shen, Z. & Liu, X. (2004). The use of Vetiver grass (*Vetiveria zizanoides*) in the phytoremediation of soils contaminated with heavy metals. *Applied Geochemistry* 19: 1553-1565.

Cheng, B.T. (1977). Soil organic matter as a plant nutrient In: Organic Matter Studies. *Proc. Ser. Soil IAEG*, Vienna 31.

Cheng, S.E., Huang, C.Y., Lin, Y.C., Lin, S.C. & Chen, K.L. (2015) Phytoremediation of lead using corn in contaminated agricultural land- An *in situ* study and benefit. *Ecotoxicology and Environmental Safety* 111:72-77.

Cheng, Y., Schwartz, J. & Sparrow, D. (2001).Bone lead and blood lead levels in relation to baseline blood pressure and the prospective development of hypertension. *American Journal of Epidemiology* 153 (2):164-171.

Cheng, Y., Schwartz, J. & Vokonas, P.S. (1998). Elecrocardiographic conduction disturbances in association with low level lead exposure. *American Journal of Cardiography* 82: 594-599.

Cherepanov, S.K. (1995). *Vascular plants of Russia and Neighbouring Countries*. St. Petersburg 732-733.

Chhotu, D.J. & Fulekar, M.H. (2009). Phytoremediation of heavy metals: recent techniques. *African Journal of Biotechnology* 8(6): 921-928.

Chimbira, C. & Moyo, .D.Z. (2009). The effect of single and mixed treatments of lead and cadmium on soil bioavailability and yield of *Brassica napus* irrigated with sewage effluent: A potential human risk. *African Journal of Agricultural Research* 4(4): 359-364.

Christina, G., Marsili-libelli, S., Baker, A. & Gabrielli, .R. (2000).Assessing plant phytoextraction potential through Mathematical modeling. *International Journal of Phytoremediation* 2(4): 343.

Clark, S., W. Menrath, M. Chen, S. Roda, & Succop, P. (1999). Use of a field portable x-ray fluorescence analyzer to determine the concentration of lead and other metals in soil samples. *Ann Agric Environ Med*. 6: 27-32.

Clavenger, T.E., Sawin, C. & Koirtyoham, S.R. (1991). Le speciation of particles on air filters collected in the vicinity of a lead smelter. *Environ. Sci. Technology* 25:1128-1133.

Cleal, Q.A. (1994) Soil geochemistry in relation to the distribution of metallophyte angiosperms and lichens. *Ph.D. Thesis*. University of Leeds. British Library ETHOS.

Clemente, R., Walker, D.J. & Bernal, M.P. (2005). Uptake of heavy metals and As by *Brassica juncea* grown in a contaminated soil in Aznalcollar (Spain): The effect of soil amendments. *Environmental Pollution* 138(1): 46-58.

Clemente, S., Paredes, C. & Bernal, M.P. (2007). A field experiment investigating the effects of olive husks and cow manure on heavy metal availability in a contaminated calcareous soil from Murcia (Spain). *Agric. Ecosys. Environ.* 118: 319-326.

Codex Alimentarius Commission [FAO/WHO]. (2001). Food additives and contaminants. Joint FAO/WHO Food Standards Program; ALINORM 01/12A, 1-289.

Cressie, N. (1993). *Statistics for Spatial Data*. John Willey, New York, USA.

Crook, M.J. & Ennos, A.R. (1993). The mechanics of root lodging in winter wheat *Triticum aestivum* L. *Journal of Experimental Botany* 44:1219-1224.

Crook, M.J. & Ennos, A.R. (1997). Scaling of anchorage in the tap rooted tree Mallous wrayi In: Jeronmidis, G., & Vincent, J.F.V. eds. *Plant biomechanics: Conference Proceedings* I. Reading UK: Centre for biomimetics, The University of Reading 31-36.

Crowe, D.R. & Parker, W.H. (1981). *Hybridization and Agamospermy of Bidens in North Western Ontario*. Taxon 30(4): 749-760.

Crowley, D.E., Wang, Y.C., Reid, C.P.P. & Szansiszlo, P.J. (1991). Mechanism of Iron acquisition from siderosphores by microorganisms and plants. *Plants and soil* 130:179-198.

Cruttwell, M.R.E. (1988). Ecology of *Chromolaena odorata* (Siam weed) in the neotropics In: Muniapppan, .R. (ed.) *Proc. First Int. Workshop on the biological control of Chromolaena odorata*. Bangkok pp13-20.

Cruttwell, M.R.E., and Skarrat, .B. (1996). Potential distribution of *Chromolaena odorata* (Siam weed) in Australia, Africa and Oceanic. *Agric. Ecosyst. Environ*. 59:89-96.

Dalenberg, J.W. & Van Driel, W. (1990). Contribution of atmospheric deposition to heavy metal concentration in field crops. *Netherlands Journal of Agric. Sci*. 38:367.

Daniela, M.M., Nicole, A. & Septimiu, M. (2010).Studies regarding the Pb toxicity accumulation in plants. *Romanian Biotechnological Letters* 15: 5240-5245.

Davidson, A. (2006). *Oxford Companion to food*. Oxford University Press, New York.

Davies, B.E (1977). Heavy metal pollution of British agricultural soils with special reference to the role of Lead and Copper mining. In: *Proc. Int. Seminar on soil Environment and Fertility Management in Intensive Agriculture*. Tokyo 394.

Davies, B.E. (1995). Heavy metal contaminated soils in an old industrial area of Wales, Great Britain: Source identification through statistical data interpretation: *Water, Air and Soil Pollution*, 94:85-98.

DEFRA & EA (2002). *The Contaminated Land Exposure Assessment (CLEA) model: technical basis and algorithms (CLR10)*. Bristol Environment Agency.

Del Rio-Celestino, M.D., Font, R., Moreno-Rojas, R. & De Haro-Basilon, A. (2006). Uptake of lead and Zinc by wild plants growing on contaminated soils. *Industrial Crops and Products* 24: 230-237.

Delorme, T.A., Gagliardi, J.V., Angle, J .S. & Chaney, R.L. (2001). Influence of the zinc hyperaccumulator *Thlaspi caerulescens* J. & C. Presl and the non-metal accumulator Trifolium pratense L. on soil microbial populations. *Can.J. Microbiol.* 47 (8): 773–776.

Deng, X., Joly, R.J. & Hahn, D.T (1990).The influence of plant water deficit on distribution of ^{14}C-labelled assimilates in cacao seedlings. *Annals of Botany* 66: 211-217.

Deo, B. & Nayak, P.K. (2011). Study of the Phytotoxicity on in vitro culture of *Musa acuminata* cv. Bantala. *Journal of Biotechnology and Sustainable Development* 3(8):136-140.

Department for Environment, Food and Rural Affairs (DEFRA) (2007). Soil guideline values for lead contamination. *R&D Publications*. SGV10

Department of Environment, Food and Rural Affairs (DEFRA) (2005). Soil guideline values and the determination of land as contaminated land under part IIA. *CLAN* 2/05.

Department of Environment, Food and Rural Affairs (DEFRA) and Environment Agency (EA) (2002a). Contaminants in soil: Collation of toxicological data and intake values for Humans Lead. *R&D Publications*. TOX6.

Department of Environment, Food and Rural Affairs (DEFRA) and Environment Agency (EA) (2002b).Soil guideline values for lead contamination. . *R&D Publications* SGV10.

Department of Environment, Food and Rural Affairs (DEFRA) and Environment Agency (EA) (2002c). Assessment of risks to human health from land contamination: An overview of the development of soil guideline values and related research. *CLR*7.

Department of Environment, Food and Rural Affairs (DEFRA) (2015). Magic maps:magic.defra.gov.uk/magic maps (OS map © Crown Copyright Ordnance Survey).

Deram, A. & Petit, .D. (1997). Ecology of bioaccumulation iin Arhenatherum elatius .L. (Poaceae) populations-applications of phytoremediation of Zinc, Lead and Cadmium contaminated soils. *Journal of Experimental Botany* 48 (Spec. Suppl.) 98.

Diehl, K.H., Rosopulo, A., Kreuzer, W. & Judel, .G.K (1983). Das Verhalten Von Bleitetralkylen im Boden Und deren Aufnahme durch die Pflanze. *Pflanzenernaeur Bodenkd*.146:551.

Djingova, R. & Kullef, I. (2000). Instrumental techniques for trace elements analysis In: *Trace Elements: Their distribution and effects on the environment*. Vernet, .J.P (Eds). Elsevier Science Ltd, United Kingdom pp146.

DoE, (1994). Contaminated land Research Project: Sampling strategies for contaminated land (CLR4) London, HMSO. Department of Environment.

Douay, F., Pelfrene, A., Planque, J., Fourrier, H., Richard, A., Roussel, H. & Girondelot, B. (2013). Assessment of potential risk for inhabitants living near a former lead smelter: Part 1: Metal concentrations in soils, agricultural crops anad homegrown vegetables. *Environmental Monitoring and Assessment* 185(5):3665-3680.

Douay, F., Roussel, H., Pruvot, C. & Waterlot, C. (2008). Impact of a smelter closdown on metal contents of wheat cultivated in the neighbourhood. *Environmental Science and Pollution Research* 15(2):162-169.

Drew, M. & Saker, L.R. (1975). Nutrient supply and growth of the seminal root system in Barley II localized, compensatory increases in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. *Journal of Experimental Botany* 26:79-90.

Duffus, J.H (2002). Heavy metals: A meaningless term? *Pure Applied Chemistry* 74:793-807.

Duggan, M.J. & Inskip, M.J. (1985). Childhood exposure to lead in surface dust and soil: A Community problem. *Public Health Rev*.13:1-4.

Duggan, M.J. (1980). Lead in Urban dust: An assessment. *Water, Air and Soil Pollution* 14:309-321.

Duke, J.A (1981). The gene revolution. In: Office of Technology Assessment, Background papers for innovative biological Technologies for lesser developed countries. *USGPO* Washington 89-150.

Duke, J.A. & Ayensu, E.S. (1985). *Medicinal plants of China*. Reference Publications, Inc. Algonac, MI.

Duke, J.A. (1978). The quest for tolerant germplasm In: *ASA Special Symposium 32, Crop tolerance to suboptimal land conditions.* American Society of Agronomists Madison.

Duke, J.A. (1982). Plant germplasm resources for breeding of crops adapted to marginal environments. In: Christiansen, M.N. and Lewis, C.F. (Eds), *Breeding plants for less favourable environments*. Wiley- Interscience, John Wiley & Sons New York.

Dushenkov, S., Vasudev, D., Kapulinik, Y., Gleba, D., Fleisher, D., Ting, K.C & Ensley, B. (1997).Phytoremediation: A novel approach to an old problem. In: Wise, .D.L (Ed). *Global Environmental Biotechnology*, Elsevier Science B.V. Amsterdam. PP 563-572.

Dushenkov, V., Nanda-Kumar, P.B.A., Motto, H. & Raskin, I. (1995). Rhizofiltration: The use of plants to remove heavy metals from aqueous streams. *Environ. Sci. Technol*. 29: 1245.

Ebbs, S.D., Lasat, M.M., Brady, D.J., Cornish, J., Gordon, R. & Kochian, L.V. (1997). Phytoextraction of cadmium and zinc from a contaminated soil. *Journal of Environmental Quality* 26 (5): 1424-1430.

Effenberger, H., Paar, W.H., Topa, D., Culleto, F.J. & Giester, G. (1999).Towards the crystal structure of nagyagite, [Pb (Pb, Sb) S2] [(Au, Te)]. *American Mineralogists* 84:669-676.

Eissenstat, D.M & Caldwell, M.M (1988). Seasonal timing of root growth in favourable microsites. Ecology 69: 870-873.

Elliott, H.A., Liberati, M.R. & Huang, C.P. (1986). Competitive adsorption of heavy metals by soils. *Journal of Environmental Quality* 15: 214-217.

Elliott, P., Arnold, R., Badtrop, D., Thornton, I., House, I.M. & Henry, J.A. (1999). Clinical lead poisoning in England: an analysis of routine sources of data. *Occupational Environmental Medicine* 56(12):820-824.

Elsokary, J.H. & Lag, J. (1978). Distribution of different fractions of Cd, Pb, Zn and Cu in industrially polluted and non-polluted soils of the Odda Region, Norway. *Acta. Agric. Scand*.28:262-268.

Ennos, A.R. & Fitter, A.H. (1992). Comparative functional morphology of the anchorage systems of annual dicots. *Functional Ecology* 6:71-78.

Ennos, A.R., Goodman, A.M. & Crook, M.J. (2001). Anchorage mechanics of the tap root system of winter-sown oilseed rape (*Brassica napus* L.). *Annals of Botany* 87: 397-404.

Environment Agency (EA) (2014). Secondary model procedure for the development of appropriate sampling strategies for land contamination. *R & D Technical Report* P5-066/TR.

Environment Agency {EA} (2006). *Performance standard for laboratories undertaking chemical testing of soil*. Environment Agency, Monitoring Certification Scheme.

Environment Agency {EA} (2009). Framework for the use of rapid measurement techniques (RMT) in the risk management of land contamination. *Science Report*. Environment Agency (Bristol) (ISBN 978-1-84432-982-3).

Environment Writer (2000). Http://www.nsc.org./ehc/ew/chems/lead/leadhtm Retrieved 6/6/2011. Lead (Pb) chemical backgrounder.

Erikson, J.E., Anderson, A. & Wenblad, A. (1990). Cadmium, nickel and zinc contents of oak grain as related to soil factors and precipitation. *Swedish J. Agric. Res*. 20:81-87.

Erwin, B., Anton, F. & Michael, K. (2005). The maize root system in situ: Evaluation of Structure and capability of utilization of phytate and inorganic soil phosphates. *Z.Pflanzenernahr. Bodenk* 152: 159-167.

Ezeibekwe, I.O., Okeke, S.E., Unamba, C.I.N. & Mmom, G.A. (2010). M orphological factors responsible for the success *of Chromolaena odorata* in Imo State. *World Rural Observations* 2(2): 21-28.

Fahr, M., Laplaze, L., Bendaou, N., Hocher, V., El Mzibri, M., Bogusz, D. & Smouni, A. (2013). Effect of lead on root growth. *Frontiers in Plant Science* 4(175):1-7.

Farago, M.E & Mehra, A. (1994). Analytical techniques for plant analysis In *Plants and the chemical elements: biochemistry, uptake, tolerance and toxicity*. New York: Basel: VCH Weinheim

Farago, M.E. & Mehra, A. (1992). Uptake of elements by the copper –tolerant plant Aimeria maritime. In Merian, .E. (Eds). Toxic metal compounds-interrelation between chemistry and biology. Merian, .E and Haerdi, W. (Eds). *Science and Technology Letters*, UK pp168-69.

Farago, M.E. (1994). Plants as indicators of mineralisation and pollution In *Plants and the chemical elements: biochemistry, uptake, tolerance and toxicity*. New York: Basel: VCH Weinheim. Pp 221

Feleafel, M. N. & Mirdad, Z. M. (2013). Hazard and effects of pollution by Pb on vegetable crops. *Journal of Agric. Environ. Ethics*, 26: 547-567.

Fent, K. (2004). Eco toxicological effects at contaminated sites. Toxicology 205(3):223-240.

Floridata.com (2003). Flowering plants. *http://www.floridata.com/ref/ann.cfm*.

Foroughi, S., Baker, A. J.M., Roessner, U., Johnson, A.A.T., Bacic, A. & Callahan, D.L. (2004). Hyperaccumulation of Zn by *Noccaea caerulenscens* results in cascade stress responses and changes in the elemental profile. *Metallomics* DOI 10.1039/c4mt00132j.

Foster, R.L & Lott, P.F. (1980). X-ray diffractometry examination of air filters for compounds emitted by lead smelting operations. Environ. Sci. Technol. 14:1240-1244.

Foy, C.D., Chaney, R.L. & White, M.C. (1978). The physiology of metal toxicity in plants. *Annu. Rev. Physiol*.29:511.

Fransen, B., De Kroon, H. & Berendse, F. (2001). Soil nutrient heterogeneity alters competition between two perennial grass species. *Ecology*, 82 (9): 2534-2546.

Freitas, H., Prasad, M.N.V. & Pratas, J. (2004). Plant community tolerant to trace elements growing on the degraded soils of Sao Domingos mine in the South East of Portugal: environmental implications. *Environ Int* 30: 65-72.

Fytianos, K., Katsianis, G., Triantafyllou, P., & Zachariadis, G. (2001). Accumulation of heavy metals in vegetables grown in an industrial area in relation to soil. B. Environ. Contam. Tox. 67:423-430.

Garret, R.G. (1969).The Determination of sampling and analytical errors in exploration Geochemistry. *Economic Geology* 64:568.

Garrett, R.G. & Goss, T.I (1979). The evaluation of sampling and analytical variation in regional surveys. In: Walterson, J.R. & Theobald, P.K (Eds). *Geochemical Exploration* 1978. Association Exploration Geochemists (Rexdale, ON, USA). 371-383.

Gaweda, M. (2009).Heavy metal content in common sorrel plants (*Rumex acetosa* .L.) obtained from natural sites in Malopolska province. *Polish Journal of Environmental Studies* 18(2): 213-218.

Gee, C., Ramsey, M.H., Maskall, J. & Thornton, .I. (1995). Factors controlling the release of heavy metals from historical smelting slags. *Heavy Metals in the Environment International Conference Hamburg*.

Gee, C., Ramsey, M.H., Maskall, J. & Thornton, .I. (1997). Mineralogy and weathering processes in historical smelting slags and their effect on the mobilization of lead. *Journal of Geochemical Exploration* 58: 249-257.

Gerr, F., Letz, R. & Stokes, L. (2002). Association between bone lead concentration and blood pressure among young adults. *American Journal of Industrial Medicine* 42:98-106.

Gilroy, S. & Jones, D.L. (2000). Through form to function: root hair development and nutrient uptake. *Trends Plant Sci* 5:56-60.

Gooverts, P. (1999).Geostatistics in soil science: state-of-the-art and perspestives. Geoderma 89:1-45.

Gosh, M., & Singh, S.P. (2005). A review on phytoremediation of heavy metals and utilization of its by-products. Asian Journal on Energy and Environment 4(6):214-231.Gross, .K.L.,

Gray, C., Mcgrath, S.P. & Sweeney, R. (2005). Phytoextraction of metals: Investigation of hyperaccumulation and field testing. Contaminated land: Applications in real Environments (*CL: AIRE*).RP6.

Greener, Y. & Kochen, J.A. (1983).Methyl mercury toxicity in the chick embryo. Teratology 8(1):23-28.

Gregoria, B. B. (2011).Comparative Lead Uptake and Responses of Some Plants Grown on Lead Contaminated Soils. *Summer Research Report*. Department of Biology, Jackson State University, Jackson, MS 39217.

Griffith, D.A. (2002). The Geographic Distribution of soil lead concentration: Description and Concerns. *URISA Journal* 14(1): 5-14.

Gross, K.L., Pregitzer, K.S & Burton, .A.J. (1995). Spatial variation in Nitrogen availability in three successional plant communities. *Journal of Ecology* 83: 357-368.

Grossgeim, V.A. (1979). The Great Soviet Encyclopedia. The Gale Group. Inc.

Ground-Water Remediation Technologies Analysis Centre (GWRTAC) (1997). Remediation of metal-contaminated soils and Groundwater. *GWRTAC Series* 97-01.

Guefarchi, I., Rejili, M., Mahdhi, M. & Mars, M. (2013). Assessing genotypic diversity and symbiotic efficiency of five rhizobial legume interactions under cadmium stress for soil phytoremediation. *International Journal of Phytoremediation* 15 (10): 938-951.

Gupta, A.K. & Sinha, S. (2006). Chemical fractionation and potentially toxic metal accumulation in plant of *Sesamum indicum* (L) var. T55 grown on soil amended with tannery sludge: Selection of single extractant. *Chemosphere* 64:161-173.

Gy, P.M. (1992). *Sampling of heterogeneous and dynamic material systems. Theories of heterogeneity, sampling and homogenizing*. Elsevier (Amsterdam), 653pp.

Haines, B.J. (2002). Zincophilic root foraging in *Thlaspi caerulescens*. *New Phytologist*, 155: 363-372.

Hall, J.L. & Williams, L. (2003). Transition metal transporters in plants. *Journal of Experimental Botany* 54 (393): 2601-2613.

Harmsen, J. (2007). Bioavailability of metals in soils to plants. *J. Environ. Qual*. 36:1420-1428.

Helgesen, H. & Larsen, E.H. (1998).Bioavailability and speciation of arsenic in carrots grown in contaminated soil. *Anal*.123:791-796.

Hemmingway, J.S. (1995). The mustard species: Condiment and Food Ingredient use and potentials oilseed crops In: *Brassica oilseeds production and utilization*. CAB International, Wallingford. pp 373-383.

Hill, J.W. & Petrucci, R.H. (1999). *General Chemistry* (2nd Ed.). Upper Saddle River, New Jersey: Prentice Hall .p781.

Hodge, A. (2004). The plastic plant: root response to heterogeneous supplies of nutrients. New Phytologist 1(162):9-24.

Horwitz, W. (1990). Protocol for design, conduct and Interpretation of collaborative studies. *Pure Applied Chemistry* 60:855.

Hu, F., Mou, P.P., Weiner, J. & Li, S. (2014). Contrast between whole-plant and local nutrient levels determine root growth and death in *Ailanthus altissima*. *American Journal of Botany* 101 (5):1-8.

Hu, H., Scheidell, J., Coatsworth, A.M. & Khan, M.R. (2014). Associations between blood lead level and substance use and sexually transmitted infection risk among adults in the United States. *Environmental Research* 135:21-30.

Hu, H., Shih, R., Rothenberg, S. & Schwartz, B.S. (2007). The Epidemiology of lead toxicity in Adults: Measuring dose and consideration of other Methodologic issues. *Environmental Health Perspective* 115 (3): 455-462.

Huang, J.W. & Cunningham, D.S. (1996). Lead phytoextraction: species variation in lead uptake and translocation. *New Phytologist,* 134(1): 75-84.

Huang, J.W., Chen, J., Berti, W.R. & Cunningham, S.D. (1997). Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead pytoextraction. Environmental Science and Technology 31(3):800-805.

Husnain, A., Ali, S.S., Zafar, Z. & Zafar, R. (2013). Phytoremediation of heavy metal contamination in industrial waste water by *Euphorbia prostrate*. *Current Research Journal of Biological Sciences* 5 (1):36-41.

Hutchings, M.J. & De Kroon, H. (1994). Foraging in plants: the role of morphological plasticity in resource acquisition. *Advances in Ecological Research,* 25: 159-238.

Hutchings, M.J. & John, E.A. (2004). The Effects of Environmental Heterogeneity on root growth and root/shoot portioning. *Annals of Botany* 94: 1-8.

Hutchings, M.J., Wijensinghe, D.K. & John, E.A. (2000). The effects of heterogeneous nutrient supply on plant performance: a survey of responses, with special reference to clonal herbs. In: Hutchings, M.J., John, E.A and Stewart, A.J.A. (Eds). *The Ecological Consequences of Environmental Heterogeneity*, Blackwell Science Ltd: 91-110.

Inez, O., Albayrak, F. & Askin, A. (1998). Copper and lead adsorption on some Bentonitic clays. *Turkish Journal of Chemistry* 22:243-252.

International Agency for Research in cancer (IARC) (2006). Inorganic lead and lead compounds probably carcinogenic to humans. IARC Monograph 87: Group 2A.

International Organization for standardization (ISO) (1993). Guide to expression of Uncertainty in measurement. 1ST Edition Geneva.

Isaaks, E.H. & Srivastava, R.M. (1989). *An Introduction to Applied Geostatistics*. Oxford University Press, Oxford, UK.

ISO 11074 (2005). Soil quality-vocabulary International Organization for standardization. *International Organization for Standardization*. Pp45-56.

ISO 3534-1: (1993).Statistics, Vocabulary and Symbols---Part 1 *Probability and General Statistical Terms*.

IST, (2007) "List of Periodic Table Elements Sorted by Abundance in Earth's crust" Israel Science and Technology. www.science.co.il Retrieved 2014-04-25.

Jackson, D.R & Watson, A.P. (1977).Disruption of nutrient pools and transport of heavy metals in a forested watershed near a lead smelter. *Journal of Environmental Quality* 6:331-338.

Jackson, M.B. (2001). Preface. In: Gasparikova, O., Ciamporova, M., Mistrick, I. (eds). *Recent Advances of Plant Root and Function*, Kluwer Academic Publishers, 1-2.

Jackson, R.B. & Caldwell, M.M. (1989).The timing and degree of root proliferation in fertile microsites for three cold desert perennials. *Oecologia* 81:149-153.

Jackson, R.B. & Caldwell, M.M. (1993). The scale of nutrient heterogeneity around individual plants and its qualifications with geostatistics. *Ecology*, 74:612-614.

Jackson, R.B. & Caldwell, M.M. (1996). Integrating resource heterogeneity and plants uptake in a patchy soil environment. *Journal of Ecology* 84:891-903.

Jain, T.B., Graham, R.T and Adams, D.L. (1997). Carbon to organic matter ratios for soils in Rocky Mountain coniferous forests. *Soil Science Society of America Journal* 61:1190-1195.

Jaisi, D.P., Dong, H.L. & Morton, J.P. (2008). Partioning of Fe^{2+} in reduced non-tronite (Nau-2) to reactive sites: reactivity in terms of Tc^{4+} reduction. *Clay Mineral* 56:175-189.

Janice, L.T., Manore, M.M. & Vaughan, L.A. (2010). *Nutrients involved in energy metabolism*. The Science of Nutrition (2nd Ed.). San Franscisco: Pearson Education pp292-321.

Jean, R. V. (1994). Phyllotaxis. *Google Books*. Retrieved 31/01/11.

Jeana, R.H. (2000). An overview of the phytoremediation of Lead and Mercury. *National Network of Environmental Management Studies (NINEMS)* 1-43.

Jean-Phillipe, B., Jean-Sebastien, D., Mirela, S. & Eric, H. (2012). Analysis of procedures of sampling contaminated soil using Gy's sampling theory. *Science of the Total Environment* 425: 199-207.

Jefferson Lab (2007). http://education.jlab.org/itselemental/index.html "It's Elemental - The Periodic Table of Elements". Archived from the original on 29 April 2007. Retrieved 2014-04-30.

Jiang, M., Guanggming, Z., Chang, Z., Xiaoying, M., Ming, C., Jiachao, Z., Lunhui, L., Qian, Y., Langping, H. & Lifeng, L. (2013). Assessment of heavy metal contamination in the surrounding soils and Surface Sediments in Xiawangang River, Qingshuitang District. *PLOS ONE*, 8(8): 1-11.

Jin, J. Martens, D.C. & Zelazny, L.W. (1987). Distribution and availability of Boron fractions. *American Journal of Science Society* 51: 1228.

Johnson, F.M. (1998).The genetic effects of environmental lead. *Mutation Research* 410(2):123-140.

Joint Nature Conservation Committee {JNCC} (2011). Gang Mine Natura 2000 data form for special protection areas (SPA).*UK SAC data form*. JNCC, Peterborough.

Juma, N.G (1999). Introduction to soil science and soil resources. Volume 1 in the Series ‘’*The Pedosphere and its dynamics: A systems approach to soil science*’’. Salman Production, Sherwood Park. PP 335.

Kabata-Pendias A. & Pendias, H. (1999). *Biogeochemistry of Trace Elements*.2nd ed. Wyd. Nauk. Warsaw. 400.

Kabata-Pendias, A. & Pendias, H. (2001). *Trace elements in soils and plants*, 3rd edition. Florida, CRC Press LLC.

Kabata-Pendias, A. (1969). Leaching of Micro-and-macro elements in columns with soil derived from granite. *Pamiet Pulawski* 38:111.

Kabata-Pendias, A. (2010). *Trace Elements in soils and plant*s. 4TH Edtion. CRC Press Boca Raton. London, New York.

