

Uptake properties of some plants species in homogenous Lead (Pb) concentration - its implication for phytoremediation.

^{1*} Ogunlade- Anibasa Grace Oyiza and ² Michael .H. Ramsey

¹ Department of Biological Sciences, University of Abuja ² School of Life Sciences, University of Sussex, UK
*grace.anibasa-ogunlade@uniabuja.edu.ng +2349030868382

Uptake properties of four plant species (*Zea mays* , *Brassica juncea* , *Brassica napus* and *Thlaspi (Nocaceae) caerulescens*) and their varieties were investigated in a greenhouse pre-phytoremediation pot trial. Varieties' abbreviations were derived either from their accession numbers or origin. *Zea mays* and *Brassica* species were obtained gratis from the United States Department of Agriculture (USDA) , while *Thlaspi* species were obtained from field sites in Gang Mine and BlackRock – Derbyshire, and the Royal Botanic Garden (KEW), United Kingdom. This pot trial was done in two stages – the seed germination experiment and pot trials in 1000 mg/kg lead (Pb) added treatment with 0 mg/kg Pb added treatment as control. Standard analytical methods were used. Lead concentration of plants and soil materials were determined using the Atomic Absorption Spectrometer Perkin Elmer 400 after acid digestion with nitric and perchloric acid. This pot experiment that assessed the effect of Pb in a fixed homogenous concentration (1000 mg/kg) and found a significant effect($p < 0.05$) 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 sixteen (16) species/varieties. The concentration and translocation factors (CF and TF) were statistically significantly different ($P < 0.05$) between species and within varieties as well as other growth parameters ,which showed the effects of the Pb added treatment. Results enhanced the determination of the uptake potentials of the plants studied and their selection for further phytoremediation pot trials.

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1.0 INTRODUCTION

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 (45; 6). 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 (60; 44).

According to (40), 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 (31). One of the most serious and long-term outcomes of environmental pollution is heavy metal contamination of soils (44). (46; 34;63; 38; 43; 40; 15; 69 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 have been widely used to refer to a group of metals and semi-metals that have been associated with contamination and potential toxicity (28). 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 (23). For example, United States Environmental Protection Agency {USEPA} (71) and USGS (73) , 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 (71; 72).

The main threats to human health from heavy metals are associated with exposure to Pb, Cd, Hg and Arsenic (As) (49; 1; 31). 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 (30). Lead pollution of soil especially in mining areas is a widespread and significant problem globally. Lead has been ranked second hazardous substances next to arsenic because of its toxicity (1). It exhibits extreme persistence and accumulation in soils, sediments and water (681; 50).

Lead has been made ubiquitous in the environment by anthropogenic activity (35). 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 (49). 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 (40; 59). 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 (73). Crustal abundance of Pb is much lower than the Pb produced by anthropogenic influences. The estimated value of Pb crustal abundance is between 10 and 14 mg/kg (47; 41; 39).

Lead contamination of soil can cause variety of environmental problems, including loss of vegetation, ground water contamination and toxicity to plants, animals and humans (19; 17; 37; 75). It has no known biological function in living organisms and is toxic at low concentrations (71;44). Lead is toxic to humans and may be implicated in systemic poisons, building up in the body over an extended period and exposure (12; 36). (53) reported that 30,000 people have been poisoned by Pb and estimated that 400 children have died due to Pb poisoning because 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 (44). 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 (44). 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 (56). In Europe, areas around metal smelting complexes have been found to be heavily contaminated by Pb, Cd, Cu and Zn (Alloway, 2; 33; 52).

Soil Pb concentration values are different for every region. A similar value (100 mg/kg) was established in China for tea garden soils (42). 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 (16). 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 **nagyárite** $\{Pb_5Au(Te, Sb)_4S_{5-8}\}$ (29). Galena may become associated with other secondary Pb minerals through weathering processes, oxidation and anthropogenic deposition (55). Lead contamination of soils and plants in gold mining areas of China and Nigeria are higher than in unmined areas (76; 58). 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 (65). 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.

(24) 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 (3) reported 180 mg/kg as the normal background concentrations (NBC) of Pb in English soils. That study (3) 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 (2); (11); (57) 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 (57). 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 (25). The highest recorded concentrations for some top and sub soils in Derbyshire were 35930 mg/kg and 24700 mg/kg respectively (25).

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 (20; 74). 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 (24). 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 (32). 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 (3). 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.

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 (21;30). 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 (21; 74; 66). 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.

Plants which accumulate heavy metals are known as metallophytes. Metallophytes can differ largely in their heavy metal contents (18). Several plants show potential for Pb accumulation from the soil (89). 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 (22). 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 (10).

(57) reported that Pb is accumulated in roots of two ecotypes of *Thlaspi caerulescens* in West Morocco. Potential hyperaccumulator species such as *Armeria maritima* (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 annuus* (sunflower), *Thlaspi rotundifolium* (round leaved pennycress), *Triticum aestivum* (bread wheat), *Vernonia amygdalina* (bitter leaf), *Occimum gratissimum* (Scent leaves) and *Zea mays* (maize or corn) have been reported (11; 26; 54; 14; 62;5; 4). The most frequently cited Pb hyperaccumulator is the cultivar *Thlaspi rotundifolium* (L). Gaud-Beaup (round leaved pennycress) which can accumulate a shoot Pb concentration of 8500mg/kg (54). 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 (NO₃)₂ as much as 1.5% (m/v) of Pb (48). It

showed little ability to translocate Pb to its shoots when grown on soils where Pb²⁺ bioavailability was limited.

(19) reported a Pb accumulation of 130-8200 mg/kg shoot dry weight of *Thlaspi rotundifolium*. (13) recorded shoot lead values of 13 to 11,750 mg/kg in *Festuca ovina*. (61) also recorded a value of 2740 mg/Kg in the roots of *Thlaspi caerulescens* colonizing a lead mine district in the Pennines, England.. (91) 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. (66) 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.

Accumulation of potentially toxic elements is one attribute of plants that can be explored to provide potential solutions to many environmental problems. The rising reports of contamination of different environmental matrices, coupled with the potential consequential health hazards of some remediation techniques have forced the drive for more effective, economical and environmentally friendly methods of remediation of contaminated. Heavy metal contamination is one of the commonest forms of environmental contamination globally. Several studies have reported heavy metal contamination of soil, water, air and food. The general picture painted is the obvious health risk posed to human by direct and indirect contact with contaminants. Using plants and biological materials to clean up the environment is beginning to gain increasing attention. Some studies have explored phytoremediation and phyto extraction in various terrestrial decontamination process. The choice of plants to use poses enormous challenge to phytoremediation. This is because not all plants can accumulate heavy metals and those who take up heavy metals from the soil or hydroponically do so in varying amount. The success of phytoremediation depends greatly on the ability of selected plants to accumulate the target heavy metal.