Kalavrouziotis, P.H., Koukoulakis, A.H., Papadopoulos, A.H. & Mehra, A. (2009). Heavy metal accumulation in Brussels sprouts after irrigation with treated municipal waste water. Journal of Plant Interactions 4(1): 41-48.

Kalnicky, D.J. & Singhvi, R. (2001). Field portable XRF analysis of environmental samples. *Journal of Hazardous materials*, 83 (1-2):63-78.

Kalogerakis, N., Eleni, C. &, Kadukova, J. (2005). A whole-plant mathematical model for the phytoextraction of lead (Pb) by maize. *Environment International* 31: 255-262.

Kanazawa, K., Higuchi, K., Nishizawa, N.K., Fushiya, S., Chino, M. & Mori, S. (1994). Nicotianamine aminotransferase activities are correlated to the phytosiderophore secretion under Fe-deficient conditions in Graminae. *Journal of Experimental Botany* 45: 1903-1906.

Kareem, S.R., Arifin, A. Abdul-Hamid, H., Dalj, S.K., Shamshuddin, J. & Aiza-Shaliha, .J. and Wong, .W. Z. (2013). Assessment of Heavy metals uptake and translocation by *Aquilaria malaccensis* planted in soils containing sewage sludge. American Journal of Applied Science. 10 (9): 952—964.

Kasheem, M.A., Singh, B.R., Kondo, S.M., Immamul-Huq, S.M. & Kawal, S. (2007). Comparison of extractability of Cd, Cu, Pb and Zn with sequential extraction in contaminated and non-contaminated soils. *Int. Journal of Environ. Sci. Tech* 4 (2):169-176.

Keever, G.J. (1994): BA-induced offset formation in Hosta. *J. Environ. Hort.* 12(1):36-39.

Kelerpteris, K., Argyraki, A. & Alexakis, D. (2006). Multivariate statistics and spatial interpretation of geochemical data for assessing soil contamination by potentially toxic elements in the mining area of Stratoni, North Greece. *Geochemistry Environment Analysis* 6: 349-355.

Kerna, T.C, Nitsche, F.O., Heron, M.M., Mailloux, B.J., Peteet, D., Sritraitrat, S., Sands, E. & Baumgarten (2011).Evaluation and calibration of field Portable X-ray Fluorescence Spectrometer for Quantitative analysis of siliciclastic soils and sediments. *Journal of Analytical Atomic Spectrometry* 26:395-405.

Kirkwood, T.B.L. (1979). Geometric means and measures of dispersion. *Biometrics* 35:908-909.

Kitagishi, K. & Yamane, I. Eds (1981). *Heavy Metal Pollution in Soils of Japan*, Japan Science Society Press, Tokyo 302.

Kopittke, P.M, Blamey, C., Pax, F. Asher, C.J. & Menzies, N.W. (2009).Trace metal phytotoxicity in solution culture: A Review. *Journal of Experimental Botany* 6(4):945-954.

Kot, A. & Namiesnik, J. (2000).The role of speciation in analytical Chemistry. *Trends in analytical Chemistry* 19: 69-79

Kramer, U. (2010). Metal hyperaccumulation in plants. *Annu. Rev. Plant Biol.* 61:517-534.

Kramer, U., Cotter-Howells, J.D., Charnock, J.M., Baker, AJ.M. & Smith, J.A.C. (1996). Free histidine as a metal chelator in plants that accumulate nickel. *Nature* 379 (6566):635-638.

Krauskopf, K.B., (1979) Crustal abundances. *Introduction to Geochemistry* (2nd ed.): New York, McGraw Hill, 617 p.

Krzeslowska, M. (2011). The cell wall in plant cell response to trace metals: polysaccharide remodelling and its role in defence strategy. *Acta Physiol.Plant*. 33:35-51.

Krzeslowska, M., Lenartowska, M., Mellerowicz, E., Samar-Dakiewicz, S. & Wozny, .A. (2009).Pectinuous cell wall thickenings formation- a response of moss protonemata cells to lead. *Environ. Exp. Bot*. 65: 119-131.

Kumagai, S., Suzuki, T., Tezuka, K., Satoh-Nagasawa, N., Takahashi, H., Sakurai, K., Watanabe, A., Fujimura, T. and Ahagi, H. (2014). Funtional analysis of C-terminal region of the vacuolar cadmium-transporting rice OsHMA3. *FEBS letters* 588(5):789-794.

Kumar, P.B.A.N.,Dushenkov, V., Motto, H. & Raskin, I.(1995). Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental Science and Technology* 29:1232-1238.

Kumar, V., Mahajan, M. & Yadav, S.K. (2012).Toxic metals accumulation, tolerance and homomeostasis in Brassicoilseed In The *plant family Brassicaceae-Contributions towards phytoremediation*. Springer Dordrecht Heidelberg, New York. PP 171-202.

Kushwaha, S.P.S., Ramakrishnan, P.S. & Tripathi, R.S. (1981). Population dynamics of *Eupatorium odoratum* in sucessional environments following slash and burn agriculture. *Journal of Applied Ecology* 18:529-533.

Laidlaw, M.A.S & Taylor, M.P (2011). Potential for childhood lead poisoning in the inner cities of Australia due to exposure lead in soil/dust (Review). *Environmental Pollution* 159 (1):1-9.

Laidlaw, M.A.S., Mielke, H.W., Filippelli, G.M., Johnson, D.L. & Christopher, R.G. (2005).Seasonality and children's blood lead levels: Developing a predictive Model using climatic variables and blood lead data from Indianapolis, Indiana, Syracuse, New York and New Orleans, Louisiana. *Environmental Health Perspectives* 113(6):1556-1665.

Lame, F., Honders, T., Derksen, G. & Gadella, M. (2005).Validated sampling strategy for assessing contaminants in soil stockpiles. *Environmental Pollution* 134: 5-11.

Lars, J. (2003). Hazards of heavy metal contamination. *British Medical Bulletin* 68(1): 167-182.

Larsen, P.B., Degenhardt, J., Tai, C.Y., Stenzler, S.L., Howell, S.H. & Kochian, L.V. (1998). Aluminium-resistant Arabdopsis mutants that exhibit altered patterns of Aluminium accumulation and organic release from roots. *Plant Physiology* 117: 19-27.

Lasat, M.M. (1998). The use of Plants for the Removal of Toxic Metals from contaminated Soil. *American Association for the Advancement of Science*.1-32.

Lasat, M.M., Baker, AJ.M. & Kochian, L.V (1998). Altered Zn compartmentation in the root symplasm and stimulated Zn absorption into the leaf as mechanisms involved in Zn hyperaccumulation in *Thlaspi caerulescens Plant Physiology* 118:875-883.

Lasat, M.M., Baker, AJ.M. & Kochian, L.V. (1996). Physiological characterization of root Zn^{2+} absorption and translocation to shoots in Zn hyperaccumulator and non-accumulator species of *Thlaspi. Plant Physiology* 112:1715-1722.

Lavania, S. & Kumar, U.C. (1998). Genomic manipulation in vetiver to realize non-seeding eco-friendly cultivars for soil water conservation and essential oil production. *Proceedings of the First International Conference on Vetiver.* Office of the Royal Development Projects Board, Bangkok. 137-140.

Lavania, S. (2003). Vetiver root oil and its utilization. *Tech. Bull.* No. 2003/1, PRVN/ORDPB, Bangkok, Thailand.

Lavania, S. Larsen, P.B., Degenhardt, J., Tai, C.Y., Stenzler, S.L., Howell, S.H. & Kochian, L.V. (1998). Aluminium-resistant Arabdopsis mutants that exhibit altered patterns of Aluminium accumulation and organic release from roots. *Plant Physiology* 117: 19-27.

Laxen, D.P.H. (1985). Trace metal adsorption/coprecipitation on hydrous ferric oxide under realistic conditions. *Water Res*.19:1229.

Lechowicz, M.J & Bell, G. (1991). The ecology and genetics of fitness in forest plants. II. Microspatial heterogeneity of the edaphic environment. *Journal of Ecology* 79: 687-696.

Lee, C. (2002). Measurement uncertainty in contaminated land investigations related to analyte concentration and cost. Ph.D Thesis. Department of Environmental Science and Technology. Imperial College, University of London. *British Library EthOS*.

Lee, K.K., Cho, H.S., Moon, Y.C., Ban, S.J. & Kim, J.Y. (2013). Cadmium and lead uptake capacity of energy crops and distribution of metals within the plant structures. KSCE *Journal of Civil Engineering* 17(1):44-50.

Leviens, C., Carleer, R., Cornelissen, T. & Yerman, J. (2009). Fast pyrolysis of heavy metal contaminated willow: Influence of plant parts. Fuel 88:1417-1425.

Leviens, C., Yperman, J., Vangronsveld, J. & Craleer, .R. (2008). Study of the potential valorisation of heavy metal contaminated biomass via phytoremediation by fast pyrolysis part I: Influence of temperature, biomass species and solid heat carrier on the behaviour of heavy metals. *Fuel* 87:1894-1905.

Levine, M.B., Stall, A.T., Barett, G.W. & Taylor, D.H (1989). Heavy metal concentration during ten years of sludge treatment to an old-field community. *Journal of Environmental Quality* 18:411-418.

Li, F., Cong-Qiang, L., Yuan-Gen, Y., Xiang-Yang, B., Tao-Ze, L. & Zhi-Qi, Z. (2012). Natural and anthropogenic lead I soils and vegetables of the Guiyang city, Southwest China: A Pb isotopic approach. *Science of the Total Environment*, 431: 339-347.

Li, X., Coles, B.J., Ramsey, M.H. & Thornton, I. (1995).Sequential extraction of soils for multielement analysis by ICP-AES. *Chemical Geology* 124:109-123.

Likar, M. & Pongrac, P. (2010). Molecular diversity and metal accumulation of different *Thlaspi praecox* populations from Slovenia. *Plant Soil* 330: 195-205.

Lin, Q., Chen, Y. X., He, Y.F. & Tian, G.M. (2004). Root-induced changes of lead availability in the rhizosphere of *Oryza sativa* .L. Agric. Ecosyst. Environ.104:605-613.

Little, P. & Martin, M.H (1972). A survey of zinc, lead and cadmium in soil and natural vegetation around a smelting complex. *Environmental Pollution*.3: 241.

Lyn, J. (2003). Optimising uncertainty from sampling and analysis of foods and Environmental samples. Dphil Thesis, Centre for Environmental Research, University of Sussex. *Sussex Research Online*

Lyn, J.A., Palestra, L.M., Ramsey, M.H., Damant, A.P. & Wood, R. (2007b). Modifying ncertainty from sampling to achieve fitness for purpose: A case study on nitrate in lettuce. *Accreditation and Quality Assurance* 12: 67-74.

Lyn, J.A., Ramsey, M.H., Coad, D.S., Damant, A.P., Wood, R. & Boon, K.A. (2007a). The duplicate method of uncertainty estimation: are eight targets enough? *Analyst* 132(11): 1147-1152.

Ma, Q. Y., Logan, T.J & Ryan, J.A (1995). "Lead immobilization from aqueous solutions and contaminated soils using phosphate rocks. *Environmental Science and Technology* 29:1118-1126.

Mackie, K.A., Schmidt, H.P., Muller, T. & Kandeler, E. (2014). Cover crops influence soil microorganisms and phytoextraction of copper from a moderately contaminated vineyard. *Science of the Total Environment* 500:34-43.

Macnair, R.M & Baker, AJ.M. (1994).The phenomenon of tolerance In *Plants and the chemical elements: biochemistry, uptake, tolerance and toxicity*. New York: Basel: VCH Weinheim.

Mahjoubet, A. R. & Ali, M. (2001). A dimeric mixed-anions lead (II) complex: Synthesis and structural characterization of Pb_2 $[(BTZ)_4$ $(NO_3)(H_2O)]$ $(CLO_4)_3$ {BTZ=4,4'-Bithiazole}. *Chemistry letters* 30(12):1234.

Maiz, I., A., Garcia, R. & Millan, E. (2000). Evaluation of heavy metal availability in polluted soils by two sequential extraction procedures using factor analysis. *Environmental Pollution* 110(1):3-9.

Malik, R.M, Husain, S.Z & Nazir, I. (2010). Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. *Pakistan Journal of Botany* 42(1):291-301.

Malone, G., Koeppe, D.E & Miller, R.J (1974).Localization of lead accumulated by corn plants. *Plant Physiol*.53:338.

Manciulea, A. & Ramsey, M.H. (2006). Effect of scale of Cd heterogeneity and timing of exposure on Cd uptake and shoot biomass, of plants with contrasting root morphology. *Science of the Total Environment* 367:958-967.

Maskall, J.E. & Thornton, I. (1993).Heavy metal contamination of soils and rocks at historical smelting sites. *Land Cont. Recl*.1:92-100.

McGrath, D. (1996).Application of single and sequential extraction procedures to polluted and unpolluted soils. *Science of the Total Environ*.178:37-44.

McGrath, S.P. & Loveland, P.J. (1992). *The Soil Geochemical Atlas of England and Wales*. Blackie Academic Professional Glasgow.

McGrath, S.P. (1998). Phytoextraction for soil remediation In Brooks, R.R. (Ed). *Plants that hyperaccumulate heavy metals; their role in phytoremediation, microbiology,*

archaeology, mineral exploration and phytomining. CAB International, Wallingford, UK. Pp 261-287.

Mei, B., Puryear, J. & Newton, R.L (2002). Assessment of Cr tolerance and accumulation in selected plant species. *Plant and Soil* 247 (2):223-231.

Melannie, L., Claude, D.C. & Rajasekaran, R.L. (2006). Effect of plant growth regulators on propagule formation in *Hemerocallis spp*. and *Hosta spp*. *HORTSCIENCE* 41(3):651-653.

Mengel, D.B. (2011).Fundamentals of soil cation exchange capacity. *Agronomy Guide* RR3/93. Department of Agronomy, Perdue University.

Menon, M., Robinson, B., Oswald, S.E., Kaestner, A., Abbapour, K.C., Lehmann, E. & Schulin, R. (2007). Visualization of root growth in heterogeneously contaminated soil using neutron radiography. *European Journal of soil Science* 58 (3): 802-810.

Menzies, N.W. Donn, M.J. & Kopitikke, M. (2007). The tolerance of extractants for the estimation of the phytoavailable traces metals in soils. *Environ. Pollut*.145:121-130.

Meyers, D.E.R., Auchterlonie, G.J., Webb, R.I. & Wood, B. (2008). Uptake and localization of Lead in the root system of Brassica juncea. *Environmental Pollution* 153:323-332.

Michal, S., Michael, P., Petra, K., Jan, H., Karel, S. & Miroslav, P. (2012). Willow trees from heavy metals phytoextraction as energy crops. *Biomass and Bioenergy* 37:106-113.

Miesch, A.T. (1976).*U.S. Geological Survey Prof. Paper* 954-A.

Miller, H.J. (2004). Tobler`s First law and spatial analysis. *Annals of the Association of American Geographers* 94: 284-289.

Millis, P.R., Ramsey, M.H. & John, E.A. (2004). Heterogeneity of cadmium concentration in soil as a source of uncertainty in plant uptake and its implication for human health risk assessment. *Science of the Total Environment*, 326: 49-53.

Millstone, E. (1997). *Lead and public Health: the dangers for children*. Earth scan Publications Limited. London. PP 74-128.

Ministry of Agriculture, Fisheries and food (1986). The *analysis of Agricultural materials*. Reference Book 427. Her Majesty Stationary Office. London. pp27.

Mishra, S., Srivastava, S., Tripathi, R.D., Kumar, R., Seth, C.S. & Gupta, D.K. (2006). Lead detoxification by coontail (*Ceratophyllum demersum* .L.) involves the induction of phytochelatins and antioxidant system in response to its accumulation. *Chemosphere* 65: 1027-1039.

Moir, A.M. & Thornton, I. (1989). Lead and Cadmium in urban allotment and garden soils and vegetables in the United Kingdom. Environ. Chem. Health 11(3-4): 113-119.

Mokany, K., Raison, J.R, & Prokushkin, A.S. (2006). Critical analysis of root-shoot ratios in terrestrial biomes. *Global Change Biology* 12:84-96.

Moradi, A.B, Oswald, S.E, Nordmeyer-Massner, J.A, Pruessmann, K.P., Robinson, B.H. & Schulin, R. (2010). Analysis of nickel concentration profiles around the roots of the hyperaccumulator plant Berkheya coddii using MRI and numerical simulations. *Plant and Soil 28*(1-2), 291 - 302.

Moradi, A.B., Conesa, H.M., Robinson, B.H., Lehmann, E., Kaestner, A. & Schulin, R. (2009). Root responses to soil Ni heterogeneity in a hyperaccumulator and non-accumulator species. *Environmental Pollution*, 157 (8-9): 2189-2196.

Morgan, R. (2013).Soil heavy metals and human health. In Brevik, .E.C., Burgess J. (Eds).Soil and Human Health.CRC Press.Boca. Raton FL: 59-82.

Morrey, D. R., Baker, A. J. M. & Cooke, J. A..(1988). Floristic variation in plant communities on metalliferous mining residues in the northern and southern Pennines, England. *Environmental Geochemistry and Health* 10:11-20.

Mossop, K.F & Davidson, C.M (2003).Comparison of original and modified BCR sequential extraction procedures for the fractionation of copper, iron, lead. Manganese and zinc in soils and sediments. *Anal. Chim. Acta*.478:111-118.

Motloch, J.L. (2000). Introduction to landscape design. *Google Books*. Retrieved 31/01/11.

Mou, P., Jones, R.H., Tan, Z., Bao, Z. & Chen, H. (2013). Morphological and physiological plasticity of pants roots when nutrients are both spatially and temporally heterogeneous. *Plant and Soil* 364(1-2):373-384.

Myers, J.C (1997). *Geostatistical error management: quantifying uncertainty for environmental sampling and mapping*. Van Nostrand, Reinhold, New York.

Nabulo, G., Oryem Origa, H., Nasinyama, G.W., & Cole, D. (2008). Assessment of Zn, Cu, Pb, Ni contamination in wetland soils and plants of Lake Victoria Basin. *Int. J. Environ. Sci. Tech*., 5(1): 65-74.

Natural England {NE} (2014). Natural Character Area Profile-Derbyshire Peak Fringe and lower Derwent. *Natural England.org.uk*. Date retrieved 4/8/14.

Needleman, H. (2004). Lead poisoning. *Annual Review of Medicine* 55:209-222.

Olajire, A.A., Bello, M.O., Abdul-Hammed, M. & Olabemiwo, O.M.(2006).Comparative evaluation of EDTA, pyridine and acetic acid for the assessment of available heavy metals from domestic and industrial sludges. *Int. J. Environ. Sci. Tech* 3: (4):341-349.

Olayinka, K.O., Oyeyiola, A.O., Odujebe, F.O. & Oboh, .B. (2011). Uptake of potentially toxic metals by vegetable plants grown on contaminated soil and their potential bioavailability using sequential extraction. *Journal of Soil Science and Environmental Management* 2(8):220-227.

Opajobi, A.O., Esume, C.O., Osasuyi, A. & Okehie, C.C. (2011). Determination of the lead content of pumpkin leaf *Telfairia occidentalis* in selected towns of Delta State covering 3 Senatorial Districts of the State. *Current World Environment*, 6(1): 39-44.

Opeolu, B.O, Adenuga, O.O, Ndakidemi, P.A. & Olujimi, O.O (2010). Assessment of phytotoxicity potential of lead on tomato (*Lycopersicum esculentum* L.) planted on contaminated soils. *International Journal of Physical Sciences* 5(2):68-73.

Oti Wilberforce, J.O & Nwabue, F.I. (2013a). Heavy metals effect due to contamination of vegetables from Enyigba lead mine in Ebonyi State, Nigeria. *Environment and Pollution*, 2(1): 19-26 Published by Canadian Centre of Science and Education.

Oti Wilberforce, J.O & Nwabue, F.I. (2013b). Uptake of heavy metals by *Dioscorea rotundata* (White yam) and *Ipomoea batatas* (sweet potatoes). *Environment and Pollution*, 2(2): 19-26 Published by Canadian Centre of Science and Education.

Panagos, P., MarcVan, L., Yusuf, Y. & Montanarella, .L. (2013). Contaminated sites in Europe: Review of the current situation based on data collected through European Network. Journal of Environmental and Public Health 15: 8764-8774.

Pandey, B.P (2005). *Plant Anatomy*. S. Chand & Company LTD. India. PP 176.

Park, C.H., Keyham, M. & Martin, M. (1999). Purification and characterization of chromate reductase in *Pseudomonas putiola*. *Abs. Gen. Meet. American Soc. Micrbiol*. 99:536.

Park, W. & Ahn, S.J (2014). How do heavy metal ATPases contribute to hyperaccumulation. Journal of *Plant Nutrition and Soil Science* 177(2):121-127.

Pawloska, T.E. & Charvat, I. (2004). Heavy metal stress and developmental patterns of arbuscular mycorrhizal fungi. *Applied and Environmental Microbiology* 70(11):6643-6649.

Pellet, M.D., Grunes, D.L. & Kochian, L.V (1995). Organic acid exudation as an Aluminium tolerance mechanism in maize (*Zea mays* .L.) *Planta* 196: 788-795.

Peterson, P.J. (1971). Unusual accumulations of elements by plants and animals. *Sci. Prog*. 59: 505.

Pierret, A. Doussan, C., Capowiez, Y., Bastardie, F. & Pages, L. (2005). Root functional Architecture: A framework for modeling the interplay between roots and soil. *Vadoze Zone Journal* .Special issue: 23-38.

Pires, A.C.D. (2004). Interacao de ions Zn^{2+} e Pb^{2+} com os constituintes organicos e minerais de solos de Curitiba, PR, *M.Sc Thesis*, Universidade Federal do Parana, Curitiba, Brasil.

Pires, A.C.D., Melo, V., Motta, A.C.V. & Lima, V.C. (2007). Major Soil classes of the Metropolitan Region of Curitiba (PR), Brazil: II- Interaction of Pb with mineral and organic Constituents. *Brazillian Archive of Biology and Technology* 50 (2): 183-192.

Podar, D., Ramsey, M.H. & Hutchings, M.J. (2004). Effect of cadmium, zinc and substrate heterogeneity on yield, shoot metal concentration and metal uptake by Brassica juncea: implications for human health risk assessment and phytoremediation. *New Phytologist*, 163 (2): 313-324.

Pollard, A.J., Powell, K.D., Harper, F.A, & Smith, J.A.C (2002). The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Science* 21(6): 539-566.

Potts, D.A., Rakow, G.W. & Males, D.R. (1999). Canola-quality *Brassica juncea*, a new oilseed crop for the Canadian Praires. *New Horizons for an old crop. Proc. 10th Intl. Rapeseed Congr.* Canberra, Australia.

Prado, C., Rosa, M., Pagano, E., Hilal, M. & Prado, F.E. (2010). Seasonal variability of physiological and biochemical aspects of chromium accumulation in outdoor-grown *Salvania minima*. *Chemosphere* 81 (5): 584-593.

Prasad, M.N.V. & Strzalka, K. (1999). Impact of heavy metals on photosynthesis In: *Heavy Metal Sress in Plants*. Prasad, .M.N.V and Hagemeyer, .J. eds. Springer, Heidelberg 117.

Prasad, M.N.V. (2007). Sunflower (*Helianthus annus* L.)- A potential crop for Environmental industry. *HELIA* 30(46): 167-174.

Pruvot, C., Douay, F., Herve, F. & Waterlot, C. (2006). Heavy metals in soil, crops and grass as a source of human exposure in the former mining areas. *Journal of soils and sediments* 6(4):215-220.

Purefoy, .C. (2010). Gold rush triggers deadly lead poisoning. *CNN* November 2010.

Quartacci, M.F., Argilla, A., Baker, AJ.M. & Navari-Izzo, F. (2006). Phytoextraction of metals from a multiply contaminated soil by Indian mustard. *Chemosphere* 63 (6): 918-925.

Quevaullier, P. (1998). Operationally defines extraction procedures for soils and sediment analysis. *Trac-Trends in Analytical Chemistry* 17(5):289-298.

Rakov, G. & Woods, D. (1987). Outcrossing in rape and mustard under Saskatchewan praire conditions. *Canada Journal of Plant Science*. 67:147-151.

Ramsey, M.H. & Argyraki, A. (1997). Estimation of measurement uncertainty from field sampling: Implications for the classification of contaminated land. *Science of the Total Environment* 198: 243-247.

Ramsey, M.H. (1998). Sampling as a source of measurement uncertainty: techniques for quantification and comparison with analytical sources. *Journal of Analytical Atomic Spectrometry* 13: 97-104.

Ramsey, M.H. (2010). Can *in situ* measurements be more fit-for-purpose than *ex situ* laboratory measurements. Society for Environmental Geochemistry and Health 27th Annual Conference. *Book of Abstract* 27:13.

Ramsey, M.H., Hartley, G.J & Rosenbaum, M.S (1994). Interpretation and source identification of heavy metal contamination of land using geographical information

system (GIS) In: Cothern, C.R. (Ed). Trace substances Environment and Health. *Science Reviews.* Northwood 95-104.

Ramsey, M.H., Solomon-Wisdom, G. & Argyraki, A. (2013). Evaluation of *in situ* heterogeneity of elements in solids: Implication for Analytical Geochemistry. *Geostandards and Geoanalytical Research* 37 (4): 379-391.

Ramsey, M.H., Thompson, M. & Hale, M. (1992). *Geochem.Explor.* 44:23-46.

Raskin, I., Nanda Kumar, P.B.A., Dushenkov, S. & Salt, D.E. (1994). Bio concentration of heavy metals by plants. *Curr. Opin. Biotechnology* 5: 285-290.

Ratti, N. & Upadhyay, A. (2013). Role of Arbuscular mycorrhizal fungi in phytoremediation. *Environews* 19 (1): 111-121.

Rauret, G., Lopez-Sanchez, J.F., Sahuquillo, A., Rubio, R., Davidson, C., Ure, A. & Qevauviller, P. (1999). Improvement of the BCR three step sequential extraction prior to the certification of new sediment and soil reference materials. *J. Environ. Monit*.1:57-61.

Reagan, P.L. & Silbergeld, E.K. (1989). Establishing a health based standard for lead in residential soils. In: Hemphill and Cotherns, eds. Trace substances in environmental health. *Environmental Geochemistry and Health* 12: 123-132.

Reddy, K.R. Member, A.S.C.E. & Jeffrey, A.A. (2001). Effects of soil heterogeneity on air flow patterns and hydrocarbon removal during *in situ* air sparging. *Journal of Geotechnical and Geo environmental Engineering* 127(3): 234-247.

Reeves, R.D & Brooks, R.R. (1983). Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. *Environmental Pollution* A, 31:277-285.

Reeves, R.D. & Baker, A.J. (2000). Metal-accumulating plants In: Raskin, .I. and Ensley, .B. ed. *Phytoremediation of Toxic Metals: Using plants to clean up the Environment.* John Wiley and Sons, Inc. New York.

Reisinger, S., Shiavon, M., Terry, N. & Pilon-Smits, E.A.H. (2008). Heavy metal tolerance and accumulation in Indian mustard (*Brassica juncea* L.) expressing bacterial gamma-glutamylcysteine synthase or glutathione synthase. *International Journal of Phytoremediation* 10(5):440-454.

Richard, T. W., Patrick, V.B. & Doughlas, B.K. (2007). Assessment for Non-radionuclides of lead. *EPA*/600/140.

Robertson, G.P., Sollins, P., Ellis, B.G. & Lajtha, K. (1999). Excahangeable ions, pH and cation exchange capacity In Robertson, G.P., Coleman, D.C., Bledsoe, C.S. & Sollins, P. eds. *Standard soil methods for long term ecological research*. New York NY: Oxford University Press. PP 106-114.