Earlier studies by (62); (5) showed that selection of plant species for phytoremediation are often based on (i) 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 that in the soil, and translocation factor (TF) as the ratio of Pb in the shoot to that in the roots, (ii) root mass, lateral size, depth and morphology in comparison with scales of heterogeneity to be investigated, (iii) whether species is native to field areas phytoremediation is to be carried out. However, some non-native plant species may be useful in pot trials and field phytoremediation (iv) practicability of obtaining seed and growing species or varieties in pot and field

trials. To identify the key characteristics of each of plant species and to develop strategies sustain actual field trials, it is very important to conduct controlled pot trials, hence the essence of this study.

This pot trial aimed to: (i) quantify and compare Pb concentration in plant shoots, and hence potential for Pb uptake, (ii) 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) Select most suitable species/varieties that can tolerate high Pb in soil for subsequent pot and field trials. This pot trial hypothesizes that (i) the 1000 mg/kg Pb in growth media influences plant performance (ii) that species/varieties differ in their tolerance to Pb in the growth media at this concentration.

2.0 METHODOLOGY

2.1 Seed Germination Experiment.

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 $20\text{ C} \pm 5^{\circ}\text{C}$.

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 1 on the result section.

2.2 Growth Medium for Pot Trial.

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.

2.2.1 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°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.

2.2.2 Preparation of Growth Media for 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). 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°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 pot trial is shown in Table 1

Table 1 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	

2.2.3 Transplanting of seedlings for 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 $20 \pm 5^\circ \text{C}$ 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 $20 \pm 5^\circ \text{C}$ 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 1). Pots were rotated clockwise by 90° 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/m² of greenhouse benches.

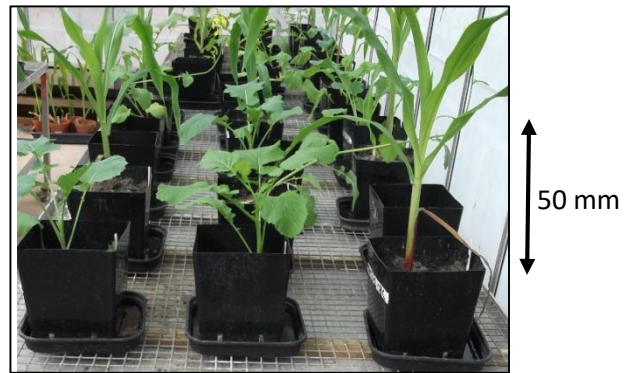


Figure 1: Randomized Block design for the pot trial. Scale bar: 17 mm represents 50 mm

2.3 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 this experiment, growth rate was expressed in terms of Growth index (GI) (79) and (87), 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 $GI = \text{height (mm)} + \text{width at widest point} + \text{width } 90^\circ \text{ to first width}/3$ (79). 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.

2.3.1 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°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.

3.0 RESULTS

3.1 Result of the seed germination experiment (prior to pot trial).

The result of the seed germination experiment is shown in Table 2. 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 caerulescens* (Table 2).

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 2.

Table 2 : Result of the seed germination experiment. Note: *Thlaspi caerulescens* recently renamed *Noccaea caerulescens*

Seed type (Species)	Accession No/Abbreviation	Origin	Plant name/ common name	Date sown	Estimated quantity sown	Supplier	No germinated	% Germination
<i>Brassica juncea</i> (BJ)	PI 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
<i>Brassica napus</i> (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
<i>Zea mays</i> (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
<i>Gentianna pennelianna</i> (GP)	Not applicable/ GP			1/8/2012	3.5 g (200 seeds)	Herbiseed	3	2
<i>Biden alba</i> (BA)	Not applicable/ BA			1/8/2012	6.3 g (200 seeds)	Herbiseed	2	1
<i>Thlaspi caerulescens</i> (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

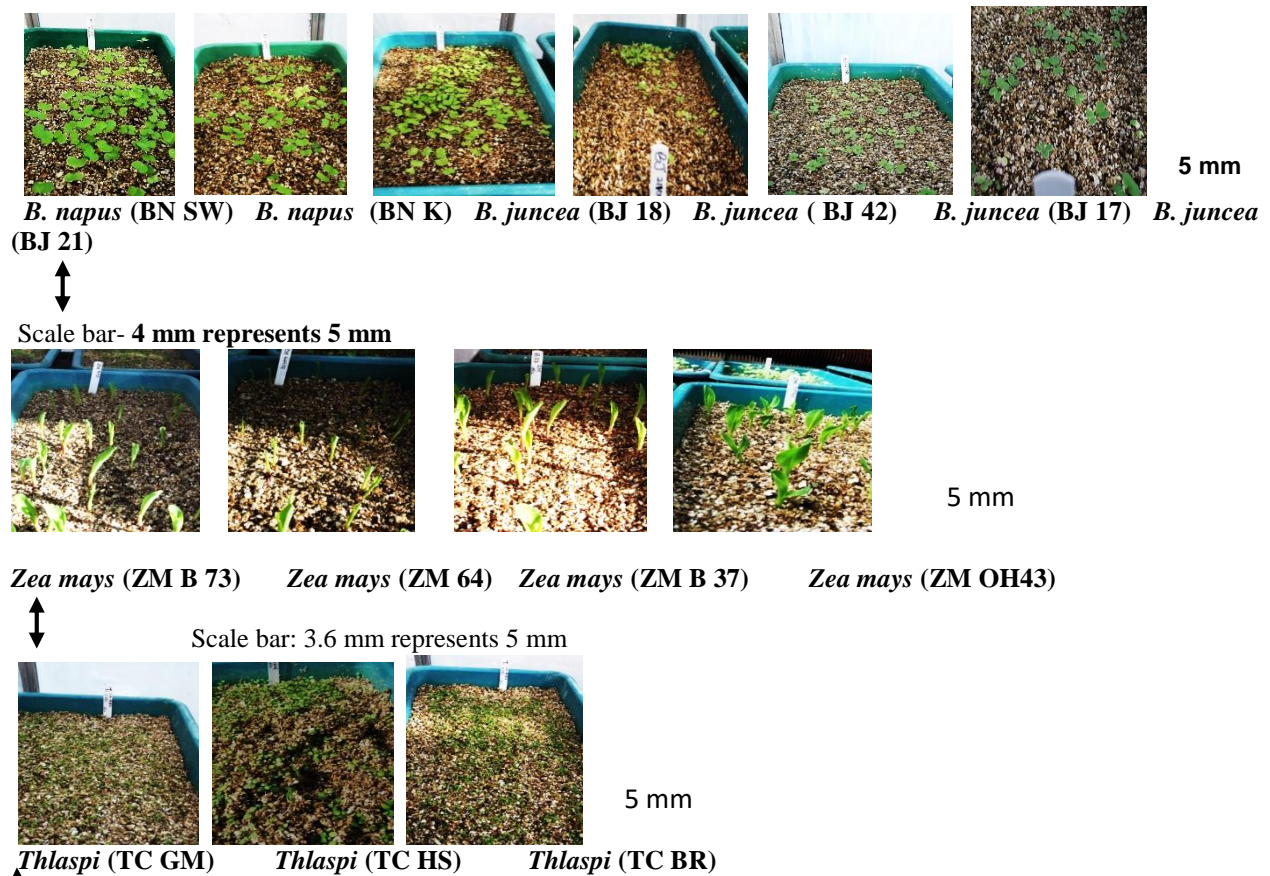


Figure 2 Some of the varieties of *Brassica napus*, *Brassica juncea*, *Zea mays* and *Thlaspi caerulescens* germinated (Species/varieties abbreviations are given in Table 2 above).