Robinson, B., Schulin, R., Nowack, B., Roulier, S., Menon, M., Clothier, B., Green, S. & Mills, T. (2006). Phytoremediation for the management of metal flux in contaminated sites. *For. Snow Landsc. Res* 80 (2): 221-234.

Robinson, B.H, Leblanc, M., Petit, D. Brooks, R.R., Kirkman, J.H. & Gregg, .P.E.H (1998).The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. *Plant and Soil* 203: 47-56.

Robinson, B.H., Brooks, R.R., Howes, A.W., Kirkman, J.H. & Gregg, P.E.H (1997). The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytoming. *Journal of Geochemical Exploration* 60: 115-126.

Robinson, B.H., Fernandez, J.E., Maranon, T., Murillo, J.M., Green, S.R. & Clothier, B.E. (2003). Phytoextraction: An assessment of biogeochemical and economic viability. *Plant and Soil* 249(1): 117-125.

Robinson, D. (1994). The response of plants to non-uniform supplies of nutrients. *New Phytologist* 127: 635-674.

Romeh, A.A. (2010). Phytoremediation of water and soil contaminated with imidacloprid pesticide by *Plantago major* L. *International Journal of Phytoremediation* 12(2): 188-199.

Romeiro, S. Lagoa, M.M.A., Furlani, P.R., De Abreu, C.A., De Abreu, M.F. & Erismann, N.M. (2006). Lead uptake and tolerance of *Ricinus communis* .L. *Brazilian Journal of Plant Physiology* 18(4):483-489.

Rothwell, J.J., Evans, M.G., Daniels, S.A. & Allott, T.E.R. (2008). Peat as a source of lead contamination to upland fluvial systems. *Environmental Pollution*. 253:582-589.

Rotkhttikhun, M.K., Ghaiyarat, C.N., Pokethitiyook, P., Paijitprapaporn, .A. & Baker, A.J.M.(2006).Uptake and accumulation of Lead by plants from the Bo Ngam lead mine area in Thailand. *Environmental Pollution* xx: 1-8.

Rouw, A.D. (1991). The invasion of *Chromolaena odorata* (L.) King & Robinson (ex *Eupatorium odoratum*), and competition with native flora, in a forest zone, South- West Cote d'Ivoire. *Journal Biogeogr*. 18:13-23.

Roy, M. & Macdonald, L.M. (2013).Metal uptake in plants and health risk assessment in metal -contaminated smelter soil. *Land Degradation Development*. DOI: 10 .1002\ldr. 2237.

Safae, B.E., Jamal, O. Nadia, S. & Abdelhak, B. (2008). Uptake and fixation of Zn, Pb and Cd by *Thlaspi caerulescens*: application in the cases of old mines of Mibladen and Zaida (West of Morocco). *Arab Journal of Geosciences* 1: 87-95.

Sakamoto, T & Matsuoka, M. (2004).Generating high-yielding varieties by genetic manipulation of plant architecture. *Curr. Opin. Biotechnol*. 15:144-147.

Salami, A.T., Jimoh, M.A & Muoghalu, J.I. (2003). Impact of gold mining on vegetation and soil in SouthWestern Nigeria. *International Journal of Environmental Studies* 60:343-352.

Salehi, H., Ransom, C.B., Oraby, H.F., Seddighi, Z. & Sticklen, M.B (2005). Delay in flowering and increase in biomass of transgenic tobacco expressing the Arabidopsis floral repressor gene flowering locus C. *Journal Plant Physiol*. 162: 711-717.

Samardakiewicz, S. Krzeslowska, M., Bilski, H., Bartosiewicz, R. & Woz'ny, .A. (2012). Is callose a barrier for lead ions entering *Lemna minor* .L. root cells? *Protoplasma* 249:347-351.

Sas-Nowosielska, A., Kucharsi, R., Malkowski, E., Pogrzeba, M., Kuperberg, J.M. & Krynski, K. (2004). Phytoextraction crop disposal- an unsolved problem. *Environmental Pollution* 28:373-379.

Schmidt, G.J. & Schilling, E.E. (2000).Phylogeny and Biogeography of Eupatorium (Asteraceae: Eupatoreae) based on Nuclear ITS Sequence. *American Journal of Botany* (*Botanical Society of America*) 87(5): 716-726.

Schwartz, C., Gerard, E., Perronnet, K. & Mrel, J.L. (1999a). Phytoextraction of metals from polluted soils by the hyperaccumulator *Thlaspi caerulescens*. *South African Journal of Science* 97(11/12):561-564.

Schwartz, C., Morel, .J.L., Saunier, S., Whiting, S.N. & Baker, A.J.M. (1999b). Root development of the Zinc –hyperaccumulator plant *Thlaspi caerulescens* as affected by metal origin, content and localization. *Plant and Soil* 208: 103-115.

Schwartz, C., Saison, C. & Jean-Louis, M. (2004). Hyperaccumulation of metals by *Thlaspi caeruslescen* as affected by root development and Cd-Zn/Ca-Mg interactions. *International Journal of Phytoremediation* 6(1):46-61.

Seeds Online (2011). *Http//www/moles seed/co/uk.* Retrieved 8/2/2012.

Seul-Ji, L., Myoung-Eun, L., Jae Woo, C., Jin Hee, P., Keun Young, H. & Gee-III, J. (2013). Immobilization of lead from Pb-contaminated soil amended with peat moss. *Journal of Chemistry* 2:1-6.

Shahid, M., Pinelli, E. & Dumat, C. (2012). Review of Pb availability and toxicity to plants in relation with metal speciation; role of synthetic and natural organic ligands. *Journal of Hazardous Materials* 12(1): 219-220.

Sharma, P. & Dubey, R. (2005). Lead toxicity in plants. *Brazilians Journal of Plant Physiology* 17: 35-52.

Sharp, R.E. (1990).Comparative sensitivity of root and shoot growth and physiology to low water potentials. In Davies, W.J., Jeffcoat, .B. (Eds) Importance of root to shoot communication in the response to Environmental stress. British Society for plant growth regulation. *Monograph* 21: 29-44.

Shella, G.S.G., Langa, A.R.G. & Salea, P.J.M. (1974). Quantitative measures of leaf orientation and heliotropic response in sunflower, bean, pepper and cucumber. *Agricultural Meteorology* 13 (1): 25-37.

Shen, Z.G. & Liu, Y.L. (1998). Progress in the study on the plants that hyperaccumulate heavy metals. *Plant Physiology Community* 34:133-139.

Shinwell, D.W. & Laurie, A.E. (1972). Lead and Zinc contamination of vegetation in the Southern Penines. *Environmental Pollution* 3: 391-301.

Sholkovitz, E.R & Copland, D. (1981). The coagulation, solubility and adsorption properties of Fe, Mn, Cu, Ni, Cd, Co and humic acids in a river water. *Geochim. Cosmochim*. Acta 45:181.

Shu, W.S., Ye, Z.H., Lan, C.Y., Zhang, Z.Q. & Wong, M.H. (2001). Acidification of lead/ Zinc mine tailings and its effect on heavy metal mobility. *Environment International* 26: 389-394.

Sinha, S. Gupta, A.K. Bhatt, K., Pandey, K., Rai, U.N. & Singh, K.P. (2006).Distribution of metals in the edible plants grown in Jajmau, Kanpur (India) receiving treated tannery waste water: relation with physic chemical properties of the soil. *Environ. Monit. Assess*. 115:1-22.

Sipos, P., .Nemeth, T. & Mochai, I. (2005). Distribution and possible immobilization of lead in a forest soil (luvisol) profile. *Environmental Geochemical Health* 27:1-10.

Sorvari, J., Antikainen, R. & Pyy, O. (2006). Environmental contamination at Finnish shooting ranges-the scope of the problem and management options. *Science of the Total Environment* 366 (1): 21-31.

Stevenson, F.J. (1983).Trace metal-organic matter interactions in geologic environments In *Trace Elements in petrogenesis*. Augustithis, .S.S. ed., Theophrastus Publications, Athens. 671.

Strubelt, O., Kremer, J., Tilse, A., Keogh, J., Pentz, R. & Younes, M. (1996). Comparative studies on the toxicity of mercury, cadmium and copper toward the isolated perfused rat liver. *Journal of Toxicology and Environmental Health* 47(3):267-283.

Stuefer, J.F. During, .H.J. & De Kroon, H. (1994). High benefits of clonal integration in two stoloniferous species, in response to heterogeneous light environments. *Journal of Ecology,* 82:511-518.

Stuefer, J.F., De Kroon, H. & During, H. (1996). Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. *Functional Ecology.* 10: 328-334.

Subramanian, M.S (2011) Analysis of soils, sediments and biological specimens. *Environmental Chemistry and Analysis* 6(1):1-10.

Suh, C.H., Park, H.S., Nahm, D.H. & Kim, H.Y. (1988). Oilseed rape allergy presented as occupational asthma in the grain industry. *Clinical and Experimental Allergy* 28 (9): 1159-1163.

Taiz, L. & Zeighler, E. (2002). *Plant Physiology*. Sinauer Associates. Sunderland. U.S.A pp 690.

Taku, D and Zheng-Hua, .Y. (2010).Regulation of plant biomass production. *Current Opinion in Plant Biology* 13:299-304.

Tan, K.H. (1998). *Principles of soil Chemistry*. 3rd edition Marcel Dekker, New York.

Tanhan, P., Kruatrachue, M., Pokethitiyook, P. & Chaiyarat, R. (2007). Uptake accumulation of cadmium, lead and zinc by Siam weed (*Chromolaena odorata* (L.) King and Robinson). *Chemosphere* 68: 323-329.

Taylor, L.C., Elrick, K.A., Horowitz, A.J. & Deocampo, D.M (2013). Environmental lead contamination: Heavy metals and cerussite in Nigerian Gold ores. *Conference Proceedings of the Geological Society of America* 12*:* 233-234.

Taylor, P. (2003). Uncertainty of geochemical measurements of contaminated land; Causes, estimationand cost-based optimisation. PH.D Thesis, School of Life Sciences, University of Sussex. *Sussex Research Online*.

Taylor, P.D., Ramsey, M.H. & Potts, P.J. (2005). Spatial contaminant heterogeneity: Quantification with scale of measurement at contrasting sites. *Journal of Environmental Monitoring* 7(12): 1364-1370.

Tessier, P., Campbell, G.C. & Blsson, M. (1979). Sequential extraction procedures for the speciation of particulate trace metals. *Analytical Chemistry* 51(7):844-859.

Thanh, N.H., Tran, T.L.H., Cao, V.H., Duc Hung, N. & Phan, Q.H. (2013). Uptake of Pb. Zn and Cu by roots and shoots of fast growing plants grown in contaminated soil in Vietnam. *Journal of Soil Science and Environmental Management* 4(6):108-115.

The Food Standards Agency (FSA) (2007). Survey of metals in a variety of foods. Food Survey *Information Sheets on the WWW: http://www.food.gov.uk/science/surveillance*.

Thomas, J.Y, Ramsey, M.H., John, E.A. & Barnes, R. (2008). Quantification of in situ heterogeneity of contaminants in soil: A fundamental prerequisite to understanding factors controlling plant uptake. Proceedings of Consoil 2008 *(10th International UfZ-Deltares/TNO Conference on soil water systems*. Milan Italy, Theme C 101-106.

Thomas, J.Y. (2010). Quantification of *in situ* heterogeneity of contaminants in soil: A fundamental prerequisite to understanding factors controlling plant uptake. *Ph.D thesis*. Department of Biology and Environmental Science. Sussex Research Online.

Thompson, M. & Walsh, J.N. (1983). *A Handbook of Inductively Coupled Plasma Spectrometry*. Blackie and Sons Ltd. London.

Thompson, M. (1999). *Journal of Environmental Monitoring* 1:19-21.

Thompson, P.L., Ramer, L.A. & Schnoor, J.L. (1998). Uptake and Transformation of TNT by hybrid poplar Trees. *Environmental Science Technology* 32:975-980.

Thornton, I. & Howarth, R.J. (1986). Applied Geochemistry in the 1980s. Graham & Trotman limited. London. Pp 4-32. 6643-6649.

Ting-nung, H., Shang-Wu, L. & Ching-ju, W. (1988) In Ting-nung, H. Gentianaceae*. Fl. Reipubl.Popularis Sin*. 62: 1-411.

Traunfield, J.H. & Clement, D. (2001). *Lead in garden soils, home and Gardens*. College Park, M.D. Maryland Cooperative Extension, University of Maryland. Pp 23-48.

Tristan-Montero, E. E. (2000). Human health risk assessment for contaminated land in historical mining areas. *T. H. Huxley School of the Environment, Earth Sciences and Engineering*. London, Imperial College of Science, Technology and Medicine**:** 346.

Troeh, F.R. & Louis, M.T. (2005). *Soils and soil fertility*. 6th ed. Blackwell Publishers Ames, Iowa.

Turan, M. & Bringu, A. (2007). Phytoremediation based on canola (*Brassica napus* L.) and Indian mustard (*Brassica juncea* L.) planted on spiked soil aliquot amount of Cd, Cu, Pb and Zn. *Plant Soil and Environment* 53(1): 7-15.

Udeigwe, T.K, Eze, P.N, Teboh, J.M. & Stietiya, M.H. (2011). Application, chemistry and Environmental implications of contaminant-immobilization amendments on agricultural soil and water quality. *Environment International* 37(1):258-267.

Ulrich S., (2003). "Enhancing Phytoextraction: The Effect of Chemical Soil Manipulation on Mobility, Plant Accumulation, and Leaching of Heavy Metals". *J. Environ. Qual.* 32 (6): 1939–54.

United States Geological Survey {USGS} (2013). Historical Statistics for Mineral and Material Commodities in the United States. *Data Series* 140. (http://minerals.usgs.gov/minerals/pubs/commodity/index.html) Date retrieved 30th April, 2014.

United States Department of Agriculture (USDA) (1993): Nitrate leaching. Extension Service. *Special Project* number 89-EWQI-1-9203.

United States Department of Agriculture (USDA) (2008). National Genetic Resources Programme. *Germplasm Resources Information Network*. 708: 1-33.

United States Environmental Protection Agency (US EPA) (1998). Standard operating procedure: In vitro method for determination of lead and arsenic bio-accessibility. Washington DC, *US Environmental Protection Agency***:** 7.

United States Environmental Protection Agency (USEPA) (1992). Selection of Control Technologies for Remediation of Lead Battery Recycling Sites. *EPA*/540/S-92/011. *Office of Emergency and Remedial Response*, Washington, D.C.

United States Environmental Protection Agency (USEPA) (1997). Cleaning up the Nation`s waste sites. Markets *and Technology Trends. EPA* 005/96/542.

United States Environmental Protection Agency (USEPA) (2000). Electro-kinetic and phytoremediation *in situ* treatment of metal contaminated soil. State of practice. *US Environmental Protection Agency*. Washington, .D.C (XXX): 542.

USEPA (1996). Soil Screening Guidance: *Technical background Document* (EPA/540/R-95/128) Washington, DC, United States Environmental Protection Agency.

Vamerali, T., Bandiera, M. & Mosca, G. (2010). Field crops for phytoremediation of metal- contaminated land—a review. *Environ. Chem. Lett*. 8:1-17.

Vanhoof, C., Corthouts, V. & Tirez, K. (2004). Energy-dispersive X-ray fluorescence systems as analytical tool for assessment of contaminated soils. *Journal of Environmental monitoring* 6(4):344-350.

Vegas, F.A., Covelo, E.F., Vazques, J.J. & Abdrade, L. (2007). Influence of mineral and organic components on copper, lead and zinc sorption by acid soils. *Journal of Environmental Science Health* Part A- *Toxic/Hazard Substance Environ. Eng*. 42:2167-2173.

Wang, H.Q., LU, S.J., Li, H. & Yao, Z.H. (2007). EDTA- enhanced phytoremediation of lead contaminated soil by *Bidens maximowicziana*. *Journal of Environ. Sci (China)*. 19(12):1496-1499.

Wang, J., Yang, L., Daxia, W., Dayuan, L., Hong, T., Jianwen, W., Yali, T., Wang, C., Yanjun, C. & Kairong, C. (2012). An evaluation approach to spatial heterogeneity of arable soil and safety in vegetable production on it. *Technological Advances in Biomedical Engineering*, 6:126-132.

Wang, L., Mou, P.P & Jones, R.H. (2006). Nutrient foraging via physiological and morphological plasticity in three plant species. *Canadian Journal of Forest Research* 36(10:164-173.

Wang, Q. & Cheng, Y. (2004). Response of fine roots to soil nutrient spatial heterogeneity. *Journal of Applied Ecology* 15(6): 1063-1068.

Wang, R.L., Cheng, R.M., Xiao, W.F., Feng, X.H., Liu, Z.B., Wang, X.R. & Wang, Z.B (2013). Spatial heterogeneity of fine root biomass of *Pinus massoniana* forests in the three Gorges Reservoir Area, China. *Forest Science and Practice* 15(1):13-23.

Wayne, S.J and Ames, .M. (2012).A review of factors affecting plant growth. *Agrikhalsa*12:26-28.

Weed Management Guide (2012). Siam weed (*Chromolaena odorata*) *Natural Heritage Trust* 2: 1-6.

Weedon, R.R. (1973). Taxonomy and distribution of the Genus *Bidens* (Compositae) in the North-Central Plain states. *PhD Dissertation*. University of Kansas.

Whiting, S.N., Leake, J.R., Mcgrath, S.P. & Baker, A.G.M. (2000). Positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. *New Phytologist* 145(2): 199-200.

Wijensinghe, D.K. & Handel, S.N. (1994). Advantages of clonal growth in heterogeneous habitats: an experiment with *Potentilla simplex*. *Journal of Ecology* 82:495-502.

Wijensinghe, D.K., John, E.A., Beurskens, S. & Hutchings, M.J. (2001). Root system size and precision in nutrient foraging: responses to spatial pattern of nutrient supply in six herbaceous species. *Journal of Ecology* 89: 927-983.

Wijesinghe, D.K & Hutchings, M.J. (1997).The effects of spatial scale of environmental factors on growth of a clonal plant: An experimental study with *Glechoma hederacea*. *Journal of Ecology*, 85:17-28.

Wijesinghe, D.K & Hutchings, M.J. (1999). The effects of environmental heterogeneity on *Glechoma hederacea*: the interactions between patch contrast and patch scale. *Journal of Ecology* 87: 2322-2334.

Wilkes, G. (2004). Corn, strange and marvellous: but is a definitive origin known In; Smith, C., Runge, B.J. *Corn: Origin, History*, *Technology and Production*. Wiley pp3-63.

Williams, S.T, McNeilly, T. & Wellington, E.M.H. (1977). The decomposition of vegetation growing on metal mine waste. *Soil Biol. Biochemistry*. 9:271.

Wilson, D.O & Cline, J.F (1996). Removal of Plutonium-239, tungsten-185 and Lead-120 from soils. *Nature* (London). 209-941.

Wong, M.H. (2003).Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere* 50: 775-780.

Woodbury, P.B. (2003). Dos and Don`ts of spatially explicit ecological risk assessments. *Environmental Toxicology and Chemistry* 22(5):977-982.

Woods, D.L., Capcara, J.J. & Downy, R.K. (1991). The potential of mustard (*Brassica juncea* (L.).Coss) as an edible oil crop on the Canadian praires. *Canada Journal of Plant Science*. 71:195-198.

World Health Organization (W H O) (1995). Lead. *Environmental Health Criteria* Vol. 165 Geneva.

www.discoverlife.org (2011). Image of *Brassica juncea*. Date retrieved 10/10/2011.

Yoon, J. Cao, X., Zhou, O. & Ma, .L. (2006). Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida Site. *Science Total Environment* 368:456-464.

Yusuf, A.A., Abdu, N. & Tanimu, .B. (2011). Mobilizing agricultural research towards attaining food security and industrial growth in Nigeria. *Proceedings of the 45th Annual Conference of the Agricultural Society of Nigeria*. 487-491.

Zabowski, D., Henry, C.L., Zheng, Z. & Zhang, X. (2001). Mining impacts on trace metal content of water, soil and stream sediments in the Hei River Basin, China. *Water, Air and Soil Pollution* 131: 261-273.

Zahran, S. Laidlaw, M.A.S, McELmurry, S.P. Filippelli, G.M. & Taylor, M.P. (2013). Linking source and effect: Re-suspended soil lead, air lead and children blood lead levels in Detroit, Michigan. Environmental Science and Technology 47 (6): 2839-2845.

Zar, J.H. (1999): *Bio-statistical Analysis*. Fourth edition. Prentice Hall International INC. Upper Saddle River New Jersey. PP 103-107.

Zhivotovsky O.P., Kuzovkina Y.A., Schulthess C.P., Morris, T. Pettinelli D. (2011). Lead uptake and translocation by willows in pot and field experiments. *International Journal of Phytoremediation* 3(8):731-49.

Zhu, X.G., Long, S.P. & Ort, .D. R. (2008). What is the maximum efficiency with which photosynthesis can convert solar energy into biomass *Current Opinion Biotechnology* 19: 153-159.

Zhuang, P., McBride, M.B., Xia, H., Li, N. & Li, Z. (2009).Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. *Science of the Total Environment* 407(5):1551-1561.

Zhukovsky, P.M. (1971). *Cultivated plants and their Congeners*. Leningrad PP 206-215.

Zimdahl, R.L (1975). *Entry and movement in vegetation of lead derived from air and soil sources*. Paper presented at 68th Annual Meeting of the Air Pollution Control Association. Boston, M.A.

Zimmerman, A.J. & Weidorf, D.C (2010).Heavy metal and trace metal analysis in soil by sequential extraction: A review of procedures. *International Journal of Analytical Chemistry* 10:1155.

Zyan, P. & Ryan, J.A (1999). Transformation of Pb (II) from cerussite to chloropyromorphite in the presence of hydroxyapatite under varying conditions of pH. *Environmental Science and Technology* 33:625-630.

APPENDICES

APPENDICES RELATED TO FIELD INVESTIGATION (CHAPTER 3).

Appendix I.1: Specification, principle of operation and calibration of the Portable X-Ray Fluorescence (P-XRF).

Specification

The Niton XL3t900 GOLDD P-XRF used represent a category of hand-held instrumentation that is capable of *in situ* simultaneous multi-element analysis outside the confines of a laboratory. It has a Ag target X-ray tube, Si drift detector, 6.2 kV Si and P X-ray tube voltage, 100µA tube current, an 8mm X-ray spot diameter and two K element lines.

Principle of operation.

The principle of operation is as described by AMC, (2009). X-rays from an excitation source interact with the test surface, causing an emission of secondary fluorescence X-rays that have energies characteristic of the atoms of the excited material. Fluorescence X-rays area detected using an energy dispersive system (Silicon drift detector {SDD}). In-built sophisticated software with high computing power compensates for background variation and line overlap and provides a comprehensive matrix correction. Qualitative analytical results can be obtained while an analysis is being undertaken or immediately after the end of a count.

Calibration.

The empirical and the fundamental approaches are two ways to calibrate the P-XRF. A site specific calibration is employed by the empirical method. Prior to analysis with the P-XRF, samples are taken and analysed using traditional laboratory techniques. They are then used to create sub-site specific reference materials (Kerna *et al*., 2011).

The fundamental method uses a theoretical approach involving inter element coefficient (Kalnicky and Singhvi, 2001). In this approach, the inverse relationship between peak intensities of the Rayleigh and Compton scatter to atomic number is used to estimate the average balance of the sample. The Niton XL3t900 GOLDD P-XRF is calibrated by analysing a range of reference material prior to *in situ* analysis.

Appendix I.2: Field mesurements data quality.

Table AI.2: Summary table showing Detection limits of *in situ* measurement using the P-XRF.

CRM	Mean measured Pb conc. (mg/kg)	median 3 s
CCRMP TILL-4	26	12
GBW 7411	2618	72
HRM 31	6754	109
NCS 73308	11	8
NIST 2710a	5447	108
NIST 2711a	1347	47
RCRA	467	36

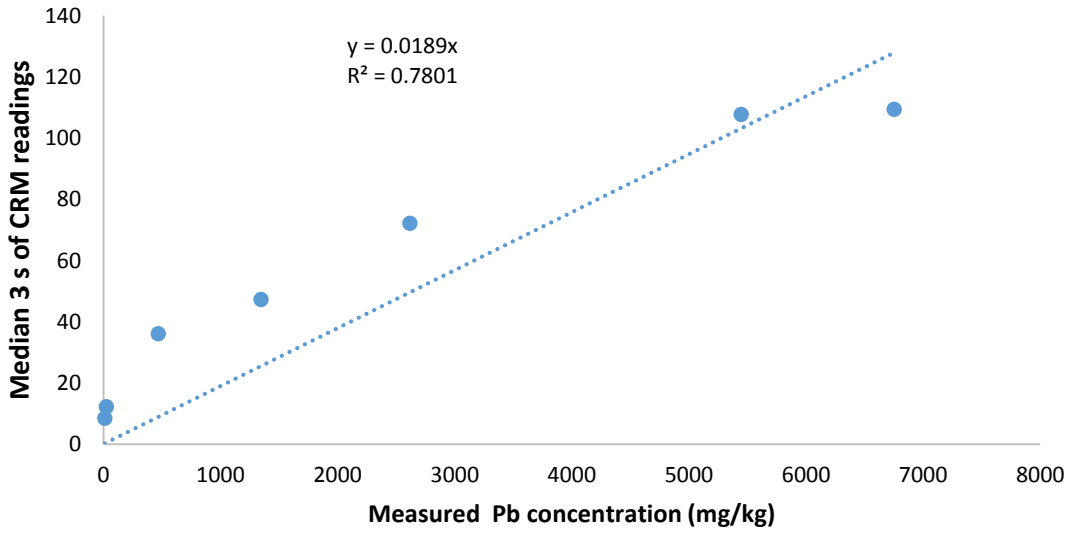


Figure AI.2 Regression model of median of 3 s of CRM readings against measured concentration. Detection limits have been calculated using median value of 3 standard deviation for each CRM readings extrapolated to zero concentration.

Appendix I.3: Data from regression analysis of P-XRF mean Pb measurements of certified reference materials against certified values.

Table AI.3: Measured and certified values of CRMs.

CRM	Mean measured concentration (mg/kg)	Certified values
NCS 73308	11	27
CCRMP TILL-4	26	50
RCRA	467	500
NIST 2711a	1347	1400
GBW 7411	2618	2759.24
NIST 2710a	5447	5552
HRM 31	6754	6895

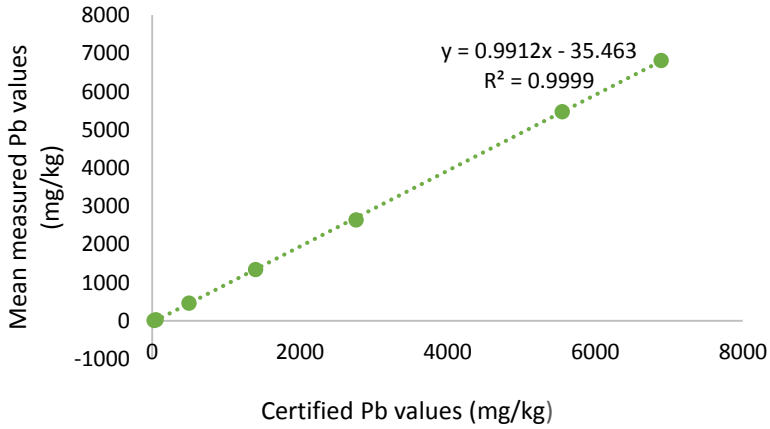


Figure AI.3: Regression of certified reference materials (CRMS) for estimation of instrumental bias for P-XRF.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	1.000[a]	1.000	1.000	28.45141

CRMs

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-35.463	14.888		-2.382	.063
	Certified Pb	.991	.004	1.000	236.313	.000

Appendix I.4: Table of Summary of *in situ* Pb measurements.

Table AI.4: Summary of RANOVA analysis for Gang Mine

Site	**GANG MINE (GM)**		**Pb**				
Scale (m)	**0.02**	**0.05**	**0.2**	**0.5**	**2.0**	**5**	**20**
	n=10	**n=8**	**n=6**	**n=8**	**n=8**	**n=360**	**n=240**
Robust Mean conc(mg/kg)	7991	5362	6401	5907	6720	5443	5617
Geometric mean (mg/kg)	6773	4547	3115	3667	3752	3348	3371
Arithmetic mean (mg/kg)	8958	7100	6401	6130	6720	6070	6210
HFsamp	1.24	1.44	1.95	1.88	2.33	2.36	3.22
Class u(1s)	18	25	54	77	97	97	118
Robust u(1s)	13	37	61	50	107	71	86

Table BI.4: Summary of RANOVA analysis for Black Rock

Site	**BLACK ROCKS (BR)**		**Pb**				
Scale (m)	**0.02**	**0.05**	**0.2**	**0.5**	**2.0**	**5**	**20**
	n=10	**n=8**	**n=6**	**n=8**	**n=8**	**n=360**	**n=240**
Robust Mean conc(mg/kg)	35107	32781	26631	33100	24627	29829	30013
Geometric mean (mg/kg)	32506	28136	19752	27024	18337	23299	23679
Arithmetic mean (mg/kg)	35107	32781	26631	31758	24374	30846	30834
HFsamp	1.17	1.23	1.31	1.21	1.97	1.81	2.22
Class u(1s)	16	24	10	22	34	42	58
Robust u(1s)	15	22	12	22	33	40	59

Footnote: **HFsamp**-Heterogeneity factor of sample

Classical u (1s)—Classical uncertainty 1standard deviation, **Robust u (1s)**–Robust uncertainty 1 standard deviation,

Appendix I.5: Regression of *in situ* Heterogeneity factor (HF) against scale for Black Rocks and Gang Mine.

Table AI.5: Black Rocks

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.373	.125		11.004	.000
	Scale	.047	.016	.797	2.954	.032

a. Dependent Variable: HF

Table BI.5: Gang Mine

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.748	.157		11.158	.000
	Scale	.079	.020	.869	3.928	.011

a. Dependent Variable: HF

Appendix I.6: Heterogeneity factor HF and soil Pb concentration (mg/kg).

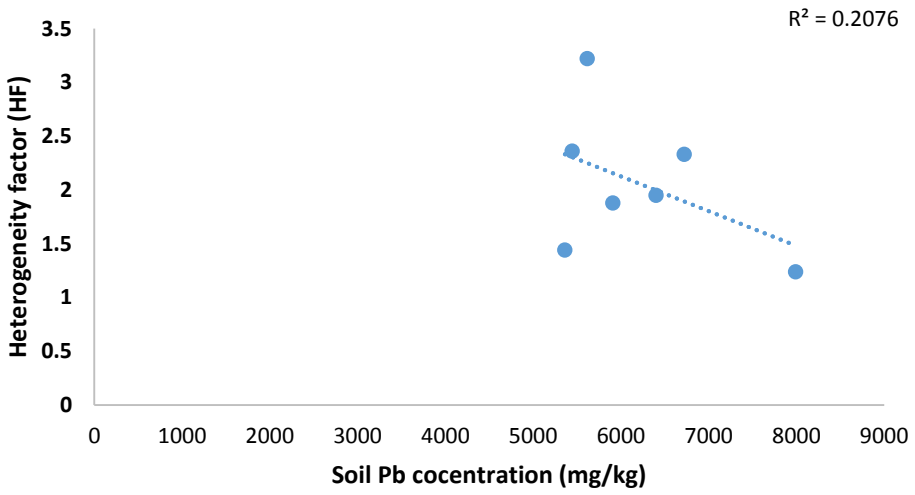


Figure AI.6: A regression model of Heterogeneity factor (HF) against soil Pb concentration (mg/kg) in Gang Mine.

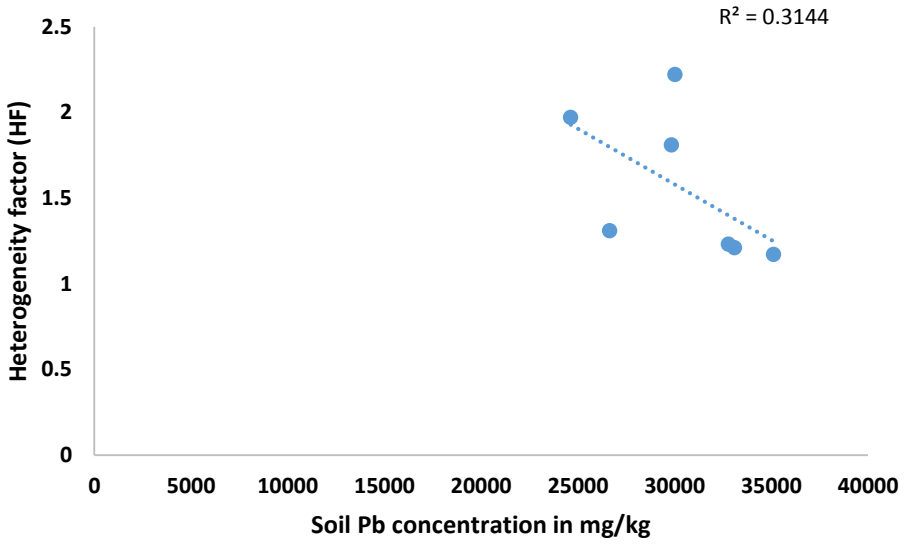


Figure BI.6: Regression model of heterogeneity factor (HF) against soil Pb concentration (mg/kg) in Black Rocks.

Appendix I.7: Test for normal distribution of log-transformed measured Pb concentration of field sites.

Table AI.7: Gang Mine

One-Sample Kolmogorov-Smirnov Test

		Gang mine Pb concentration
N		100
Normal Parameters[a,b]	Mean	3.5122
	Std. Deviation	.51132
Most Extreme Differences	Absolute	.070
	Positive	.066
	Negative	-.070
Test Statistic		.070
Asymp. Sig. (2-tailed)		.200[c,d]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

d. This is a lower bound of the true significance.

Table BI.7: Black Rocks.

One-Sample Kolmogorov-Smirnov Test

		BlackrockPbconcentration
N		100
Normal Parameters[a,b]	Mean	4.3632
	Std. Deviation	.38885
Most Extreme Differences	Absolute	.107
	Positive	.093
	Negative	-.107
Test Statistic		.107
Asymp. Sig. (2-tailed)		.006[c]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

Appendix I.8: Copyright permission for maps used in Chapter 3

Send Options... HTML

To...

Cc...

Subject: FW: GV-156781 C Grace Solomon-Wisdom - OS OpenData

Tahoma 10 **B** *I* U

From: Customer Services [CustomerServices@os.uk]
Sent: Tuesday, March 10, 2015 10:43 AM
To: Grace Oyiza Solomon-Wisdom
Subject: RE: GV-156781 C Grace Solomon-Wisdom - OS OpenData

Dear Grace

Thank you for contacting Ordnance Survey to request permission to use mapping in your thesis.

I am pleased to inform you that the Ordnance Survey has no objection to you using the mapping for this purpose, and on this occasion royalties have been waived providing the following conditions are met:

- The maps are for sole use as part of your thesis.
- The maps will not be used for any commercial purpose.
- The maps will not be passed, sold or demonstrated to any third party without our prior consent.
- All plot/copies must show the following acknowledgement:

  "Reproduced from (insert date of publication) Ordnance Survey map with the permission of the Controller of Her Majesty's Stationery Office, Crown Copyright NC/insert month/insert year."

I hope this is helpful to you, and may I wish you luck with your thesis.

Kind regards

Jane Chiplin
Customer Service Adviser
Customer Service Centre, Ordnance Survey

Ordnance Survey

Adanac Drive, Southampton, United Kingdom, SO16 0AS
Phone +44 (0)3456 050505 | Fax +44 (0)3450 990494
www.os.uk | customerservices@os.uk
Please consider your environmental responsibility before printing this email.

From: Grace Solomon-Wisdom [mailto:G.Solomon-Wisdom@sussex.ac.uk]
Sent: 09 March 2015 14:15
To: Customer Services
Subject: GV-156781 Grace Solomon-Wisdom - OS OpenData

APPENDICES RELATED TO POT TRIALS 1 AND 2 (CHAPTER 4) ON REWRITABLE CD ATTACHED TO THESIS.

Appendix II.3: First pot trial test for significance shoot dry biomass between treatments of statistically significant species/varieties (BJ 18, BJ 42, BN SW, BNK, ZM B73 and ZM 64)-----CD

Appendix II. 4: First pot trial test for significance of root and total dry biomass between treatments of statistically significant species/varieties (BJ 18, BJ 42ZM B73, ZM OH43, ZM 64, BNK & BN SW)-----CD

Appendix II.6: Table of significance and Tukey HSD homogeneous subset of comparison of shoot, root, and total plant Pb concentrations mg/kg DW between species/varieties (First pot trial)-----CD

Appendix II.7: Tukey H.S.D comparison of shoot, root and total plant Pb mg/kg within species/varieties (Pot trial 1) ---CD

Appendix II. 9: Tukey HSD test of significance of shoot, root, total dry biomass and Growth Index (Pot trial 2) ----CD

Appendix II. 10: Table of homogeneous subsets of Tukey HSD test for shoot, root and total plant Pb mg/kg DW (Pot trial 2) -----CD

Appendix II. 15: ANOVA of shoot, root and total biomass of species/varieties in pot trial 2----CD

Appendix II. 16: Regression of biomass against lead concentration gradient for all species and varieties (2nd pot trial) -----CD

APPENDICES RELATED TO POT TRIALS 1 AND 2 (CHAPTER 4) ON HARD COPY

Appendix II.5:Pot trial 1 test for significance of shoot and root Concentration factors.

AII.5: One-way ANOVA: CFshoot between Species/varieties

Source	DF	SS	MS	F	P
Species or varieties	12	2.321149	0.193429	371.57	0.000
Error	26	0.013535	0.000521		
Total	38	2.334684			

S = 0.02282 R-Sq = 99.42% R-Sq(adj) = 99.15%

Grouping Information Using Tukey Method

Species or varieties	N	Mean	Grouping
BJ 21	3	0.9931	A
TC HS	3	0.2895	B
BJ 42	3	0.1576	C
ZM 64	3	0.1381	C D
TC BR	3	0.1320	C D
BJ 17	3	0.1295	C D
BJ 18	3	0.0910	C D E
ZM 73	3	0.0906	C D E
BN K	3	0.0723	D E
ZM 37	3	0.0572	E
BN SW	3	0.0530	E
ZM OH43	3	0.0489	E
TC GM	3	0.0468	E

Means that do not share a letter are significantly different.

BII.5: One-way ANOVA: CFroot between Species/varieties

Source	DF	SS	MS	F	P
Species or varieties	12	1.51690	0.12641	58.75	0.000
Error	26	0.05595	0.00215		
Total	38	1.57285			

S = 0.04639 R-Sq = 96.44% R-Sq(adj) = 94.80%

Grouping Information Using Tukey Method

Species or varieties	N	Mean	Grouping
BJ 18	3	0.70533	A
TC BR	3	0.69194	A
ZM 73	3	0.63370	A
BJ 42	3	0.49525	B
ZM 64	3	0.45902	B C
BN K	3	0.42206	B C
ZM 37	3	0.41124	B C
TC HS	3	0.39331	B C D
BN SW	3	0.33430	C D E
ZM OH43	3	0.26820	D E
BJ 17	3	0.21591	E F
TC GM	3	0.12508	F G
BJ 21	3	0.04195	G

Means that do not share a letter are significantly different.

Appendix II.1: Data from first pot trial.

Table AII.1: Simple randomized block design for the first pot trial.

Treatment Block 1													
1	BN SW	TC BR	ZM 64	BJ 18	ZM 73	BJ 17	TC HS	ZM 64	ZM OH43	BJ 21	TC BR	BJ17	ZM73
2	BJ 21	ZM 37	BJ 42	BN K	TC GM	BJ 21	BJ 18	BN SW	BJ 42	BN SW	ZM OH43	ZM 64	BJ 42
3	BN K	ZM OH43	TC HS	BJ 17	TC HS	ZM 37	TC GM	ZM 73	TC BR	ZM 37	BN K	TC GM	BJ 18

Treatment Block 2													
1	TC BR	BJ 42	TC GM	BN K	BJ 18	TC HS	ZM OH43	TC GM	ZM 64	BJ 42	TC BR	ZM OH43	BN SW
2	ZM 73	BJ 21	ZM 37	TC HS	TC GM	BJ 21	BJ 17	ZM73	TC BR	BN K	BJ 17	BJ 21	BJ 18
3	BJ 18	ZM OH43	BJ 17	ZM 64	BJ 42	ZM 37	BN SW	BN K	TC HS	ZM 37	BN SW	ZM 64	ZM 73

Table BII.1: Estimated water content of silver sand of growth media (First and second pot trial).

Dish No	Sand bag No	Dish WT (g)	Dish + FW(g)	Dish + DW (g)	FW(g)	DW (g)	% Moisture
1	1	42.48	196.93	196.82	154.45	154.34	0.07
2	1	54.40	199.16	199.04	144.76	144.64	0.08
3	2	51.19	183.49	183.42	132.30	132.23	0.05
4	2	61.55	205.81	205.73	144.26	144.18	0.05
5	3	42.48	196.93	196.82	154.45	154.34	0.07
6	3	63.83	188.49	188.40	124.66	124.57	0.07
7	4	54.19	205.39	205.21	151.20	151.02	0.12
8	4	51.20	201.59	201.41	150.39	150.21	0.12
9	5	61.55	139.40	139.32	77.85	77.77	0.10
10	5	31.31	108.22	108.12	76.91	76.81	0.13
11	6	32.39	101.88	101.80	69.49	69.41	0.11
12	6	32.04	99.23	99.04	67.19	67.00	0.28
13	7	101.79	180.51	180.37	78.72	78.58	0.18
14	7	113.03	194.93	194.78	81.90	81.75	0.18
15	8	61.55	140.84	140.73	79.29	79.18	0.21
16	8	31.31	118.61	118.50	87.30	87.19	0.13
17	9	32.39	115.03	114.92	82.64	82.53	0.13
18	9	32.04	112.06	111.92	80.02	79.88	0.18
19	10	31.30	135.42	135.35	104.12	104.05	0.06
20	10	58.48	109.66	109.58	51.18	51.10	0.20
						Mean	0.12
						SD	0.06
						%RSD	49

Table CII.1: Estimated water content of John Innes compost 2.

Dish No	Compost bag No	Dish WT (g)	Dish + FW(g)	Dish + DW (g)	FW(g)	DW (g)	% Moisture
1	1	58.49	128.83	113.80	70.34	55.31	27.17
2	1	92.09	162.67	149.38	70.58	57.29	23.19
3	2	58.45	136.23	116.76	77.78	58.31	33.39
4	2	62.85	133.29	115.84	70.44	52.99	32.93
5	3	62.12	145.14	123.36	83.02	61.24	35.56
6	3	42.45	116.22	98.64	73.77	56.19	31.29
7	4	58.50	142.36	121.10	83.86	62.60	33.96
8	4	51.40	122.83	106.46	71.43	55.06	29.73
9	5	63.54	139.71	121.44	76.17	57.90	31.55
10	5	55.00	126.40	108.53	71.40	53.53	33.38
11	6	54.21	90.61	82.89	36.40	28.68	26.92
12	6	42.45	82.62	73.97	40.17	31.52	27.44
13	7	61.55	97.78	90.07	36.23	28.52	27.03
14	7	62.87	96.65	88.90	33.78	26.03	29.77
15	8	61.22	96.81	88.20	35.59	26.98	31.91
16	8	149.51	184.57	175.64	35.06	26.13	34.17
17	9	42.50	82.29	73.41	39.79	30.91	28.73
18	9	54.21	86.95	79.89	32.74	25.68	27.49
19	10	58.57	96.02	85.57	37.45	27.00	38.70
20	10	62.93	100.21	90.21	37.28	27.28	36.66
						Mean	31.05
						SD	4.00
						%RSD	13

Table DII.1 Calculation of PbO for pot trials 1 and 2.

Concentration mg/kg	PbO required in mg/kg/treatment	PbO required in g/kg	g required per fresh weight of pot . 1 pot=2.8 kg DW	PbO required per treatment by Nos of pot. n=3	PbO required per treatment by pot and species (g)
100	107. 72	0.108	0.302	0.905	5.429
300	323.16	0.323	0.905	2.715	16.287
1000	1,077.20	1.077	3.016	9.048	54.291
3000	3,231.60	3.232	9.048	27.145	162.873
10000	10,772.00	10.772	30.162	90.485	542.909

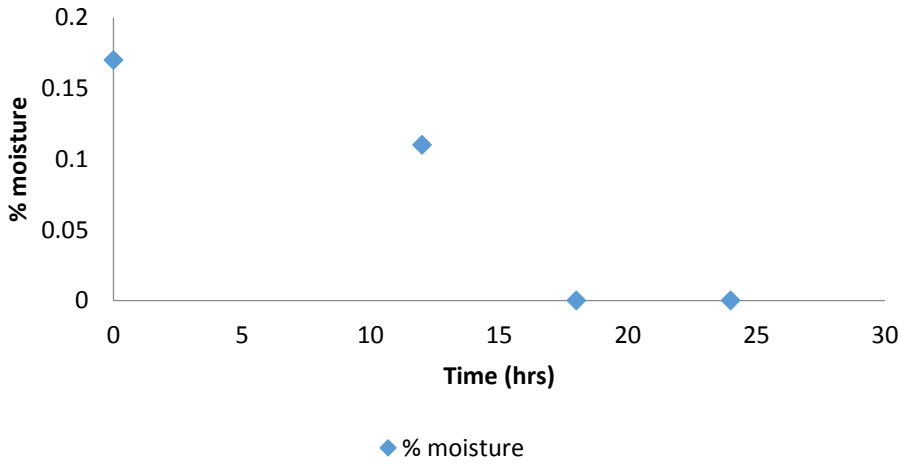


Figure AII.1: Percentage moisture content of dried PbO against time (hours).

Appendix II.2: Growth and biomass data from first pot trial.

Table AII.1: Summary table of test of significance of growth and data biomass between treatment for each variety and species (First pot trial) with P values in brackets.

BIOMASS DATA	BJ 18	BJ 42	BJ 17	BJ 21	ZM B73	ZM B37	ZM OH43	ZM 64	BN SW	BN K	TC BR	TC GM	TC HS
Height	NS (0.315)	NS (0.273)	NS (0.480)	**S (0.021)**	NS (0.718)	S (0.012)	NS (0.668)	S (0.015)	NS (0.380)	NS (0.106)	NS (0.080)	S (0.000)	**S** (0.005)
Shoot dry biomass	S (0.012)	S (0.006)	NS (0.101)	NS (0.185)	S (0.036)	NS (0.238)	NS (0.351)	S (0.007)	S (0.002)	S (0.012)	NS (0.725)	NS (0.519)	NS (0.163)
Root dry biomass	S (0.001)	NS (0.498)	NS (0.624)	NS (1.00)	S (0.004)	NS (0.267)	S (0.002)	NS (0.267)	S (0.000)	NS (0.670)	NS (0.678)	NS (0.374)	S (0.030)
Total dry biomass	S (0.007)	S (0.009)	NS (0.115)	NS (0.184)	S (0.003)	NS (0.185)	NS (0.047)	S (0.012)	S (0.001)	S (0.068)	NS (0.692)	NS (1.00)	NS (0.051)
NTL	NS (0.275)	NS (0.070)	NS (0.116)	S (0.014)	NS (0.422)	NS (0.643)	NS (0.101)	NS (1.00)	NS (1.00)	NS (1.00)	NS (0.101)	S (0.025)	NS (0.101)
LL	S (0.016)	S (0.001)	NS (0.644)	S (0.042)	NS (0.056)	NS (0.310)	S (0.029)	NS (0.215)	NS (0.079)	NS (1.00)	NS (0.069)	NS (0.116)	NS (0.089)
Growth Index	NS (0.325)	NS (0.264)	NS (0.469)	S (0.017)	NS (0.403)	S (0.001)	NS (0.707)	S (0.008)	NS (0.071)	NS (0.150)	NS (0.078)	S (0.000)	S (0.002)

KEY:

NS = Not significant, S- = Significant, NTL= Number of true leaves, NDL-= Number of dead leaves, LL= Longest leaf length,

Appendix II. 8: Randomized block design for pot trial 2.

Treatment Block 1						
Control (0 mg/kg Pb)						
	BN SW	**BJ 18**	**ZM OH43**	**BJ 17**	**ZM B73**	**TC BR**
	TC BR	**ZM B73**	**BJ 17**	**ZM OH43**	**BJ 18**	**BN SW**
	ZM OH43	**BJ 17**	**BN SW**	**BJ 18**	**TC BR**	**ZM B73**
Treatment Block 2						
(100 mg/kg Pb)						
	TC BR	**ZM B73**	**BJ 17**	**ZM OH43**	**BJ 18**	**BN SW**
	BN SW	**BJ 18**	**ZM OH43**	**BJ 17**	**ZM B73**	**TC BR**
	ZM B73	**BJ 17**	**TC BR**	**BJ 18**	**ZM OH43**	**BN SW**
Treatment Block 3						
(300 mg/kg Pb)						
	ZM B73	**BJ 17**	**TC BR**	**BJ 18**	**ZM OH43**	**BN SW**
	BN SW	**ZM OH43**	**BJ 18**	**TC BR**	**BJ 17**	**ZM B73**
	BJ 17	**TC BR**	**BN SW**	**ZM OH43**	**ZM B73**	**BJ 18**
Treatment Block 4						
(3000 mg/kg Pb)						
	BJ 17	**TC BR**	**BN SW**	**ZM OH43**	**ZM B73**	**BJ 18**
	BJ 18	**ZM B73**	**ZM OH43**	**BJ 17**	**TC BR**	**BN SW**
	BN SW	**BJ 17**	**TC BR**	**ZM B73**	**BJ 18**	**ZM OH43**
Treatment Block 5						
(10000 mg/kg Pb)						
	ZM OH43	**BJ 18**	**ZM B73**	**TC BR**	**BJ 17**	**BN SW**
	BJ 17	**TC BR**	**BN SW**	**ZM B73**	**ZM OH43**	**BJ 18**
	BJ 18	**ZM OH43**	**TC BR**	**BJ 17**	**BN SW**	**ZM B73**

Appendix II. 11: Pot trial 2 Analysis of variance for shoot, root and total plant Pb mg/kg between species.

Table AII.11: Shoot Pb ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
BJ 18 shoot Pb	Between Groups	8636.987	3	2878.996	70.036	.000
	Within Groups	328.860	8	41.108		
	Total	8965.847	11			
BJ 17 shoot Pb	Between Groups	10172.889	3	3390.963	203.082	.000
	Within Groups	133.580	8	16.698		
	Total	10306.469	11			
ZM OH43 shoot Pb	Between Groups	199724.769	3	66574.923	99.578	.000
	Within Groups	5348.540	8	668.567		
	Total	205073.309	11			
ZM B73 shoot Pb	Between Groups	25613.563	3	8537.854	71.085	.000
	Within Groups	960.867	8	120.108		
	Total	26574.429	11			
BN SW shoot Pb	Between Groups	10787.107	3	3595.702	126.154	.000
	Within Groups	228.020	8	28.503		
	Total	11015.127	11			
TC BR shoot Pb	Between Groups	559443.333	2	279721.667	17.338	.022
	Within Groups	48400.667	3	16133.556		
	Total	607844.000	5			

Table BII.11: ANOVA Root Pb

		Sum of Squares	df	Mean Square	F	Sig.
BJ 18 root Pb	Between Groups	748729.209	3	249576.403	36.850	.000
	Within Groups	54182.540	8	6772.818		
	Total	802911.749	11			
BJ 17 root Pb	Between Groups	193168.727	3	64389.576	42.708	.000
	Within Groups	12061.400	8	1507.675		
	Total	205230.127	11			
ZM OH43 root Pb	Between Groups	5419441.629	3	1806480.543	1074.027	.000
	Within Groups	13455.760	8	1681.970		
	Total	5432897.389	11			
ZM B73 root Pb	Between Groups	5287797.083	3	1762599.028	12.007	.002
	Within Groups	1174380.167	8	146797.521		
	Total	6462177.249	11			
BN SW root Pb	Between Groups	162668.756	3	54222.919	58.497	.000
	Within Groups	7415.467	8	926.933		
	Total	170084.223	11			

Table CII.11: ANOVA Total plant Pb mg/kg DW

		Sum of Squares	df	Mean Square	F	Sig.
BJ 18 total plant Pb	Between Groups	16830.000	3	5610.000	98.277	.000
	Within Groups	456.667	8	57.083		
	Total	17286.667	11			
BJ 17 total plant Pb	Between Groups	18960.917	3	6320.306	267.055	.000
	Within Groups	189.333	8	23.667		
	Total	19150.250	11			
ZM OH43 total plant Pb	Between Groups	629914.917	3	209971.639	234.933	.000
	Within Groups	7150.000	8	893.750		
	Total	637064.917	11			
ZM B73 total plant Pb	Between Groups	403108.250	3	134369.417	20.990	.000
	Within Groups	51212.667	8	6401.583		
	Total	454320.917	11			
BN SW total plant Pb	Between Groups	18699.000	3	6233.000	134.525	.000
	Within Groups	370.667	8	46.333		
	Total	19069.667	11			

Appendix II. 12: Other information from the second pot trial.

Figure AII.12: Caterpillar on *B. juncea* (pot trial 2)

Appendix II. 13: Analytical/Laboratory methods.

AII.13. NITRIC AND PERCHLORIC ACID DIGESTION (Thompson and Walsh, 1983).

DESCRIPTION: Nitric and Perchloric acid attack.

Sample types: Soil, herbage, silage, animal faeces.

Sample weight: 0.010 - 0.500 g for herbage and 0.250g for soil.

Final volume: 10.0 ml

Dilution Factor: 20-1000 ml g^{-1} (herbage), 40 ml g^{-1} (soil)

COSSH Assessment

Hydrochloric Acid AR 36% w/w

Nitric Acid AR 70% w/w

Perchloric Acid 60% w/w

SAFETY POINTS:

1. Do not add perchloric acid to samples in the absence of Nitric acid.
2. Samples with high organic content may react vigorously with Nitric and Perchloric acids. Watch for frothing when adding Nitric acid. If frothing occurs increase step 1 dwell time to 12 hours.
3. This method must not be attempted on samples containing oil or bitumen.

BATCH ORGANISATION

Maximum batch size: 214 samples (252 solutions).

Block Time: 36 hours (or 5 pm day 1 to 9 am day 3).

Total Prep Time: 3 days

Solution preferably less than 3 months (if capped).

QUALITY CONTROL

Reagent Blanks: 5% (of total number of samples) ≥2

Duplicated Samples: 10% (of total number of samples)

Reference materials: 4% (all RM's should be duplicated)

Possible Reference Materials: (HRM1, HRM2, HRM 31+ NIST SRM 2709a, 2710a, 2711a for soil) and (HRM14, HRM11+ Certified RM BCR 60).

EQUIPMENT

Test tubes 18 mm o.d. x 180 mm (PYREX)

Wire test tube racks (plastic coated).

Stainless steel test tube racks

Aluminium heating block (deep, 252 holes).

Shallow aluminium heating block (315 holes)

Oxford dispensers

Centrifuge tubes 18 mm x 110 mm (polystyrene).

Vortex tube mixer

Balance, top pan.

Centrifuge GF8

REAGENTS

Water-Reverse Osmosis (RO)

Nitric Acid A.R. 70% w/w

Perchloric Acid A.R. 60% w/w

Hydrochloric Acid A.R. 36% w/w

5M HCl (Dilute 430 ml of hydrochloric Acid A.R. 36% w/w to 1 litre with DIW).