3.2 Results of the 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 3. 1 to 3. 4.

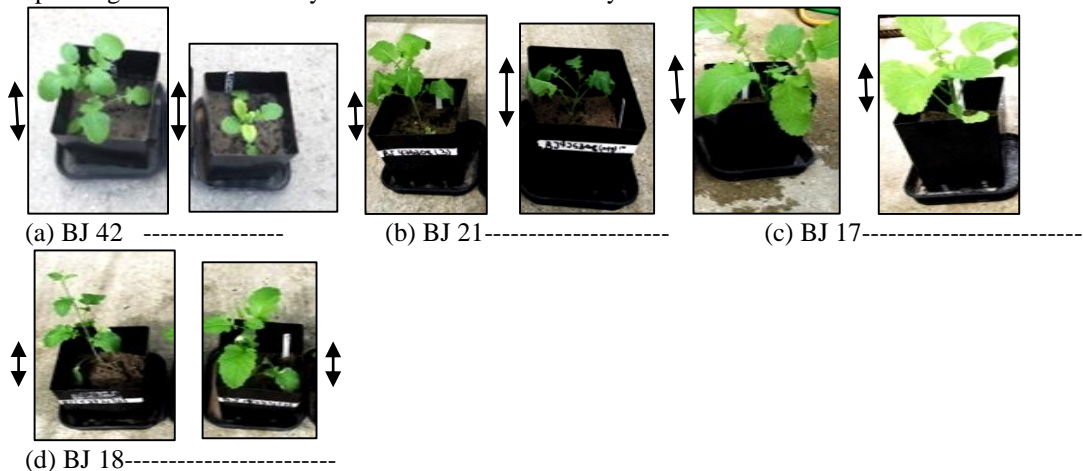


Figure 3.1: *Brassica juncea* BJ 42, 21,17 and 18 (from left to right) in the control (Left) and Pb added (right) treatments at harvest respectively. BJ 42, BJ 21 and BJ 18 showed chlorosis, reduced height and wilting of leavest. Arrow represents scale bar. See scale bar information on key below.

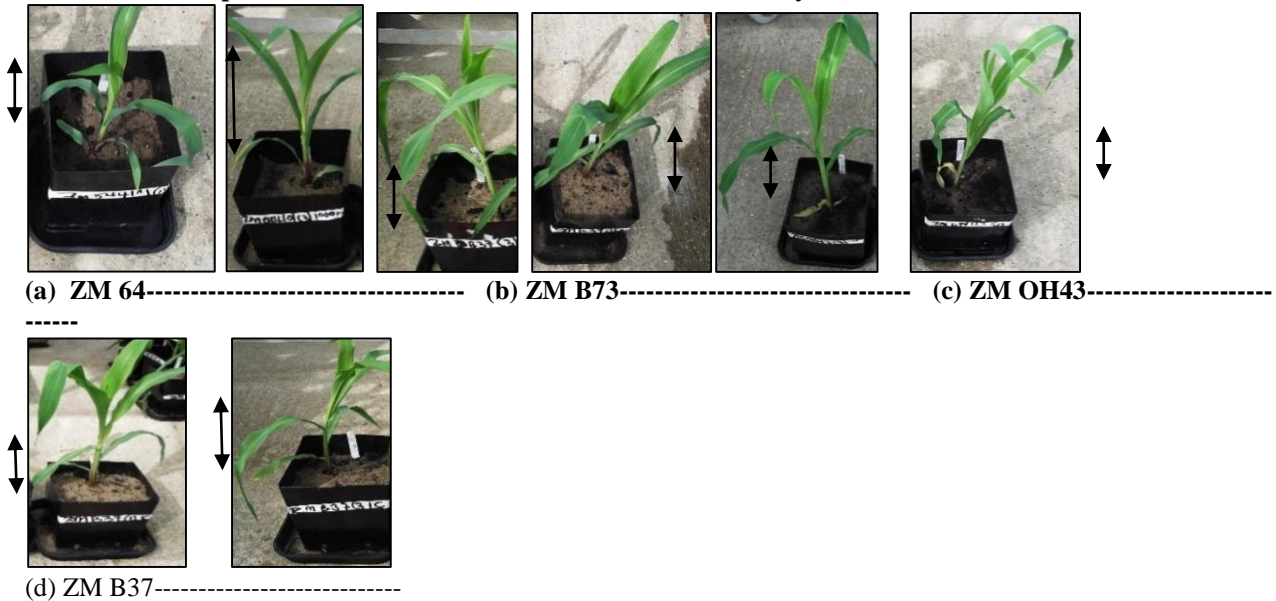


Figure 3.22: *Zea mays* ZM 64, B37, OH43 and B73 varieties (from left to right) in the control and Pb added treatments at harvest respectively. Arrows represents scale bars. See scale bar information on key below.