PROCEDURE

1. Prepare a weighing list
2. Number a set of test tubes using a waterproof marker pen.
3. Weigh 0.500g or 0.250g (±0.001) of sample (oven dried and milled) onto a clean piece of weighing paper using a top pan balance. Transfer carefully into clean, dry, numbered test tubes (in wire test tube racks).
4. Add 4.0 ml Nitric Acid into each clean tube from an Oxford dispenser.
5. For herbage samples place tubes in the aluminium heating block and leave overnight at 50ºC.
6. For soil samples, add 1.0 ml Perchloric Acid from an Oxford dispenser.
7. Place tubes of soil samples in the aluminium heating block. Switch the block programmer to Manual mode, then set up as follows:

Programme 4: Total metal soil attack

Rise Rate sec/deg	Dwell time hrs	Dwell time ºC
001	3.0	50
001	3.0	150
001	18.0	190
001	0.1	200

8. For herbage samples, remove tubes from heating block and add 1.0 ml Perchloric Acid from an Oxford dispenser.

9. Place tubes in the aluminium heating block. Switch Programmer to Manual mode, then set up as follows:

Programme 3- Herbage attack

Rise Rate sec/deg	Dwell time hrs	Dwell Temp ºC
0.001	0.1	50
0.001	3.0	150
0.001	18.0	190
0.001	0.1	195

10. Check the fume cupboard is on and switch the block programmer to ‘Auto’ and press ‘Reset’ button.

11. When attack cycle is complete, check each tube to ensure that residue is dry. If any liquid remains, continue heating at 195ºC until dry. Transfer tubes to stainless steel rack.

12. When tubes are cool. Add 2.0 ml of 5M HCl to each tube from Oxford dispenser (Calibrated gravimetrically).

13. Place tubes in shallow heating block and leave to leach for one hour at 60ºC.

14. Transfer tubes to wire racks and allow to cool.

15. Add 8.0 ml DIW from Oxford dispenser (calibrated gravimetrically) and mix each tube using a vortex mixer.

16. Decant into polystyrene tubes and cap.

17. Centrifuge at 2000 rpm for 2 minutes.

18. Deliver the tubes (with Analytical Request form) to room 4.59 at least 12 hours before analysis, to allow solutions to equilibrate at 21ºC.

Appendix II. 14: Quality control data for pot trials 1 and 2.

Table AII.14: Certified CRMs for herbage analysis --Pot trial 1

Sample		BCR-60	HRM 11	HRM 14
x1		57.30	33.25	11.3
x2		65.26	20.1	7.06
MEAN		61.28	26.68	9.18
CERTIFIED VALUES		64.00	26.00	9.00
BIAS		-2.7	0.68	0.18
BIAS%		-4.3	2.6	2.0

BII.14: Regression Analysis: Mean measured values versus certified values of CRMs {mg/kg} - (Pot trial 1)

The regression equation is

Measured values = 1.32 + 0.941 Certified values

Predictor	Coef	SE Coef	T	P
Constant	1.323	1.124	1.18	0.448
Certified values	0.94111	0.02796	33.66	0.019

S = 1.11339 R-Sq = 99.9% R-Sq(adj) = 99.8%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	1404.7	1404.7	1133.15	0.019
Residual Error	1	1.2	1.2		
Total	2	1405.9			

Table CII.14: Test for significance of blanks for herbage analysis (Pot trial 1)

SAMPLE	mg/kg Pb
RBLK 1	1.76
RBLK 2	1.28
RBLK 3	-0.04
RBLK 4	0.28
RBLK 5	-0.22
Mean	0.61
Std	0.86
μ	0
SEM	0.39
mean-μ	0.61
t-test	1.59

T-TAB.	2.78		df(n-1) =4
two tailed test		1.59<2.78	Not significant

P>0.05 at 95% confidence interval

Table DII.14: Certified CRMs for herbage analysis --Pot trial 2

Sample	**HRM 11**	**HRM 14**	**BCR-60**
X1	34.30	15.50	70.80
X2	29.30	15.60	69.3
MEAN	31.80	15.55	71.03
CERTIFIED VAL.	26.00	9.00	64.00
BIAS	5.80	6.55	7.03
BIAS%	22.3	72.8	11.0

Table EII.14: Regression Analysis: Mean measured values versus certified values of CRMs {mg/kg} - (Pot trial 2)

The regression equation is

Measured values = 6.04 + 1.01 Certified values

Predictor	Coef	SE Coef	T	P
Constant	6.0412	0.7234	8.35	0.076
Certified values	1.01269	0.01799	56.30	0.011

S = 0.716313 R-Sq = 100.0% R-Sq(adj) = 99.9%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	1626.5	1626.5	3169.95	0.011
Residual Error	1	0.5	0.5		
Total	2	1627.0			

Table FII.14: Test for significance of blanks for herbage analysis (Pot trial 2).

BLANK CORRECTION	
SAMPLE	mg/kg Pb
RBLK	-8.3
RBLK	-4.5
RBLK	-10.7
RBLK	-5.3
RBLK	-2.8
Mean	-6.32
STD	3.16
μ	0
SEM	0.36
mean-μ	-6.32
t-test	-17.56

T-TAB.	2.78	P>0.05	df(n-1) 4
two tailed test		17.56>2.78	Significant

Table GII.14: Certified CRMs for Soil Pb analysis (Pot trials 1&2).

Sample	HRM 1	HRM 2	SRM 2709	SRM 2710	SRM 2711	HRM 31
x1	16.2	599.6	36.84	6300	1284.4	7272
x2	19.84	414.8	32.56	6032	1276.8	6668
mean	18.02	507.2	34.7	6166	1280.6	6970
Certified value	13	510	17.3	5552	1400	6895
BIAS	5.02	-2.8	17.4	614	-119.4	75
BIAS%	39	-1	101	11	-9	1

I II.14: Regression Analysis: Mean measured values versus certified values of CRMs {mg/kg} - (Soil Pb analysis Pot trials 1& 2)

The regression equation is

Measured values = - 24 + 1.05 Certified values

Predictor	Coef	SE Coef	T	P
Constant	-23.8	126.6	-0.19	0.860
Certified values	1.05088	0.03455	30.42	0.000

S = 234.541 R-Sq = 99.6% R-Sq (adj) = 99.5%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	50896444	50896444	925.23	0.000
Residual Error	4	220037	55009		
Total	5	51116481			

Table J II.14: Test for significance of Blanks for soil Pb analysis (pot trial 2)

Samples	Pb (mg/kg)		
RBLK 1	1.28		
RBLK 2	-9		
RBLK 3	-6.24		
RBLK 4	-1		
RBLK 5	4		
Mean	-2.19		
Std	5.35		
μ	0.00		
SEM	2.39		
mean-μ	-2.19		
t-test	-0.92		
T-TAB.	2.78		df(n-1)=4
two tailed test		0.92<2.78	Not significant

Table KII.14: Precision and detection limit of the first and second pot trials analysis.

Experiment	Precision	Detection limits
Herbage analysis pot trial 1	6.8%	1.72 mg/kg
Herbage analysis pot trial 2	6.4%	3.15 mg/kg

Detection limit of herbage analysis in all pot trials were calculated by using 3 standard deviation of 11 blank analysis multiplied by the dilution factor.

APPENDICES RELATED TO THIRD POT TRIAL (CHAPTER 5).

Appendix III. 1: Data from pot third pot trial (Simplistic binary heterogeneity experiment).

Table AIII.1: Randomized block design

Treatment block 1 (*Brassica napus*)			Treatment block 2 (*Brassica juncea*)		
A	B	C	A	B	C
BN B 4	BN H10	BN C8	BJ H2	BJ B8	BJ H7
BN C2	BN B6	BN H1	BJ B10	BJ C10	BJ B7
BN B7	BN C4	BN B3	BJ C3	BJ B4	BJ C4
BN H3	BN B5	BN C9	BJ H8	BJ C6	BJ H5
BN B2	BN H6	BN B9	BJ B1	BJ H6	BJ C5
BN C7	BN C10	BN H7	BJ H4	BJ C1	BJ B3
BN H9	BN H4	BN C3	BJ B9	BJ H9	BJ H1
BN C1	BN B8	BN H5	BJ C7	BJ B 5	BJ C8
BN B10	BN C6	BN B1	BJ H3	BJ C2	BJ B2
BN C5	BN H8	BN H2	BJ C9	BJ B6	BJ H10

BNB-------*Brassica napus* Binary, BJB---------*Brassica juncea* binary.

BNC--------*Brassica napus* control, BJC---------*Brassica juncea* control

BNH------- *Brassica napus* homogeneous, BJH------- *Brassica juncea* homogeneous

Pot numbers are added to treatment names and treatments are coloured tagged e.g blue for homogeneous, red for binary and black for control.

Table BIII.1: Estimate of compost, sand and PbO (Analytical grade supplied Sigma Aldrich was used throughout the pot trial) required in growth media.

Sand	Compost	PbO	Carrier Sand	Growth Media
192 kg sand (DW) per batch in all 60 pots (3.2 kg per pot)	54 L compost per batch in all 60 pots (0.78 kg per pot)	31 g dry PbO per batch of growth media to make 1000 mg/kg (DW) Pb treatment (total required for 2 species = 62 g 15.5 g per batch of growth media to make 2000 mg/kg Pb treatment (2 species=31 g)	10 g per batch	2.88 kg (DW) per pot. %Moisture =26%

Appendix III.2: Statistical analysis for simplistic binary experiment (Third pot trial).

Table AIII.2: ANOVA of Shoot, root and total dry biomass between control, homogeneous and binary treatments of *B. napus* and *juncea*.

		Sum of Squares	df	Mean Square	F	Sig.
BNSW Shoot DW	Between Groups	70.098	2	35.049	48.969	.000
	Within Groups	19.325	27	.716		
	Total	89.423	29			
BJ18 Shoot DW	Between Groups	97.395	2	48.698	23.969	.000
	Within Groups	54.857	27	2.032		
	Total	152.252	29			
BN Root DW	Between Groups	4.856	2	2.428	27.710	.000
	Within Groups	2.366	27	.088		
	Total	7.222	29			
BJ Root DW	Between Groups	7.602	2	3.801	64.112	.000
	Within Groups	1.601	27	.059		
	Total	9.203	29			
BN Total DW	Between Groups	110.546	2	55.273	64.782	.000
	Within Groups	23.037	27	.853		
	Total	133.583	29			
BJ Total DW	Between Groups	158.386	2	79.193	32.384	.000
	Within Groups	66.026	27	2.445		
	Total	224.411	29			

Table BIII.2: Independent Samples Test for root ball diameter in binary quarters between species.

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
BN root ball diameter	Equal variances assumed	17.718	.000	34.728	38	.000	52.8500	1.52182	49.76925	55.93075
	Equal variances not assumed			34.728	21.839	.000	52.8500	1.52182	49.69260	56.00740
BJ root ball diameter	Equal variances assumed	31.716	.000	23.406	38	.000	23.3250	.99656	21.30758	25.34242
	Equal variances not assumed			23.406	20.727	.000	23.3250	.99656	21.25088	25.39912

Table CIII.2: Summary of homogeneous subset –Tukey H.S.D post-hoc test (Simplistic binary experiment)

Treatments	Parameters	Species	Subset for	Alpha	p=0.05
			C	B	A
Binary	Shoot dry biomass	*B. napus*	9.1890		
Homogeneous				11.6190	
Control					12.8710
Homogeneous	Shoot dry biomass	*B. juncea*	10.0320		
Binary				13.1640	
Control				14.2910	
Binary	Root dry biomass	*B. napus*	1.5950		
Homogeneous				2.0140	
Control					2.5770
Homogeneous	Root dry biomass	*B. juncea*	1.3250		
Binary				2.0150	
Control					2.5550
Binary	Total dry biomass	*B. napus*	10.7840		
Homogeneous				13.6330	
Control					15.4480
Homogeneous	Total dry biomass	*B. juncea*	11.3570		
Binary				15.1790	
Control				16.8460	

N=10 95% confidence interval.

DIII.2: One-way ANOVA: *B. juncea* root-shoot biomass ratio versus Treatment (pot trial 3).

```
Source      DF        SS        MS      F      P
Treatment    2  0.011771  0.005885  16.74  0.000
Error       27  0.009495  0.000352
Total       29  0.021265

S = 0.01875   R-Sq = 55.35%   R-Sq(adj) = 52.04%


Grouping Information Using Tukey Method

Treatment     N     Mean  Grouping
Control      10  0.17979  A
Binary       10  0.15401    B
Homogeneous  10  0.13131      C

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment
```

EIII.2:One-way ANOVA: *B. napus* root-shoot biomass ratio versus Treatment (pot trial 3).

```
Source     DF        SS        MS     F      P
Treatment   2  0.004666  0.002333  2.65  0.089
Error      27  0.023768  0.000880
Total      29  0.028433

S = 0.02967   R-Sq = 16.41%   R-Sq(adj) = 10.22%


Grouping Information Using Tukey Method

Treatment     N    Mean  Grouping
Control      10  0.20078  A
Binary       10  0.17450  A
Homogeneous  10  0.17415  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Treatment.
```

FIII.2: Shoot, root and total plant Pb concentration (mg/kg) between homogeneous and binary treatments of *B. juncea* (pot trial 3).

Two-Sample T-Test : *B. juncea* shoot Pb

```
Two-sample T for BJ shoot Pb

Treatment     N   Mean  StDev  SE Mean
Binary       10  22.04   2.50     0.79
Homogeneous  10  41.01   5.57      1.8

T-Test of difference = 0 (vs not =): T-Value = -9.83 P-Value = 0.000 DF = 12
```

Two-Sample T-Test: *B. juncea* root Pb

```
Two-sample T for BJ root Pb

Treatment     N  Mean  StDev  SE Mean
Binary       10  1065    295       93
Homogeneous  10  1664    332      105

T-Test of difference = 0 (vs not =): T-Value = -4.26  P-Value = 0.001  DF = 17
```

Two-Sample T-Test: *B. juncea* total plant Pb

```
Two-sample T for BJ total plant Pb

                                SE
Treatment     N   Mean  StDev  Mean
Binary       10  161.0   40.3    13
Homogeneous  10  227.8   41.4    13

T-Test of difference = 0 (vs not =): T-Value = -3.66  P-Value = 0.002  DF = 17
```

GIII.2: Shoot, root and total plant Pb concentration (mg/kg) between homogeneous and binary treatments of *B. napus* (pot trial 3).

Two-Sample T-Test *B. napus* shoot Pb

Treatment	N	Mean	StDev	SE Mean
Binary	10	31.43	9.85	3.1
Homogeneous	10	43.2	16.6	5.3

T-Test of difference = 0 (vs not =): T-Value = -1.93 P-Value = 0.074 DF = 14

Two-Sample T-Test *B. napus* root Pb

Two-sample T for BN root Pb

Treatment	N	Mean	StDev	SE Mean
Binary	10	1166	429	136
Homogeneous	10	1845	456	144

T-Test of difference = 0 (vs not =): T-Value = -3.43 P-Value = 0.003 DF = 17

Two-Sample T-Test *B. napus* total plant Pb, Treatment

Two-sample T for BN total plant Pb

Treatment	N	Mean	StDev	SE Mean
Binary	10	198.6	68.8	22
Homogeneous	10	316	106	34

T-Test of difference = 0 (vs not =): T-Value = -2.93 P-Value = 0.010 DF = 15

Two-Sample T-Test *B. napus* TF

Two-sample T for BN TF

Treatment	N	Mean	StDev	SE Mean
Binary	10	0.0312	0.0160	0.0050
Homogeneous	10	0.0250	0.0119	0.0038

T-Test of difference = 0 (vs not =): T-Value = 0.98 P-Value = 0.343 DF = 16

HIII.2: T-test for *B. juncea* shoot and total concentration factor and translocation factor.

Two-Sample T-Test B. *juncea*

Two-sample T for BJ CFshoot

Treatment	N	Mean	StDev	SE Mean
Binary	10	0.005444	0.000618	0.00020
Homogeneous	10	0.01013	0.00138	0.00043

T-Test of difference = 0 (vs not =): T-Value = -9.83 P-Value = 0.000 DF = 12

Two-Sample T-Test B. *juncea* CFtotal

Two-sample T for BJ CFtotal

Treatment	N	Mean	StDev	SE Mean
Binary	10	0.03976	0.00996	0.0032
Homogeneous	10	0.0563	0.0102	0.0032

T-Test of difference = 0 (vs not =): T-Value = -3.66 P-Value = 0.002 DF = 17

Two-Sample T-Test B. *juncea* TF

```
Two-sample T for BJ TF

Treatment     N     Mean    StDev  SE Mean
Binary       10  0.02211  0.00608   0.0019
Homogeneous  10  0.02567  0.00678   0.0021

T-Test of difference = 0 (vs not =): T-Value = -1.24  P-Value = 0.233  DF = 17
```

I III.2: T-test for *B. napus* shoot and total concentration factor and translocation factor (Pot trial 1).

Two-Sample T-Test *B. napus CFshoot*

```
Two-sample T for BN CFshoot

Treatment     N     Mean    StDev  SE Mean
Binary       10  0.00777  0.00243  0.00077
Homogeneous  10  0.01068  0.00411   0.0013

T-Test of difference = 0 (vs not =): T-Value = -1.93 P-Value = 0.074 DF = 14
```

Two-Sample T-Test *B. napus CFtotal*, Treatment

```
Two-sample T for BN CFtotal

Treatment     N    Mean   StDev  SE Mean
Binary       10  0.0491  0.0170   0.0054
Homogeneous  10  0.0781  0.0263   0.0083


T-Test of difference = 0 (vs not =): T-Value = -2.93  P-Value = 0.010  DF = 15
```

Table J III.2: Mixed model ANOVA for *B. juncea* (pot trial 3).

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Treatment	Pillai's Trace	1.869	42.899	10.000	30.000	.000	.935
	Wilks' Lambda	.001	105.370[b]	10.000	28.000	.000	.974
	Hotelling's Trace	193.096	251.024	10.000	26.000	.000	.990
	Roy's Largest Root	186.120	558.359[c]	5.000	15.000	.000	.995

a. Design: Treatment b. Exact statistic c. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects for *B. juncea*

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	Shoot Pb	21677.837[a]	2	10838.918	581.768	.000
	Root Pb	39026517.663[b]	2	19513258.831	197.700	.000
	Total plant Pb	778460.500[c]	2	389230.250	232.975	.000
	Shoot biomass	2739.319[d]	2	1369.660	554.710	.000
	Root biomass	58.159[e]	2	29.079	435.862	.000
	Total biomass	3593.835[f]	2	1796.917	582.619	.000
Treatment	Shoot Pb	21677.837	2	10838.918	581.768	.000
	Root Pb	39026517.662	2	19513258.831	197.700	.000
	Total plant Pb	778460.500	2	389230.250	232.975	.000
	Shoot biomass	2739.319	2	1369.660	554.710	.000
	Root biomass	58.158	2	29.079	435.862	.000
	Total biomass	3593.835	2	1796.917	582.619	.000
Error	Shoot Pb	335.358	18	18.631		
	Root Pb	1776625.213	18	98701.401		
	Total plant Pb	30072.500	18	1670.694		
	Shoot biomass	44.445	18	2.469		
	Root biomass	1.201	18	.067		
	Total biomass	55.516	18	3.084		
Total	Shoot Pb	22013.195	20			
	Root Pb	40803142.875	20			
	Total plant Pb	808533.000	20			
	Shoot biomass	2783.764	20			
	Root biomass	59.359	20			
	Total biomass	3649.351	20			

Table KIII.2: Mixed model ANOVA for *B. napus* (pot trial 3).

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Treatment	Pillai's Trace	1.179	4.310	10.000	30.000	.001	.590
	Wilks' Lambda	.002	55.475[b]	10.000	28.000	.000	.952
	Hotelling's Trace	353.544	459.607	10.000	26.000	.000	.994
	Roy's Largest Root	353.321	1059.964[c]	5.000	15.000	.000	.997

a. Design: Treatment b. Exact statistic c. The statistic is an upper bound on F that yields a lower bound on the significance level

Tests of Between-Subjects Effects for *B. napus*

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	F	Sig.
Model	Shoot Pb	28563.870[a]	2	14281.935	76.381	.000
	Root Pb	47631594.698[b]	2	23815797.349	121.445	.000
	Total plant Pb	1394244.000[c]	2	697122.000	86.994	.000
	Shoot biomass	2194.389[d]	2	1097.194	1715.322	.000
	Root biomass	66.002[e]	2	33.001	409.587	.000
	Total plant biomass	3021.533[f]	2	1510.767	2083.808	.000
Treatment	Shoot Pb	28563.870	2	14281.935	76.381	.000
	Root Pb	47631594.698	2	23815797.349	121.445	.000
	Total plant Pb	1394244.000	2	697122.000	86.994	.000
	Shoot biomass	2194.389	2	1097.194	1715.322	.000
	Root biomass	66.002	2	33.001	409.587	.000
	Total plant biomass	3021.533	2	1510.767	2083.808	.000
Error	Shoot Pb	3365.704	18	186.984		
	Root Pb	3529875.202	18	196104.178		
	Total plant Pb	144242.000	18	8013.444		
	Shoot biomass	11.514	18	.640		
	Root biomass	1.450	18	.081		
	Total plant biomass	13.050	18	.725		
Total	Shoot Pb	31929.573	20			
	Root Pb	51161469.900	20			
	Total plant Pb	1538486.000	20			
	Shoot biomass	2205.902	20			
	Root biomass	67.452	20			
	Total plant biomass	3034.583	20			

Table LIII.2: Test for normal distribution for *B. juncea* and *B. napus* variables

One-Sample Kolmogorov-Smirnov Test for *B. juncea* shoot, root and total plant Pb

		Shoot Pb	Root Pb	Total plant Pb
N		20	20	20
Normal Parameters[a,b]	Mean	31.5260	1364.4250	194.4500
	Std. Deviation	10.60144	433.47016	52.47403
Most Extreme Differences	Absolute	.209	.164	.145
	Positive	.209	.150	.143
	Negative	-.138	-.164	-.145
Test Statistic		.209	.164	.145
Asymp. Sig. (2-tailed)		.022[c]	.168[c]	.200[c,d]

a. Test distribution is Normal.

b. Calculated from data.

c. Lilliefors Significance Correction.

d. This is a lower bound of the true significance.

One-Sample Kolmogorov-Smirnov Test for *B. napus* shoot, root and total plant Pb

		Shoot Pb	Root Pb	Total plant Pb
N		20	20	20
Normal Parameters[a,b]	Mean	37.3290	1505.4300	257.4000
	Std. Deviation	14.61882	554.17460	105.97686
Most Extreme Differences	Absolute	.182	.098	.138
	Positive	.182	.098	.138
	Negative	-.139	-.084	-.078
Test Statistic		.182	.098	.138
Asymp. Sig. (2-tailed)		.083[c]	.200[c,d]	.200[c,d]

a. Test distribution is Normal.

b. Calculated from data.

One-Sample Kolmogorov-Smirnov Test for *B. juncea* shoot, root, total biomass and root-shoot biomass ratio.

		shoot biomass	Root biomass	Total biomass	Root-shoot biomass ratio
N		30	30	30	30
Normal Parameters[a,b]	Mean	12.4957	1.9650	14.4607	.1550
	Std. Deviation	2.29130	.56333	2.78178	.02713
Most Extreme Differences	Absolute	.140	.093	.111	.160
	Positive	.096	.068	.093	.160
	Negative	-.140	-.093	-.111	-.127
Test Statistic		.140	.093	.111	.160
Asymp. Sig. (2-tailed)		.138[c]	.200[c,d]	.200[c,d]	.048[c]

a. Test distribution is Normal.

b. Calculated from data.

One-Sample Kolmogorov-Smirnov Test for *B. napus* shoot, root, total biomass and root-shoot biomass ratio

		Shoot biomass	Root biomass	Total biomass	Root shoot biomass
N		30	30	30	30
Normal Parameters[a,b]	Mean	11.2263	2.0620	13.2883	.1837
	Std. Deviation	1.75601	.49904	2.14624	.03222
Most Extreme Differences	Absolute	.117	.099	.140	.145
	Positive	.117	.099	.140	.145
	Negative	-.081	-.092	-.078	-.070
Test Statistic		.117	.099	.140	.145
Asymp. Sig. (2-tailed)		.200[c,d]	.200[c,d]	.138[c]	.106[c]

a. Test distribution is Normal.

b. Calculated from data.

Appendix III 3: Raw data from third pot trial.

Table AIII.3: Raw data of shoot, root/ total biomass and growth variables of *B. juncea* from third pot trial.

	Treatments	Shoot biomass DW (g)	Root biomass DW (g)	Total biomass (g)	Root-shoot biomass ratio (g)	Growth index	Height (mm)	Number of true leaves	Number of dead leaves	Longest leaf length (mm)
1	Control	14.1	2.22	16.32	0.16	474	1390	50	0	232
2	Control	14.11	2.55	16.66	0.18	439	1290	40	0	250
3	Control	13.02	2.63	15.65	0.20	427	1260	45	0	222
4	Control	14.75	2.48	17.23	0.17	448	1320	52	0	210
5	Control	15.67	2.53	18.20	0.16	416	1220	43	0	224
6	Control	12.33	2.52	14.85	0.20	499	1472	40	0	250
7	Control	14.56	2.32	16.88	0.16	441	1290	52	0	226
8	Control	15.93	2.55	18.48	0.16	523	1540	47	0	210
9	Control	14.2	2.96	17.16	0.21	424	1250	46	0	175
10	Control	14.24	2.79	17.03	0.20	421	1230	48	0	224
11	Homogeneous	10.29	1.11	11.40	0.11	346	1010	36	5	240
12	Homogeneous	10.02	1.5	11.52	0.15	444	1310	36	3	220
13	Homogeneous	4.84	0.58	5.42	0.12	278	820	25	4	185
14	Homogeneous	10.26	1.24	11.50	0.12	353	1040	38	5	230
15	Homogeneous	10.24	1.13	11.37	0.11	455	1335	35	4	245
16	Homogeneous	11.46	1.69	13.15	0.15	320	940	33	4	178
17	Homogeneous	10.19	1.66	11.85	0.16	355	1040	35	4	210
18	Homogeneous	11.38	1.54	12.92	0.14	461	1360	34	4	246
19	Homogeneous	10.81	1.21	12.02	0.11	391	1150	36	3	190
20	Homogeneous	10.83	1.59	12.42	0.15	410	1200	38	4	245
21	Binary	14.1	2.19	16.29	0.16	484	1425	46	4	225
22	Binary	11.54	1.86	13.40	0.16	417	1224	36	4	200
23	Binary	14.64	1.96	16.60	0.13	452	1330	39	3	260
24	Binary	11.79	1.9	13.69	0.16	433	1280	47	3	225
25	Binary	13.97	1.97	15.94	0.14	476	1400	41	2	220
26	Binary	13.05	2.21	15.26	0.17	429	1260	39	2	230
27	Binary	12.1	2.18	14.28	0.18	416	1220	42	2	226
28	Binary	13.09	1.99	15.08	0.15	456	1340	38	4	275
29	Binary	12.61	1.94	14.55	0.15	435	1285	36	3	220
30	Binary	14.75	1.95	16.70	0.13	454	1338	40	2	200

Table BIII.3: Raw data of shoot, root/ total biomass and growth variables of B. *napus* from third pot trial.