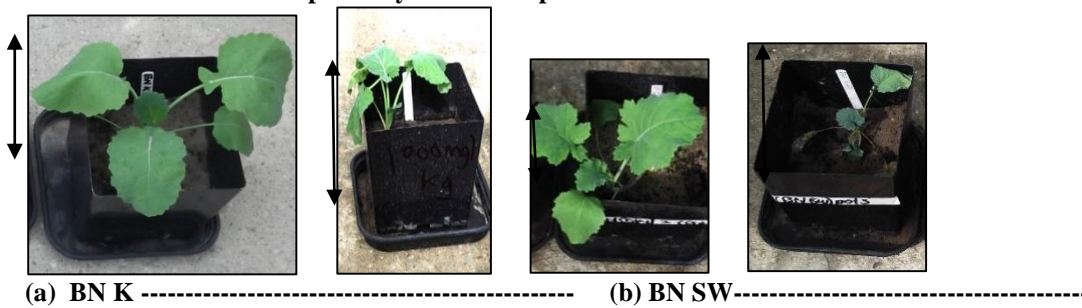
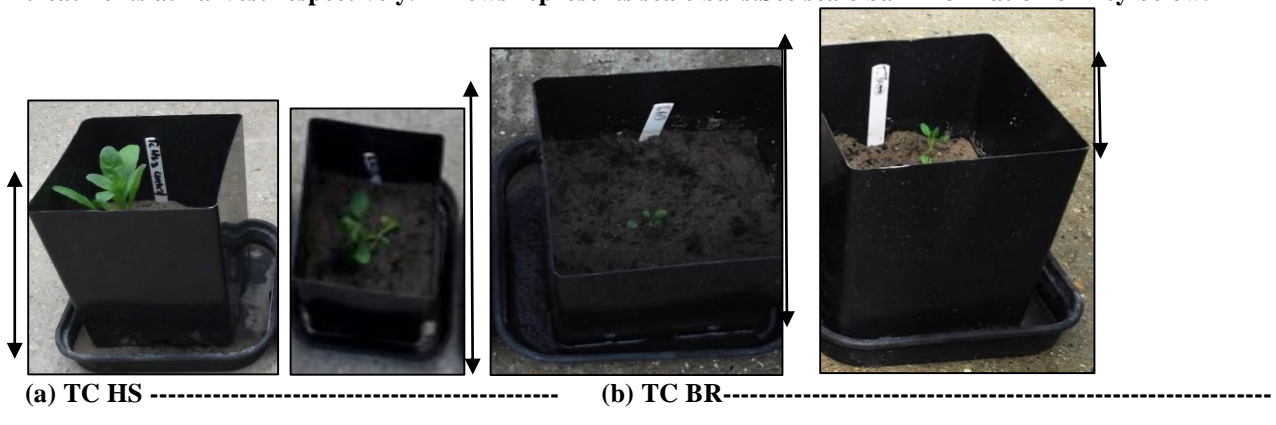
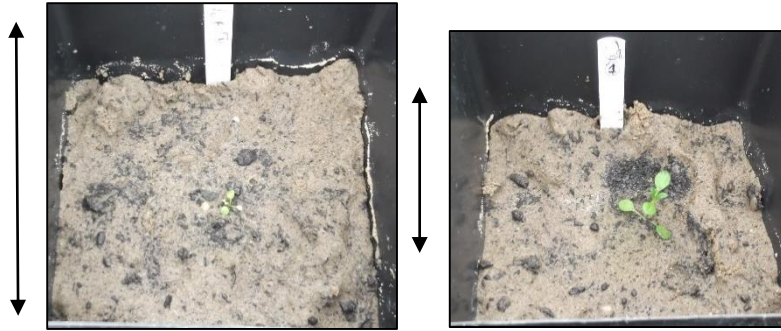


Figure 3.3: *Brassica napus* varieties, BN K and BN SW (from left to right) in the control and Pb added treatments at harvest respectively. Arrows represents scale bars. See scale bar information on key below.





(c) TC GM-----
 Figure 3.4: *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 3.1 to 3.4.

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

3.2.1 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 4.1-4.3). 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. 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 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 3.1.).

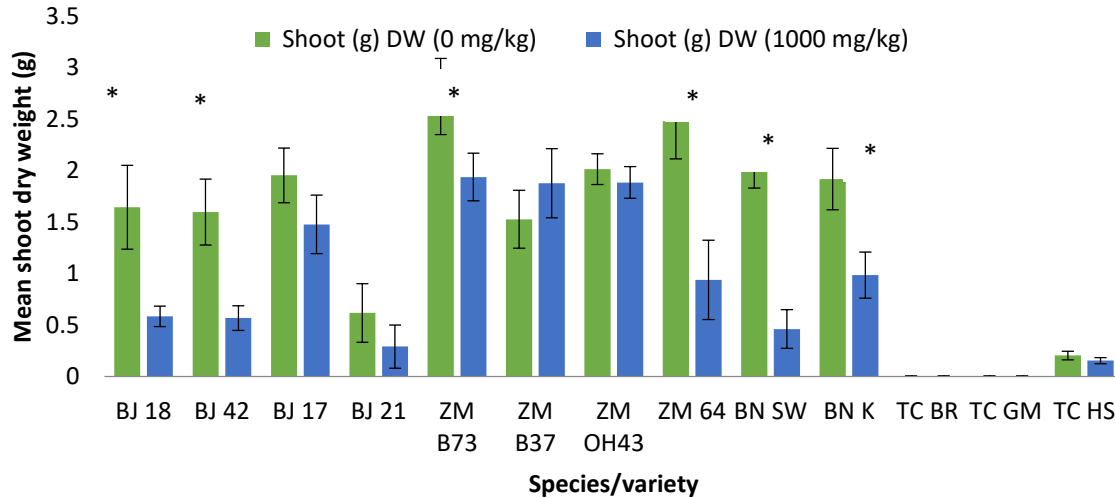


Figure 4.1: Mean shoot biomass DW between treatments for each species and variety in the 1st pot trial. Error bars represent 1 standard error on the mean where n=3. *-----Significant at $P < 0.05$.

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 4.2).

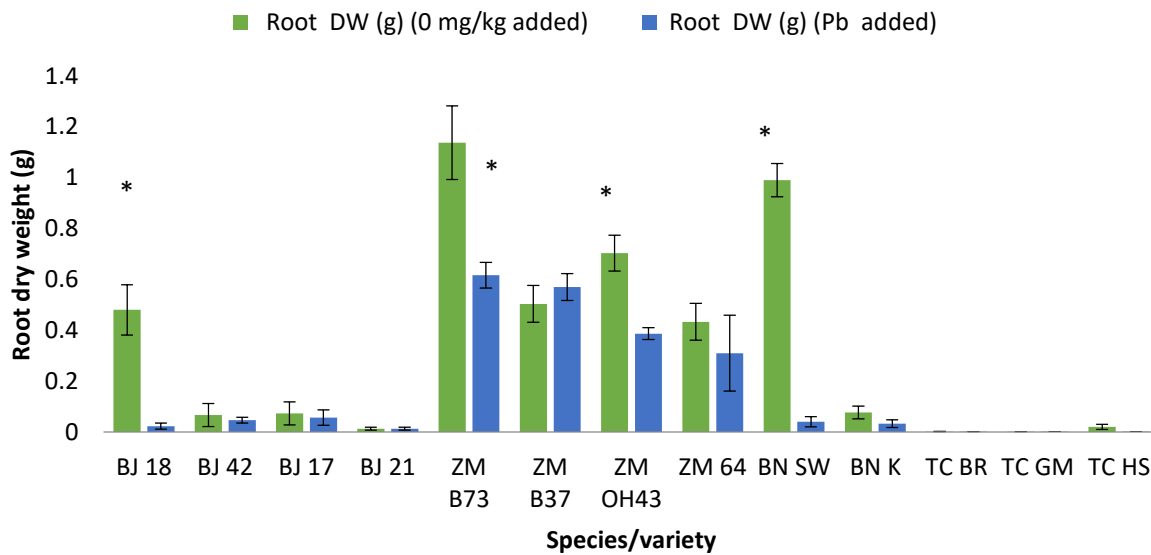


Figure 4.2: Root biomass DW between treatments for each species and variety in the 1st pot trial. Error bars represent 1 standard error on the mean where n=3. *-----Significant at $P < 0.05$.