	Treatments	Shoot biomass DW (g)	Root biomass DW (g)	Total biomass (g)	Root-shoot biomass ratio (g)	Growth index	Height (mm)	Number of true leaves	Number of dead leaves	Longest leaf length (mm)
1	Control	14.22	2.51	16.73	0.18	26	45	14	0	333
2	Control	14.35	2.78	17.13	0.19	27	45	13	0	310
3	Control	13.83	2.93	16.76	0.21	31	55	14	0	310
4	Control	11.85	2.36	14.21	0.20	21	35	15	0	315
5	Control	12.00	2.98	14.98	0.25	23	40	15	0	311
6	Control	12.25	2.09	14.34	0.17	23	40	14	0	267
7	Control	12.12	2.81	14.93	0.23	29	50	15	0	332
8	Control	12.85	2.33	15.18	0.18	24	45	13	0	302
9	Control	12.68	2.78	15.46	0.22	30	52	15	0	352
10	Control	12.56	2.20	14.76	0.18	21	40	14	0	278
11	Homogeneous	10.86	1.98	12.84	0.18	15	26	11	3	298
12	Homogeneous	11.60	1.92	13.52	0.17	17	30	12	2	315
13	Homogeneous	11.52	2.40	13.92	0.21	12	20	12	4	310
14	Homogeneous	11.36	1.74	13.10	0.15	13	25	12	2	315
15	Homogeneous	11.29	1.60	12.89	0.14	16	30	11	2	311
16	Homogeneous	10.80	2.55	13.35	0.24	17	30	12	4	267
17	Homogeneous	11.58	2.10	13.68	0.18	12	20	10	4	332
18	Homogeneous	11.82	1.86	13.68	0.16	18	30	12	2	302
19	Homogeneous	11.03	1.78	12.81	0.16	15	24	10	3	352
20	Homogeneous	14.33	2.21	16.54	0.15	17	31	11	3	332
21	Binary	9.22	1.42	10.64	0.15	11	21	13	3	311
22	Binary	9.56	1.87	11.43	0.20	13	20	13	4	332
23	Binary	8.50	1.42	9.92	0.17	13	21	13	4	328
24	Binary	9.07	1.60	10.67	0.18	12	18	13	4	300
25	Binary	9.85	1.22	11.07	0.12	13	19	13	3	295
26	Binary	9.75	1.52	11.27	0.16	12	20	12	2	333
27	Binary	9.09	1.96	11.05	0.22	12	20	13	3	278
28	Binary	9.42	1.77	11.19	0.19	14	20	12	3	276
29	Binary	9.19	1.28	10.47	0.14	11	19	13	3	304
30	Binary	8.24	1.89	10.13	0.23	12	21	12	3	288

Table CIII.3: Shoot, root, total plant Pb concentrations (mg/kg), Shoot, total concentration and translocation factors of *B. juncea*.

	Treatment	Shoot Pb concentration (mg/kg)	Root Pb concentration (mg/kg)	Total plant Pb (mg/kg) DW	Shoot CF	Total CF	Translocation factor
1	Homogeneous	42.78	2056	239	0.011	0.06	0.02
2	Homogeneous	52.7	1960	301	0.013	0.07	0.03
3	Homogeneous	43.16	1118	158	0.011	0.04	0.04
4	Homogeneous	37.4	1724	219	0.009	0.05	0.02
5	Homogeneous	40	1952.25	230	0.010	0.06	0.02
6	Homogeneous	36.12	1476.5	221	0.009	0.05	0.02
7	Homogeneous	32.26	1763	275	0.008	0.07	0.02
8	Homogeneous	45.34	1675.5	240	0.011	0.06	0.03
9	Homogeneous	40.1	1799	217	0.010	0.05	0.02
10	Homogeneous	40.27	1114.5	178	0.010	0.04	0.04
11	Binary	19	1080	162	0.005	0.04	0.02
12	Binary	24.35	841.5	138	0.006	0.03	0.03
13	Binary	21.7	1197.5	161	0.005	0.04	0.02
14	Binary	23.4	1065.75	168	0.006	0.04	0.02
15	Binary	19.76	705	104	0.005	0.03	0.03
16	Binary	20.68	768	129	0.005	0.03	0.03
17	Binary	22.05	1277.5	214	0.005	0.05	0.02
18	Binary	18.77	1706	241	0.005	0.06	0.01
19	Binary	25	869.5	138	0.006	0.03	0.03
20	Binary	25.68	1139	156	0.006	0.04	0.02

Table DIII.3: Shoot, root, total plant Pb concentrations (mg/kg), Shoot, total concentration and translocation factors of *B. napus*.

	Treatments	Shoot Pb concentration (mg/kg)	Root Pb concentration (mg/kg)	Total plant Pb (mg/kg) DW	Shoot CF	Total CF	Translocation factor
1	Homogeneous	87.62	1650.5	329	0.02	0.08	0.05
2	Homogeneous	45.18	2008	324	0.01	0.08	0.02
3	Homogeneous	35.86	1758	333	0.01	0.08	0.02
4	Homogeneous	36.83	1851.2	278	0.01	0.07	0.02
5	Homogeneous	45.78	1559	234	0.01	0.06	0.03
6	Homogeneous	28.32	2934	583	0.01	0.14	0.01
7	Homogeneous	35.44	2182	365	0.01	0.09	0.02
8	Homogeneous	44.82	1318.6	218	0.01	0.05	0.03
9	Homogeneous	40.16	1678.4	268	0.01	0.07	0.02
10	Homogeneous	32.22	1509.6	230	0.01	0.06	0.02
11	Binary	33.52	789.6	134	0.01	0.03	0.04
12	Binary	27.42	511.2	107	0.01	0.03	0.05
13	Binary	26.98	1102.4	181	0.01	0.04	0.02
14	Binary	19.8	1227.1	201	0.00	0.05	0.02
15	Binary	29.72	1882.4	234	0.01	0.06	0.02
16	Binary	37.7	637.6	119	0.01	0.03	0.06
17	Binary	18.28	1394.6	262	0.00	0.06	0.01
18	Binary	50.61	1625.6	300	0.01	0.07	0.03
19	Binary	28.56	1164.4	167	0.01	0.04	0.02
20	Binary	41.76	1324.4	281	0.01	0.07	0.03

Table EIII.3: Shoot and root Pb mass of *B. juncea* and *B. napus* between treatments in third pot trial.

	Species	Treatments	Shoot Pb mass (µg)	Root Pb mass (µg)		Species	Treatments	Shoot Pb mass (µg)	Root Pb mass (µg)
1	*B. juncea*	Homogeneous	440.21	2282.16	1	*B. napus*	Homogeneous	440.21	2282.16
2		Homogeneous	528.05	2940.00	2		Homogeneous	528.05	2940.00
3		Homogeneous	208.89	648.44	3		Homogeneous	208.89	648.44
4		Homogeneous	383.72	2137.76	4		Homogeneous	383.72	2137.76
5		Homogeneous	409.60	2206.04	5		Homogeneous	409.60	2206.04
6		Homogeneous	413.94	2495.29	6		Homogeneous	413.94	2495.29
7		Homogeneous	328.73	2926.58	7		Homogeneous	328.73	2926.58
8		Homogeneous	515.97	2580.27	8		Homogeneous	515.97	2580.27
9		Homogeneous	433.48	2176.79	9		Homogeneous	433.48	2176.79
10		Homogeneous	436.12	1772.06	10		Homogeneous	436.12	1772.06
11		Binary	267.90	2365.20	11		Binary	267.90	2365.20
12		Binary	281.00	1565.19	12		Binary	281.00	1565.19
13		Binary	317.69	2347.10	13		Binary	317.69	2347.10
14		Binary	275.89	2024.93	14		Binary	275.89	2024.93
15		Binary	276.05	1388.85	15		Binary	276.05	1388.85
16		Binary	269.87	1697.28	16		Binary	269.87	1697.28
17		Binary	266.81	2784.95	17		Binary	266.81	2784.95
18		Binary	245.70	3394.94	18		Binary	245.70	3394.94
19		Binary	315.25	1686.83	19		Binary	315.25	1686.83
20		Binary	378.78	2221.05	20		Binary	378.78	2221.05

Table GIII.3: Root ball diameter (mm) of *B. juncea* and *B. napus* in binary quadrants (patches).

B. *juncea*	0 mg/kg quadrant 1	0 mg/kg Quadrant 2	2000 mg/kg quadrant 1	2000 mg/kg Quadrant 2	B. napus	0 mg/kg quadrant 1	0 mg/kg Quadrant 2	2000 mg/kg quadrant 1	2000 mg/kg Quadrant 2
1	33.00	36.00	10.00	10.00	1	62.00	60.00	15.00	15.00
2	30.00	30.00	10.00	10.00	2	70.00	66.00	16.00	18.00
3	27.00	27.00	9.00	9.00	3	61.00	63.00	15.00	16.00
4	32.00	33.00	7.50	7.50	4	65.00	69.00	15.00	15.50
5	30.00	30.00	8.50	8.50	5	78.00	80.00	15.00	16.00
6	38.00	37.00	10.00	10.50	6	61.00	70.00	19.00	18.50
7	30.00	30.00	9.00	9.50	7	71.00	74.00	17.50	18.00
8	30.00	30.00	10.00	10.00	8	80.00	80.00	20.00	20.00
9	40.00	41.00	10.00	10.00	9	72.00	70.00	15.50	15.00
10	35.00	40.00	10.50	11.00	10	65.00	70.00	14.50	15.50

Table HIII.3: Root ball diameter (mm) for *B. juncea* and *B. napus* in homogeneous quadrants (patches).

B. *juncea*	1000 mg/kg quadrant 1	1000 mg/kg Quadrant 2	1000 mg/kg quadrant 1	1000 mg/kg Quadrant 2	B. *napus*	1000 mg/kg quadrant 1	1000 mg/kg Quadrant 2	1000 mg/kg quadrant 1	1000 mg/kg Quadrant 2
1	10.00	10.00	20.00	10.00	1	32.00	30.00	31.00	30.00
2	12.00	12.50	12.50	12.50	2	25.00	24.00	25.00	25.00
3	9.50	9.00	9.50	9.50	3	30.00	30.00	30.00	31.00
4	15.00	15.00	15.00	15.00	4	29.00	28.00	29.00	29.00
5	13.00	13.00	13.00	13.00	5	28.00	25.00	25.00	25.00
6	10.00	10.50	10.50	10.00	6	28.00	29.00	29.00	27.00
7	10.00	10.00	10.00	10.00	7	25.00	25.00	24.00	25.00
8	11.50	12.50	12.50	12.50	8	26.00	26.00	26.00	26.00
9	10.50	11.00	10.50	10.00	9	22.00	23.00	22.00	22.00
10	12.50	12.50	12.50	12.50	10	30.00	31.00	30.00	30.00

Appendix III.4: Quality control data for third pot trial.

Table AIII.4: Certified reference materials for extractable Pb analysis

	BCR 142	BCR 143	SRM-2709	SRM 2710	SRM 2711	HRM 1	HRM 2	HRM 31
X1	30.27	29.71	15.65	533.50	105.06	12.72	99.10	687.98
X2	29.34	30.48	15.41	593.66	105.30	11.78	111.66	612.38
Mean	29.80	30.10	15.53	563.58	105.18	12.25	105.38	650.18
Stdev	0.66	0.55	0.17	42.54	0.17	0.66	8.88	53.46
Certified values	37.8	1,333	17.3	1400	5552	13	510	6895
% Recovery	78.85	2.26	89.77	40.26	1.89	94.23	20.66	9.43

Table BIII.4: Certified reference materials for third pot trial herbage analysis

Sample	BCR-60	HRM 11	HRM 14
x1	78.58	27.05	10.3
x2	76.12	25.08	8.45
MEAN	77.35	26.07	9.38
CERTIFIED VALUES	64.00	26.00	9.00
BIAS	13.4	0.06	0.38
BIAS%	20.9	0.2	4.2

Table CIII.4: Blank analysis for third pot trial herbage analysis

BLANK ANALYSIS	
SAMPLE	**mg/kg Pb**
RBLK 1	-0.06
RBLK 2	0.74
RBLK 3	1.4
RBLK 4	1.8
RBLK 5	-0.68
Mean	0.64
Std	1.02
μ	0
SEM	0.46
mean-μ	0.64
t-test	1.40

T-TAB.	2.78		df(n-1)	4
two tailed test		1.40<2.78	Not significant	**P >0.05**

Table DIII.4: Precision and detection limit of third pot trial analysis.

Experiment	Precision	Detection limits
Herbage analysis pot trial 3	3.6%	2.65 mg/kg
Extractable Pb analysis	7.4%	3.01 mg/kg

Appendix III. 5: Test for significance of shoot and root Pb masses (µg) (Third pot trial).

AIII.5: Two-Sample T-Test of B. napus HO shoot Pb (µg), and B. juncea HO shoot Pb (µg)

```
Two-sample T for B. Napus HO shoot Pb (ug) vs B.juncea HO shoot Pb (ug)

                                        SE
                          N   Mean  StDev  Mean
B. Napus HO shoot Pb (ug  10    497    173    55
B.juncea  HO  shoot Pb (  10  409.9   91.3    29


T-Test of difference = 0 (vs not =): T-Value = 1.41 P-Value = 0.181 DF = 13
```

BIII.5: Two-Sample T-Test of B. napus binary shoot Pb (µg) and B. juncea binary shoot Pb (µg)

```
Two-sample T for B. napus binary shoot Pb (ug) vs B.juncea binary shoot Pb
(ug)

                                        SE
                          N   Mean  StDev  Mean
B. napus binary shoot Pb  10  289.0   92.4    29
B.juncea binary shoot Pb  10  289.5   38.2    12


T-Test of difference = 0 (vs not =): T-Value = -0.02 P-Value = 0.988 DF = 11
```

CIII.5: Two-Sample T-Test of B. napus HO shoot Pb (µg) and B. napus binary shoot Pb (µg)

```
Two-sample T for B. Napus HO shoot Pb (ug) vs B. napus binary shoot Pb (ug)

                                        SE
                          N   Mean  StDev  Mean
B. Napus HO shoot Pb (ug)  10    497    173    55
B. napus binary shoot Pb  10  289.0   92.4    29

T-Test of difference = 0 (vs not =): T-Value = 3.36  P-Value = 0.005  DF = 13
```

Two-Sample T-Test of B. napus HO root Pb (µg) and B. napus binary root Pb (µg)

```
Two-sample T for B. n HO root Pb (ug) vs B. n binary root Pb (ug)

                          N  Mean  StDev  SE Mean
B. n HO root Pb (ug)      10  3790   1467      464
B. n binary root Pb (ug)  10  1848    730      231

T-Test of difference = 0 (vs not =): T-Value = 3.75  P-Value = 0.002  DF = 13
```

DIII.5: Two-Sample T-Test of B. juncea HO shoot Pb (µg) and B. juncea binary shoot Pb (µg)

```
Two-sample T for B.juncea  HO  shoot Pb (ug) vs B.juncea binary shoot Pb (ug)

                                        SE
                          N   Mean  StDev  Mean
B.juncea  HO  shoot Pb (  10  409.9   91.3    29
B.juncea binary shoot Pb  10  289.5   38.2    12
```

T-Test of difference = 0 (vs not =): T-Value = 3.84 P-Value = 0.002 DF = 12

Two-Sample T-Test B. napus HO root Pb (µg), B. juncea HO root Pb (µg)

Two-sample T for B. n HO root Pb (ug) vs B.j HO root Pb (ug)

	N	Mean	StDev	SE Mean
B. n HO root Pb (ug)	10	3790	1467	464
B.j HO root Pb (ug)	10	2217	659	208

T-Test of difference = 0 (vs not =): T-Value = 3.09 P-Value = 0.009 DF = 12

EIII.5: Two-Sample T-Test of B. napus binary root Pb (µg), B. juncea binary root Pb (µg)

Two-sample T for B. n binary root Pb (ug) vs B.j binary root Pb (ug)

	N	Mean	StDev	SE Mean
B. n binary root Pb (ug)	10	1848	730	231
B.j binary root Pb (ug)	10	2148	615	194

T-Test of difference = 0 (vs not =): T-Value = -0.99 P-Value = 0.334 DF = 17

Two-Sample T-Test of B. juncea HO root Pb (µg) and B. juncea binary root Pb (µg)

Two-sample T for B.j HO root Pb (ug) vs B.j binary root Pb (ug)

	N	Mean	StDev	SE Mean
B.j HO root Pb (ug)	10	2217	659	208
B.j binary root Pb (ug)	10	2148	615	194

T-Test of difference = 0 (vs not =): T-Value = 0.24 P-Value = 0.812 DF = 17

APPENDICES RELATED TO FOURTH POT TRIAL (CHAPTER 6).

Appendix IV.1: Randomized Block design

Table AIV.1: Treatment blocks for *Brassica napus* and *Brassica juncea*

	Treatment block for Brassica napus					Treatment block for Brassica juncea			
Columns	A	B	C	D	Columns	A	B	C	D
1	BNMH4	BNHO3	BNHH1	BNMH3	1	BJLH8	BJHO10	BJMH6	BJHH6
2	BNHO10	BNHH7	BNHO2	BNHL6	2	BJHO8	BJLH9	BJHO3	BJMH5
3	BNHH4	BNHL5	BNHH9	BNHO7	3	BJHH8	BJHH3	BJMH3	BJLH1
4	BNMH5	BNMH1	BNHL2	BNMH2	4	BJMH8	BJHO7	BJLH5	BJMH2
5	BNHL10	BNHO6	BNHH2	BNHL8	5	BJLH10	BJMH1	BJHO9	BJHH1
6	BNMH8	BNHH10	BNMH7	BNHH3	6	BJHH2	BJHO4	BJHH4	BJLH7
7	BNHH8	BNMH9	BNHL7	BNMH6	7	BJMH7	BJLH4	BJLH6	BJHO2
8	BNHO5	BNHL4	BNHH6	BNHL3	8	BJHH7	BJHH10	BJHH5	BJMH4
9	BNHL9	BNHO1	BNHO9	BNHH5	9	BJHO1	BJLH2	BJMH10	BJHO6
10	BNHO4	BNMH10	BNHL1	BNHO8	10	BJHH9	BJMH9	BJHO5	BJLH3

Appendix IV.2: Tests of significance of biomass for *B. napus*.

One-way ANOVA: Shoot dry biomass versus Treatment

Source	DF	SS	MS	F	P
Treatment	3	259.880	86.627	687.40	0.000
Error	36	4.537	0.126		
Total	39	264.416			

S = 0.3550 R-Sq = 98.28% R-Sq(adj) = 98.14%

Grouping Information Using Tukey Method

Treatment	N	Mean	Grouping
HO	10	8.1497	A
MH	10	6.0447	B
LH	10	3.3902	C
HH	10	1.4478	D

Means that do not share a letter are significantly different.

One-way ANOVA: Root dry biomass versus Treatment

Source	DF	SS	MS	F	P
Treatment	3	9.44493	3.14831	536.71	0.000
Error	36	0.21118	0.00587		
Total	39	9.65611			

S = 0.07659 R-Sq = 97.81% R-Sq(adj) = 97.63%

Grouping Information Using Tukey Method

Treatment	N	Mean	Grouping
MH	10	1.9051	A
HO	10	1.5458	B
LH	10	1.1086	C
HH	10	0.6060	D

Means that do not share a letter are significantly different.

One-way ANOVA: Total dry biomass versus Treatment

Source	DF	SS	MS	F	P
Treatment	3	352.750	117.583	917.05	0.000
Error	36	4.616	0.128		
Total	39	357.366			

S = 0.3581 R-Sq = 98.71% R-Sq(adj) = 98.60%

Grouping Information Using Tukey Method

Treatment	N	Mean	Grouping
HO	10	9.6955	A
MH	10	7.9498	B
LH	10	4.4988	C
HH	10	2.0538	D

Means that do not share a letter are significantly different.

Appendix IV.3: Test of significance of uptake for *B. napus*

AIV.3: Analysis of variance.

One-way ANOVA: Shoot Pb versus Treatment

Source	DF	SS	MS	F	P
Treatment	3	264451	88150	164.38	0.000
Error	36	19305	536		
Total	39	283756			

S = 23.16 R-Sq = 93.20% R-Sq(adj) = 92.63%

Grouping Information Using Tukey Method

Treatment	N	Mean	Grouping
HO	10	269.18	A
MH	10	169.94	B
LH	10	147.50	B
HH	10	40.36	C

Means that do not share a letter are significantly different.

One-way ANOVA: Root Pb versus Treatment

Source	DF	SS	MS	F	P
Treatment	3	11833490	3944497	259.20	0.000
Error	36	547847	15218		
Total	39	12381337			

S = 123.4 R-Sq = 95.58% R-Sq(adj) = 95.21%

Grouping Information Using Tukey Method

Treatment	N	Mean	Grouping
MH	10	1708.8	A
LH	10	1496.5	B
HO	10	1042.4	C
HH	10	289.5	D

Means that do not share a letter are significantly different.

One-way ANOVA: Total plant Pb versus Treatment

Source	DF	SS	MS	F	P
Treatment	3	13942306	4647435	287.31	0.000
Error	36	582316	16175		
Total	39	14524622			

S = 127.2 R-Sq = 95.99% R-Sq(adj) = 95.66%

Treatment	N	Mean	Grouping
MH	10	1878.7	A
LH	10	1644.0	B
HO	10	1311.6	C
HH	10	329.9	D

Means that do not share a letter are significantly different.

One-way ANOVA: B. napus CFshoot versus Treatment

Source	DF	SS	MS	F	P
Treatment	3	0.0017229	0.0005743	51.53	0.000
Error	36	0.0004013	0.0000111		
Total	39	0.0021242			

```
S = 0.003339   R-Sq = 81.11%   R-Sq(adj) = 79.54%
Grouping Information Using Tukey Method

Treatment   N     Mean  Grouping
LH         10  0.045306  A
HO         10  0.034491    B
MH         10  0.029381      C
HH         10  0.029084      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: B. napus CFroot versus Treatment

```
Source     DF       SS       MS       F      P
Treatment   3  4.61293  1.53764  195.68  0.000
Error      36  0.28288  0.00786
Total      39  4.89582

S = 0.08864   R-Sq = 94.22%   R-Sq(adj) = 93.74%

Grouping Information Using Tukey Method

Treatment   N    Mean  Grouping
LH         10  1.4118  A
MH         10  0.9380    B
HO         10  0.7064      C
HH         10  0.4989        D

Means that do not share a letter are significantly different.
```

Table BIV.3: Mixed model ANOVA for *B. napus* (shoot, root and total plant Biomass and uptake)

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Treatment	Pillai's Trace	3.602	81.491	16.000	144.000	.000	.901
	Wilks' Lambda	.000	614.539	16.000	101.454	.000	.970
	Hotelling's Trace	890.410	1752.994	16.000	126.000	.000	.996
	Roy's Largest Root	837.479	7537.311[b]	4.000	36.000	.000	.999

a. Design: Treatment

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects for *B. napus*

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	F	Sig.
Model	Shoot DW	1165.463[a]	4	291.366	2312.058	.000
	Root DW	76.150[b]	4	19.038	3245.452	.000
	Shoot Pb	45282.790[c]	4	11320.698	1112.447	.000
	Root Pb	33098580.337[d]	4	8274645.084	1152.026	.000
Treatment	Shoot DW	1165.463	4	291.366	2312.058	.000
	Root DW	76.150	4	19.038	3245.452	.000
	Shoot Pb	45282.790	4	11320.697	1112.447	.000
	Root Pb	33098580.337	4	8274645.084	1152.026	.000
Error	Shoot DW	4.537	36	.126		
	Root DW	.211	36	.006		
	Shoot Pb	366.350	36	10.176		
	Root Pb	258576.753	36	7182.688		
Total	Shoot DW	1170.000	40			
	Root DW	76.362	40			
	Shoot Pb	45649.140	40			
	Root Pb	33357157.090	40			

Appendix IV.4: Test of significance of biomass for *B. juncea*

One-way ANOVA: Shoot dry biomass versus Treatment

```
Source     DF      SS      MS       F      P
Treatment   3   50.071 16.690 165.38 0.000
Error      36   3.633   0.101
Total      39   53.704

S = 0.3177   R-Sq = 93.23%   R-Sq(adj) = 92.67%

Grouping Information Using Tukey Method

Treatment   N Mean Grouping
HO         10 3.6783 A
LH         10 2.0305    B
MH         10 1.0891      C
HH         10 0.8183      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: Root dry biomass versus Treatment

```
Source     DF    SS       MS       F      P
Treatment   3 1.13660 0.37887 181.36 0.000
Error      36 0.07520 0.00209
Total      39 1.21180

S = 0.04571   R-Sq = 93.79%   R-Sq(adj) = 93.28%


Grouping Information Using Tukey Method

Treatment   N Mean Grouping
HO         10 4.4513 A
LH         10 2.6328    B
MH         10 1.5868      C
HH         10 1.1262        D

Means that do not share a letter are significantly different.
```

One-way ANOVA: Total dry biomass versus Treatment

```
Source     DF      SS      MS       F      P
Treatment   3    65.359 21.786   201.64 0.000
Error      36    3.890   0.108
Total      39   69.248

S = 0.3287   R-Sq = 94.38%   R-Sq(adj) = 93.91%


Grouping Information Using Tukey Method

Treatment   N    Mean Grouping
HO         10 4.4513 A
LH         10 2.6328    B
MH         10 1.5868      C
HH         10 1.1262        D

Means that do not share a letter are significantly different.
```

Appendix IV.5: Test of significance of uptake for *B. juncea*

AIV.5: Analysis of variance.

One-way ANOVA: Shoot Pb versus Treatment

```
Source     DF   SS      MS      F      P
Treatment   3 46513    15504 55.37 0.000
Error      36 10081    280
Total      39 56593

S = 16.73   R-Sq = 82.19%   R-Sq(adj) = 80.70%

Grouping Information Using Tukey Method

Treatment   N   Mean   Grouping
HO         10   129.38 A
LH         10   70.38    B
HH         10   46.32      C
MH         10   45.54      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: Root Pb versus Treatment

```
Source     DF   SS      MS       F      P
Treatment   3   834751   278250 116.40 0.000
Error      36   86059    2391
Total      39 920811

S = 48.89   R-Sq = 90.65%   R-Sq(adj) = 89.88%


Grouping Information Using Tukey Method

Treatment   N Mean Grouping
MH         10 577.79 A
HO         10 399.27    B
LH         10 235.01      C
HH         10 222.57      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: Total plant Pb versus Treatment

```
Source     DF     SS      MS       F      P
Treatment   3   885809    295270   106.66 0.000
Error      36   99663    2768
Total      39   985473

S = 52.62   R-Sq = 89.89%   R-Sq (adj) = 89.04%

Grouping Information Using Tukey Method

Treatment   N Mean Grouping
MH         10 623.32 A
HO         10 528.65    B
LH         10 305.39      C
HH         10 268.90      C

Means that do not share a letter are significantly different.
```

One-way ANOVA: B. juncea CFshoot versus Treatment

```
Source     DF         SS         MS      F      P
Treatment   3  0.0035915  0.0011972  14.36  0.000
Error      36  0.0030019  0.0000834
Total      39  0.0065934

S = 0.009132   R-Sq = 54.47%   R-Sq(adj) = 50.68%

Grouping Information Using Tukey Method

Treatment   N      Mean  Grouping
HH         10  0.059540  A
MH         10  0.043895    B
HO         10  0.036493    B
LH         10  0.036172    B
```

One-way ANOVA: B. juncea CFroot versus Treatment

```
Source     DF       SS       MS       F      P
Treatment   3  3.66352  1.22117  313.54  0.000
Error      36  0.14021  0.00389
Total      39  3.80374

S = 0.06241   R-Sq = 96.31%   R-Sq(adj) = 96.01%

Grouping Information Using Tukey Method

Treatment   N     Mean  Grouping
MH         10  1.20853  A
HH         10  0.75729    B
HO         10  0.54093      C
LH         10  0.41241        D

Means that do not share a letter are significantly different.
```

Two-Sample T-Test and CI: B. napus CFshoot, B. juncea CFshoot

```
Two-sample T for B. napus CFshoot vs B. juncea CFshoot

                    N     Mean    StDev  SE Mean
B. napus CFshoot   40  0.03457  0.00738   0.0012
B. juncea CFshoot  40   0.0440   0.0130   0.0021


Difference = mu (B. napus CFshoot) - mu (B. juncea CFshoot)
Estimate for difference:  -0.00946
95% CI for difference:  (-0.01419, -0.00473)
T-Test of difference = 0 (vs not =): T-Value = -4.00  P-Value = 0.000  DF = 61
```

Two-Sample T-Test and CI: B.napus CFroot, B. juncea CFroot

```
Two-sample T for B.napus CFroot vs B. juncea CFroot

                   N   Mean  StDev  SE Mean
B.napus CFroot    40  0.889  0.354    0.056
B. juncea CFroot  40  0.730  0.312    0.049


T-Test of difference = 0 (vs not =): T-Value = 2.13 P-Value = 0.037 DF = 76
```

Table BIV.5: Mixed model ANOVA for *Brassica juncea*

Multivariate Tests[a]

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Treatment	Pillai's Trace	3.135	32.642	16.000	144.000	.000	.784
	Wilks' Lambda	.000	277.562	16.000	101.454	.000	.945
	Hotelling's Trace	463.055	911.640	16.000	126.000	.000	.991
	Roy's Largest Root	417.424	3756.812[b]	4.000	36.000	.000	.998

a. Design: Treatment

b. The statistic is an upper bound on F that yields a lower bound on the significance level.

Tests of Between-Subjects Effects for *B. juncea*

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Model	Shoot DW	195.085[a]	4	48.771	483.265	.000
	Root DW	13.029[b]	4	3.257	1559.200	.000
	Shoot Pb	74156.989[c]	4	18539.247	243.205	.000
	Root Pb	22824165.240[d]	4	5706041.310	1602.590	.000
Treatment	Shoot DW	195.085	4	48.771	483.265	.000
	Root DW	13.029	4	3.257	1559.200	.000
	Shoot Pb	74156.989	4	18539.247	243.205	.000
	Root Pb	22824165.240	4	5706041.310	1602.590	.000
Error	Shoot DW	3.633	36	.101		
	Root DW	.075	36	.002		
	Shoot Pb	2744.241	36	76.229		
	Root Pb	128178.404	36	3560.511		
Total	Shoot DW	198.718	40			
	Root DW	13.104	40			
	Shoot Pb	76901.230	40			
	Root Pb	22952343.645	40			

Appendix IV.6: Tests for significance of root placement data.

AIV.6: Analysis of variance.

One-way ANOVA: *B. juncea* root biomass versus Patch with same nominal Pb concentration.

```
Source                DF        SS        MS      F      P
Patch with  same Pb    9  0.017729  0.001970  13.77  0.000
Error                 90  0.012875  0.000143
Total                 99  0.030604

S = 0.01196   R-Sq = 57.93%   R-Sq(adj) = 53.72%
```

One-way ANOVA: B. juncea root Pb versus Nominal soil Pb

```
Source            DF        SS       MS       F      P
Nominal soil Pb    4  16000700  4000175 946.49 0.000
Error             45    190185     4226
Total             49  16190885

S = 65.01   R-Sq = 98.83%   R-Sq (adj) = 98.72%

Grouping Information Using Tukey Method
Nominal
soil Pb   N    Mean  Grouping
10000    10  1551.3   A
 3000    10   415.9   B
  100    10   134.6     C
 1000    10    81.0     C
  300    10    62.2     C

Means that do not share a letter are significantly different.
```

Regression Analysis: B. juncea root Pb versus Nominal soil Pb

```
The regression equation is
BJ root Pb = 12.21 + 0.1517 Nominal soil Pb


S = 91.9045   R-Sq = 97.5%   R-Sq(adj) = 97.4%


Analysis of Variance

Source      DF        SS        MS        F      P
Regression   1  15785457  15785457  1868.89  0.000
Error       48    405429      8446
Total       49  16190885
```

BIV.6: Patch comparison for *B. juncea*

Two-Sample T-Test and CI: B. juncea 100, Patch

```
Two-sample T for BJ 100

Patch    N     Mean    StDev  SE Mean
middle  10  0.01683  0.00466   0.0015
Outer   10   0.0259   0.0355    0.011

T-Test of difference = 0 (vs not =): T-Value = -0.80  P-Value = 0.445  DF = 9
```

Two-Sample T-Test and CI: B. juncea 300, Patch

```
Two-sample T for BJ 300

Patch    N     Mean    StDev  SE Mean
middle  10  0.01238  0.00725   0.0023
Outer   10  0.01305  0.00210  0.00066

T-Test of difference = 0 (vs not =): T-Value = -0.28  P-Value = 0.784  DF = 10
```

Two-Sample T-Test and CI: B. juncea 1000, Patch

```
Two-sample T for BJ 1000

Patch    N     Mean    StDev  SE Mean
middle  10  0.00856  0.00231  0.00073
Outer   10  0.01374  0.00288  0.00091


T-Test of difference = 0 (vs not =): T-Value = -4.44  P-Value = 0.000  DF = 17
```

Two-Sample T-Test and CI: B. juncea 3000, Patch

```
Two-sample T for BJ 3000

Patch    N     Mean    StDev  SE Mean
middle  10  0.00522  0.00351   0.0011
Outer   10  0.00476  0.00104  0.00033

T-Test of difference = 0 (vs not =): T-Value = 0.40  P-Value = 0.699  DF = 10
```

Two-Sample T-Test and CI: B. juncea 100 and 300

```
Two-sample T for BJ 100 vs BJ 300

         N     Mean    StDev  SE Mean
BJ 100  20   0.0214   0.0251   0.0056
BJ 300  20  0.01272  0.00521   0.0012


T-Test of difference = 0 (vs not =): T-Value = 1.51  P-Value = 0.147  DF = 20
```

Two-Sample T-Test and CI: B. juncea 100 and 1000

```
Two-sample T for BJ 100 vs BJ 1000

          N     Mean    StDev  SE Mean
BJ 100   20   0.0214   0.0251   0.0056
BJ 1000  20  0.01115  0.00367  0.00082

T-Test of difference = 0 (vs not =): T-Value = 1.80  P-Value = 0.088  DF = 19
```

Two-Sample T-Test and CI: B. juncea 100 and 3000

```
Two-sample T for BJ 100 vs BJ 3000

          N     Mean    StDev  SE Mean
BJ 100   20   0.0214   0.0251   0.0056
BJ 3000  20  0.00499  0.00253  0.00057
```

```
T-Test of difference = 0 (vs not =): T-Value = 2.90  P-Value = 0.009  DF = 19
```

Two-Sample T-Test and CI: B. juncea 100 and 10000

```
Two-sample T for BJ 100 vs BJ 10000

           N      Mean     StDev  SE Mean
BJ 100    20    0.0214    0.0251   0.0056
BJ 10000  10  0.002470  0.000650  0.00021


T-Test of difference = 0 (vs not =): T-Value = 3.36  P-Value = 0.003  DF = 19
```

Two-Sample T-Test and CI: B. juncea 300 and 1000

```
Two-sample T for BJ 300 vs BJ 1000

          N     Mean    StDev  SE Mean
BJ 300   20  0.01272  0.00521   0.0012
BJ 1000  20  0.01115  0.00367  0.00082


T-Test of difference = 0 (vs not =): T-Value = 1.10  P-Value = 0.280  DF = 34
```

Two-Sample T-Test and CI: B. juncea 300 and 3000

```
Two-sample T for BJ 300 vs BJ 3000

          N     Mean    StDev  SE Mean
BJ 300   20  0.01272  0.00521   0.0012
BJ 3000  20  0.00499  0.00253  0.00057


T-Test of difference = 0 (vs not =): T-Value = 5.97  P-Value = 0.000  DF = 27
```

Two-Sample T-Test and CI: B. juncea 300 and 10000

```
Two-sample T for BJ 300 vs BJ 10000

           N      Mean     StDev  SE Mean
BJ 300    20   0.01272   0.00521   0.0012
BJ 10000  10  0.002470  0.000650  0.00021


T-Test of difference = 0 (vs not =): T-Value = 8.67  P-Value = 0.000  DF = 20
```

Two-Sample T-Test and CI: B. juncea 3000 and 10000

```
Two-sample T for BJ 3000 vs BJ 10000

           N      Mean     StDev  SE Mean
BJ 3000   20   0.00499   0.00253  0.00057
BJ 10000  10  0.002470  0.000650  0.00021


T-Test of difference = 0 (vs not =): T-Value = 4.19  P-Value = 0.000  DF = 23
```

Two-Sample T-Test and CI: B. juncea 1000 and 3000

```
Two-sample T for BJ 1000 vs BJ 3000

          N     Mean    StDev  SE Mean
BJ 1000  20  0.01115  0.00367  0.00082
BJ 3000  20  0.00499  0.00253  0.00057


T-Test of difference = 0 (vs not =): T-Value = 6.18  P-Value = 0.000  DF = 33
```

Two-Sample T-Test and CI: B. juncea 1000, BJ 10000

```
Two-sample T for BJ 1000 vs BJ 10000

           N      Mean     StDev  SE Mean
BJ 1000   20   0.01115   0.00367  0.00082
BJ 10000  10  0.002470  0.000650  0.00021


T-Test of difference = 0 (vs not =): T-Value = 10.26  P-Value = 0.000  DF = 21
```

CIV.6: Analysis of variance and Regression

One-way ANOVA: *B. napus* root biomass versus Patch with same nominal Pb Concentration.

```
Source                DF        SS        MS       F      P
Patch with same Pb    9  0.973881  0.108209  585.02  0.000
Error                 90  0.016647  0.000185
Total                 99  0.990528

S = 0.01360   R-Sq = 98.32%   R-Sq(adj) = 98.15%
```

One-way ANOVA: B. napus root Pb versus Nominal soil Pb

```
Source            DF       SS       MS        F      P
Nominal soil Pb    4  5110863  1277716  3007.41  0.000
Error             45    19119      425
Total             49  5129981

S = 20.61   R-Sq = 99.63%   R-Sq(adj) = 99.59%

Grouping Information Using Tukey Method

Nominal
soil Pb   N    Mean  Grouping
10000    10   1551.3  A
 3000    10   415.9    B
  100    10   134.6      C
 1000    10    81.0      C
  300    10    62.2      C

Means that do not share a letter are significantly different.
```

Regression Analysis: B. napus root Pb versus Nominal soil Pb

```
The regression equation is
BN root Pb = 8.674 + 0.08598 Nominal soil Pb


S = 34.1943   R-Sq = 98.9%   R-Sq(adj) = 98.9%
```

```
Analysis of Variance

Source      DF       SS       MS        F      P
Regression   1  5073857  5073857  4339.41  0.000
Error       48    56124     1169
Total       49  5129981
```

DIV.6: T-test patch comparison for *B. napus*

One-Sample T: B. napus 100

```
Variable   N     Mean    StDev  SE Mean        95% CI
BN 100    20  0.01733  0.01967  0.00440  (0.00812, 0.02653)
```

Two-Sample T-Test and CI: B. napus 100, Patch

```
Two-sample T for BN 100

Patch    N     Mean    StDev  SE Mean
middle  10   0.0190   0.0279   0.0088
Outer   10  0.01570  0.00574   0.0018

T-Test of difference = 0 (vs not =): T-Value = 0.36  P-Value = 0.725  DF = 9
```

Two-Sample T-Test and CI: B. napus 300, Patch

```
Two-sample T for BN 300

Patch    N     Mean    StDev  SE Mean
middle  10  0.00673  0.00219  0.00069
Outer   10  0.01457  0.00187  0.00059


T-Test of difference = 0 (vs not =): T-Value = -8.60  P-Value = 0.000  DF = 17
```

Two-Sample T-Test and CI: B. napus 100 and 300

```
Two-sample T for BN 100 vs BN 300

         N     Mean    StDev  SE Mean
BN 100  20   0.0173   0.0197   0.0044
BN 300  20  0.01065  0.00448   0.0010


T-Test of difference = 0 (vs not =): T-Value = 1.48  P-Value = 0.154  DF = 20
```

Two-Sample T-Test and CI: B. napus 300 and 3000

```
Two-sample T for BN 300 vs BN 3000

          N     Mean    StDev  SE Mean
BN 300   20  0.01065  0.00448   0.0010
BN 3000  20  0.00534  0.00444  0.00099


T-Test of difference = 0 (vs not =): T-Value = 3.76 P-Value = 0.001 DF = 37
```

Two-Sample T-Test and CI: B. napus 100 and 3000

```
Two-sample T for BN 100 vs BN 3000

          N     Mean    StDev  SE Mean
BN 100   20   0.0173   0.0197   0.0044
BN 3000  20  0.00534  0.00444  0.00099


T-Test of difference = 0 (vs not =): T-Value = 2.66  P-Value = 0.015  DF = 20
```

Two-Sample T-Test and CI: B. napus 3000 and 10000

```
Two-sample T for BN 3000 vs BN 10000

           N     Mean    StDev  SE Mean
BN 3000   20  0.00534  0.00444  0.00099
BN 10000   2  0.00255  0.00106  0.00075


T-Test of difference = 0 (vs not =): T-Value = 2.24  P-Value = 0.066  DF = 6
```

Two-Sample T-Test and CI: B. napus 100 and 1000

```
Two-sample T for BN 100 vs BN 1000

          N     Mean    StDev  SE Mean
BN 100   20   0.0173   0.0197   0.0044
BN 1000  20  0.00848  0.00542   0.0012


T-Test of difference = 0 (vs not =): T-Value = 1.94  P-Value = 0.066  DF = 21
```

Two-Sample T-Test and CI: B. napus 300 and 1000

```
Two-sample T for BN 300 vs BN 1000

          N     Mean    StDev  SE Mean
BN 300   20  0.01065  0.00448   0.0010
BN 1000  20  0.00848  0.00542   0.0012


T-Test of difference = 0 (vs not =): T-Value = 1.38  P-Value = 0.177  DF = 36
```

Two-Sample T-Test and CI: B. napus 3000 and 1000

```
Two-sample T for BN 3000 vs BN 1000

          N     Mean    StDev  SE Mean
BN 3000  20  0.00534  0.00444  0.00099
BN 1000  20  0.00848  0.00542   0.0012


T-Test of difference = 0 (vs not =): T-Value = -2.00  P-Value = 0.053  DF = 36
```

Two-Sample T-Test and CI: B. napus 10000 and 1000

```
Two-sample T for BN 10000 vs BN 1000

           N     Mean    StDev  SE Mean
BN 10000   2  0.00255  0.00106  0.00075
```

```
BN 1000   20  0.00848  0.00542   0.0012


T-Test of difference = 0 (vs not =): T-Value = -4.16  P-Value = 0.002  DF = 9
```

Two-Sample T-Test and CI: B. napus 1000 outer and Patches

```
Two-sample T for BN 1000

Patch    N     Mean    StDev  SE Mean
middle  10  0.00401  0.00346   0.0011
Outer   10  0.01294  0.00240  0.00076

T-Test of difference = 0 (vs not =): T-Value = -6.70  P-Value = 0.000  DF = 16
```

EIV.6: Central (1000 mg/kg Pb) patch comparison for *B. napus* and *B. juncea*

One-way ANOVA: B. napus 1000 versus Patch

```
Source  DF        SS        MS        F      P
Patch    2  0.724834  0.362417  1096.40  0.000
Error   27  0.008925  0.000331
Total   29  0.733759

S = 0.01818   R-Sq = 98.78%   R-Sq(adj) = 98.69%


Grouping Information Using Tukey Method

Patch     N     Mean  Grouping
Central  10  0.33812  A
Outer    10  0.01294    B
middle   10  0.00401    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Patch
```

One-way ANOVA: B. juncea 1000 versus Patch

```
Source  DF         SS         MS       F      P
Patch    2  0.0103149  0.0051575  199.76  0.000
Error   27  0.0006971  0.0000258
Total   29  0.0110120

S = 0.005081   R-Sq = 93.67%   R-Sq(adj) = 93.20%


Grouping Information Using Tukey Method

Patch     N      Mean  Grouping
Central  10  0.050230  A
Outer    10  0.013737    B
middle   10  0.008565    B

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Patch
```

Two-Sample T-Test: B. juncea central root biomass, B. napus central root biomass

```
Two-sample T for B.juncea central root biomass vs B.napus central root biomass

                                N     Mean    StDev  SE Mean
B.juncea central root biomass  10  0.05023  0.00799   0.0025
B.napus central root biomass   10   0.3381   0.0312   0.0099


T-Test of difference = 0 (vs not =): T-Value = -28.26  P-Value = 0.000  DF =
10
```

FIV.6: Comparison of *B. napus* and *B. juncea* root response.

Two-Sample T-Test and CI: B. napus root biomass, B. juncea root biomass

```
Two-sample T for B.napus root biomass vs B. juncea root biomass

                          N    Mean   StDev  SE Mean
B.napus root biomass    100   0.042   0.100    0.010
B. juncea root biomass  100  0.0153  0.0176   0.0018


T-Test of difference = 0 (vs not =): T-Value = 2.68  P-Value = 0.009  DF = 105
```

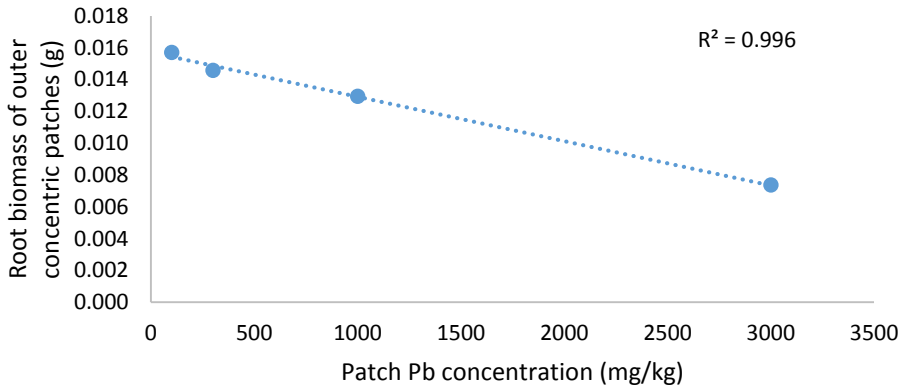


Figure AIV.6: Regression model of root biomass of outer concentric patches against Patch Pb concentration of *B.napus*.

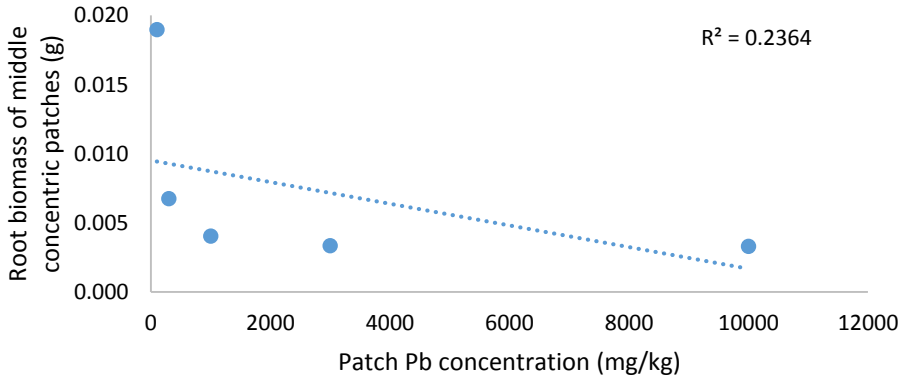


Figure BIV.6: Regression model of root biomass of middle concentric patches against Patch Pb concentration of *B. napus*.

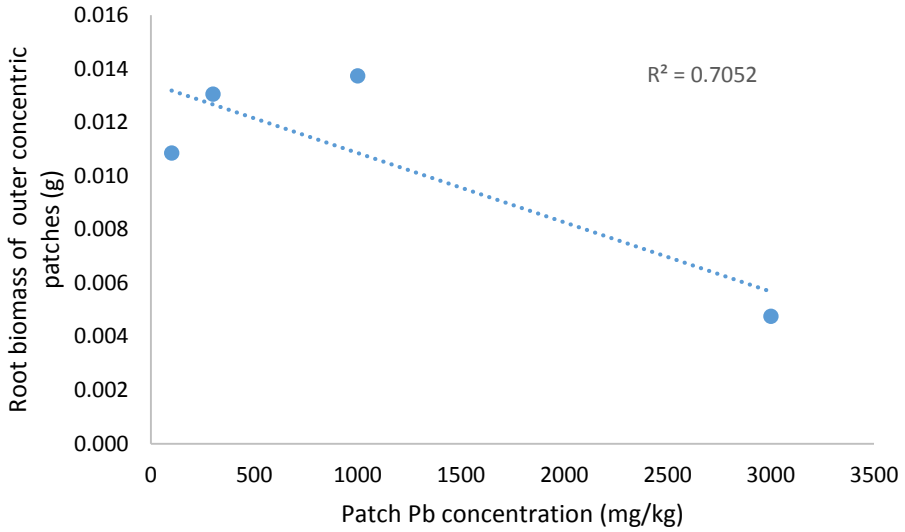


Figure CIV.6: Regression model of root biomass of outer concentric patches against Patch Pb concentration of *B. juncea*.

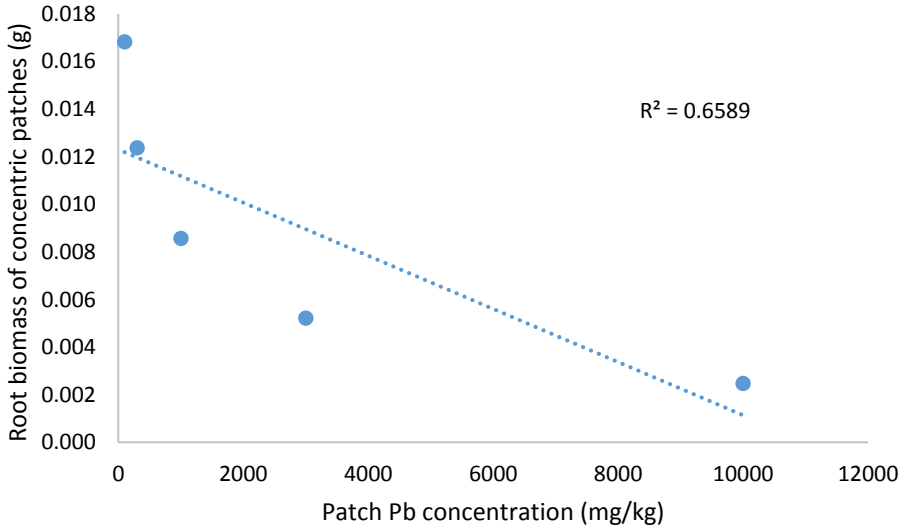


Figure DIV.6: Regression model of root biomass of middle concentric patches against Patch Pb concentration of *B. juncea*.

Appendix IV.7: Other information related to pot trial four (pot trial simulating *in situ* heterogeneity).

(a) (b) (c)

(d) (e)

Figure AIV.1: Pot trial 4 experimental method showing (a) pot filled with heterogeneous distribution of Pb, (b) Filling of pots with growth media (c) PTEG pot divider (d) Root extraction showing wooden box and sleeve (e) customized metal blade.

Appendix IV.8: Raw data for pot trial four.

Table AIV.8: Biomass and Pb uptake data for *B. napus*

S/N	Pot No	Species & treatment	Shoot DW (g)	Root DW (g)	Total DW (g)	Shoot Pb (mg/kg)	Shoot Pb (µg)	Root Pb (mg/kg)	Root Pb (µg)	Total plant Pb (µg)
1	1	BNHO	8.2921	1.5438	9.8359	31.17	258.4648	676.53	1044.4270	1302.8918
2	2	BNHO	7.7833	1.3921	9.1754	28.08	218.5551	763.74	1063.2025	1281.7575
3	3	BNHO	8.4376	1.5193	9.9569	33.37	281.5627	610.24	927.1376	1208.7003
4	4	BNHO	8.7488	1.4376	10.186	34.9	305.3331	701.99	1009.1808	1314.5139
5	5	BNHO	8.3025	1.5873	9.8898	33.36	276.9714	648.24	1028.9514	1305.9228
6	6	BNHO	8.2843	1.5711	9.8554	28.32	234.6114	641.74	1008.2377	1242.8491
7	7	BNHO	8.0268	1.563	9.5898	33.72	270.6637	690.74	1079.6266	1350.2903
8	8	BNHO	8.2727	1.647	9.9197	37.1	306.9172	674.24	1110.4733	1417.3905
9	9	BNHO	8.3767	1.5894	9.9661	37.27	312.1996	638.24	1014.4187	1326.6183
10	10	BNHO	6.9725	1.6073	8.5798	32.48	226.4668	708.24	1138.3542	1364.8210
11	11	BNLH	3.8722	1.1071	4.9793	49.58	191.9837	1446.7	1601.6859	1793.6695
12	12	BNLH	3.4174	1.1017	4.5191	42.66	145.7863	1206.7	1329.4655	1475.2517
13	13	BNLH	3.0378	1.0489	4.0867	33.37	101.3714	1167.7	1224.8425	1326.2139
14	14	BNLH	3.6345	1.1079	4.7424	41.68	151.4860	1167.1	1292.9858	1444.4717
15	15	BNLH	3.6213	1.1004	4.7217	40.08	145.1417	1378.2	1516.6153	1661.7570
16	16	BNLH	3.5239	1.2158	4.7397	48.28	170.1339	1215.2	1477.4888	1647.6227
17	17	BNLH	3.5064	1.1156	4.622	42.82	150.1440	1471.7	1641.8731	1792.0172
18	18	BNLH	3.5212	1.1164	4.6376	47.84	168.4542	1580.2	1764.1799	1932.6341
19	19	BNLH	2.9426	1.1527	4.0953	43	126.5318	1465.7	1689.5585	1816.0903
20	20	BNLH	2.8246	1.0195	3.8441	43.87	123.9152	1399.2	1426.5252	1550.4404
21	21	BNMH	6.1651	1.8308	7.9959	27.4	168.9237	774.24	1417.4786	1586.4023
22	22	BNMH	6.1303	1.9475	8.0778	30.1	184.5220	879.24	1712.3199	1896.8419
23	23	BNMH	5.1469	1.9378	7.0847	26.72	137.5252	952.24	1845.2507	1982.7758
24	24	BNMH	6.2564	2.1008	8.3572	29.56	184.9392	900.05	1890.8250	2075.7642
25	25	BNMH	6.1468	1.7262	7.873	29.18	179.3636	960.24	1657.5663	1836.9299
26	26	BNMH	6.0528	1.8623	7.9151	29.06	175.8944	808.24	1505.1854	1681.0797
27	27	BNMH	6.1136	1.9893	8.1029	28.68	175.3380	915.24	1820.6869	1996.0250
28	28	BNMH	6.1568	1.8937	8.0505	24.12	148.5020	955.24	1808.9380	1957.4400
29	29	BNMH	6.1457	1.9702	8.1159	27.04	166.1797	908.53	1789.9858	1956.1655
30	30	BNMH	6.1329	1.7922	7.9251	29.06	178.2221	914.74	1639.3970	1817.6191
31	31	BNHH	1.8607	0.4775	2.3382	28.74	53.4765	450.32	215.0278	268.5043
32	32	BNHH	1.338	0.6157	1.9537	23.16	30.9881	508.79	313.2620	344.2501
33	33	BNHH	1.2802	0.6287	1.9089	28.01	35.8584	415.91	261.4826	297.3410
34	34	BNHH	1.7349	0.6283	2.3632	25.69	44.5696	477.52	300.0258	344.5954
35	35	BNHH	1.0782	0.647	1.7252	26.43	28.4968	496.17	321.0220	349.5188
36	36	BNHH	1.2831	0.6284	1.9115	27.22	34.9260	509.34	320.0693	354.9952
37	37	BNHH	1.5267	0.6313	2.158	27.51	41.9995	500.41	315.9088	357.9084
38	38	BNHH	1.5174	0.6221	2.1395	31.24	47.4036	476.03	296.1383	343.5418
39	39	BNHH	1.4804	0.6429	2.1233	30.42	45.0338	465.06	298.9871	344.0208
40	40	BNHH	1.3782	0.5382	1.9164	29.66	40.8774	470.19	253.0563	293.9337