Similarly, the total dry biomass differed significantly between treatments for BJ 18, BJ 42, ZM B73, ZM 64, BN SW and BNK (Figure 4. 3).

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.

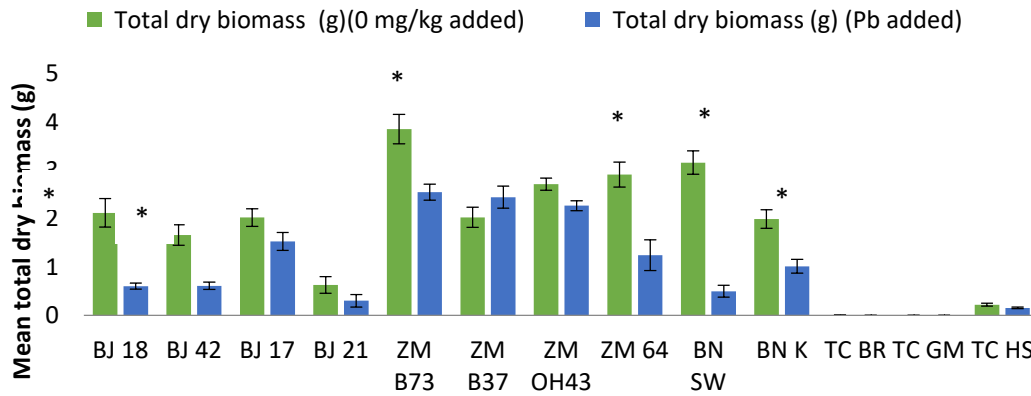


Figure 4.3: Mean total dry biomass DW between treatments for each species and variety in the 1st pot trial. Error bars represent 1 standard error on the mean where n=3. *-----Significant.

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

Comparison of the shoot, root and total plant Pb (mg/kg) DW between species/varieties are shown in Figures 5.1 to 5.4 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 5.1 to 5.4). *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.

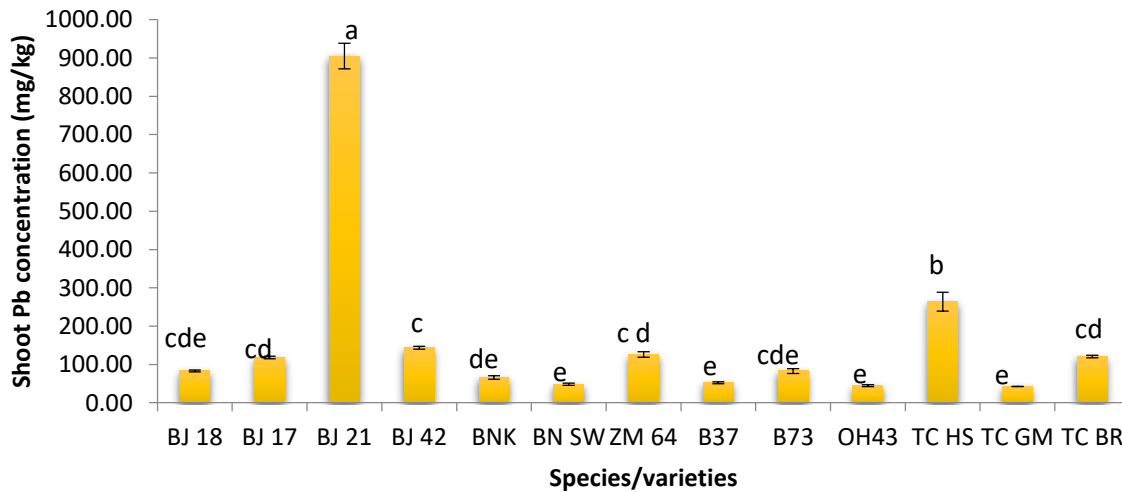


Figure 5.1 :Shoot Pb concentration (mg/kg) across species and varieties in the 1000 mg/kg Pb added treatment. Tukey post-hoc test, sharing letters means not significantly different. Error bar represent 1 standard error on the mean where n=3).

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 5. 3). 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 5.1). 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.

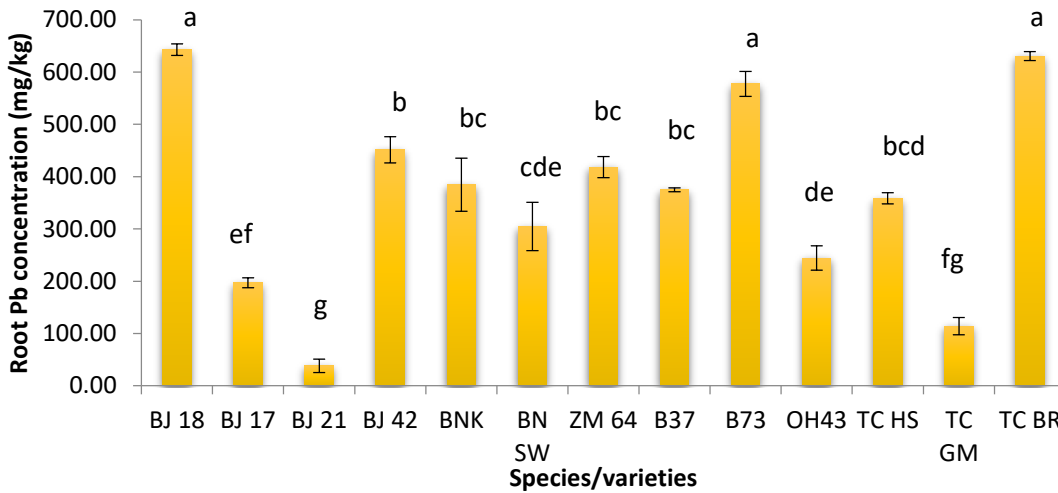


Figure 5.3: Root Pb concentration (mg/kg) across species and varieties in the Pb added treatment. Tukey post-hoc test, sharing letters means not significantly different. Error bars represent 1 standard error on the mean where n=3.