Table BIV.8: Biomass and Pb uptake data for *B. juncea*

S/N	Pot No	Species & treatment	Shoot DW (g)	Root DW (g)	Total DW (g)	Shoot Pb (mg/kg)	Shoot Pb (µg)	Root Pb (mg/kg)	Root Pb (µg)	Total plant Pb (µg)
1	1	BJHO	3.8502	0.7997	4.6499	35.41	136.3356	487.69	390.0057	526.3413
2	2	BJHO	3.6778	0.7527	4.4305	36.22	133.2099	497.54	374.4984	507.7083
3	3	BJHO	2.6016	0.8052	3.4068	31.72	82.5228	444.94	358.2657	440.7884
4	4	BJHO	4.0583	0.7547	4.813	37.96	154.0531	593.74	448.0956	602.1486
5	5	BJHO	3.5058	0.7878	4.2936	34.26	120.1087	519.34	409.1361	529.2448
6	6	BJHO	3.952	0.7343	4.6863	35.27	139.3870	502.29	368.8315	508.2186
7	7	BJHO	3.8807	0.7926	4.6733	38	147.4666	484.54	384.0464	531.5130
8	8	BJHO	3.5142	0.7421	4.2563	31.82	111.8218	557.74	413.8989	525.7207
9	9	BJHO	3.4553	0.7827	4.238	28.54	98.6143	565.34	442.4916	541.1059
10	10	BJHO	4.2871	0.7778	5.0649	39.72	170.2836	518.74	403.4760	573.7596
11	11	BJLH	1.4502	0.6465	2.0967	32.14	46.6094	409.54	264.7676	311.3770
12	12	BJLH	2.4085	0.6677	3.0762	35.38	85.2127	307.94	205.6115	290.8243
13	13	BJLH	1.9312	0.526	2.4572	34.02	65.6994	470.39	247.4251	313.1246
14	14	BJLH	2.2554	0.5147	2.7701	35.49	80.0441	378.24	194.6801	274.7243
15	15	BJLH	2.2761	0.6078	2.8839	30.62	69.6942	427.44	259.7980	329.4922
16	16	BJLH	2.3221	0.6513	2.9734	35.16	81.6450	342.89	223.3243	304.9693
17	17	BJLH	1.275	0.509	1.784	34.53	44.0258	438.79	223.3441	267.3699
18	18	BJLH	2.0389	0.5418	2.5807	37.88	77.2335	450.54	244.1026	321.3361
19	19	BJLH	2.1917	0.6713	2.863	37.36	81.8819	367.74	246.8639	328.7458
20	20	BJLH	2.156	0.687	2.843	33.26	71.7086	349.59	240.1683	311.8769
21	21	BJMH	1.1545	0.5398	1.6943	40.38	46.6187	1176.7	635.2043	681.8230
22	22	BJMH	0.8154	0.4671	1.2825	39.42	32.1431	1113.2	519.9944	552.1375
23	23	BJMH	1.4865	0.5267	2.0132	38.82	57.7059	1281.7	675.0925	732.7984
24	24	BJMH	0.9781	0.4878	1.4659	38.99	38.1361	1191.7	581.3308	619.4669
25	25	BJMH	1.1332	0.5177	1.6509	41.08	46.5519	1169.2	605.3155	651.8674
26	26	BJMH	1.1459	0.4911	1.637	47.86	54.8428	1154.7	567.0928	621.9356
27	27	BJMH	1.1751	0.5722	1.7473	37.32	43.8547	1249.7	715.1012	758.9560
28	28	BJMH	0.9299	0.4756	1.4055	48.26	44.8770	971.86	462.2166	507.0936
29	29	BJMH	1.11	0.4864	1.5964	43.36	48.1296	1208.7	587.9311	636.0607
30	30	BJMH	0.9621	0.4132	1.3753	44.2	42.5248	1037.2	428.5876	471.1124
31	31	BJHH	0.7094	0.3383	1.0477	99.5	70.5853	693.59	234.6415	305.2268
32	32	BJHH	0.6083	0.3268	0.9351	42.88	26.0839	735	240.1980	266.2819
33	33	BJHH	0.9935	0.3097	1.3032	49.36	49.0392	739.12	228.9055	277.9446
34	34	BJHH	0.8414	0.2834	1.1248	66.52	55.9699	741.07	210.0192	265.9892
35	35	BJHH	0.9254	0.3038	1.2292	60.98	56.4309	738.45	224.3411	280.7720
36	36	BJHH	0.8256	0.3023	1.1279	47.94	39.5793	708.62	214.2158	253.7951
37	37	BJHH	0.8508	0.3434	1.1942	48.8	41.5190	683	234.5422	276.0612
38	38	BJHH	0.8596	0.2614	1.121	52.92	45.4900	768.59	200.9094	246.3995
39	39	BJHH	0.7592	0.2929	1.0521	54.36	41.2701	703.04	205.9204	247.1905
40	40	BJHH	0.8094	0.3178	1.1272	46.02	37.2486	730.14	232.0385	269.2871

Appendix IV.9: Result of test of experimental data for normal distribution (fourth pot trial).

Table AIV.9:One-Sample Kolmogorov-Smirnov Test for shoot, root and total DW

		Shoot DW	Root DW	Total DW
N		40	40	40
Normal Parameters[a,b]	Mean	4.7581	1.2914	6.0495
	Std. Deviation	2.60383	.49759	3.02708
Most Extreme Differences	Absolute	.165	.152	.202
	Positive	.142	.152	.142
	Negative	-.165	-.127	-.202
Kolmogorov-Smirnov Z		1.047	.963	1.275
Asymp. Sig. (2-tailed)		.223	.311	.078

Table BIV.9: One-Sample Kolmogorov-Smirnov Test for shoot, root and total Pb (mg/kg & µg)

		Shoot Pb µg	Root Pb µg	Total Pb µg	Shoot Pb mg/kg	Root Pb mg/kg
N		40	40	40	40	40
Normal Parameters[a,b]	Mean	156.7430	1134.2975	1291.0395	33.0488	849.7600
	Std. Deviation	85.29774	563.44510	610.26686	7.05625	338.76018
Most Extreme Differences	Absolute	.137	.176	.196	.176	.137
	Positive	.137	.176	.187	.176	.137
	Negative	-.096	-.136	-.196	-.099	-.100
Kolmogorov-Smirnov Z		.866	1.110	1.242	1.114	.866
Asymp. Sig. (2-tailed)		.441	.170	.092	.167	.441

a. Test distribution is Normal.

b. Calculated from data.

Appendix IV.11: Quality control data for pot trial four soil and herbage analysis.

Table AIV.11: Analysis of certified reference materials for pot trial four herbage analysis.

Sample	BCR-60	HRM 11	HRM 14
x1	43.96	27.46	9.52
x2	86.04	25.74	11.21
MEAN	65.00	26.60	10.37
CERTIFIED VALUES	64.00	26.00	9.00
BIAS	1.0	0.60	1.37
BIAS%	1.6	2.3	15.2

Regression Analysis: Measured values versus certified values

```
The regression equation is
Measured values = 1.12 + 0.996 Certified values


Predictor            Coef  SE Coef      T      P
Constant           1.1165   0.5280   2.11  0.281
Certified values  0.99617  0.01313  75.89  0.008

S = 0.522773   R-Sq = 100.0%   R-Sq(adj) = 100.0%
```

Table BIV.11: Blank analysis for pot trial 4 herbage analysis.

BLANK					
SAMPLE	mg/kg Pb				
RBLK 1	4.94				
RBLK 2	12.14				
RBLK 3	10.44				
RBLK 4	6.96				
RBLK 5	4.24				
Mean	7.74				
Std	3.44				
μ	0				
SEM	1.54				
mean-μ	7.74				
t-test	5.04				
	T-TAB.	2.78		df(n-1)	4
	two tailed test		5.04>2.78	significant	

Table CIV.11: Analysis of Certified Reference Materials for root placement herbage analysis (pot trial 4)

Sample	BCR-60	HRM 11	HRM 14
x1	48.44	28.26	7.88
x2	78.82	24.56	10.62
MEAN	63.63	26.41	9.25
CERTIFIED VALUES	64.00	26.00	9.00
BIAS	-0.4	0.41	0.25
BIAS%	-0.6	1.6	2.8

Regression Analysis: Measured values versus certified values

```
The regression equation is
Measured values = 0.520 + 0.987 Certified values


Predictor             Coef   SE Coef       T      P
Constant            0.5199    0.2832    1.84 0.318
Certified values 0.987175   0.007040 140.22 0.005


S = 0.280378   R-Sq = 100.0%   R-Sq(adj) = 100.0%
```

Table DIV.11: Blank analysis for root placement herbage analysis (Pot trial 4).

BLANKS	
SAMPLE	mg/kg Pb
RBLK 1	0.28
RBLK 2	3.68
RBLK 3	2.40
RBLK 4	4.00
RBLK 5	0.08
Mean	2.09
Std	1.84
μ	0
SEM	0.82
mean-μ	2.09
t-test	2.54

	T-TAB.	2.78		df(n-1)	4
	two tailed test		2.54<2.78	Not significant	

Table EIV.11: Analysis of Certified Reference Materials for growth media soil analysis (pot trial 4).

Sample		HRM 31	SRM-2711	SRM-2709	HRM 1	HRM 2	SRM-2710
x1		7196.00	1340.80	20.08	8.80	567.20	5884.00
x2		6716.00	1418.40	16.88	17.60	447.20	4452.00
MEAN		6956.00	1379.60	18.48	13.20	507.20	5168.00
CERTIFIED VALUES		6895.00	1400.00	17.30	13.00	510.00	5552.00
BIAS		61.0	-20.40	1.18	0.2	-2.80	-384.00
BIAS%		0.9	-1.5	6.8	1.5	-0.5	-6.9

Regression Analysis: Measured values versus certified values

The regression equation is
Measured values = - 9.8 + 0.980 Certified values

Predictor	Coef	SE Coef	T	P
Constant	-9.83	90.99	-0.11	0.919
Certified values	0.98015	0.02483	39.48	0.000

S = 168.556 R-Sq = 99.7% R-Sq (adj) = 99.7%

Table FIV.11: Blank analysis for growth media soil analysis (Pot trial 4).

BLANK	
SAMPLE	mg/kg Pb
RBLK 1	8.28
RBLK 2	-3.80
RBLK 3	2.92
RBLK 4	5.64
RBLK 5	7.44
mean	4.10
std	4.87
µ	0
SEM	2.17
mean-µ	4.10
t-test	1.88

T-TAB.	2.78		df(n-1)
two tailed test		1.88<2.78	Not significant

Table GIV.11: Precision and detection limits of herbage and soil Pb analysis (Pot trial 4).

Analysis	Precision%	Detection limit (mg/kg)
Herbage analysis 1	6.6	0.81
Root placement herbage analysis	3.5	0.91
Growth media soil analysis	5.2	12.96

Appendix IV.10: Growth media concentration check data analysis

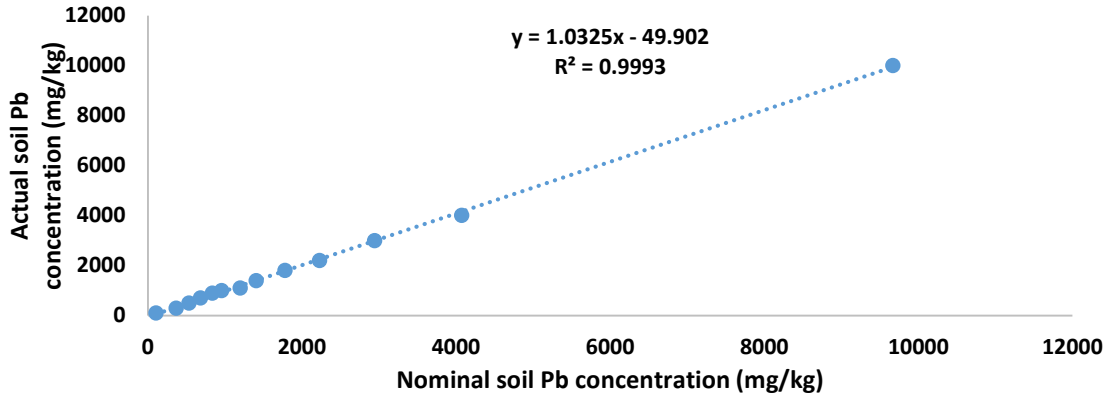


Figure AIV.10: Regression model of Actual soil Pb concentration against Nominal soil Pb concentration.

Table BIV.10: Summary of the amount of PbO used to make grow media.

Pb conc. (mg/kg)	No. of cells	No. of pots/treatment	No of species	g of PbO for mg/kg (DW in whole PT4	Kg of growth media for both species (DW)	Kg of growth media (FW)
100	3	10	2	0.81	7.52	8.16
300	12	10	2	9.72	30.08	32.64
500	9	10	2	12.15	22.56	24.48
700	3	10	2	5.67	7.52	8.16
900	9	10	2	21.87	22.56	24.48
1000	36	10	2	97.22	90.25	97.92
1100	16	10	2	47.53	40.11	43.52
1400	3	10	2	11.34	7.52	8.16
1800	1	10	2	4.86	2.51	2.72
2200	3	10	2	17.82	7.52	8.16
3000	3	10	2	24.30	7.52	8.16
4000	1	10	2	10.80	2.51	2.72
10000	1	10	2	27.00	2.51	2.72

Table CIV.10: Growth media pH.

TUBE NO	SAMPLE ID	MASS (g)	pH
1	1000	4	6.62
2	700	4	6.27
3	500	4	6.73
4	1000	4	6.54
5	100	4	6.22
6	900	4	6.49
7	1100	4	6.25
8	4000	4	6.36
9	1800	4	6.60
10	2200	4	6.68
11	500	4	6.35
12	300	4	6.28
13	1400	4	6.43
14	3000	4	6.15
15	10000	4	6.57
		MEAN	6.44
		STDEV	0.18
		SEM	0.05

Table DIV.10: **Gang Mine soil pH determination**

SAMPLE ID	MASS (g)	pH
GM-E8	4	6.84
GM-A1	4	6.11
GM-J6	4	6.36
GM-B2	4	5.95
GM-F1	4	6.27
GM-G6	4	6.41
	MEAN	6.32
	STDEV	0.30
	SEM	0.12

STDEV--- Standard deviation

SEM----Standard error on the mean

APPENDIX RELATED TO PUBLICATIONS AND PRESENTATIONS

Appendix V: List of Publications and presentations from the thesis.

Publications

1. Michael. H. Ramsey, **Solomon-Wisdom, G.O.** & Ariadne Argyraki (2013). Evaluation of *in situ* heterogeneity of elements in solids: (Implications for Analytical Geochemistry). *Geostandard and Geoanalytical Research* 37 (4):379-391.
2. **Solomon-Wisdom, G.O**., Ramsey, M.H. & John, E.A (2014). The effects of more realistic forms of lead heterogeneity in soil on uptake and biomass of two Brassica species. In the press.

Conferences (Oral and poster presentations).

1. **Solomon-Wisdom, G.O**., John, E.A & Ramsey, M.H. (2014). Improved estimation of plant uptake of Pb in soil by two *Brassica* species using a more realistic simulation of *in situ* heterogeneity. Paper orally presented at the 30th Annual International Conference of the Society of Environmental Geochemistry and Health. Newcastle 30th June—4th July, 2014.
2. **Solomon-Wisdom, G.O**., John, E.A & Ramsey, M.H. (2014). Improved estimation of plant uptake of Pb in soil by two *Brassica* species using a more realistic simulation of *in situ* heterogeneity. Paper orally presented at the 2014 Life Sciences Posgraduate Research Colloquium. University of Sussex, 8th-9th September, 2014.
3. **Solomon-Wisdom, G.O**. & Ramsey, M.H. (2013). The effect of *in situ* heterogeneity of Pb on biomass and uptake of two *Brassica* species. Poster presented at the Annual Life Sciences Postgraduate Research Colloquium 1st-3rd September, 2013.
4. **Solomon-Wisdom, G.O.** & Ramsey, M.H (2012**).** Preliminary studies for assessment of heterogeneity of lead in soil and its implications for uptake by plants. *Book of abstracts* 9: 306 Poster presented at the 9TH International Symposium for Environmental Geochemistry conference, Aveiro – Portugal.

APPENDIX RELATED TO COPYRIGHT

Appendix VI: Copyright permission of some figures used in the thesis and seeds used in pot trials.

Send Options... HTML

To...

Cc...

Subject: FW: Permission to use map.

Tahoma 10 **B** *I* U

From: McAuliffe, Elizabeth [elizabeth@bgs.ac.uk]
Sent: Wednesday, March 25, 2015 3:26 PM
To: Grace Oyiza Solomon-Wisdom
Subject: RE: Permission to use map.

Dear Grace

Thank you for your email.

BGS would consider this to be an academic us and are happy for it to be reproduced in your thesis, subject to the correct acknowledgment. Please see our terms and conditions of use as posted on the BGS website here http://www.bgs.ac.uk/about/copyright/

BGS always asks for a copyright acknowledgement, such as "Reproduced by permission of the British Geological Survey © NERC" but please note that you must make your own arrangements with regards to reproduction of the Ordnance Survey topography as the BGS licence does not extend to third parties. Here is the link to the Ordnance Survey website http://www.ordnancesurvey.co.uk/.

Thank you for your interest in BGS material and I wish you good luck with your thesis.

Kind regards

Elizabeth

Elizabeth McAuliffe
Copyright Assistant
British Geological Survey
Environmental Science Centre
Keyworth
Nottingham
NG12 5GG

Tel: 0115 936 3120
Email: elizabeth@bgs.ac.uk
Website: www.bgs.ac.uk/

From: Grace Oyiza Solomon-Wisdom [mailto:G.Solomon-Wisdom@sussex.ac.uk]
Sent: 25 March 2015 14:36
To: McAuliffe, Elizabeth
Subject: RE: Permission to use map. (to Elizabeth)

June 8, 2012 General Packing Order: 232147 Local: 20120482

From:

USDA, ARS, NCRPIS
Iowa State University
Regional Plant Introduction Station
Ames, Iowa, United States, 50011-1170
(515)294-3255

Final Destination:

Grace Solomon-Wisdom
University of Sussex
Environmental Research Unit
JMS Building 5D23
Brighton, East Sussex
Falmer, England, United Kingdom, BN1 9QG

Ship Through:

C/O: Alan Bone/Jason Lebo
USDA, APHIS
Plant Germplasm Inspection Station
Bldg 580, BARC-East
Beltsville, Maryland, United States, 20705
301-313-9341

Brassica juncea

Item	Accession	Origin	Plant name	Amount
1	PI 173874	India, Delhi		200 ct sd
2	PI 182921	India, Gujarat		200 ct sd
3	PI 211000	Afghanistan, Badakhshan		200 ct sd
4	PI 426308	Pakistan	K-100	200 ct sd

Brassica napus

Item	Accession	Origin	Plant name	Amount
5	PI 601261	Sweden, Malmohus	Crystal	200 ct sd

Zea mays subsp. mays

Item	Accession	Origin	Plant name	Amount
6	Ames 19288	United States, Ohio	Oh43	200 ct sd
7	Ames 26798	United States, Indiana	H98	200 ct sd
8	PI 550467	United States, Iowa	B37	200 ct sd
9	PI 550473	United States, Iowa	B73	200 ct sd
10	PI 558532	United States, Missouri	Mo17	200 ct sd
11	PI 561620	Cameroon	ATP	200 ct sd
12	PI 587139	United States, Minnesota	A619	200 ct sd
13	PI 644101	United States, Iowa	LH1	200 ct sd

Total items ordered: 13

This germplasm is being freely distributed by the U.S. National Plant Germplasm System (NPGS) for educational, agricultural research or breeding purposes.

Notes:

No phytosanitary certificate can be issued until an application is completed (7 CFR 353)

FORM APPROVED
OMB NO. 0579-0052

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

PHYTOSANITARY CERTIFICATE

FOR OFFICIAL USE ONLY

PLACE OF ISSUE
Beltsville, Maryland

NO.
F-F-24033-03173468-7-N

DATE INSPECTED
May 22, 2013

TO: THE PLANT PROTECTION ORGANIZATION(S) OF
United Kingdom

CERTIFICATION

This is to certify that the plants, plant product or other regulated articles described herein have been inspected and/or tested according to approp
official procedures and are considered to be free from the quarantine pests, specified by the importing contracting party and to conform with the cu
phytosanitary requirements of the importing contracting party including those for regulated non-quarantine pests.

DISINFESTATION AND/OR DISINFECTION TREATMENT

1. DATE	2. TREATMENT
**********	**********
3. CHEMICAL (active ingredient)	4. DURATION AND TEMPERATURE
**********	**********
5. CONCENTRATION	6. ADDITIONAL INFORMATION
**********	**********

DESCRIPTION OF THE CONSIGNMENT

7. NAME AND ADDRESS OF THE EXPORTER	8. DECLARED NAME AND ADDRESS OF THE CONSIGNEE
USDA, ARS, North Central Regional Plant Introduction Station Exporter address information is printed on the attachment page.	Grace Solomon-Wisdom University of Sussex Environmental Research Unit, JMS Building 5D23 Brighton, England BN1 9QG United Kingdom
9. NAME OF PRODUCE AND QUANTITY DECLARED	10. BOTANICAL NAME OF PLANTS
(1) 15 Grams Brassica sp. (Seeds) **********	(1) Brassica sp. **********
11. NUMBER AND DESCRIPTION OF PACKAGES	12. DISTINGUISHING MARKS
(1) 1 Envelope **********	(1) None **********
13. PLACE OF ORIGIN	14. DECLARED MEANS OF CONVEYANCE
(1) Iowa, USA **********	Air Mail
	15. DECLARED POINT OF ENTRY
	United Kingdom

WARNING: Any alteration, forgery, or unauthorized use of this phytosanitary certificate is subject to civil penalties of up to $250,000 (7 U.S.C. Section 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. Section 1001).

ADDITIONAL DECLARATION

Page 1 of

16. DATE ISSUED	17. NAME OF AUTHORIZED OFFICER *(Type or Print)*	18. SIGNATURE OF AUTHORIZED OFFICER
May 22, 2013	Mark Thurmond	Mark Thurmond

No liability shall attach to the United States Department of Agriculture or to any officer or representative of the Department with respect to this certifica

PPQ Form 577 FEB 2001 Previous editions are obsolete after 6/30/01 SHIPPER'S ORIGIN

No phytosanitary certificate can be issued until an application is completed (7 CFR 353)

FORM APPROVED
OMB NO. 0579-0052

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

PHYTOSANITARY CERTIFICATE

FOR OFFICIAL USE ONLY

PLACE OF ISSUE
Beltsville, Maryland

NO.
F-F-24033-02494717-7-N

DATE INSPECTED
June 25, 2012

TO: THE PLANT PROTECTION ORGANIZATION(S) OF
United Kingdom

CERTIFICATION

This is to certify that the plants, plant product or other regulated articles described herein have been inspected and/or tested according to appropriate official procedures and are considered to be free from the quarantine pests, specified by the importing contracting party and to conform with the curren phytosanitary requirements of the importing contracting party including those for regulated non-quarantine pests.

DISINFESTATION AND/OR DISINFECTION TREATMENT

1. DATE	2. TREATMENT
**********	**********
3. CHEMICAL (active ingredient)	4. DURATION AND TEMPERATURE
**********	**********
5. CONCENTRATION	6. ADDITIONAL INFORMATION
**********	**********

DESCRIPTION OF THE CONSIGNMENT

7. NAME AND ADDRESS OF THE EXPORTER
USDA, ARS, North Central Regional Plant
Introduction Station
Exporter address information is printed on
the attachment page.

8. DECLARED NAME AND ADDRESS OF THE CONSIGNEE
Grace Solomon-Wisdom
University of Sussex
Environmental Research Unit, JMS Building 5D23
Brighton, England BN1 9QG United Kingdom

9. NAME OF PRODUCE AND QUANTITY DECLARED
(1) 55 Grams Mustard (Seeds)
(2) 590 Grams Corn (Seeds)

10. BOTANICAL NAME OF PLANTS
(1) Brassica juncea
(2) Zea mays

11. NUMBER AND DESCRIPTION OF PACKAGES
(1-2) 1 Box

12. DISTINGUISHING MARKS
(1-2) None

13. PLACE OF ORIGIN
(1-2) Iowa, USA

14. DECLARED MEANS OF CONVEYANCE
Air Mail

15. DECLARED POINT OF ENTRY
United Kingdom

WARNING: Any alteration, forgery, or unauthorized use of this phytosanitary certificate is subject to civil penalties of up to $250,000 (7 U.S.C. Section 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C. Section 1001).

ADDITIONAL DECLARATION

Six of the eight corn seed lots originated in areas known to be free from Erwinia stewartii. The other two seed lots had a representative sample taken and were tested and found free from Erwinia stewartii.

16. DATE ISSUED	17. NAME OF AUTHORIZED OFFICER *(Type or Print)*	18. SIGNATURE OF AUTHORIZED OFFICER
June 25, 2012	Mark Thurmond	Mark Thurmond

No liability shall attach to the United States Department of Agriculture or to any officer or representative of the Department with respect to this certificate.

PPQ Form 577 FEB 2001 Previous editions are obsolete after 6/30/01

Send Options... HTML

To...

Cc...

Subject: FW: Permission to use diagram

Tahoma 10 B I U

From: Surabhi Khare [skhare@schandpublishing.com]
Sent: Monday, April 27, 2015 1:33 PM
To: Grace Oyiza Solomon-Wisdom
Subject: RE: Permission to use diagram

Hi Grace,

Thanks for the confirmation. We grant you permission to use the said figure in your PhD thesis for non-commercial use. The Figure should be acknowledged as Pandey, 2005 with permission from the S. Chand Publishing. In the reference list, the author's name, date of publication, title of the book, publishers name, place of Publication and page number are duly referenced.

Best wishes,
Surabhi

From: Grace Oyiza Solomon-Wisdom [mailto:G.Solomon-Wisdom@sussex.ac.uk]
Sent: 23 April 2015 16:53
To: skhare@schandpublishing.com
Subject: RE: Permission to use diagram

Dear Mr Surabhi,

Many thanks for your mail. I confirm that the Figure 9.1 on page 176 of the textbook Plant Anatomy by B.P Pandey will not be used for commercial purpose. It is solely for educational purpose and would only be used in my Ph.D thesis. The Figure is acknowledged as Pandey, 2005 with permission from the Schand Publishers April 2015. In the reference list, the author's name, date of publication, title of the book, publishers name, place of Publication and page number are duly referenced.

I look forward to hearing from you soon.

Many thanks for your kind consideration.

Best Regards,
Grace

From: Surabhi Khare [skhare@schandpublishing.com]
Sent: Thursday, April 23, 2015 9:06 AM
To: Grace Oyiza Solomon-Wisdom
Cc: spatki@schandgroup.com; 'Santosh Verma'
Subject: RE: Permission to use diagram

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

ATTACHMENT SHEET FOR PHYTOSANITARY CERTIFICATE OR PHYTOSANITARY CERTIFICATE FOR REEXPORT

TO: THE PLANT PROTECTION ORGANIZATION(S) OF
United Kingdom

FOR OFFICIAL USE ONLY

PLACE OF ISSUE
Beltsville, Maryland

NO. **F-F-24033-03173468-7-N**

Page 2 of 2

Exporter Details (Name, Address)
USDA, ARS, North Central Regional Plant Introduction Station
NCRPIS
Ames, Iowa 50011-1070

16. DATE ISSUED	17. NAME OF AUTHORIZED OFFICER *(Type or Print)*	18. SIGNATURE OF AUTHORIZED OFFICER
May 22, 2013	Mark Thurmond	Mark Thurmond

SHIPPER'S ORIGI