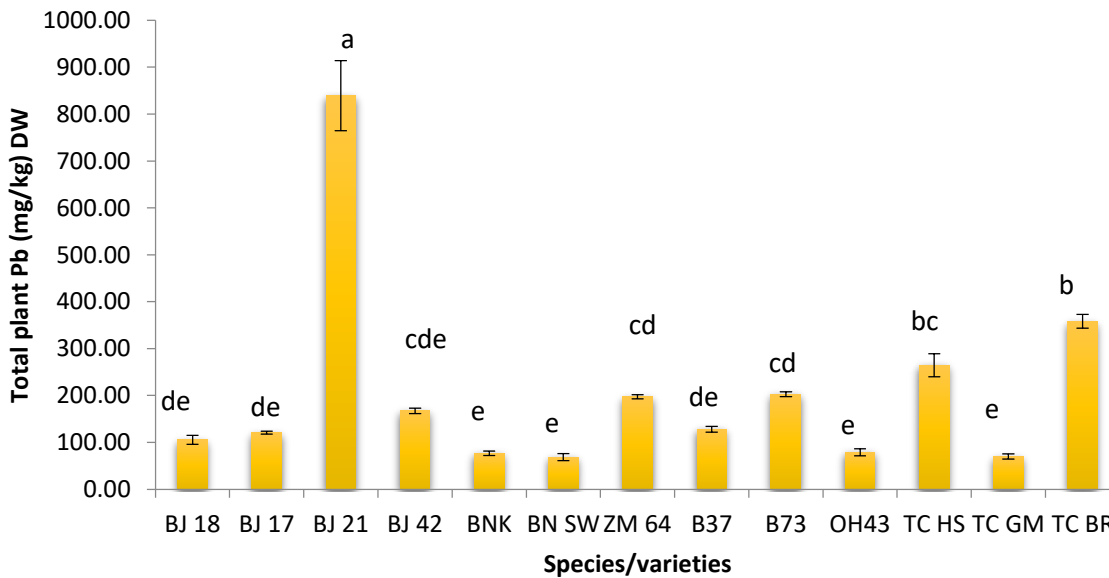


Figure 5.1: Total plant Pb concentration (mg/kg) dry weight across species and varieties in the Pb added treatment. Tukey post-hoc test, sharing letters means not significantly different. Error bars represent 1 standard error on the mean where n=3.

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 5.1 to 5.4). 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 4.1 to 4.4).

3.2.3 Comparison of Concentration Factor between species and varieties.

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 6.1) .. while the root concentration factor (CF_{root}) ranged from 0.04 to 0.70 (Figure 6.2). 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 6). 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.

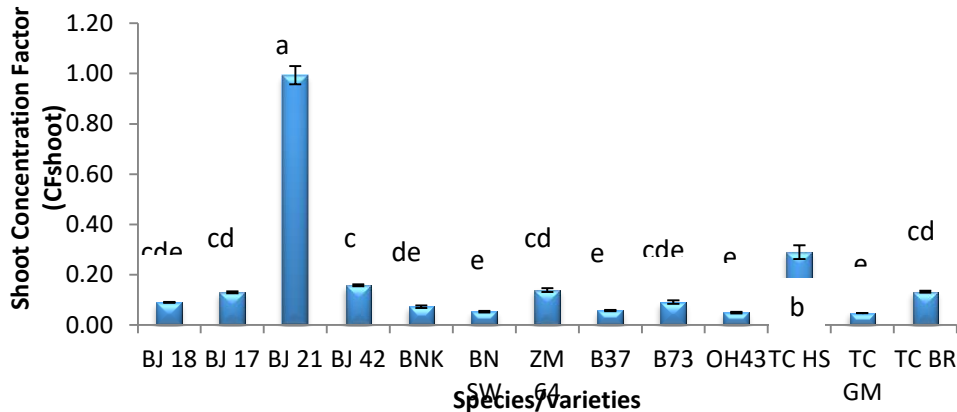


Figure 6.1 : Mean Shoot Concentration factor (CF_{shoot}) between species and varieties in the Pb added treatment. Error bars represent 1 standard error on the mean where n=3. Means sharing letters are not significantly different as judged by the Tukey post-hoc test.

The CF_{root} of most species were generally higher than the CF_{shoot} , which was 73 to 75% higher (Figure 6) 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.

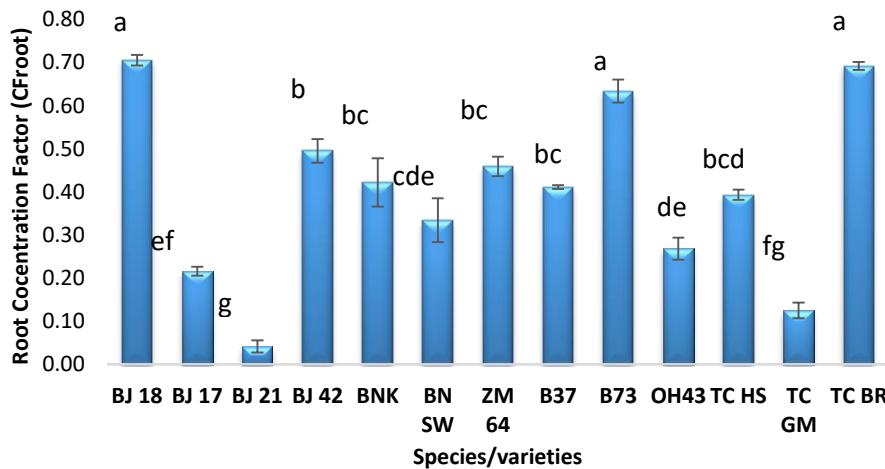


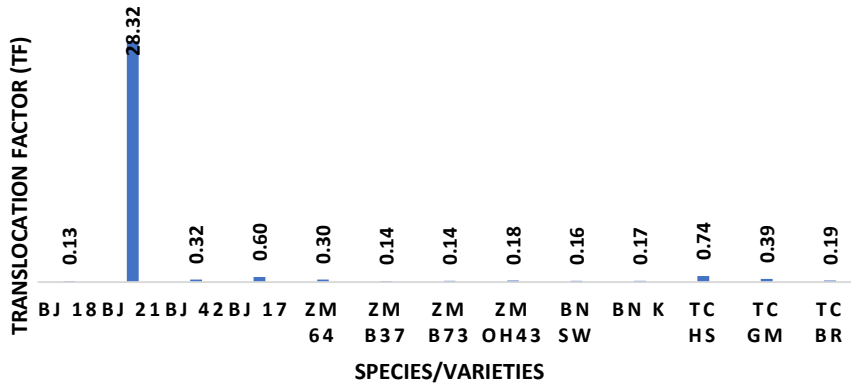
Figure 6.2: Root Concentration factor (CF_{root}) across species and varieties in the Pb added treatment. Error bars represent 1 standard error on the mean where n=3. Mean sharing letters means are not significantly different as judged by the Tukey post-hoc test.

3.2.4 Translocation factor of species/varieties in the 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 7a, 7b and 8). 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 6.1). The histogram of TF (Figure 7b) and the Log10 transformation of TF (Figure 8) 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)

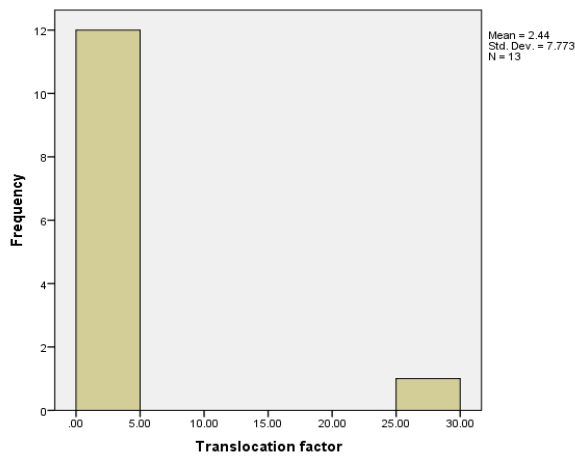


Figure 8 (a) Translocation factor (TF) across species and varieties in the Pb added treatment. (Shoot Pb DW mg/kg/ root Pb concentration mg/kg) (b) Histogram of translocation factor.

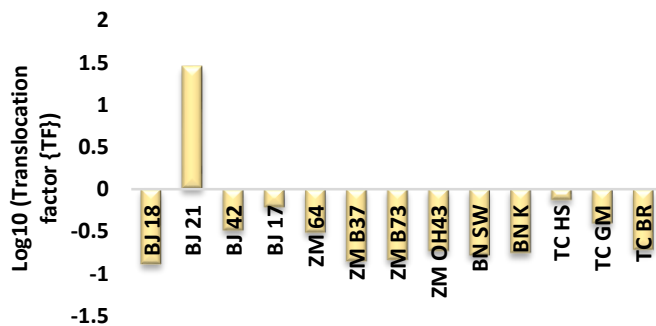


Figure 8 : Log10 transformation of the translocation factor (TF) across species and varieties in the Pb added treatment.

Table 3: Mean values of variables for each species/variety compared in the 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 further pot trial are highlighted in red.

4.0 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. Biomass, uptake and growth in contaminated media are key qualities that can influence phytoremediation. 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 (77) and on a range of toxic metals in soils (78).

For most of the plant species, more Pb was accumulated in the root than in the shoot. This is in line with findings of, (11); (80); (81). 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 potential Pb hyperaccumulation by these varieties. This ability to accumulate more Pb in the shoot is an advantage in terms of phytoremediation.

(82) stated that hyperaccumulators have potential roles in the mining industry where they may be found useful in phytoremediation/Phyto management and Phyto mining. A few plant species such as *Parthenium hysterophorus* {L} (Whiteweed 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 (83). 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. (10) and (11) 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 Concentration factor (CF) < 1 might be excluders, indicators or tolerant species whilst $CF \geq 1$ might be classified as accumulators supported by literature discussed criteria for classifying plant species as excluders, accumulators or hyperaccumulators. 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 (84).

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 of BJ 18. However, BJ 18 showed tolerance to high Pb in the soil. The *Brassica juncea* varieties BJ 18 and BJ 17 can be selected for phytoremediation 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 to 16 % 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 that plant death might be recorded with higher Pb concentration (Figure 2).

Identification of suitable plant species for pre phytoremediation trial also considered plants which can concentrate metal contaminant without completely inhibiting growth. (85) 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 pot experiment showed that the amount of biomass these species/varieties produced affected the shoot and root Pb mass (μg) (Table 4), which was generally low (ranged from 0.11 to 95 μg), apart from BJ 17, BJ 21, ZM B37, ZM B73 and ZM 64. The duration of growth might have partially contributed to the generally lower biomass of most species/varieties in the control and Pb added treatments. This is supported by findings in pot trials (5), 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. 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.

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 the hypothesis that the 1000 mg/kg Pb added treatment had a significant effect on such plant species was accepted.

Similarly, when a statistically significant difference ($P < 0.05$) in metal uptake is found within and between species/varieties, the hypothesis that these plants can take up Pb is accepted. The summary of hypothesis testing for each species and varieties is shown in Table 3.

From the results of this pot trial and in line with stated objectives i.e to select plant species/varieties for a further pre phytoremediation pot trial in a range of Pb-concentration, and field trials, 4 species made up of 6 varieties could be selected for further 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 (*Brassica napus*) and TC BR (*nocacae 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 (85) 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.

Table 3: Summary of hypotheses tested in the pot trial for each species/variety based upon independent sample t-test for (i) and Tukey H.S.D for (ii) comparison of means where $p < 0.05$. Varieties that could be potentially elected for further pot trials 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

5.0 CONCLUSION

This pot trial showed that specific differences between plants influences their ability to take up metals from the soil and that the presence of heavy metals in the soil could trigger accumulative responses of varying degrees in plants. The plant-soil interaction is key to the success of *in situ* phytoremediation. However, little is known about the molecular and genetic basis of such responses in plants. The overall result of this pot experiment informed the selection of species/varieties that could be used for further pot trials to ensure suitable species are used for phytoremediation.

6.0 ACKNOWLEDGMENT

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References

- [1]. 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).
- [2]. Alloway, B.J (1990). Introduction In: B.J. Alloway (Ed). *Heavy metals in soils*. Blackie Academic Professional.
- [3]. 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.
- [4]. **Anibasa G.O** and Oladele, M.O. (2019). An Assessment of the lead contamination of two species of frozen fishes sold in Gwagwalada, Federal Capital Territory. *Zuma Journal of Pure and Applied Sciences*. 10(1):90-102 ISSN 11196548.
- [5]. **Anibasa, G.O. (2016)**. *In situ metal heterogeneity- its implication for plant uptake*. Michael. H. Ramsey & Elizabeth. A. John (eds). Lambert Academic Publishing Company, Germany ISBN 978-3330-00833-5
- [6]. **Anibasa, Grace Oyiza & Ejeikwu, Emmanuel.O. (2017)** *A handbook of Soil Ecology*. Omonowo Academic Publication Limited, Abuja Nigeria ISBN 978-978-962-999-2
- [7]. 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*.
- [8]. Argyraki, A. (2014). Garden soil and house dust as exposure media for lead uptake in the mining village of Straton, Greece. *Environmental Geochemistry and Health* 36:677-692.
- [9]. 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.
- [10]. Baker, A.J.M. (1981). Accumulators and Exuders-strategies in response of plants to heavy metals. *Journal of Plant Nutrition*, 3: 643-654.
- [11]. 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.
- [12]. Barkerly, J.B. (1978). Lead nitrate as an oxidizer in blackpowder. *Pyrotechnica Publications* IV: 16-18.
- [13]. 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.
- [14]. Bert, V. Macnair, M.R., De Langurie, P., Saumitoy-Laprade, P. & Petit, D. (2000). Zinc tolerance and accumulation in metallicolous and non-metallicolous populations of *Arabidopsis halleri* (*Brassicaceae*). *New Phytologist* 146: 225-233.
- [15]. 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.
- [16]. Blacksmith Institute {BI} (2011). UNICEF programme Cooperation Agreement. Environmental Remediation—Lead poisoning in Zamfara. *Final Report*. Pp 57.
- [17]. Body, P.E., Dolan, P.R., & Mulcahy, D.E. (1991). Environmental lead –A review. *Critical Reviews in Environmental Control* 20:229-310.
- [18]. 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.
- [19]. 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.
- [20]. 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.
- [21]. 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.
- [22]. Chhotu, D.J. & Fulekar, M.H. (2009). Phytoremediation of heavy metals: recent techniques. *African Journal of Biotechnology* 8(6): 921-928.
- [23]. Chimbara, 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.
- [24]. 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.
- [25]. Department for Environment, Food and Rural Affairs (DEFRA) (2007). Soil guideline values for lead contamination. *R&D Publications*. SGV10
- [26]. Deram, A. & Petit, D. (1997). Ecology of bioaccumulation in *Arhenatherum elatius* L. (Poaceae) populations-applications of phytoremediation of Zinc, Lead and Cadmium contaminated soils. *Journal of Experimental Botany* 48 (Spec. Suppl.) 98.
- [27]. 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.
- [28]. Duffus, J.H (2002). Heavy metals: A meaningless term? *Pure Applied Chemistry* 74:793-807.
- [29]. 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.
- [30]. Environment Writer (2000). <http://www.nsc.org/ehc/ew/chems/lead/leadhtm> Retrieved 6/6/2011. Lead (Pb) chemical background.
- [31]. 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.
- [32]. 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
- [33]. Fent, K. (2004). Eco toxicological effects at contaminated sites. *Toxicology* 205(3):223-240.
- [34]. Greener, Y. & Kochen, J.A. (1983). Methyl mercury toxicity in the chick embryo. *Teratology* 8(1):23-28.
- [35]. Griffith, D.A. (2002). The Geographic Distribution of soil lead concentration: Description and Concerns. *URISA Journal* 14(1): 5-14.
- [36]. Hill, J.W. & Petrucci, R.H. (1999). *General Chemistry* (2nd Ed.). Upper Saddle River, New Jersey: Prentice Hall .p781.
- [37]. Huang, J.W. & Cunningham, D.S. (1996). Lead phytoextraction: species variation in lead uptake and translocation. *New Phytologist*, 134(1): 75-84.
- [38]. Huang, J.W., Chen, J., Berti, W.R. & Cunningham, S.D. (1997). Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction. *Environmental Science and Technology* 31(3):800-805.
- [39]. 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.
- [40]. Jeana, R.H. (2000). An overview of the phytoremediation of Lead and Mercury. *National Network of Environmental Management Studies (NINEMS)* 1-43.
- [41]. 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.
- [42]. Jin, J. Martens, D.C. & Zelazny, L.W. (1987). Distribution and availability of Boron fractions. *American Journal of Science Society* 51: 1228.
- [43]. Johnson, F.M. (1998). The genetic effects of environmental lead. *Mutation Research* 410(2):123-140.
- [44]. Kabata-Pendias, A. (2010). *Trace Elements in soils and plants*. 4TH Edition. CRC Press Boca Raton. London, New York.
- [45]. 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.

- [46]. Kitagishi, K. & Yamane, I. Eds (1981). *Heavy Metal Pollution in Soils of Japan*, Japan Science Society Press, Tokyo 302.
- [47]. Krauskopf, K.B., (1979) Crustal abundances. *Introduction to Geochemistry* (2nd ed.): New York, McGraw Hill, 617 p.
- [48]. 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.
- [49]. Lars, J. (2003). Hazards of heavy metal contamination. *British Medical Bulletin* 68(1): 167-182.
- [50]. 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.
- [51]. McGrath, S.P. & Loveland, P.J. (1992). *The Soil Geochemical Atlas of England and Wales*. Blackie Academic Professional Glasgow.
- [52]. 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.
- [53]. Purefoy, C. (2010). Gold rush triggers deadly lead poisoning. *CNN* November 2010.
- [54]. 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.
- [55]. Richard, T. W., Patrick, V.B. & Douglas, B.K. (2007). Assessment for Non-radionuclides of lead. *EPA/600/140*.
- [56]. 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.
- [57]. 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.
- [58]. 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.
- [59]. 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.
- [60]. Sharma, P. & Dubey, R. (2005). Lead toxicity in plants. *Brazilians Journal of Plant Physiology* 17: 35-52.
- [61]. Shinwell, D.W. & Laurie, A.E. (1972). Lead and Zinc contamination of vegetation in the Southern Penines. *Environmental Pollution* 3: 391-301.
- [62]. Solomon-Wisdom, G.O., Michael. H. Ramsey and Elizabeth. A. John (2015). The Effects of More Realistic forms of lead heterogeneity in soil on uptake, biomass and root response of two Brassica species. *Advances in Research* 5(1): 1-26. ISSN 2348-0394 Doi: 10:9734/AIR/205/17975 www.sciencedomain.org
- [63]. 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.
- [64]. Subramanian, M.S (2011) Analysis of soils, sediments and biological specimens. *Environmental Chemistry and Analysis* 6(1):1-10.
- [65]. 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.
- [66]. 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.
- [67]. Thompson, M. & Walsh, J.N. (1983). *A Handbook of Inductively Coupled Plasma Spectrometry*. Blackie and Sons Ltd. London.
- [68]. 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.
- [69]. 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.
- [70]. United States Department of Agriculture (USDA) (2008). National Genetic Resources Programme. *Germplasm Resources Information Network*. 708: 1-33.

- [71]. United States Environmental Protection Agency (USEPA) (1997). Cleaning up the Nation's waste sites. *Markets and Technology Trends*. EPA 005/96/542.
- [72]. 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.
- [73]. 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.
- [74]. Vameralli, T., Bandiera, M. & Mosca, G. (2010). Field crops for phytoremediation of metal-contaminated land—a review. *Environ. Chem. Lett.* 8:1-17.
- [75]. 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.
- [76]. 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.
- [77]. 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.
- [78]. 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.
- [79]. Keever, G.J. (1994): BA-induced offset formation in *Hosta*. *J. Environ. Hort.* 12(1):36-39.
- [80]. 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.
- [81]. 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.
- [82]. 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.
- [83]. 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.
- [84]. 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.
- [85]. **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.
- [86]. United States Department of Agriculture (USDA) (2008). National Genetic Resources Programme. *Germplasm Resources Information Network*. 708: 1-33.
- [87]. 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.
- [88]. 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.
- [89]. 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.
- [90]. 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.

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